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World Journal of Gastroenterology

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AIMS AND SCOPE

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TOPIC HIGHLIGHT

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

Anti-TNF alpha in the treatment of ulcerative colitis: A valid approach for organ-sparing or an expensive option to delay surgery?

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Abstract

Ulcerative colitis (UC) is an inflammatory bowel disease affecting large bowel with variable clinical course. The history of disease has been modified by the introduction of biologic therapy, in particular Infliximab (IFX), that has demonstrated efficacy in inducing fast symptoms remission, promoting mucosal healing and maintaining long-term remission. However, surgery is still needed for UC patients: in case of failure of medical therapy and if acute complications or a malignancy occurred. Surgical treatment is associated with a short-term post-operative mortality and morbidity respectively of 0%-4% and 30%. In this study we systematically analyzed: the role of IFX in reducing the colectomy rate, the risk of post-operative morbidity in pre-operatively IFX-treated patients and the cost-effectiveness of IFX therapy. Four of 5 analyzed randomized controlled trials demonstrated that therapy with IFX significantly reduces the colectomy rate. Moreover, pre-operative treatment with IFX doesn't seem to increase post-operative infectious complications. By an economic point of view, the cost-effectiveness of IFX-therapy was demonstrated for UC patients suffering from moderate to severe UC in a study based on a cost estimation of the National Health Service of England and Wales. However, the argument is debated.

Key words: Ulcerative colitis; Infliximab; Colectomy; Post-operative complications; Cost-effectiveness; Inflammatory bowel disease

Core tip: The introduction of biologic therapy with Infliximab (IFX) has significantly modified the clinical course of ulcerative colitis. In this study we systematically analyzed the role of IFX in reducing the colectomy rate, the risk of post-operative morbidity in pre-operatively IFX-treated patients and the cost-effectiveness of IFX therapy.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease, of an unknown etiology, affecting the large bowel. It is characterized by a contiguous mucosal inflammation

starting in the rectum and proximally progressing in continuity in the colon for a different distance. According to the Montreal Classification, which describes the maximal macroscopic extent of the disease at colonoscopy, the distribution of UC is commonly classified as: proctitis, left-sided and extensive colitis[1-4]. Disease activity is grouped into remission, mild, moderate, or severe but the clinical course of UC is variable and can range from a long-standing remitting to a refractory or fulminant disease[5-11]. Solberg *et al*[5], in a population-based cohort study of 843 patients with inflammatory bowel disease, enrolled in South-Eastern Norway and systematically followed-up at 1, 5 and 10 years after diagnosis, identified 4 different clinical patterns: (1) initial high activity to remission or mild severity (55%); (2) chronic intermittent symptoms (37%); (3) continuous symptoms (6%); and (4) initial low activity to increased severity (1%)[12]. The main symptoms of UC are bloody diarrhea and abdominal pain, associated with urgency and tenesmus. UC is conventionally treated with a step-up approach, based on the severity and extent of the disease, including various agents such as 5-aminosalicylates, corticosteroids and immunosuppressants (including thiopurines and cyclosporine). The primary aims of medical therapy in UC should be inducing and maintaining long-term remission, achieving mucosal healing, minimizing steroid-dependence, avoiding serious complications (hospitalization and surgery) and improving patients' quality of life[13-17]. However, standard therapy is not always able to achieve these goals and patients become steroid-dependent or experience frequent or severe relapse, with consequent increased risk of hospitalization and surgery. The history of disease has been partially modified by the introduction of biologic therapies. Infliximab (IFX) has demonstrated efficacy in inducing fast symptoms-remission, promoting mucosal healing and maintaining long-term remission[17-20]. It is currently approved for patients with moderate to severe UC who have incomplete response, are intolerant or have any medical contraindications to corticosteroids and/or immunomodulators[21-24]. It is also recommended as rescue therapy in severe steroid-refractory disease[25-27] and in steroid-dependent patients[28,29].

Surgery is still needed for UC patients, in case of failure of medical therapy, occurrence of acute complications (such as fulminant colitis, toxic megacolon and bowel perforation) or development of malignancy. Since its first description (1978), restorative proctocolectomy with ileal pouch anal anastomosis (IPAA) represents the gold standard of surgical treatment of UC: all the colon and rectum are removed and a J pouch is created with terminal ileum and anatomized to the anal canal. This restorative operation, avoiding a permanent stoma, maintains intestinal continuity and preserves the patient's body image[30-33]. Moreover, the introduction of minimally invasive approach significantly contributed in improving the acceptance and tolerability of this procedure[34-37], reducing the rate of post-operative adhesions and post-operative hospital stay and improving cosmetic results[38-41]. However, even if in skilled hands, proctocolectomy with IPAA is not without risk and is associated with an estimated short-term mortality ranged between 0% and 4% and a morbidity rate of about 30%, with an incidence of pelvic sepsis ranging from 5% to 24% and a re-surgery risk of about 16%[42,43].

If surgery represents a definitive solution for cessation of symptoms, withdrawing medical therapies and reducing the cancer-risk, it is not free of long-term post-operative morbidity (as pouchitis, fecal incontinence, female fecundity or fertility) with a relevant impact on patients' quality of life[44-46]. Population-based studies have reported a 10-years cumulative risk of colectomy ranging between 9%-30%, with some differences among countries. Approximately, 4% to 9% of UC patients will require colectomy within the first year of diagnosis and, subsequently, the risk of colectomy increases of 1% per year[47-50].

It is still under debate whether, in the long-term, the biological therapies could be a valid approach for organ-sparing, rather than an expensive option to delay surgery. Aim of this review was to evaluate the real impact of biological therapy on the rate of colectomy in UC patients.

A review of the literature searching for the terms "anti-tumor necrosis factor-α (TNF-α)", "infliximab" matched with the terms "ulcerative colitis" and "surgery" was performed, using PubMed, MEDLINE, EMBASE and Cochrane databases. All relevant articles (both experimental and observational studies) in English between January 2000 and July 2013 were reviewed.

Therapy with infliximab and rate of colectomy

We identified five randomized controlled trials[51-54], one meta-analysis[55] and six observational studies[56-61] following literature search. Characteristics of the studies are summarized in Table 1.

RANDOMIZED CONTROLLED TRIALS AND META-ANALYSIS

Two randomized, double-blind, placebo-controlled trials, ACT 1 and ACT 2, demonstrated the efficacy of IFX for induction (week 8) and maintenance (week 30 and week 54 for ACT1) of clinical response and remission in patients with moderate-to-severe UC, despite the use of conventional therapy[51]. Further analysis, from ACT 1-2 open-label extension phase, focused on colectomy and hospitalization rates during follow-up to 54 wk. Compared with placebo, the cumulative incidence of colectomy through 54 wk for IFX was significantly lower (10% *vs* 17%, $P = 0.02$), with an absolute risk reduction of 7% (95%CI: 0.01-0.12, HR = 0.59). Moreover, in IFX-treated patients were recorded fewer (compared to placebo group) UC-related hospitalizations and surgical procedures per 100 patient-years of treatment (40 *vs* 20, $P = 0.003$; 34 *vs* 21, $P = 0.03$ respectively)[52].

Previous controlled smaller studies have addressed the risk of colectomy in patients with severe UC treated

Table 1 Rate of colectomy after therapy with Infliximab

Ref.	RCT	Pts (*w*)	Tipe off disease	FU	Rate of colectomy in IFX Pts	Rate of colectomy in control Pts	*P* value
Rutgeerts *et al*[51] Sandborn *et al*[52]	Y	364 (IFX) 364 (control)	Moderate to severe UC	54 wk (ACT1) 30 wk (ACT2)	9.5%	14.7%	< 0.05
Sands *et al*[53]	Y	8 (IFX) 3 (control)	Severe active steroid-refractory	2 wk	50%	100%	NS
Järnerot *et al*[25]	Y	24 (IFX) 21 (control)	Severe to moderate UC not responding to conventional therapy	3 mo	29.2%	66.7%	0.017
Gustavsson *et al*[54]	Y	24 (IFX) 21 (control)	Severe to moderate UC not responding to conventional therapy	36 mo	50%	76%	0.012
Aratari *et al*[56]	N	15 (IFX)	Severe steroid-refractory UC	26 mo	18%		
Teisner *et al*[57]	N	52 (IFX)	Acute, severe and chronic refractory UC	22 mo	27%		
Ferrante *et al*[58]	N	121 (IFX)	Acute severe refractory UC	33 mo	17%	-	-
Oussalah *et al*[59]	N	191 (IFX) Multicenter	UC	18 mo	18.8%	-	-
Desmond *et al*[60]	N	21 (IFX)	UC	14 mo	9.5%	-	-
Garcia-Planella *et al*[61]	N	22 (IFX)	UC	84 mo	27.3%	-	-

RCT: Randomized controlled trial; Pts: Patients; FU: Follow-up; IFX: Infliximab; UC: Ulcerative colitis; Y: Yes; N: Not.

with IFX as rescue therapy. In 2001 Sands reported data on 11 patients with severe steroid-refractory disease, of whom 8 treated with IFX and 3 with placebo. After 2 wk, all patients treated with placebo underwent to surgery, while only 50% of patients receiving IFX needed surgery; however, the sample size was too small to detect a statistically significant benefit[53]. Later, 45 patients with moderate to severe UC were randomized to IFX or placebo (24 *vs* 21 respectively) both after four day from the start of corticosteroid treatment. In the placebo group more patients (14/21, 66.7%) than IFX group (7/24, 29.2%) had a colectomy (P = 0.017; OR = 4.9; 95%CI: 1.4-17) within 3 mo after randomization[25]. After a follow-up of 3 years, 50% of patients in the IFX group and 76% in the placebo group had a colectomy (P = 0.012)[54].

Recently, Costa *et al*[55] presented data from a meta-analysis on the benefit of IFX in reducing hospitalization and/or major surgeries in patients with inflammatory bowel disease. They analyzed 11 studies: 5 randomized controlled trials (RCTs) and 6 observational studies. In the RCTs, IFX treatment was associated with a significant 43% odds reduction of overall major surgery risk (OR = 0.57; 95%CI: 0.37-0.88) with a number-to-treat to avoid colectomy of 11 (95%CI: 6-51) for 1.2 years. However, a not significant increase was found in pooled results from observational studies (OR = 1.43; 95%CI: 0.65-3.13). The authors concluded that this discrepancy could be explained by the heterogeneity of observational studies, including patients at high risk of colectomy due to more severe disease and refractoriness to previous treatment.

OBSERVATIONAL STUDY

The first data on the long-term risk of colectomy were reported in a study of 314 UC patients from Italy. Among them, 52 (16.5%) patients had severe UC and were treated with intravenous corticosteroids for a median of 7 days. Of 15 patients who did not respond, 11 received IFX with short-term clinical benefit and 4 underwent urgent colectomy. In the long-term follow-up, another 6 patients underwent elective colectomy for a disease relapse, with a total colectomy rate, following the acute flare-up, of 19%. The long-term colectomy risk was not different between patients treated with IFX and steroid-responsive patients (18% *vs* 11%, respectively), as IFX was able to avoid urgent colectomy, but not to reduce the risk of elective surgery[56]. The risk of long-term colectomy in severe UC was also evaluated in a smaller Danish study of 52 UC patients. Nineteen (37%) patients had severe UC and 7 of them (37%) underwent colectomy after a median follow-up of 22 mo (range 4-57 mo). Among the remaining patients with a chronic refractory UC, the colectomy rate was 21%. The authors concluded that IFX can avoid colectomy in two-thirds of the patients with acute, severe UC, but the beneficial effect on colectomy rate in chronic, refractory UC seems less convincing[57]. Long-term data on colectomy in UC patients treated with IFX come from referral centers studies. In the Leuven's cohort of 121 refractory UC patients (patients with acute severe attack, refractory to intravenous steroids were excluded), 21 patients (17%) came to colectomy and 68% of initial responders achieved a sustained clinical response during a median follow-up of 33 mo (IQR 17-49.8). Lack of short-term clinical benefit, high values of baseline C-reactive protein (CRP) and previous intravenous steroid or cyclosporine treatment were identified as independent predictors of colectomy[58]. These results are similar to those reported in a French multicenter study, in which, among 191 patients who received at least one IFX infusion, 36 patients (18.8%) underwent colectomy during a median follow-up of 18 mo (IQR 8-32). Independent predictors of colectomy were: no clinical response after IFX induction, high baseline CRP value, previous treatment with cyclosporine and IFX indication for acute severe UC[59]. Furthermore, other experience supported history of hospital admission as an indepen-

Table 2 Rate of overall post-operative morbidity and post-operative infectious complications in patients pre-operatively treated with Infliximab

Ref.	RCT	Pts (IFX *vs* control) (*n*)	PO morbidity (IFX *vs* control)	*P* value	PO infectious complications (IFX *vs* control)	*P* value
Schluender *et al*[64]	N	17 *vs* 134	36% *vs* 28%	> 0.05	18% *vs* 8%	> 0.05
Selvasekar *et al*[62]	N	47 *vs* 254	62% *vs* 49%	0.10	28% *vs* 10%	< 0.01
Mor *et al*[63]	N	46 *vs* 46	34.8% *vs* 15.2%	0.004	21.7% *vs* 2.2%	0.011
Ferrante *et al*[65]	N	22 *vs* 119	11.1% *vs* 28.6%	> 0.05	9% *vs* 24%	0.161
Gainsbury *et al*[66]	N	29 *vs* 52	44.8% *vs* 44.2%	0.96	17.2% *vs* 26.9%	0.32
Rizzo *et al*[67]	N	16 *vs* 22	37.5% *vs* 22.7%	> 0.05	18.7% *vs* 18.2%	> 0.05

RCT: Randomized controlled trial; Pts: Patients; PO: Post-operative; IFX: Infliximab; Y: Yes.

dent predictor of the need of colectomy[60,61].

Peri-operative infliximab and post-operative outcome

An increasing number of patients undergo to surgery after experienced biologic therapy. There is an emerging concern on the safety profile of IFX in the peri-operative setting in potentially pre-surgical patients. Many groups have reported their experiences for UC patients and there is not an agreement on the impact of these drugs on post-operative complications[62-67]. The main characteristics of the studies are summarized in Table 2. Recently, Yang *et al*[68] performed a high quality meta-analysis based on five studies, including 706 patients, who were treated with IFX before restorative procto-colectomy with IPAA. The authors did not find a strong association between pre-operative treatment with IFX and short-term infectious complications (OR = 2.24), but it was associated with a significantly increased risk of short-term overall post-operative complications (OR = 1.80). However, these results need to be interpreted with caution. The subgroup analysis was underpowered to assess the nature of these complications because of the small sample size and heterogeneity of the included studies (end-points, patients' characteristics and indication, type and timing of surgery).

Infliximab and surgery: Cost-effectiveness

An important issue of IFX therapy is its cost-effectiveness. IFX is often perceived to be an expensive treatment option for patients with IBD. Tsai *et al*[69] made a cost-effectiveness analysis in UC patients based on a cost estimation of the National Health Service of England and Wales for the year 2006-07. At analysis of responders patients only, therapy with IFX was associated at an additional 0.753 quality-adjusted life year (QALYs) at an additional cost of £20662 compared to standard care without IFX; the estimated incremental cost per QALY gained for IFX against standard care was £27424. At analysis of remission patients, therapy with IFX derived an additional 0.387 QALYs at an additional cost of £7615 compared with standard care without IFX. The estimated incremental cost per QALY gained for IFX against standard care was £19696. The authors conclude that therapy with IFX appears to be a cost-effective treatment option for adult patients suffering from moderate to severe UC. In a recent study, Park *et al*[70] created a Markov model simulating 2 cohorts of 21-year-old patients with severe UC, following them until 100 years of age or death, comparing early colectomy with IPAA strategy to the standard medical therapy strategy (including IFX). In this study standard medical therapy accrued a discounted lifetime cost of $236370 per patient; in contrast, early colectomy with IPAA accrued a discounted lifetime cost of $147763 per patient. QALY-gained for standard medical therapy was 20.78, while QALY-gained for early colectomy with IPAA was 20.72; the resulting incremental cost-effectiveness ratio was approximately $1.5 million per QALY-gained. So, the authors concluded that early colectomy with IPAA after diagnosis of severe UC reduce health care expenditures and provides comparable quality of life compared exhaustive standard medical therapy. Only an extremely low quality of life after IPAA could maintain the standard medical therapy strategy as the optimal management strategy in severe UC.

CONCLUSION

IFX has demonstrated efficacy in inducing and maintaining clinical and endoscopic remission in the long run. IFX can also avoid urgent colectomy in patients with severe acute UC refractory to intravenous steroids. The real impact of biological therapy on the natural history of UC is still controversial, whereas it is not clear if it allows avoidance of colectomy or rather than a delay. The median colectomy risk for UC patients treated with IFX is about 10%-20% in both RCTs and observational studies, with higher rate for patients with severe acute attack. Data from RCTs support the efficacy of IFX in reducing the risk of surgeries in the long-term, but none was designed to assess IFX effects on surgeries. Real life data from referral centers do not confirm this issue, but each study includes patients with different baseline characteristics and risks of colectomy. From these evidences, it seems that patients with more severe disease, high inflammation burden, refractoriness to intravenous steroids and/or cyclosporine and history of hospitalizations, have higher risk of colectomy. So, it is necessary for physicians taking in account the risks and the benefits of medical

versus surgical therapy, concerning about cost and side effects of medications, cost and morbidity of surgery and patients' quality of life. Prospective, specifically designed studies are necessary to assess the long-term risk of colectomy in UC patients treated with IFX.

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TOPIC HIGHLIGHT

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

Inflammatory bowel disease and celiac disease: Overlaps and differences

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Abstract

Recent findings demonstrate the common genetic basis for many immune-mediated diseases, and consequently, the partially shared pathogenesis. We collected these findings and reviewed the extension of these overlaps to other disease characteristics. Two autoimmune diseases were selected that also share the specific target organ, the bowel. The etiology and immunopathogenesis of both conditions characterized by chronic intestinal inflammation, inflammatory bowel disease (IBD) and celiac disease (CeD), are not completely understood. Both are complex diseases with genetics and environment contributing to dysregulation of innate and adaptive immune responses, leading to chronic inflammation and disease. CeD constitutes a particular disease because the main environmental and genetic triggers are largely known. IBD comprises two main clinical forms, Crohn's disease and ulcerative colitis, which most likely involve a complex interplay between some components of the commensal microbiota and other environmental factors in their origin. These multifactorial diseases encompass a broad spectrum of clinical phenotypes and ages of onset, although the clinical presentation often differs depending on childhood or adult onset, with greater heterogeneity commonly observed in adults.

Key words: Disease susceptibility; Gene-environment interaction; Immune system; Inflammation; Microbiota; Inflammatory bowel disease

Core tip: Inflammatory bowel disease and celiac disease are two immune-mediated diseases characterized by chronic intestinal inflammation. Recent findings demonstrate shared genetics and functional pathways. We reviewed the extension of these overlaps to other disease features and suggest future research approaches.

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INTRODUCTION

The immune system is essential for defense self against external pathogens, and to maintain homeostasis. Dysregulation between those two processes contributes to development of immune-mediated diseases. These diseases represent an important cause of chronic illness, with a consequent high impact on public health. Different diseases can be found under this term, including autoimmune and inflammatory conditions. In these cases, it is common to find a specific organ affected, as occurs in inflammatory bowel disease (IBD) and celiac disease (CeD), both involving damage of the gastrointestinal tract. The gut is highly exposed to exogenous and endogenous antigens and controlled inflammation is a key process in maintaining homeostasis. Different factors can contribute to alter this equilibrium and to disrupt health status. Decades of research have focused on identifying those contributing factors and the reason why one specific disease develops. Recent findings demonstrate the extensive overlap in the genetic basis of immune-mediated diseases, including IBD and CeD.

The present review summarizes the current knowledge of different features related to the two major clinical forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC), and CeD, paying special attention to the overlaps and differences between them. These two diseases share genetic risk factors, and it would be interesting to know whether this overlap also extend to other disease characteristics in order to gain knowledge about common pathogenic mechanisms and possible shared treatments.

CeD is one of the most frequent immune-mediated diseases. In Europe and the US the current prevalence of CeD is around 1 per 100 individuals[1,2] and a similar prevalence probably exists worldwide, although it has not been as extensively studied[3,4]. IBD shows a lower prevalence with values ranging from 26 to 199 per 100000 individuals in CD and from 37 to 246 per 100000 individuals in UC[5]. The prevalence of IBD is higher in developed countries and urban areas. A recent increase in the prevalence of both CeD and IBD has been described as a consequence of several factors[6-8]. In CeD these factors include the development of more effective diagnostic tools[9].

IBD affects both sexes similarly and the highest incidence is found between the second and the fourth decade of life. CeD seems to be more frequent in women[10], although this depends on the age at onset[11]. CeD can be diagnosed in individuals at any age, but it appears more frequently during childhood[12].

The prevalence of CeD in patients with IBD is not clear. There are several cases in the literature describing the coexistence of both diseases in the same family or even in the same patient[13-18]. However, some authors consider that this is an incidental association and the prevalence of CeD is similar between IBD patients and the general population[19]. In fact, CD and UC patients are not considered a risk group for routine CeD screening.

CLINICAL PRESENTATION

A wide spectrum of clinical symptoms characterizes IBD and CeD. In addition, differences in the clinical presentation can be found depending on the age at diagnosis.

IBD manifests during childhood or adolescence in at least 20% of patients. This presentation is commonly more severe and extensive than that observed in adult-onset disease[20]. In CD, involvement of the upper gastrointestinal tract is more frequently observed at early onset[21]. Bloody, mucous diarrhea is the almost universal hallmark of UC, although additional symptoms may be also present. The initial symptoms of CD are more subtle and varied, partly as a result of its diffuse and diverse anatomical location. The constellation of abdominal pain, diarrhea, poor appetite and weight loss constitutes the classical presentation of CD in all age groups, and is the mode of presentation in nearly 80% of children and adolescents (with or without extraintestinal manifestations). Abdominal pain is the most common single symptom at presentation[22,23].

In CeD, the presentation may be variable, but diarrhea, which may be acute or insidious in onset, is the most common presenting symptom in children. On the contrary, mild and nonspecific gastrointestinal symptoms are common in adults with CeD, with intermittent diarrhea, diffuse abdominal pain, dyspepsia, constipation, asthenia, flatulence, bloating or abdominal discomfort being the most frequently observed symptoms. In adults, iron-deficiency anemia without response to any appropriate treatment is a frequently observed sign[24,25].

Extraintestinal manifestations may be present in IBD and CeD. In IBD, most of the extraintestinal manifestations are shared by CD and UC. They may accompany intestinal symptoms or, less commonly, precede or overshadow them. These manifestations seem to be related to activity (relapse/remission) and location of the disease. Although children and adults may share extraintestinal manifestations, their frequency is usually different. Ocular lesions seem to be less common in young patients and a childhood-onset of IBD, particularly CD, may represent a specific risk factor for long-term morbidity from clinical osteoporosis[26].

In CeD, extraintestinal manifestations are usually a consequence of nutrient malabsorption and may coexist with digestive symptoms. It is currently accepted that extraintestinal signs and symptoms are common and may be the only presenting manifestation, mainly in adults[25,27].

Table 1 summarizes the main clinical features for IBD and CeD. Several overlapping characteristics can be observed. Diarrhea and abdominal pain are digestive symptoms commonly observed in both groups, but they also share several extraintestinal manifestations, as iron-deficiency anemia, short stature or osteoporosis. Some

Table 1 Main clinical features associated with inflammatory bowel disease and celiac disease

IBD	CeD
Intestinal mucosal involvement	Intestinal mucosal involvement
Clinical heterogeneity	Clinical heterogeneity
Depending on location and severity	Depending on degree of gluten sensitivity and amount of gluten ingested
Symptomatic (relapses/remission)	Commonly symptomatic (early onset)
	Mono or oligosymptomatic (late onset)
Digestive signs or symptoms	Digestive signs or symptoms
Diarrhea (± rectorrhagia)	Diarrhea
Abdominal pain (less predominant in UC)	Abdominal distension
	Abdominal pain
	Constipation
	Dyspepsia
	Recurrent vomiting
	Pyrosis and regurgitation
	Irritable bowel syndrome with diarrhea predominance
Extraintestinal manifestations	Extraintestinal manifestations
Refractory iron-deficiency anemia	Refractory iron-deficiency anemia
Short stature	Short stature
Poor appetite	Failure to thrive
Weight loss (less prevalent and extreme in UC)	Dermatitis herpetiformis
Sexual maturation delay	Vitamin B12 deficiency
Pneumopathies	Neurological symptoms
Psychological syndromes	Menstrual disturbances
Joints: arthritis and arthralgias (the most common in both CD and UC)	Bleeding diathesis (malabsorption of vitamin K)
Ocular: acute episcleritis, uveitis, orbital myositis	Paresthesia, cramps and tetany (hypocalcemia)
Skin: erythema nodosum, pyoderma gangrenosum	Hepatobiliary system: hypertransaminasemia
Hepatobiliary system: primary sclerosing cholangitis (less predominant in CD), autoimmune hepatitis (unusual)	Osteopenia, osteomalacia and osteoporosis
	Edema, ascites and anasarca (hypoproteinemia)
Renal system: ureteral obstruction, hydronephrosis, urinary stones	Hypopituitarism and adrenal insufficiency
Vascular system: thrombocytosis, hyperfibrinogenemia, elevated factor Ⅴ-Ⅶ, depression antithrombin Ⅲ	Recurrent mouth ulcers
Bone: osteoporosis (less predominant in UC)	
Severe complications	Severe complications
Malnutrition with weight loss and emaciation	(In refractory CeD or in patients who do not follow a GFD)
Fistulae	Collagenous CeD
Abscesses	Ulcerative jejunitis
Obstruction	T cell lymphomas
Perforation	
Dysplasia and colorectal cancer	

IBD: Inflammatory bowel disease; CeD: Celiac disease; UC: Ulcerative colitis; CD: Crohn's disease; GFD: Gluten-free diet.

differences are also observed. CeD patients can remain asymptomatic, in contrast with the symptomatic IBD patients.

The major complications of CeD, ulcerative jejunitis and intestinal lymphoma, cause more severe clinical manifestations that may resemble CD, such as acute and persistent abdominal pain, weight loss, signs of intestinal obstruction or gastrointestinal bleeding, fever or signs of marked malnutrition[28]. It has been suggested that complicated CeD should be considered in CD patients who do not respond to immunosuppressive or biological treatments[29].

SEROLOGY

As frequently observed in autoimmune diseases, CeD is characterized by the presence of autoantibodies, which are currently included in the definition and diagnostic guidelines for CeD[30]. Transglutaminase type 2 (TG2) is the major autoantigen in CeD and the target antigen for endomysial antibodies (EMA) and anti-TG2 antibodies. Therefore, those two antibodies are the most specific for CeD diagnosis. Although a high correlation exists between anti-TG2 and EMA antibodies, the highest specificity is observed for EMA, because anti-TG2 can be present in individuals with other conditions, including CD and UC. However, it is difficult to know the frequency of anti-TG2 antibodies in IBD patients because the studies developed with that aim have yielded different results, probably partially caused by the wide variety of commercial kits used[18,19,31-35].

On the contrary, the presence of specific antibodies is not a common feature in IBD. Two major groups of serological markers have been described in these patients: those against microbial antigens and autoantibodies. Their relevance for IBD diagnosis is not as strong as for CeD, but they are useful to differentiate CD from UC patients. Among the antibodies against microbial agents, those against *Saccharomyces cerevisiae* (ASCAs) are the most extensively studied and they are related to CD. These anti-

bodies have also been described in CeD patients but they disappear after taking a gluten-free diet (GFD), which is supposedly due to the association of ASCAs with inflammation of the small bowel, and therefore, questions their specificity for CD[36]. Regarding autoantibodies, antineutrophil cytoplasmic antibodies have a high prevalence in UC[37].

ETIOLOGY

The causes underlying the development of IBD and CeD have not been completely unraveled, but both diseases show a multifactorial origin with a complex genetic and environmental involvement.

Environment

In this regard, CeD is the best-understood immune-mediated disease because the main environmental factor involved is largely known. CeD is triggered by ingestion of dietary wheat gluten or analogous proteins present in other cereal grains, mainly rye and barley.

Although gluten intake is necessary to develop CeD, other environmental factors may play a role. Infections have been related to CeD development. Specifically, rotavirus[38] and hepatitis B and C virus infections have been observed in CeD patients[39]. A protector role for breastfeeding at the moment of gluten introduction has also been described[40].

Viruses have also been implicated in the origin of IBD. The hygiene hypothesis establishes that lack of early exposure to microbial agents due to severe hygienic conditions could increase the likelihood of developing autoimmune and allergic disorders, and it has been used to explain the rising prevalence of IBD observed in industrialized countries[41]. Although to a lesser extent, this hypothesis has also been proposed for CeD[42]. Recent genetic findings support a role of pathogens in IBD and CeD.

Other environmental factors contributing to IBD risk are smoking and appendectomy. The effect of cigarette smoking is opposite in both forms of IBD: beneficial in UC and harmful in CD[43,44]. For appendectomy, it seems that it reduces the risk of UC[45,46].

Vitamin D levels, diet, hormone use and stress have also been postulated as risk factors for one or both main forms of IBD, but they need to be further investigated[47].

The environmental influence in IBD pathogenesis seems to involve complex mechanisms because its role in disease risk may be modified by other factors such as sex, geographic region, or genetic background[47]. Moreover, environmental factors are probably influencing the natural history in addition to the origin of these diseases.

Microbiota

An altered microbiota composition seems to be a common phenomenon in intestinal inflammatory disorders. In IBD, an abnormal response to the normal commensal flora of the bowel is considered to cause the disease[48,49]. The role of the microbiota in the pathogenesis of IBD was first suggested by studies in mice, which showed a lack of experimental colitis in animals kept in a germ-free environment[50]. Since then, numerous works have been published in this field. Although no final conclusions can be drawn, it is clear that no single pathogen is associated with the disease, and quantitative besides qualitative changes in the microbiota influence disease development[51,52].

Microbiota alterations have also been related to CeD risk, again with quantitative and qualitative changes reported[53,54]. In CeD, an altered microbial diversity depending on the clinical presentation has been recently described, with marked differences between patients showing classical gastrointestinal symptoms and those with extraintestinal manifestations[55].

The influence of diet (including breastfeeding) and cigarette smoking or the increased risk of disease in children born by Cesarean section have been postulated to be mediated through changes in the microbiota. These changes have also been linked to the increasing incidence of IBD and CeD in recent decades.

Nowadays it is clearly accepted that the intestinal microflora differs between healthy individuals and those showing CeD or IBD. It has been claimed that those differences could be a consequence of the disease. Among other functions, commensal bacteria of the gut contribute to protection against external pathogens and participate in the maturation of the mucosal immune system, supporting their role in the etiology of these diseases. Recent genetic studies are also concordant with a causal role[56]. Nevertheless, a complex situation exists because the microbiome can be altered as a result of infection or pathological processes.

Genetics

The genetic contribution to disease risk differs between CeD and IBD. The highest values are observed for CeD (75% of concordance between monozygotic twins)[57], followed by CD (44%-50%) and UC (16%)[58].

Knowledge of the genetic basis of immune-mediated diseases has dramatically increased in recent years with the advent of genome-wide association studies (GWAS). These studies analyze hundreds of thousands of common [minor allele frequency (MAF) > 5%] genetic variants (single nucleotide polymorphisms, SNPs) across the human genome, looking for variants with a different frequency between individuals showing the disease and the general population. They need high numbers of affected and unaffected individuals that provide enough statistical power to find significant associations. Initially, GWAS included around 1000 individuals with each phenotype, but this number has been increased in recent GWAS. Moreover, follow-up of the nonsignificant most associated SNPs and meta-analysis of previously published largescale studies have also been performed. Additionally, cross-disease meta-analyses that combine data of previous GWAS have been performed to identify susceptibility loci common to different immune-mediated diseases. Looking for variants shared between CD and CeD, these

studies have identified four new shared loci[59]. New approaches to study the genetic basis of IBD and CeD include the Immunochip Project, also based on the presence of a common genetic basis for immune-mediated diseases but focused on deep replication and fine mapping[56,60]; and the recently published high-throughput exon-sequencing of 25 GWAS risk genes[61].

GWAS are not based on prior hypothesis determined by previously available information (*e.g.,* gene function, previous association studies, and animal models) and the studied SNPs are selected to cover a high proportion of gene variation across the genome. With this approach, unexpected genes have been identified as related to disease susceptibility. The newly identified genes point out functional pathways involved in particular phenotypes, some of them also previously unexpected.

IBD and CeD show both a complex genetic basis that is characterized by the presence of numerous common susceptibility factors contributing a small risk to disease susceptibility. In IBD, no factor seems either necessary or sufficient to develop the disease, as it is commonly observed in complex diseases. However, CeD constitutes a particular case. It is commonly accepted that the main genetic risk alleles, those coding the HLA-DQ2 or -DQ8 heterodimers, are necessary although not sufficient to develop CeD, because they are present in almost all CeD patients.

The influence of the HLA region in disease risk marks more differences between IBD and CeD. This region, located on 6p21, contains hundreds of genes with immunological functions and it is responsible for the strongest association signals observed in most immune-mediated diseases. However, HLA influence is different between IBD and CeD, and these two diseases are at opposite ends of the spectrum. HLA loci are the main genetic susceptibility factors for CeD and they are responsible for 40% of the genetic risk; in addition, their functional involvement in disease pathogenesis is well established. On the contrary, a weak and a weak-moderate association is found in CD and UC, respectively. Moreover, the HLA alleles associated with IBD are markers of still unknown HLA risk variants[62].

Additional to the HLA region, 163 loci have been associated with IBD risk: 110 common to CD and UC, 30 specific for CD and 23 for UC[56]. For most of the specific loci, the same direction of effect exists in the two forms of IBD and only two loci (*NOD2* and *PTPN22*) have shown significant opposite effects between CD and UC[56]. In CeD, 40 susceptibility loci have been described[60,61]. The number of associated SNPs is even higher, because one SNP does not always account for the risk overall attributed to one locus. The causal variant or even the genes responsible for the reported associations remain unknown for many of these regions. In CeD, the individual gene involved is known for half of the associated regions. In both diseases, independent effects of the associated variants have been reported, as well as correlation of genotypes for many SNPs with expression levels, and an important role of noncoding variants. This last observation has been recently underscored by the discovered negligible impact of rare variants (MAF < 5%) within exons[61], until now considered as potential relevant contributors to disease risk. The different number of variants associated with IBD and CeD is probably mirroring the different sample sizes used in the studies.

The large sample sizes required by GWAS have been achieved thanks to international collaborations, but this necessary effort may overlook genetic factors associated with specific populations. A new genetic region (22q13.2) has been recently associated with CD risk in a GWAS performed in a Southern European population[63]. This encourages us to study homogeneous groups of patients (in terms of ethnicity or clinical features) to look for new genetic susceptibility factors.

GWAS have found similar genes associated with IBD when considering childhood or adult onset[56], although the severe inflammation that mutations in interleukin (*IL*)-*10RB* cause in children suffering extreme phenotypes, identified through other kind of genetic studies, must be highlighted[64]. In CeD, studies considering the age of onset remain to be performed.

Seventy percent (113/163) of the IBD loci are shared with other complex diseases, and 12% (20) with CeD. The picture is different when the number of CeD risk regions is used as a reference; 50% of the CeD loci are shared with IBD. Independent of the real percentage, which is impossible to ascertain until scientific advances provide us with a full knowledge of the genetic basis of these diseases, the existence of a common genetic background is evident.

Despite the huge advance in the genetic basis of these chronic diseases, only 14% of the genetic variance is known in CD, 7.5% in UC, and approximately 50% in CeD. The highest values of CeD are due to the strong influence of the HLA, which accounts for 40% of the genetic variance.

IMMUNOPATHOLOGY

The model of immunopathogenesis for CeD has long been established. Dietary gluten induces innate and adaptive immune responses. The innate immune response is characterized by the gluten-induced production of IL-15, which acts on intraepithelial lymphocytes and licenses them to kill epithelial cells. This increases permeability and facilitates that gluten peptides pass through the impaired epithelial barrier into the lamina propria. In this compartment, TG2 induces deamidation of gluten-derived peptides, creating epitopes that bind efficiently to HLA-DQ2/DQ8 heterodimers on antigen-presenting cells, and thus elicits a T-cell response[65,66].

GWAS findings suggest four main processes underlying CeD: T-cell development in the thymus, innate immune detection of viral RNA, T and B cell co-stimulation (or co-inhibition) and cytokines, chemokines and their receptors. It seems now that a specific enrichment for genes involved in natural killer (NK) cell activation

and interferon γ production also exists. Thus, besides T cells, other cell types may have special relevance in the pathological process, as B cells, NK cells or neutrophils, but the previous model of pathogenesis remains valid and basically unchanged.

In IBD, the model of pathogenesis is based on the dysregulation of the normally controlled immune response to commensal bacteria, which could be precipitated by infection or by defects in the mucosal barrier. This involves infiltration of several cells of the immune system and chemokine and cytokine production, which in turn exacerbate the dysfunctional immune response and activate either T helper (Th)1 or Th2 cells in the gut mucosa, associated with CD and less conclusively with UC, respectively[67].

GWAS results have been crucial in advancing our understanding of IBD pathogenesis. Two major findings were the unsuspected role of autophagy and the implication of the Th17 immune response. The genes associated with CD and UC risk point to shared pathways involved in the pathogenesis of these two inflammatory conditions.

The genes shared between IBD and CeD are mainly related to the innate immune response against pathogens and to the activation of the immune system to produce inflammation, including T-cell differentiation and immune-cell signaling. Specific pathways like autophagy and Th17 response seem to be only involved in IBD. Autophagy is responsible for degradation of intracellular structures, but it is also important in removal and recognition of invasive pathogens. Its involvement in CD etiology was suggested after the association of *ATG16L1, LRRK2* and *IRGM* with CD risk. The implication of Th17 cells marks an important difference between IBD and CeD, because they have been associated with susceptibility to numerous immune-related diseases but not to CeD[60,68], which is still considered to be a Th1-mediated disease. Th17 cells are involved in defense against extracellular pathogens but they act as potent inducers of autoimmunity through their involvement in tissue inflammation and are probably linked to innate and adaptive responses[69]. *IL23R* was the first Th17 gene found in genetic association studies, but it was followed by numerous related loci: *IL22*, *IL17A*, *IL17F*, *TYK2*, *JAK2*, *CCR6* and *STAT3*.

In Figure 1, all the genes associated with IBD and CeD in large scale studies are shown. They have been grouped according to their predominant role in three major functions: innate immune response, adaptive immune response, and epithelial barrier function. All the genes with a different role or with still unknown function have been grouped as "others". The highest number of shared genes between IBD and CeD is observed for genes involved in adaptive immunity. The specific association with UC risk for most of the loci involved in barrier function is noteworthy.

Both CeD and IBD need an environmental stimulus that activates the immune system and leads to the pathological process. The amplification of the immune response involves release of cytokines, molecules involved in intracellular signaling, and transcription factors. GWAS have found many genes coding for products related to these processes. The initial stimulus to trigger the disease is different and it seems to be crucial in developing one or other disease, probably in combination with the genes specific for each disease.

Despite the scientific revolution prompted by GWAS, some issues still hamper a direct translation to disease pathogenesis: (1) in many regions, the genetic variant or even the gene responsible for the association remain to be identified; (2) many of the proteins detected are pleiotropic, with different possible roles and with still unknown functions; and (3) the immune responses, with complex networks of interactions that include specific molecules inducing up- or downregulation of the same functional pathway depending on the microenvironment.

TREATMENT

There is no single effective treatment for all IBD patients. Therefore, different treatments are used to manage the disease, often several of them in the same individual. Conventional immunosuppressive drugs, including azathioprine, mercaptopurine and methotrexate, are the initial treatment for IBD. When the immunosuppressive therapy losses efficacy or the patient continues with active disease, alternative biological therapies based on tumor necrosis factor (TNF)-α blockade are commonly used in IBD. In recent years, infliximab and adalimumab became standardized biological therapies in IBD. Infliximab is a chimeric monoclonal IgG1 anti-TNFα and it is indicated in refractory CD[70,71] and in acute severe UC[72]. Despite the high efficacy of infliximab, some patients do not respond to this treatment. An alternative therapy for these patients is adalimumab, an humanized TNF-α antibody, which decreases the risk of developing antibodies[73-75]; one of the causes of non-response to infliximab[76]. Based on the results of genetic studies, new biological therapies are currently being tested or are already in clinical use in IBD patients[77-80].

In CeD, treatment is a lifelong GFD. This is an effective and safe treatment, but there is a small group of patients who do not respond. Refractory CeD patients (RCeD) are defined as those showing persistent villous atrophy, crypt hyperplasia, and high levels of intraepithelial lymphocytes despite strict adherence to a GFD for > 12 mo[81,82]. There are two categories of RCeD, depending on the presence (type Ⅰ) or not (type Ⅱ) of aberrant intraepithelial T cells[83]. RCeD patients develop higher severe malnutrition combined with an increased risk for developing enteropathy-associated T-cell lymphoma[84]. Immunosuppressive therapy, similar to that used in IBD patients and based on azathioprine, cyclosporine or anti-TNF-α, is the current treatment for RCeD[84,85]. In the bad prognosis of RCeD, primarily type Ⅱ RCeD, chemotherapy shows moderate clinical, histological and hematological efficacy[86,87].

Figure 1 Immunopathology. Overlap between inflammatory bowel disease (IBD) [considering the two major forms Crohn's disease (CD) and ulcerative colitis (UC)] and celiac disease (CeD) for the loci identified by large scale genetic studies. Genes are grouped according to their participation in three main functional blocks: innate immunity, adaptive immunity, and intestinal barrier. All the genes with still unknown function are grouped as "others". Note that some genes can be found in more than one functional group.

New therapeutic approaches in CeD have increased in recent years. These therapies are focused on engineering gluten-free grains, decreasing the intestinal permeability by blockade of the epithelial zonulin receptor, inhibiting gluten peptide presentation by HLA-DQ2 antagonists, and inducing oral tolerance to gluten[88].

In both disorders, a delay in diagnosis or in proper treatment carries an increased risk of future complications. However, individuals with different autoimmune diseases can present similar symptoms, which sometimes makes it difficult to establish early diagnosis and treatment.

Reconstitution of the physiological flora remains an interesting therapeutic aim for both IBD and CeD.

FUTURE PERSPECTIVES

Despite the great advances in our understanding of IBD and CeD, we are still far from being able to anticipate who will develop some of these immune-mediated conditions. Ingestion of gluten and alterations in the commensal microbiota seem to be the main environmental triggers for CeD and IBD, respectively. However, it remains to be understood what is further needed to break the tolerance in specific individuals and develop disease.

Nevertheless, current advances related to the functional pathways involved in IBD are being useful in finding new drug targets. The shared molecular pathways between IBD and CeD open new possibilities for therapy in RCeD. With the identification of the causal variants in all the associated regions new clues about disease pathogenesis will be obtained and new treatment targets will appear.

Future epidemiological studies are necessary to gain knowledge about the genetic and environmental interactions that contribute to disease development. Better knowledge of the role of pathogens will also be useful to look for new therapies or prevent disease. Although numerous factors make it difficult to study the environmental contributing factors, some genes seem to point to specific triggers. Prospective as well as retrospective studies involving individuals with alterations in those genes could be performed, which aim to advance the role of those specific environmental triggers.

The multifactorial nature of the etiology of CeD and IBD likely hides great complexity due to the interplay among all the factors involved. The role of the microbiota seems to be influenced by interaction with external pathogens, but also by host genetic factors. More research is needed in this field, which will likely contribute to identifying where the missing heritability lies and a better understanding of the immunopathogenesis.

A broad range of symptoms characterize CeD and IBD. Subgroups of patients combining their clinical features with the presence of a similar genetic profile may help to establish more homogeneous groups to perform the next steps in research.

CONCLUSION

IBD and CeD are two immune-mediated disorders with a partially common genetic background. Overlaps between both disorders are also observed for other specific features; however, these conditions do not seem to be more strongly correlated with each other than with other immune-related disorders. The common clinical manifestations are probably a consequence of the target organ affected: the gut. Shared genetics originates the altered immune response and the inflammation characterizing both diseases. Nevertheless, comparison of these two diseases helps to understand what is specific for each disease and what is common. Common features may be useful to understand better the inflammatory processes and to look for new shared therapies.

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TOPIC HIGHLIGHT

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

Venous thrombosis and prothrombotic factors in inflammatory bowel disease

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Abstract

Patients with inflammatory bowel disease (IBD) may have an increased risk of venous thrombosis (VTE). PubMed, ISI Web of Knowledge and Scopus were searched to identify studies investigating the risk of VTE and the prevalence of acquired and genetic VTE risk factors and prothrombotic abnormalities in IBD. Overall, IBD patients have a two- to fourfold increased risk of VTE compared with healthy controls, with an overall incidence rate of 1%-8%. The majority of studies did not show significant differences in the risk of VTE between Crohn's disease and ulcerative colitis. Several acquired factors are responsible for the increased risk of VTE in IBD: inflammatory activity, hospitalisation, surgery, pregnancy, disease phenotype (*e.g.*, fistulising disease, colonic involvement and extensive involvement) and drug therapy (mainly steroids). There is also convincing evidence from basic science and from clinical and epidemiological studies that IBD is associated with several prothrombotic abnormalities, including initiation of the coagulation system, downregulation of natural anticoagulant mechanisms, impairment of fibrinolysis, increased platelet count and reactivity and dysfunction of the endothelium. Classical genetic alterations are not generally found more often in IBD patients than in non-IBD patients, suggesting that genetics does not explain the greater risk of VTE in these patients. IBD VTE may have clinical specificities, namely an earlier first episode of VTE in life, high recurrence rate, decreased efficacy of some drugs in preventing further episodes and poor prognosis. Clinicians should be aware of these risks, and adequate prophylactic actions should be taken in patients who have disease activity, are hospitalised, are submitted to surgery or are undergoing treatment.

Key words: Acquired; Genetic; Prothrombotic; Venous thrombosis; Risk of venous thrombosis; Inflammatory bowel disease

Core tip: In inflammatory bowel disease (IBD), there is an increased risk of venous thrombosis (VTE) due to inflammatory activity, hospitalisation, surgery, pregnancy, disease phenotype and drug therapy. Classical genetic alterations are not generally found more often in IBD patients than in non-IBD patients, suggesting that genetics does not explain the greater risk of VTE in these patients. IBD VTE may have clinical specificities, namely an earlier first episode of VTE in life, high recurrence rate, decreased efficacy of some drugs in preventing further episodes and poor prognosis.

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INTRODUCTION

The possible association between inflammatory bowel disease (IBD) and venous thrombosis (VTE) was first reported in 1936 by Bargen *et al*[1], who described 18 patients with thromboembolic disease (predominantly venous) from among more than 1000 patients treated for IBD at the Mayo Clinic. Since that time, several publications have suggested that patients with IBD have an increased risk of VTE, including deep venous thrombosis (DVT), pulmonary emboli, portal vein thrombosis, cerebral venous sinus thrombosis, Budd Chiari syndrome and retinal vein thrombosis[2-5]. The overall incidence rate of VTE in IBD patients has been estimated to be 1%-8%, although necropsy studies report an incidence of 39%-41%[2-5]. One systematic review[4] and one meta-analysis[3] showed a higher VTE risk in IBD patients, even after correction for known prothrombotic factors such as smoking and obesity[3]. Nevertheless, other studies, such as that of Grip *et al*[6], show a similar risk between IBD and the background population. However, in that report, where the incidence of VTE in the IBD cohort (0.15% per year) was comparable with that of the background population, the differences in age between the groups could have affected the conclusions[6].

It is important to stress that most of the evaluated studies were retrospective. When the IBD population was compared with other patients or healthy controls, most of the classical prothrombotic risks were not assessed, and therefore a bias could have been present. Therefore, the aim of this review was to assess the risk of VTE and the prevalence of acquired and genetic VTE risk factors and prothrombotic abnormalities in IBD.

SEARCH STRATEGY

A systematic review was conducted on published articles that assessed the risk of VTE and the prevalence of acquired and genetic VTE risk factors and prothrombotic abnormalities in IBD through a literature search of PubMed, ISI Web of Knowledge and Scopus. This search was performed in September 2013 using the following medical terms: "venous thrombosis IBD", "acquired venous thrombosis risk factor IBD", "genetic venous thrombosis risk factor IBD", "coagulation IBD", "fibrinolysis IBD", "platelets IBD" and "endothelium IBD". Additionally, a comprehensive search of reference lists of all review articles and original papers achieved by this method was performed to identify additional reports that could be included in the final analysis. Potential studies were initially screened by title and abstract. Potential exclusion criteria to reduce the risk of bias and unnecessary observations included case reports on single patients, book chapters and studies exclusively on arterial thrombosis. A total of 207 articles were studied to construct this review.

RISK OF VTE IN IBD

A summary of the controlled studies comparing the risk of VTE in IBD patients with the risk of VTE in non-IBD patients is presented in Table 1.

General risk

In one of the earliest studies evaluating the incidence of VTE in IBD patients, 61 out of 7199 patients (0.84%) developed VTE during an 11-year period from January 1970 to December 1980 at the Mayo Clinic, with similar rates of VTE observed in patients with Crohn's disease (CD) and ulcerative colitis (UC)[7]. In 2001, Bernstein *et al*[8] published the first study on the risk of VTE in IBD in a large population-based study using health administrative data from the province of Manitoba, Canada, in which they applied validated case ascertainment definitions of CD and UC (Table 1). The incidence rate for VTE in IBD patients was 45.6 per 10000 persons-year of follow-up, and IBD patients were 3.5 times more likely to develop VTE than the controls. Similar rates of VTE were observed in CD and UC and in males and females. The highest rates of VTE were observed among patients over 60 years old; however, the highest incidence rate ratio (IRR) for VTE was among patients younger than 40 years old (IRR = 6.02, 95%CI: 3.92-9.12). In 2004, Miehsler *et al*[9] compared the risk of VTE in patients with IBD and other chronic inflammatory diseases (rheumatoid arthritis and coeliac disease) with matched controls (Table 1). The subjects with IBD had a significantly higher risk of VTE compared with the matched controls [prevalence: 6.15% *vs* 1.62%; odds ratio (OR) = 3.6, 95%CI: 1.7-7.8], whereas the subjects with rheumatoid arthritis or coeliac disease had a risk of VTE similar to that of the controls. In 2007, the risk of VTE among 17 chronic illnesses was evaluated (Table 1)[10]. The relative risk (RR) of VTE was nearly twofold higher in IBD patients than in the matched controls (OR = 1.84, 95%CI: 1.29-2.63), with only cancer and heart failure carrying a greater risk of VTE than IBD.

IBD, activity, hospitalisation and surgery

Some studies have shown that the risk of VTE may be higher in UC than in CD [11,12], with other showing the opposite[13]; however, the majority of did not show significant differences in the risk of VTE between CD and UC[3,4,8,14,15]. A recent meta-analysis showed similar risks in patients with UC (RR = 2.57, 95%CI: 2.02-3.28; n = 6 studies) and CD (RR = 2.12, 95%CI: 1.40-3.20; n = 5 studies)[3].

Several studies reported IBD activity in 45% to 90% of patients at the time of VTE diagnosis[8,9,16-18]. The asso-

Table 1 Risk of venous thrombosis in inflammatory bowel disease patients relative to non-inflammatory bowel disease patients

Ref.	Design	Population		Risk measure (95%CI)	Controlled variables
		IBD	Controls		
Grip *et al*[6], 2000 Sweden	Retrospective cohort study Inpatients Records from 2 university hospitals	1253 patients	387 (significant age differences between the IBD cohort and controls)	Incidence rate of VTE 1.5/1000 IBD per year (comparable to the background population)	
Bernstein *et al*[8], 2001 Canada	Retrospective cohort study Inpatients Manitoba Health administrative 1984-1997	5529 patients	Approximately 55000 year, age, gender and postal area of residence matched members of the general population	DVT RR 4.7 (3.5-6.3) CD RR 2.8 (2.1-3.7) UC PE RR 2.9 (1.8-4.7) CD RR 3.6 (2.5-5.2) UC	
Miehsler *et al*[9], 2004 Austria	Retrospective cohort study Outpatients and inpatients Three outpatient clinics of Division of Gastroenterology and Hepatology	618 patients	707 age and gender matched controls	Incidence rate of VTE 6.2% IBD 1.6% Controls VTE aOR 3.6 (1.7-7.8) IBD	Operation, injuries, oral contraceptive use, pregnancy, body mass index and smoking
Bernstein *et al*[20], 2007 Canada	Retrospective cohort study Inpatients The Statistics Canada's Health Person Oriented Information database 1994-2004	About 22000 to 25000 patients	About 2.5 to 3.2 million age and gender matched controls	VTE ⩾ 50 yr old RR 1.3 (1.23-1.37) IBD < 50 yr old RR 1.57 (1.42-1.72) IBD	
Huerta *et al*[10], 2007 United Kingdom	Prospective cohort study with nested case-control analysis Outpatients and inpatients General Practice Research Database - GPRD 1994-2000	6550 patients	10000 age, gender and year matched controls	VTE OR 1.84 (1.29-2.63) IBD	
Nguyen *et al*[12], 2008 United States	Retrospective cohort study Inpatients Nationwide Inpatient Sample 1998-2004	116842 patients (73197 CD and 43645 UC patients)	522703 controls	VTE aOR 1.48 (1.35-1.62) DC aOR 1.85 (1.70-2.01) UC	Age, gender, calendar year, health insurance payer, comorbidity, presence of IBD related surgery, geographic location, and hospital characteristics
Ha *et al*[148], 2009 United States	Retrospective cohort study Outpatients and inpatients MarketScan Commercial Claims and Encounters database - Thomson Reuters 2001-2006	17487 patients (7480 CD and 9 968 UC patients)	69948 age, gender and index date matched controls	PVT aHR 6.2 ($P < 0.05$) IBD DVT aHR 2.3 ($P < 0.0001$) IBD PE aHR 1.7 ($P < 0.001$) IBD	Hypertension, diabetes, hyperlipidemia, and, in women, the use of contraceptives
Nguyen *et al*[14], 2009 United States	Retrospective cohort study Pregnant hospitalized women Nationwide Inpatient Sample 2005	3740 patients (2372 CD and 1368 UC patients)	4.21 million pregnant women	VTE aOR 6.12 (2.91-12.9) CD aOR 8.44 (3.71-19.2) UC	Maternal age, race/ethnicity, median neighbourhood income, comorbidity, health insurance, geographical region, hospital location and teaching status and caesarean delivery
Grainge *et al*[19], 2010 United Kingdom	Retrospective cohort study Outpatients and inpatients General Practice Research Database 1987-2001	13 756 patients (4835 CD and 6765 UC patients)	71672 age, gender, and general practice matched controls	VTE aHR 3.4 (2.7-4.3) IBD	Age, sex, body-mass index, smoking, cancer diagnosis and history of pulmonary embolism or deep vein thrombosis
Novacek *et al*[16], 2010 Austria	Retrospective cohort study Outpatients IBD patients from 14 Austrian centers specializing in the treatment of patients with IBD (2006-2008) and controls patients from 4 centers in Austria (1992-2008) 2006-2008	86 patients with history of unprovoked VTE	1255 controls with unprovoked VTE	Recurrence 5 yr after discontinuation of anticoagulation therapy aRR 2.5 (1.4-4.2) IBD	Age, gender, factor V Leiden, prothrombin G20210A mutation, high factor VIII (> 234 IU/dL), duration of anticoagulation and body mass index

Scarpa *et al*[147], 2010 Italy	Prospective case-control study Hospitalized patients who had major colo-rectal surgery Patients admitted for colorectal surgery in the institute of Clinica Chirurgica I of the University of Padova (Italy) 2004-2006	323 patients	432 controls	Incidence rate of VTE in surgical IBD patients *vs* surgical non IBD patients (both with prophylactic therapy) 1.9% *vs* 0% VTE with prophylactic therapy OR 5.9 (0.9-39.7) UC	
Kappelman *et al*[13], 2011 Denmark	Retrospective cohort study and nested case-control study Danish National Patient Registry 1980-2007	49799 patients (14211 CD and 35 229 UC patients)	477504 age and gender matched members of the general population	All VTE HR 2.0 (1.8-2.1) IBD HR 2.2 (2.0-2.5) CD HR 1.9 (1.8-2.0) UC Unprovoked VTE HR 1.6 (1.5-1.8) IBD HR 2.0 (1.6-2.5) CD	Comorbidities and medications
Merrill *et al*[23], 2011 United States	Retrospective cohort study Surgical patients National Surgical Quality Improvement Program 2008	2249 patients	269119 patients without IBD who were hospitalized and underwent surgery	HR 1.5 (1.4-1.7) UC aOR 1.7 (1.3-2.2) IBD VTE aOR 2.03 (1.52-2.70) IBD	Age, gender, race/ethnicity, admitted from home, smoker, BMI > 30, medical history, clinical factor
Rothberg *et al*[22], 2011 United States	Retrospective cohort study Inpatients 374 US hospitals 2004-2005	814 patients	241924 controls	VTE aOR 3.11 (1.59-6.08) IBD	Age, gender, VTE prophylaxis, length of stay ⩾ 6 d, primary diagnosis, comorbidities, cancer and treatments
Saleh *et al*[11], 2011 United States	Retrospective cohort study Inpatients National Hospital Discharge Survey 1979-2005	2932000 patients (1803000 CD and 1129000 UC patients)	918570000 age, gender matched controls	VTE HR 1.08 (1.06-1.09) CD HR 1.64 (1.62-1.66) UC	
Sridhar *et al*[21], 2011 United States	Cross-sectional study Inpatients Nationwide Inpatient Sample 2010	148229 patients	17261952 controls	VTE (DVT, PE and/or PVT) aOR 1.38 (1.25-1.53) IBD	Hypertension, diabetes mellitus and hyperlipidemia
Bröms *et al*[15], 2012 Sweden	Retrospective cohort study Pregnant women Medical, Patient, and Prescribed Drug Registers of all residents in Sweden 2006-2009	1996 patients (787 CD and 1209 UC patients) who gave birth to a single infant	10773 women without IBD who gave birth to a single infant	VTE aRR 2.65 (0.65-10.1) CD (with inactive disease) aRR 3.78 (1.52-9.38) UC	Age, parity, smoking, body mass index and comorbidities

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; VTE: Venous thrombosis; DVT: Deep venous thrombosis; PE: Pulmonary emboli; PVT: Portal vein thrombosis; aRR: Adjusted relative risk; aOR: Adjusted odds ratio; aHR: Adjusted hazard ratio.

ciation of VTE and IBD flares was assessed using a large primary care database from the United Kingdom (Table 1)[19]. According to the data from this assessment, the risk of VTE was increased most prominently during a flare of IBD [hazard risk (HR) = 8.4, 95%CI: 5.5-12.8], compared with periods of chronic activity (HR = 6.5, 95%CI: 4.6-9.2) and periods of clinical remission (HR = 2.1, 95%CI: 1.6-2.9). The RR at the time of a flare, compared with a matched control, was higher during non-hospitalised periods (HR = 15.8, 95%CI: 9.8-25.5 *vs* HR = 3.2, 95%CI: 1.7-6.3). However, this finding must be interpreted with caution because the lower RR during hospitalised periods is related to a higher absolute risk (37.5 *vs* 6.4 per 1000 persons-years), and the treatment with corticosteroids in patients with active disease may also be an additional risk factor for the development of VTE. Moreover, the use of VTE prophylaxis in hospitalised patients can also contribute to a lower RR of VTE during hospitalisation. Bernstein *et al*[20] showed higher VTE rates in hospitalised IBD patients than in non-IBD hospitalised patients regardless of age (Table 1). IBD patients who were younger than 50 years had a higher RR than those who were older than 50 years (RR = 1.57, 95%CI: 1.42-1.72 *vs* RR = 1.30, 95%CI: 1.23-1.37)[20]. Nguyen *et al*[12] compared the risk of VTE between hospitalised IBD patients and randomly selected hospitalised non-IBD patients (Table 1) and reported that IBD patients had an adjusted 1.7-fold [adjusted OR (aOR) = 1.66, 95%CI: 1.33-2.06] increased rate of VTE compared with non-IBD patients. In 2011, three studies were published showing a 1.1- to 3.1-fold higher risk of VTE in hospitalised IBD patients (Table 1)[11,21,22].

The risk of VTE in IBD was also evaluated in the surgical setting. Merrill *et al*[23] compared the risk of VTE between patients with IBD and patients without IBD who underwent surgery in 211 hospitals participating in the American College of Surgeons National Surgical Quality Improvement Program (Table 1). The incidence of VTE was 2.5% in IBD patients (n = 57) *vs* 1.0% (n = 2608)

Table 2 Prothrombotic risk factors and abnormalities associated with inflammatory bowel disease

Acquired prothrombotic risk factors
Infection or inflammation, previous thromboembolism, age, smoking, malignancy, central venous catheter, surgery, trauma, immobilization, Pregnancy, drugs (oral contraceptives, steroids), antiphospholipid antibody syndrome, hyperhomocysteinemia, fluid depletion
Genetic prothrombotic risk factors
Factor V Leiden, prothrombin mutation, deficiency of protein C, deficiency of protein S, deficiency of antithrombin, PAI-1 mutation, factor XII mutation and MTHFR mutation
Abnormalities of coagulation
↑ TF, factors VII, FXII, FXI, FX and FV, prothrombin and fibrinogen
↓ AT, protein C, protein S, EPCR, TM and TFPI
↑ Prothrombin fragment 1+2, TAT complexes, fibrinopeptide A and fibrinopeptide B
↓ Factor XIII
Abnormalities of fibrinolysis
↓ t-PA
↑ PAI-1 and TAFI
↑ D-dimer
Abnormalities of platelets
↑ Number, activation (CD40L and P-selectin) and aggregation
Abnormalities of endothelium
↓ NO
↑ vWF

PAI-1: Plasminogen activator inhibitor 1; MTHFR: Methylenetetrahydrofolate reductase; TF: Tissue factor; AT: Antithrombin; EPCR: Endothelial cell protein C receptor; TM: Thrombomodulin; TFPI: Tissue factor-pathway inhibitor; TAT: Thrombin-antithrombin; t-PA: Tissue plasminogen activator; TAFI: Thrombin-activatable fibrinolysis inhibitor; CD40L: CD40 ligand; vWF: von Willebrand factor.

in the controls. IBD remained a significant predictor of VTE after multivariate adjustment (OR = 2.03, 95%CI: 1.52-2.70). Another interesting finding of this was the observation that the risk persisted even when procedures on small and large bowels were excluded, with IBD patients undergoing non-intestinal procedures having a 4.45-fold increased risk of VTE compared with non-IBD patients. Furthermore, in a large cohort of surgical IBD patients, bleeding disorders, steroid use, anaesthesia time, emergency surgery, haematocrit < 37%, malnutrition and functional status were identified as potentially modifiable risk factors for postoperative VTE in IBD patients[24].

Pregnancy

The risk of VTE in IBD was also evaluated during pregnancy and puerperium. According to the Nguyen *et al*[14] study, based on nationwide inpatient sample data (database containing discharge abstracts from 1054 hospitals in the United States), the aOR of VTE was substantially higher in women with CD (aOR = 6.12, 95%CI: 2.91-12.9) and UC (aOR = 8.44, 95%CI: 3.71-19.2) compared with the non-IBD obstetric population, and this increased risk was independent of whether the women underwent a caesarean section (Table 1). A similar study conducted by Bröms *et al*[15] in Sweden also showed an increased risk of VTE in pregnant IBD patients compared with non-IBD pregnant patients but showed a lower odds ratio (aOR = 2.65, 95%CI: 0.65-10.1 for CD; aOR = 3.78, 95%CI: 1.52-9.38 for UC) (Table 1). For women with UC, the increased risk of VTE seemed to be highest during pregnancy and not during puerperium like in the general population of women giving birth.

IBD-phenotype risk factors

Several IBD-phenotype risk factors have been shown to affect the risk of VTE. Nguyen *et al*[12] reported that fistulising disease was independently associated with a greater VTE risk (OR = 1.39, 95%CI: 1.13-1.70). Colonic involvement in CD patients or extensive disease in UC patients was also associated with an increased VTE risk. A study by Solem *et al*[17] showed that CD patients with VTE typically had colonic disease involvement (ileocolonic in 56% and colonic in 23%), and most UC patients with VTE (76%) had pancolonic disease. Nguyen *et al*[12] found a higher risk of VTE in CD patients with colonic-only disease that was 40% higher than the risk of VTE in those with small bowel-only disease. In UC patients, 25% experienced VTE after proctocolectomy, and VTE recurrence rates were not improved by proctocolectomy[17].

IBD-nonspecific acquired risk factors

In IBD, several nonspecific acquired risk factors, other than those previously discussed, often increase the risk of VTE, such as oral contraceptive/hormone substitution use, smoking and drug therapy (Table 2). In most studies, at least one of the known clinical risk factors for VTE was present in approximately 20%-50% of IBD patients with VTE at the time a TE occurred[9,16,18,25]. Approximately 30% of these patients may have two or more risk factors[9].

PROTHROMBOTIC ABNORMALITIES IN IBD

Impact of inflammation on coagulation

Although the causes of the increased risk of VTE in IBD are not yet completely understood, most studies suggest that this risk is largely dependent on the biological and biochemical effects exerted by the activation of the inflammatory pathways (*e.g.*, cells and cytokines) in

the haemostatic system. In fact, there is now convincing evidence from clinical, epidemiological and experimental studies that inflammation and VTE are related[5,26-28]. Many inflammatory diseases other than IBD, such as systemic lupus erythematosus, Behçet's disease, polyarteritis nodosa and polymyositis/dermatomyositis, have been associated with an increased risk of VTE in several clinical and epidemiological studies[5,26-28]. This association appears to be the strongest when the time between the exposure and the outcome is short, *e.g.*, when the inflammatory disease was experienced recently or, more specifically, in the active stage of an inflammatory disease (flare-up)[5,26-28]. In many inflammatory diseases, such as systemic lupus erythematosus and Behçet's disease, VTE may be part of the presentation of those diseases. VTE in IBD may complicate the differential diagnosis with other inflammatory diseases that may also lead to VTE and intestinal inflammation, such as Behçet's disease.

The impact of inflammation on coagulation has been confirmed by several experimental studies showing that inflammatory mechanisms shift the haemostatic balance to favour the activation of coagulation and, in the extremes, VTE[27].

Tumour necrosis factor-alpha (TNF-α) and CD40 ligand (CD40L), two inflammatory cytokines, and C-reactive protein (CRP), a liver-synthesised acute phase protein, have been shown to induce the expression of tissue factor (TF) on the cell surface of leucocytes[29-31]. Interleukin (IL)-6, an inflammatory cytokine, and TNF-α have been shown to lead to thrombin generation[32,33]. Of the natural anticoagulant pathways, the protein C pathway and heparin-antithrombin pathway have been shown to be downregulated by IL-1β and TNF-α[34,35], whereas the tissue factor pathway inhibitor (TFPI) has been shown to be inhibited by CRP[36]. There is also evidence that CRP increases the expression of plasminogen activator inhibitor type 1 (PAI-1) and decreases the expression of tissue plasminogen activator (t-PA)[37,38].

Inflammatory mediators, such as IL-6, increase platelet production. The newly formed platelets appear to be more thrombogenic. For example, the newly formed platelets activate at lower concentrations of thrombin[39]. Thus, both the platelet count and platelet reactivity are increased in response to inflammatory mediators.

Some authors have proposed the term "endothelial stunning" for the endothelial dysfunction/activation that may be induced by inflammatory cytokines and may thus play a key-role in the association between inflammation and VTE[40]. For example, CRP has been shown to induce the release of von Willebrand factor (vWF)[41] and to reduce the production of nitric oxide (NO) by endothelial cells[42].

As discussed above, in general for inflammatory diseases, there is also convincing evidence from basic science as well as clinical and epidemiological studies that IBD is associated with several prothrombotic abnormalities, including the initiation of the coagulation system, downregulation of natural anticoagulant mechanisms, impairment of fibrinolysis, increase in the platelet count and reactivity and dysfunction of the endothelium (Table 3)[43-45].

The mechanisms underlying IBD-associated prothrombotic abnormalities have been the subject of recent experimental and clinical studies. For example, Yoshida *et al*[46,47] showed that TNF-α and IL-1β are both implicated in the enhanced extra-intestinal thrombosis that accompanies experimental colitis [mice with dextran sodium sulphate (DSS)-induced colitis]. The authors noted that exogenous TNF-α and IL-1β enhanced thrombosis in the arterioles of control mice and that the enhanced thrombus formation in the arterioles of mice with DSS-induced colitis was significantly attenuated in wild-type colitic mice treated with TNF-α or IL-1β blocking antibodies and in colitic mice deficient for the TNF-α receptor or the IL-1 receptor. The IL-6 concentrations were positively correlated with disease activity and thrombocytosis in patients with UC[48]. Taken together, these data suggest that inflammatory cytokines such as TNF-α, IL-1β and IL-6 may play an important role in the inflammation-mediated risk of VTE in IBD.

Abnormalities of haemostasis associated with IBD

Abnormalities of coagulation: Quantitative alterations in key sites of the coagulation cascade that favour clot formation occur in patients with IBD[44]. These alterations include the elevation of circulating microparticles (including TF-rich microparticles)[49,50], factor Ⅶa[51], factors XIIa and XIa[52], factors Xa and Va[52,53], prothrombin[7,54-56] and fibrinogen[56,57]. The levels of antithrombin (AT) are significantly lower in the plasma of patients with IBD[56,58]. Reports on protein C and S deficiency in IBD are conflicting[48,59-61]. There is also a significantly lower expression of endothelial protein C receptor (EPCR) and thrombomodulin (TM), which impairs protein C activation leading to lower effective protein C activity[62]. TFPI levels were also shown to be reduced in patients with IBD[63,64]. Alterations suggestive of the activation of coagulation have also been reported in IBD. These include elevated prothrombin fragment 1+2 (prothrombin F1+2), thrombin-antithrombin (TAT) complexes, fibrinopeptide A (FPA) and B (FPB)[7,54-56] and decreased factor XIII levels[57,65].

IBD treatment was also found to influence coagulation abnormalities associated with IBD. For example, the treatment with infliximab induced a significant decrease in the amounts of circulating microparticles in IBD patients[49].

Abnormalities of fibrinolysis: The circulating concentration of factors involved in the lysis of clots also favours thrombosis in IBD. The plasma levels of t-PA are significantly lower in IBD patients than these levels in the general population[66,67]. There is also a significant absolute increase in urokinase-type plasminogen activator (u-PA) activity and a decrease in t-PA activity in the inflamed mucosa of IBD patients compared with the control group[66]. Two proteins that inhibit fibrinolysis,

Table 3 Controlled studies on the prevalence of inherited thrombophilias in inflammatory bowel disease

Ref.	Compared groups	Results							
		Mutation[1]	CD	UC	IBD	IBD-VTE	HC	C-VTE	Significance
Liebman *et al*[101], 1998 United States	11 IBD-VTE patients and 51 IBD patients without VTE	Factor V Leiden			4%	36%			Significant difference (OR = 14.00, 95%CI: 1.55-169.25)
Over *et al*[107], 1998 Turkey	63 IBD patients (20 CD and 43 UC patients) and 36 HC	Factor V Leiden	50%	20%			11%		Significant difference for CD *vs* HC (OR = 6.5, 95%CI: 1.3-18.0)
Haslam *et al*[112], 1999 United Kingdom	54 IBD patients (30 CD and 24 UC patients) and 55 HC	Factor V Leiden			9.3%		3.6%		Difference not significant
Heliö *et al*[111], 1999 Finland	563 IBD patients (235 CD and 328 UC patients) and 142 HC	Factor V Leiden	3.4%	5.2%	4.5%		2.1%		Differences not significant
		Factor XIII mutation	5.0%	6.1%	5.7%		3.3%		Differences not significant
Grip *et al*[6], 2000 Sweden	16 IBD-VTE patients, 99 C-VTE and 288 HC	Factor V Leiden				27%	11%	28%	Significant difference for IBD-VTE *vs* HC (OR = 3.0, 95%CI: 0.8-11.9)
		Prothrombin mutation				0%	1.8%	7.10%	Differences not significant
Koutroubakis *et al*[61], 2000 Greece	84 IBD patients (36 CD and 48 UC patients) and 61 HC	Factor V Leiden			8.3%		4.9%		Difference not significant
Papa *et al*[113], 2000 Italy	52 IBD patients (19 CD and 33 UC patients) and 156 HC	Factor V Leiden			1.9%		1.9%		Difference not significant
		Prothrombin mutation			1.9%		2.6%		Difference not significant
Vecchi *et al*[110], 2000 Italy	102 IBD (51 CD and 51 UC patients) and 204 HC	Factor V Leiden			1.5%		1.2%		Difference not significant
		Prothrombin mutation			1.1%		0.7%		Difference not significant
		MTHFR mutation			41.1%		47.4%		Difference not significant
Guédon *et al*[102], 2001 France	15 IBD-VTE, 58 IBD patients without VTE, 110 C- VTE and 84 HC	Factor V Leiden			0%	14.3%	3.6%	15.50%	Significant difference for IBD-VTE *vs* IBD ($P < 0.05$)
		Prothrombin mutation			1.7%	14.3%	3.6%	11.80%	Differences not significant
		MTHFR mutation			0%	0%	1.2%	0.90%	Differences not significant
Mózsik *et al*[106], 2001 Hungary	84 IBD patients (49 CD and 35 UC patients) and 57 HC	Factor V Leiden	14.3%	27.5%			5.3%		Significant difference for CD and UC *vs* HC ($P < 0.05$)
Nagy *et al*[108], 2001 Hungary	78 IBD patients (49 CD and 29 UC patients) and 57 HC	Factor V Leiden	14.3%	27.6%			5.3%		Significant difference for CD and UC *vs* HC ($P < 0.05$)
Turri *et al*[103], 2001 Italy	18 IBD patients with arterial or venous thrombosis, 45 IBD patients without thromboembolic events and 100 HC	Factor V Leiden			2.2%	0%	5%		Differences not significant
		Prothrombin mutation			0%	0%	2%		Differences not significant
Bjerregaard *et al*[120], 2002 Denmark	106 IBD patients and 4188 HC	Factor V Leiden			5.7%		6.7%		Difference not significant
		Prothrombin mutation							Difference not significant
Magro *et al*[119], 2003 Portugal	116 IBD patients (74 CD and 42 UC) and 141 healthy controls	Factor V Leiden	7%	2%			1%		Differences not significant
		G20210A Prothrombin	4%	0%			3%		Differences not significant
		MTHFR mutation	14%	12%			10%		Differences not significant
		PAI-1 mutation	11%	14%			24%		Differences not significant
Saibeni *et al*[121], 2003 Italy	152 IBD patients (62 CD and 90 UC patients) and 130 HC	Factor XIII mutation			5.3%		5.4%		Difference not significant
Törüner *et al*[118], 2004 Turkey	62 IBD patients (28 CD and 32 UC patients) and 80 HC	Factor V Leiden			3.2%		6.3%		Difference not significant
		Prothrombin mutation			0%		2.5%		Difference not significant
		MTHFR mutation			11.3%		6.3%		Difference not significant
Mahmood *et al*[117], 2005 United Kingdom	68 IBD patients (31 CD and 37 UC patients) and 30 HC	Factor V Leiden	0%	1.5%	1.5%		0%		Differences not significant
		Prothrombin mutation	0%	1.5%	1.5%		0%		Differences not significant

Oldenburg *et al*[98], 2005 Netherlands	22 IBD-VTE patients and 23 IBD patients without VTE	Factor V Leiden			0%	20%		Difference not significant
		Prothrombin mutation			8.7%	4.5%		Difference not significant
Spina *et al*[105], 2005 Italy	47 IBD-VTE patients and 94 C-VTE	Factor V Leiden				2.1%		13.8%	Significant difference for C-VTE *vs* IBD-VTE ($P < 0.05$)
		Prothrombin mutation				8.5%		12.8%	Difference not significant
Yilmaz *et al*[116], 2006 Turkey	27 IBD patients and 27 HC	Factor V Leiden			6.7%		5%		Difference not significant
		Prothrombin mutation			3.3%		6.7%		Difference not significant
		Factor XIII mutation			5%		0%		Difference not significant
		MTHFR mutation			3.3%		0%		Difference not significant
		PAI-1 mutation			11.7%		8.3%		Difference not significant
Bernstein *et al*[109], 2007 Canada	492 IBD patients (327 CD and 165 UC) and 412 HC	Factor V Leiden	6.4%	4.2%			6.1%		Differences not significant
		Prothrombin mutation	1.8%	1.2%			1.2%		Differences not significant
		Factor XIII mutation	8.7%	7.1%			4.3%		Significant difference for CD *vs* HC ($P < 0.05$)
		MTHFR mutation	12.5%	9.9%			11.7%		Differences not significant
Koutroubakis *et al*[104], 2007 Greece	30 IBD patients with vascular complications, 60 IBD patients without vascular complications, 30 controls with vascular complications and 54 HC	Factor V Leiden			6.7%	20.0%	3.7%	16.7%	Significant difference for IBD-VTE *vs* HC ($P < 0.05$)
		Prothrombin mutation			5.0%	10.0%	1.9%	13.3%	Differences not significant
		Factor XIII mutation			3.3%	0%	1.90%	0%	Differences not significant
		MTHFR mutation			11.6%	6.6%	7.5%	13.3%	Differences not significant
		PAI-1 mutation			20%		13.0%		Significant difference for IBD-VTE *vs* HC ($P < 0.05$)
Yasa *et al*[115], 2007 Turkey	27 IBD patients and 47 HC	Factor V Leiden			11.1%	43.3%	4.3%	36.7%	Difference not significant
		Prothrombin mutation			7.4%		0%		Difference not significant
		MTHFR mutation			14.9%		6.3%		Difference not significant
Maher *et al*[114], 2010 Saudi Arabia	26 IBD patients (7 CD and 19 UC patients) and 40 HC	Factor V Leiden			3.8%		2.5%		Difference not significant
Novacek *et al*[16], 2010 Austria	102 IBD patients (77 CD and 25 UC) and 102 HC	Factor V Leiden			16.1%		26.1%		Difference not significant
		Prothrombin mutation			1.7%		6%		Difference not significant

VTE: Venous thrombosis; CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; IBD-VTE: Inflammatory bowel disease with venous thrombosis; HC: Healthy controls; C-VTE: Controls with venous thrombosis; OR: Odds ratio. [1]Presented prevalence data refer to heterozygous and homozygous carrier for FV Leiden and prothrombin mutation and to homozygous carrier for the other mutations.

PAI-1[66] and thrombin-activatable fibrinolysis inhibitor (TAFI)[68], were found in higher concentrations in the plasma of IBD patients. Increasing levels of D-dimer, a fibrin degradation product, were also found in IBD, mainly in patients with active disease[58]. As D-dimer is generated from cross-linked fibrin, but not from fibrinogen, and elevated plasma concentration of D-dimer indicates recent or ongoing intravascular blood coagulation.

Abnormalities of platelets: In patients with IBD, there are often increased circulating platelet numbers and platelet and leukocyte-platelet aggregates (PLAs)[69]. Greater platelet aggregation has also been demonstrated in the mesenteric vasculature compared with non-IBD controls, supporting the hypothesis that platelet activity is stimulated in the mesenteric microcirculation[70]. Indeed, spontaneous platelet aggregation or platelet hypersensitivity to low levels of aggregating agents occurs in nearly one-half of patients with IBD[71] and appears to be independent of the disease activity[57,71]. Such platelet hyperactivation is mediated at least partly by the CD40-CD40L pathway, a key regulator and amplifier of immune-inflammatory reactivity and inducer of TF, which initiates the extrinsic coagulation pathway. Evidence for the involvement of CD40L includes a markedly elevated expression of CD40L protein by platelets from patients with IBD and the release of larger amounts of soluble CD40L into the plasma, leading to an approximately 15-fold increase in the CD40L plasma levels[72,73]. There are also elevated levels of CD40L in the mucosa that appear to be proportional to the degree of inflammation[72,74]. The activation of platelets in IBD may also be mediated by P-selectin.

IBD patients have been reported to have more platelets expressing P-selectin (marker of platelet activation) than healthy controls[69]. In the DSS model of murine colonic inflammation, colonic inflammation has been reported be associated with an increased number of circulating activated platelets, along with the formation of PLAs, which can be inhibited by selectin blockade with fucoidin[75].

The IBD treatment may influence platelet abnormalities associated with IBD. Infliximab significantly reduced plasma-soluble CD40L levels and eliminated CD40 from mucosal microvessels[76], whereas IBD patients on thiopurines had fewer PLAs than those not taking them[69]. These findings suggest that IBD treatment may influence platelet abnormalities associated with IBD.

Abnormalities of endothelium: Endothelial dysfunction has been clearly demonstrated in IBD patients and involves several aspects of endothelium biochemical physiology[77]. In particular, such dysfunction involves an alteration in the NO and reactive oxygen species (ROS) balance, which occurs when the endothelium fails to generate NO, a potent vasodilator and anti-aggregating agent, and instead forms elevated levels of superoxide anion[78]. Decreased NO generation may result from an acquired deficient transcription of nitric oxide synthase 2 (NOS2) in chronically inflamed IBD endothelium[79] and from the induction, by many inflammatory cytokines (*e.g.*, IL-2 and TNF-α), of the enzyme arginase, which competes with NOS[80]. The increased production of ROS in the inflamed endothelium may also contribute to oxidative stress in vWF molecules, which become unresponsive to proteolysis by ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), leading to the accumulation of ultra-large vWF multimers[81]. The latter are the most haemostatically active forms of vWF and, by favouring platelet adhesion and aggregation, may contribute to microvascular thrombosis in IBD. Increased levels of vWF were reported in IBD patients, especially in those with active disease[58,82].

Thrombophilia and IBD

Acquired risk factors: Antiphospholipid antibodies (APLA) and hyperhomocysteinaemia are two acquired thrombophilias associated with arterial and venous thrombosis. APLA are a group of prothrombotic antibodies directed against plasma proteins that are bound to anionic phospholipids. Patients with APLA may present with venous or arterial thrombosis, recurrent foetal loss and/or thrombocytopaenia. The disorder may be primary or may be associated with pregnancy or with inflammatory, post infectious and other disease states. This group of antibodies includes anti-cardiolipin (aCL), anti-β2-glycoprotein 1 (b2-GPI) and lupus anticoagulants, each of which requires specific testing. Available studies in IBD vary in the assessment of different antibodies, types of IBD and disease activity level; therefore, the true prevalence of APLA in IBD patients remains unclear. The prevalence of aCL antibodies in IBD patients is higher than in the control population, with an average incidence of 20%-30%, but the association with thrombosis in IBD patients is less clear[83]. Similarly, the levels of antibodies against b2-GPI, the cofactor that mediates the binding of aCL antibodies to cardiolipin and a more specific measure of the risk associated with thrombosis, has been detected in 9% of patients with IBD compared with its absence in healthy controls[84]. Lupus anticoagulants were not detected in a small series of 16 patients with CD[85]. The levels of aCL antibodies and anti-b2-GPI antibodies and of lupus anticoagulants were similar in a population of IBD patients with and without current or past VTE events[84,86]. One of the causes of the appearance of APLA in IBD may be anti-TNF-α therapy because this therapy has been associated with the development of APLA[87,88].

Hyperhomocysteinaemia may be both a genetic and acquired abnormality. The most common genetic defect is homozygosity for a thermolabile mutant of the enzyme methylenetetrahydrofolate reductase (MTHFR)[89]. Plasma homocysteine concentrations can also be increased by deficiencies in vitamin B6, B12 or folic acid (dietary, genetic or drug-associated)[90]. Hyperhomocysteinaemia is an independent risk factor for atherosclerotic vascular disease, with the risk increasing in a graded fashion with increasing plasma homocysteine concentrations[91,92]. Hyperhomocysteinaemia has also been associated with an increased risk of VTE[93,94]. Reducing levels of homocysteine with B vitamin supplements, however, has not resulted in a reduction in the incidence of recurrent VTE or arterial thrombotic complications[95,96]. The association of folate deficiency and hyperhomocysteinaemia has been evaluated in IBD patients. One study reported elevated serum homocysteine and low folate in 63 patients with IBD, but these levels were not observed in 183 matched controls without thrombotic complications[97]. In a small study, fasting homocysteine levels in IBD patients with a history of arterial or venous thrombosis tended to be higher (although not significantly) than in IBD controls[98]. Recently, Oussalah *et al*[99] conducted a systematic review and meta-analysis to evaluate the association between homocysteine metabolism and IBD and the association between hyperhomocysteinaemia and thrombosis in IBD. The mean plasma homocysteine level was significantly higher in IBD patients compared with the controls. The mean plasma homocysteine level did not differ between patients with UC and CD. The risk of hyperhomocysteinaemia was significantly higher in IBD patients compared with controls (OR = 4.65, 95%CI: 3.04-7.09). The risk of hyperhomocysteinaemia was not higher among IBD patients who experienced thromboembolic complications (OR = 1.97, 95%CI: 0.83-4.67), suggesting that hyperhomocysteinaemia may not be a major contributor to VTE in IBD. Medication-associated folate deficiency (*e.g.*, methotrexate or sulfasalazine) may be the most common explanation for hyperhomocysteinaemia in IBD patients, although deficiencies in vitamin B6 and B12 and a MTHFR mutation may also play a role

(see below). Taken together, the current data do not support a major role for APLA or hyperhomocysteinaemia in IBD-associated VTE, but studies with a higher number of patients and a prospective design are needed.

Inherited risk factors: A summary of controlled studies on the prevalence of inherited thrombophilias in IBD is presented in Table 3. The rate of inherited thrombophilias in patients with IBD and VTE is estimated to be 15%-30%, which is similar to the rate in non-IBD patients and VTE in most studies[6,17,25,98,100-104], although one study has reported a lower rate of inherited thrombophilias in patients with IBD and VTE[105]. Data comparing the prevalence of inherited thrombophilias in the overall IBD population and in the general population are conflicting. Although some cohorts reported a higher prevalence in the overall IBD population[106-109], most reported a similar prevalence[6,16,61,102-104,109-121]. These data suggest that the role of inherited thrombophilias in VTE in IBD patients is similar to that in the general population.

The most prevalent thrombophilia reported in IBD patients is factor V mutation. Other genetic variants that have been found in IBD patients include the prothrombin G20210A mutation, deficiencies in protein C, protein S and antithrombin, the PAI-1 4G mutation, the factor XII val34leu mutation and the MTHFR C677T mutation (Tables 2 and 3). Factor V mutation increases the risk of thrombosis five- to eightfold for heterozygous carriers and 50- to 80-fold for homozygous carriers[122]. A similar frequency of factor V Leiden was reported in IBD patients with thrombosis compared with thrombotic controls[6,102,104] and in the overall IBD population compared with the general population[61,109]. However, the prevalence of factor V mutation in thrombotic IBD patients has been shown to be significantly higher than that in IBD patients without thrombosis, suggesting that factor V Leiden, when present, increases the risk of IBD-associated VTE[101,102]. Two recent meta-analyses confirmed this conclusion. In the meta-analysis of Zhong *et al*[123], the OR of VTE in IBD patients with factor V mutation was higher than in IBD patients (OR = 4.00, 95%CI: 2.04-7.87) and healthy controls (OR = 3.19, 95%CI: 1.38-7.36). Liang *et al*[124] showed a similar prevalence of factor V mutation in IBD patients and the general population (summary OR = 1.13, 95%CI: 0.87-1.46). Of note, the factor V Leiden mutation was associated with a significantly higher risk of thromboembolism in IBD patients (summary OR = 5.30, 95%CI: 2.25-12.48)[124].

The prothrombin G20210A mutation leads to greater prothrombin plasma levels (heterozygous carriers have approximately 30% higher PT levels than healthy controls) and increases the risk of VTE approximately threefold[122]. There is no difference in the prevalence of the prothrombin G20210A mutation between IBD patients and normal controls[110,120], between IBD patients with thrombosis and non-IBD patients with thrombosis[6,102] or between IBD patients with and without thrombosis[102,104]. Protein C, protein S and antithrombin Ⅲ deficiencies also have no increased prevalence among patients with IBD, regardless of whether they have had a VTE[52,125-127].

The factor XIII val34leu mutation is associated with a greater FXIII activation rate and leads to a 20%-40% reduction of the risk of VTE for homozygous carriers[111,121]. A slightly greater prevalence of factor XIII (val34leu) mutation carriers in CD was found in a recent population-based study[109], but this prevalence could not explain the greater risk of VTE in CD. Available data suggest that there is no difference in the prevalence of homozygous carriers of the factor XIII val34leu mutation between IBD patients and healthy controls[111,116,121]. Finally, the prevalence of the factor XIII val34leu mutation was similar in thrombotic IBD patients and non-IBD thrombotic patients[104].

The MTHFR C677T mutation leads to a 25% increase in homocysteine plasma levels in homozygous carriers[128]. The effect of the MTHFR C677T mutation on the risk of VTE varies among studies, and a recent meta-analysis found a weak effect (20% risk increase)[128]. The prevalence of the MTHFR C677T mutation in IBD has shown discordant results, most likely because of regional and ethnic variations in the prevalence of this polymorphism in the general population. The allelic frequency of MTHFR C677T has been reported to be higher in IBD patients than in the reference population[119]. In a recent population-based case-control study, some differences were observed between patients with IBD and healthy controls (with a decreased number of mutant allele carriers in UC); however, these differences did not explain the excess risk of thrombosis[109]. No difference in the prevalence of homozygous carriers of the MTHFR C677T mutation was found between the IBD patients and healthy controls in most studies[102,104,109,110,115,116,118,119]. The prevalence of C677T homozygosity between IBD thrombotic patients and non-IBD thrombotic patients showed no significant difference[102,104].

Several studies have demonstrated that the PAI-1 (4G) homozygosity is associated with enhanced PAI-1 expression[129] and contributes as an additional risk factor towards the development of VTE[130]. However, the evidence regarding the relationship between an elevated PAI-1 plasma level or PAI-1 4G polymorphism and the risk of VTE is rather conflicting. The allelic frequency of PAI-1 4G has been reported as being higher in IBD patients than in the reference population[119]. Moreover, a recent study showed a significantly higher allelic frequency of PAI-1 4G in IBD patients with vascular complications compared with IBD patients and healthy controls[104]. No difference in the prevalence of homozygous carriers of the PAI-1 4G mutation was found between IBD patients and healthy controls in most studies[104,116,119]. The prevalence of this genotype does not differ in thrombotic IBD patients compared with non-IBD thrombotic patients[104].

As in the general population, more than one thrombotic defect can occur among IBD patients with inherited thrombophilia, particularly factor V Leiden[104,119]. A higher prevalence of the carriage of two or more throm-

bogenic polymorphisms has been found in IBD patients compared with the reference population[119], but no significant difference has been found between thrombotic and non-thrombotic IBD patients[104].

Taken together, these data show that genetic risk factors are generally not found more often in IBD patients than in others, suggesting that genetics does not explain the greater risk of VTE in CD and UC. However, when genetic risk factors occur, patients with IBD (compared with healthy controls) are more likely to suffer thromboembolic complications, suggesting that hereditary thrombophilia and inflammation-associated thrombogenicity have at least an additive effect for the risk of VTE in IBD.

PHARMACOLOGICAL EFFECT ON RISK FACTORS

Almost all drugs used in the treatment of IBD have been associated with abnormalities in the haemostatic system in experimental and clinical studies. Corticosteroids have been associated with both hypo- and hypercoagulating alterations[131,132]. A meta-analysis demonstrated that dexamethasone-based chemotherapy was a risk for VTE in patients with multiple myeloma[133]. Furthermore, in a large cohort of surgical IBD patients, the use of steroids was identified as a potentially modifiable risk factor for postoperative VTE in IBD patients[24]. Studies of platelets from IBD patients treated with 5-aminosalicylic acid (5-ASA) agents have shown conflicting results. *In vitro*, 5-ASA significantly reduced both spontaneous and thrombin-induced platelet activation[134]. *In vivo,* platelets from IBD patients taking 5-ASA have decreased expression levels of P-selectin, a surface marker for platelet activation[134], and lower plasma levels of RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted), a prothrombotic platelet cytokine[135]. In contrast to these findings, a study of six patients with IBD (four with UC and two with CD) treated with 5-ASA showed no changes in platelet aggregation or fibrinolytic activity[136]. Sulfasalazine inhibits dihydrofolate reductase leading to folate deficiency, which is a cause of hyperhomocysteinaemia (acquired thrombophilia; see above). Plasma homocysteinemia levels have been reported to be significantly increased in patients with ankylosing spondylitis under sulfasalazine therapy[137].

Azathioprine has been shown to inhibit platelet aggregation *in vitro*[138]. IBD patients taking thiopurines experienced fewer PLAs than patients who were not taking them[69]. The *in vitro* data suggest an antithrombotic effect from azathioprine and 6-mercaptopurine. Methotrexate, a folate antagonist, is a well-established contributor to hyperhomocysteinaemia (which is associated with thrombosis risk) when used in patients with rheumatoid arthritis[139]. Nonetheless, no study associating methotrexate with hyperhomocysteinaemia is available for IBD. Cyclosporine has been associated with thrombogenicity *in vitro* and *in vivo*. *In vitro* studies showed an increased platelet aggregation[138] and activation of endothelial cells[140] induced by cyclosporine. Cyclosporine has also been associated with impaired fibrinolysis through a decrease in PAI-1 activity[141]. The *in vitro* thrombogenicity of cyclosporine has been confirmed *in vivo* by several studies showing thrombotic events in patients taking cyclosporine[142].

Infliximab, an antibody against anti-TNF-α, may decrease platelet activity through the downregulation of the CD40/CD40L pathway in the mucosal microcirculation[76]. Additionally, in patients with active rheumatoid arthritis, infliximab treatment has been shown to normalise the disease-associated impairment of the coagulation and fibrinolytic systems by decreasing the levels of prothrombin F1+2 and D-dimer[143], t-PA antigen, PAI-1 antigen and PAI-1 activity[144]. Finally, infliximab induced a significant decrease in the amounts of circulating microparticles in IBD patients[49]. Despite these potential anticoagulant effects of the TNF-α blockade, there are also case reports of thrombosis at several sites, such as the retinal vein, in patients under anti-TNF-α therapy[145]. Moreover, the prothrombotic effects of anti-TNF-α therapy may be mediated by antiphospholipid antibodies (acquired thrombophilia) as anti-TNF-α therapy has been associated with the development of APLA[87,88]. Nonetheless, a recent prospective observational cohort study of biological safety in patients with rheumatoid arthritis showed that VTE events are not increased in patients with rheumatoid arthritis who are treated with anti-TNF therapy[146].

CONCLUSION

In IBD, there is an increased risk of thromboembolic events due to inflammation, nutritional deficiencies, hospitalisations, surgery and inherited prothrombotic factors. Moreover, beyond an increased risk, VTE may have clinical specificities in IBD. There is evidence that subjects with IBD experience the first episode of VTE early in life[6]. In IBD, the RR of VTE is inversely correlated with age (*i.e.*, younger IBD patients have a higher RR of VTE); nevertheless, the actual incidence increases with age[8,11,13,21]. The rate of recurrent VTE in the five years after the discontinuation of anticoagulation therapy is also increased in IBD [adjusted RR (aRR) = 2.5, 95%CI: 1.4-4.2][16]. Even with continued prophylaxis for VTE, the risk of recurrence of VTE in IBD patients has been reported to be 13%[17]. Low molecular weight heparin (LMWH) has been shown to be less effective in preventing DVT in hospitalised subjects undergoing surgery for IBD than in patients with non-IBD conditions, including colorectal cancer and diverticular disease (aOR = 5.9, 95%CI: 0.9-39.7, for UC and DVT postoperatively)[147]. Importantly, VTE appears to carry a poorer prognostic outcome for patients with IBD than for the general population. In a hospitalised cohort, the rate of VTE was not only higher in IBD subjects than in the controls, but the admissions for IBD subjects were also longer (11.7 *vs*

6.1 d, $P < 0.0001$) and were associated with higher costs ($47515 *vs* $21499, $P < 0.0001$) and higher mortality (aOR = 2.5, 95%CI: 1.83-3.43)[12].

Therefore, clinicians should be aware of these risks so that adequate prophylactic actions can be taken in all IBD patients with flares, particularly in patients who are hospitalised, submitted to surgery or undergoing treatment.

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TOPIC HIGHLIGHT

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New serological markers in pediatric patients with inflammatory bowel disease

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Abstract

The spectrum of serological markers associated with inflammatory bowel disease (IBD) is rapidly growing. Due to frequently delayed or missed diagnoses, the application of non-invasive diagnostic tests for IBD, as well as differentiation between ulcerative colitis (UC) and Crohn's disease (CD), would be useful in the pediatric population. In addition, the combination of pancreatic autoantibodies and antibodies against *Saccharomyces cerevisiae* antibodies/perinuclear cytoplasmic antibody (pANCA) improved the sensitivity of serological markers in pediatric patients with CD and UC. Some studies suggested that age-associated differences in the patterns of antibodies may be present, particularly in the youngest children. In CD, most patients develop stricturing or perforating complications, and a significant number of patients undergo surgery during the disease course. Based on recent knowledge, serum antibodies are qualitatively and quantitatively associated with complicated CD behavior and CD-related surgery. Pediatric UC is characterized by extensive colitis and a high rate of colectomy. In patients with UC, high levels of anti-CBir1 and pANCA are associated with the development of pouchitis after ileal pouch-anal anastomosis. Thus, serologic markers for IBD can be applied to stratify IBD patients into more homogeneous subgroups with respect to disease progression. In conclusion, identification of patients at an increased risk of rapid disease progression is of great interest, as the application of early and more aggressive pharmaceutical intervention could have the potential to alter the natural history of IBD, and reduce complications and hospitalizations.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Pediatric; Serologic markers; Antimicrobial antibodies; Anti-glycan antibodies; Pancreatic antibodies; Inflammatory bowel disease

Core tip: Application of non-invasive diagnostic tests for the diagnosis of inflammatory bowel disease (IBD) and differentiation between ulcerative colitis (UC) and Crohn's disease (CD) would be useful in the pediatric population. The combination of pancreatic autoantibodies and antibodies against *Saccharomyces cerevisiae* antibodies/perinuclear cytoplasmic antibody improved the sensitivity of serological markers in pediatric patients with CD and UC. In addition, serologic markers for IBD can be applied to stratify IBD patients into more homogeneous subgroups with respect to disease progression. With this knowledge, clinicians will be able to stratify patients accordingly with regards to the risk of disease progression, create a personalized treatment strategy, and attempt to modify disease course, thereby

improving long-term prognosis.

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INTRODUCTION

Inflammatory bowel diseases (IBD), Crohn's disease (CD), and ulcerative colitis (UC) are chronic relapsing and remitting disorders of the digestive tract with unknown etiology[1]. Previous studies suggested that IBD results from an aberrant innate and acquired immune response to commensal microorganisms in genetically susceptible individuals[2,3]. This hypothesis is supported by the presence of antibodies directed to microbial antigens and by the identification of genetic polymorphisms, such as *NOD2/CARD15* and toll-like receptor 4 variants in CD[4]. Besides genetic predisposition and environmental factors, innate immunity is assumed to be another major contributor to pathogenesis in IBD.

Incidence of IBD is increasing, especially in pediatric patients with CD[5]. It is estimated that 15%-25% of IBD patients present in childhood. Recent studies showed that up to 20% of pediatric patients and 5%-15% of adult patients with colon only involvement had diagnostic difficulties if they had UC or colonic CD[6]. Serologic markers may help to establish diagnosis of IBD and to differentiate CD from UC, particularly when they are combined. It is especially important in the pediatric population, where invasive diagnostic testing is less desirable. In CD, most patients develop stricturing or perforating complications, and a significant number of patients undergo surgery during the disease course. Pediatric UC is more often associated with pancolitis and colectomy. Besides their diagnostic significance, current knowledge suggests that serologic markers can be a valuable aid in stratifying patients according to disease phenotype and risk of complications in IBD.

Several circulating autoantibodies have been described in IBD. The two most intensively studied conservative antibodies are atypical perinuclear anti-neutrophil cytoplasmic antibodies (atypical pANCA), which are primarily associated with UC and anti-*Saccharomyces cerevisiae* antibodies (ASCA), which are primarily associated with CD[4,7]. In pediatric IBD, sensitivity/specificity of pANCA in UC ranged between 57% to 83% and 65% to 97%, respectively, whereas in CD, ASCA showed a sensitivity/specificity in the range of 44% to 76% and 88% to 95%, respectively[8,9]. ASCA positivity or high titers are associated with complicated CD behavior (penetrating or stenosing disease) and could be useful markers for predicting the need for surgery in adults and children[10-12]. In pediatric studies, ASCA positivity increased with age at diagnosis[13] and was predictive for a more relapsing disease course [OR 2.9 (95%CI: 1.33-6.33)] in CD[14]. In addition, Trauernicht and Steiner[15] reported that serum ASCA antibodies are associated with lower anthropometric data (lower mean weight and height *Z*-scores) at the diagnosis of pediatric CD. pANCA is noted for its association with the "UC-like" phenotype in patients with CD[16,17]. Testing for ASCA and pANCA alone may have limited usefulness; therefore additional seromarkers are needed to improve the diagnosis, differentiation, and stratification of IBD, as well as prediction of disease course.

NEW SEROLOGICAL MARKERS

Crohn's disease

Antibodies to *Escherichia coli* outer membrane porin C, *Pseudomonas*-associated sequence I2, and bacterial flagellin CBir: Several antibodies against microbial components have been detected in serum samples of patients with IBD, including ones against outer membrane porin C (anti-OmpC) of *Escherichia coli*, against *Pseudomonas*-associated sequence I2 (anti-I2), and against bacterial flagellin CBir (anti-CBir1). Adherent-invasive *E. coli* has been found in ileal CD lesions, and OmpC has been shown to be required for these organisms to adhere to intestinal epithelial cells[18,19]. I2 was identified as a bacterial sequence from lamina propria mononuclear cells of active CD patients, and was shown to be associated with *Pseudomonas fluorescens*[20]. CBir1 is a flagellin related antigen that was initially identified in the gut flora of mice, and has the ability to induce colitis in immunodeficient mice[21].

Approximately 50% of adult patients with CD were positive for these markers, which were insignificant in adult patients with UC and healthy subjects[22,23]. The prevalence of anti-OmpC and anti-I2 was found to be 11% and 56% in pediatric CD, respectively[10,13,24-28]. The occurrence of antibodies varies in children of different ages: children younger than 8 years old at diagnosis are predominantly anti-CBir1 positive and ASCA and anti-OmpC negative, while those older than 8 are more commonly both ASCA and anti-CBir1 positive[13]. In children with CD, these strong serological responses to bacterial flagellin CBir antigens suggest that this antigen may have a potential role in the immunopathology of the disease.

Anti-glycan antibodies: The most recently described serum markers directed against microbial antigens are anti-glycan antibodies. Glycans are predominant cell surface oligosaccharides found on microorganisms, immune cells, erythrocytes, and tissue matrices. In IBD, the presence of anti-glycan antibodies results from the interaction between the immune system and the glycosylated cell wall components of such pathogens as fungi, yeast, and bacteria. Besides gASCA (which is very similar to conventional ASCA IgG), certain novel anti-glycan

antibodies were identified and associated with CD: anti-mannobioside carbohydrate antibodies (AMCA), anti-laminaribioside carbohydrate antibodies (ALCA), anti-chitobioside carbohydrate antibodies (ACCA), anti-laminarin carbohydrate antibodies (anti-L), and anti-chitin (anti-C) carbohydrate antibodies.

Anti-glycan markers are significantly increased in CD compared to UC and healthy controls[29,30]. However, only 16.9%-30.5% of patients were positive for each of AMCA, ALCA, ACCA, anti-L, and anti-C markers in pediatric CD[31]. Since the presence of anti-L and anti-C is low in ASCA-negative patients with CD, it has been proposed that these markers may bind different epitopes. Interestingly, the optimal cutoff values for anti-glycan markers were different in children than in adult populations in a serological study by Rieder *et al*[31]; strikingly lower cutoff points of gASCA, ACCA, ALCA, AMCA, anti-L, and anti-C were observed in children compared to adult patients with CD.

Pancreatic autoantibodies: Autoantibodies against exocrine pancreas (PAB) were described for the first time in 1984[32], but the autoantigenic targets of PAB were identified only in 2009[33,34]. The recognition of glycoprotein 2 (GP2) as a major target antigen of the droplet-like PAB (type I PAB) has been followed by the identification of CUB/zona pellucida-like domain-containing protein 1 (CUZD1) as another major antigenic target of PAB giving the reticulogranular, cytoplasmic pattern by indirect immunofluorescence (type II PAB). Both GP2 and CUZD1 are glycosylated membrane proteins residing in the acinar secretory storage granules of the pancreas. It was previously believed that GP2 is exclusively expressed by pancreatic acinar cells, but recent studies have shown that GP2 is also present as a specific membrane-anchored receptor on the microfold (M) intestinal cells of intestinal Peyer's patches, and is essential for host-microbial interaction and the initiation of bacteria-specific mucosal immune responses[35,36]. Notably, GP2 overexpresses at the site of CD inflammation in contrast to UC[33,37]. Respective data regarding CUZD1 expression in the intestine are sparse, with further research being needed to evaluate the relevance of these autoantibodies in CD. Combined determination of GP2 and CUZD1-specific autoantibodies by indirect immunofluorescence using recombinantly expressed human embryonic-kidney cell autoantigens represents a new method in the serological diagnosis of IBD. Discrimination between positive and negative reactions is considered to be easier in transfected cells than in primate tissues. The selective detection of anti-GP2 and CUZD1 autoantibodies by enzyme-linked immunosorbent assay (ELISA) has also been recently developed[34].

PAB have been reported to be pathognomonic markers of CD. A prevalence of 27% to 39% of PAB was present in patients with CD, compared with only 0% to 5% in patients with UC[38-40]. Increased prevalence of PAB has been found in unaffected first-degree relatives[41]. Stöcker *et al*[38] reported that PAB could only be determined in the serum of patients with CD. However, other studies found much higher (22%-24%) prevalence of PAB in UC[42-44]. Although anti-GP2 only represents a small proportion of PAB seropositive cases, anti-GP2 autoantibodies are detected in about 30% of patients with CD and in 5%-12% of patients with UC[45-47].

Ulcerative colitis

Autoantibodies against intestinal goblet cells: Serological markers have been far less extensively studied in UC than in CD. Autoantibodies against different colonic antigens have been found in patients with UC [*e.g.*, goblet cell autoantibodies (GAB)]. In previous studies, GAB has been detected in adult patients with UC, with a prevalence of 28% to 30%. In contrast, other studies suggested a much lower prevalence in both diseases[42-44]. These conflicting results are likely due to methodological differences, such as enzyme-linked immunosorbent assay antigen substrates and the evaluation of fluorescence patterns. GAB produce mucin that has multiple functions: it serves as a lubricant, provides nonspecific protection against unwanted microbial agents, and hosts the normal bacterial flora. Through complicated and strictly regulated glycosylation, mucins act as a decoy in binding a range of different microbes and maintaining the normal intestinal flora. The significance of these antibodies, however, has not been established and thus remains unclear.

DIAGNOSTIC VALUE OF NEW SEROLOGIC MARKERS IN IBD

In diagnostic workup of IBD, a serologic test with high sensitivity and specificity is desired. The diagnostic value of the new serologic markers for IBD is limited due to their low sensitivity and presence in other conditions, such as celiac disease, autoimmune diseases, and liver cirrhosis[48-50]. Sensitivity can be increased by the combination of different antibodies. A role for serological testing in screening for IBD was suggested by several studies, but the low sensitivity of these assays only provide a modest contribution to the identification of IBD[8,24,51-53]. The diagnostic value of the new serologic markers in children with IBD is shown in Table 1. A retrospective study of 300 pediatric patients tested in the IBD7 panel (anti-OmpC, anti-CBir-1, ASCA, and ANCA, Serology 7, Prometheus, Sandiego, CA, United States) for the evaluation of pediatric IBD resulted in a 67% sensitivity and 76% specificity. Consequently, this panel has a limited clinical utility in screening for pediatric IBD[53].

In pediatric CD, each anti-OmpC, anti-I2, or anti-CBir1 antibody was detected in 11%-55% of patients as a single marker. In a prospective pediatric study using combined analysis (anti-OmpC, anti-I2, anti-CBir1 or ASCA), 77% of patients with CD were positive for at least one microbial-driven antibody[26]. Therefore this method provided modest support for the diagnosis of CD.

Single glycan markers have limited clinical value for the primary diagnostic workup for CD due to their low

Table 1 Diagnostic value of the new serologic markers in children with inflammatory bowel disease

Marker	Sensitivity		Specificity	PPV	NPV	Ref.
	CD	UC		CD *vs* UC		
Anti-Omp	11%-34%	5%-25%	75%-95%	57.9%-69%	51.6%-53.3%	[8,24,25]
Anti-CBir	52%-56%	ND	ND	ND	ND	[10,13,26]
Anti-I2	44.4%-50%	41.7%-42%	58%-58.3%	51.6%-54.3%	51.1%-53.7%	[27,28]
gASCA	60.7%-62.7%	11.1%-14.6%	85.4%-88.9%	87.1%-92.5%	52.2%-55.9%	[30[1],31]
ACCA	8.7%-22%	3%-18.5%	81.5%-97%	72.2%-83.3%	32.4%-38.2%	[30[1],31]
ALCA	19.7%-30.5%	7.6%-14.8%	85.2%-92.4%	81.8%-81.6%	35.9%-40.1%	[30[1],31]
AMCA	12.2%-16.9%	7.6%-14.8%	85.2%-96.7%	71.4%-86.3%	31.9%-39.06%	[30[1],31]
Anti-L	18%-22%	3.3%-14.8%	85.2%-96.7%	76.5%-90.3%	33.3%-40.07%	[30[1],31]
Anti-C	10.2%-22%	2.3%-14.8%	85.2%-97.7%	76.5%-83.3%	33.3%-38.8%	[30[1],31]
PAB	34%-38.5%	20.4%-20.6%	79.4%-79.6%	62.5%-65.1%	54.7%-56.5%	[44,45[1]]
Anti-GP2	30.2%	8.8%	91.2%	77.4%	56.7%	[45][1]
GAB	12.2%	1.9%	98.1%[2]	86.5%[2]	52.7%[2]	[44]

[1]Mixed pediatric and adult cohort; [2]Ulcerative colitis (UC) *vs* Crohn's disease (CD). ND: No data available; Anti-OmpC: Antibodies against outer membrane porin C of *Escherichia coli*; Anti-CBir1: Antibodies against bacterial flagellin CBir1; Anti-I2: Antibodies against the *Pseudomonas*-associated sequence; ASCA: Antibodies against *Saccharomyces cerevisiae*; AMCA: Anti-mannobioside carbohydrate antibodies; ALCA: Anti-laminaribioside carbohydrate antibodies; ACCA: Anti-chitobioside carbohydrate antibodies; Anti-L: Anti-laminarin carbohydrate antibodies; Anti-C: Anti-chitin carbohydrate antibodies; PAB: Pancreatic antibodies; Anti-GP2: Antibodies against glycoprotein 2; GAB: Antibodies against intestinal goblet cells; IBD: Inflammatory bowel disease; PPV: Positive predictive value; NPV: Negative predictive value.

sensitivity. From the entire panel, gASCA came out as the most accurate for the diagnosis of pediatric CD (sensitivity: 62.7%, specificity: 95.6% CD *vs* controls, and 88.9% CD *vs* UC)[31]. With respect to the latest two novel markers, the addition of Anti-L and Anti-C to gASCA and pANCA further improved discrimination between CD and UC ($P < 0.001$) in a large pediatric and adult cohort with IBD ($n = 818$, 517 CD, 301 UC)[30]. More specifically, nearly three-quarters of the patients with CD showed seropositivity for at least one of the aforementioned seven anti-glycan antibodies[30,31]. Anti-glycan antibodies may be particularly important in ASCA-negative patients with CD. Rieder *et al*[31] found that 40.9% of ASCA-negative pediatric patients with CD were positive for at least one other anti-glycan marker, suggesting that these novel antibodies may further improve serological diagnosis for CD. Similarly, other studies found that about half of ASCA negative adult patients were positive for ALCA, ACCA, or AMCA[29,54]. In concordance with the results published by Rieder *et al*[55], Seow *et al*[30] demonstrated that all the anti-glycan antibodies were highly specific for IBD, particularly for CD (85.4%-97.7%), and were more prevalent in CD *vs* UC ($P < 0.0015$). In this large pediatric and adult cohort with IBD, anti-C showed the highest specificity of 97.7, followed by ACCA at 97%, then anti-L at 96.7%. Due to the combined use of these markers, the specificity for CD increases up to 100%[29,55].

While the specificity of PAB for CD is high, its sensitivity is low. In our study the presence of PAB was significantly higher in CD (34%) and UC (20.4%) compared with the pediatric control cohort (0%, $P < 0.0001$). Specificity of PAB was 100%; however, sensitivity was low. The combination of PAB and antibodies against ASCA/pANCA improved the sensitivity of serological markers in CD (87.4%) and in UC (79.6%); specificity was 89.3% and 93.2%, respectively[44]. Combinations of these antibodies, particularly with ASCA, have shown increased sensitivity; therefore, it may be recommended in the diagnostic procedure of IBD[42,44]. Diagnostic accuracy of the combined novel antibodies with conventional serological markers in children with IBD is shown in Table 2[44].

In a recent study, Bogdanos *et al*[45] observed a significantly higher prevalence of PAB compared to anti-GP2 in UC (20.6% *vs* 8.8%, $P < 0.003$), whereas the difference between PAB and anti-GP2 did not reach a statistically significance level in CD (38.5% *vs* 30.2%, $P = 0.108$), respectively. Thus, anti-GP2 testing by ELISA assay seems to be more specific for CD than for PAB testing, so it may improve the differentiation between CD and UC.

In UC, the most frequently studied serological marker is pANCA. Besides pANCA, in our study the prevalence of GAB was significantly increased in patients with UC in comparison to CD and controls (UC, 12.2%; CD, 1.9%; controls, 1.9%; $P = 0.02$). Sensitivity can be significantly increased with combinations of different antibodies. For example, pANCA and/or GAB together had a sensitivity of approximately 80% for UC[44].

ASSOCIATION WITH IBD PHENOTYPES AND PROGNOSIS

In patients with CD at diagnosis, most patients have inflammatory type disease[56,57]. Nevertheless, during the disease course the development of complicated behavior in the pediatric population is a common feature[58]. In the largest pediatric cohort with CD ($n = 989$), the cumulative incidence of stricturing or penetrating complications was found to be 13%, 27%, and 38%, 1, 5, and 10 years after the diagnosis of IBD, respectively[58]. Furthermore, small bowel disease is more frequently correlated with the development of complicated disease behavior than in isolated colonic disease. Based on these observations,

Table 2 Diagnostic accuracy of the combined novel antibodies with conventional serological markers in children with inflammatory bowel disease[44,45]

	Marker	Sensitivity	Specificity	PPV	NPV	Ref.
CD *vs* controls	ASCA	35.5%-72.8%	95.2%-96.5%	91%-93.8%	59.9%-77.8%	[44,45[1]]
	PAB	34.0%-43.8%	100%	100%	60.2%	[44,45[1]]
	Anti-GP2	30.2%	96%	88.3%	57.9%	[45]
	pANCA	33.0%	94.2%	85.1%	58.4%	[44]
	GAB	1.9%	98.1%	50.0%	50.0%	[44]
	PAB and/or ASCA	79.6%	95.2%	94.3%	82.3%	[44]
	Anti-GP2 and/or ASCA	50.9%	92.9%	87.8%	65.4%	[45]
	PAB and/or ASCA and/or pANCA	87.4%	89.3%	89.1%	87.6%	[44]
	PAB and /or ASCA/ pANCA-	53.4%	95.2%	91.8%	67.1%	[44]
	ASCA+/pANCA-	51.5%	95.2%	91.5%	66.2%	[44]
UC *vs* controls	pANCA	77.5%	94.2%	93.0%	80.9%	[44]
	GAB	12.2%	98.1%	86.5%	52.8%	[44]
	PAB	20.4%-23.5%	100%	100%	55.6%	[44,45[1]]
	Anti-GP2	8.8%	96%	68.8%	51.3%	[45][1]
	ASCA	6.9%-26.5%	95.2%-96.5%	66.3%-84.7%	50.9%-56.4%	[44,45[1]]
	ASCA[2]	16.3%	95.2%	77.3%	53.2%	[44]
	PAB and/or pANCA	79.6%	94.2%	93.2%	82.2%	[44]
	PAB and/or pANCA and/or GAB	79.6%	94.2%	93.2%	82.2%	[44]
	Anti GP2 and/or ASCA	14.7%	92.9%	67.4%	52.1%	[45]
	GAB+/pANCA+	12.2%	98.1%	86.5%	52.8%	[44]
	PAB+/pANCA+	18.4%	100%	100%	55.1%	[44]
	rPAB+/pANCA+	22.4%	100%	100%	56.3%	[44]
	GAB+/PAB+/pANCA+	4.1%	100%	100%	51.0%	[44]

[1]Mixed pediatric and adult cohort; [2]Diagnostic value of antibodies against *Saccharomyces cerevisiae* (ASCA) antibodies in ulcerative colitis (UC) patients without primary sclerosing cholangitis (PSC). PAB: Pancreatic antibodies; Anti-GP2: Antibodies against glycoprotein 2; pANCA: Perinuclear anti-neutrophil cytoplasmic antibodies; GAB: Antibodies against intestinal goblet cells; IBD: Inflammatory bowel disease; CD: Crohn's disease; PPV: Positive predictive value; NPV: Negative predictive value.

a more aggressive treatment should be considered in this large subgroup of pediatric patients with CD. Consequently, the evaluation of relevant phenotype-serotype correlations may provide important prognostic information. Association of the new serologic markers with phenotype in pediatric CD is summarized in Table 3.

Antibodies directed to bacterial antigens were reported as being qualitatively (presence) and quantitatively (titer) associated with aggressive disease behavior in both children and adults[10,26,59,60]. The first prospective pediatric study conducted by Dubinsky and co-workers demonstrated that the degree of the immune response to ASCA, anti-I2, anti-OmpC, and anti-CBir1 correlated with internal penetrating, stricturing disease, and the need for surgery in a large cohort with CD (n = 196). The risk of developing penetrating and/or stricturing CD was increased 11-fold in those subjects with immune responses to all four antigens (anti-12, anti-OmpC, anti-CBir1, and ASCA) compared to seronegative cases (OR = 11, 95%CI: 1.5-80.4, P = 0.03). Moreover, in this study, the highest antibody sum group and quartile sum score group showed the most rapid disease progression[26]. These initial findings were confirmed in another larger study of 796 pediatric CD patients using ASCA, anti-OmpC, and anti-CBir1[10].

Recent studies demonstrated that seropositivity for anti-glycan antibodies was associated with early disease onset, small bowel disease, complicated disease behavior, and CD-related surgery in both adult and pediatric CD[4,29,30,31,54,55,61,62]. This was also found in both qualitative (number of positive antibodies) and quantitative (antibody titers) immune response. In a cross-sectional pediatric study, ALCA and anti-L had the strongest association with complications[31]. In this pediatric population, most of the anti-glycan markers, except for ACCA and anti-C, were associated with complicated disease behavior and ALCA with CD-related surgery. Only gASCA was associated with terminal ileal disease location. Surprisingly, gASCA was inversely correlated with early disease onset in this pediatric cohort[31], but this link was found to be positive in adult CD[4,55,63]. This difference may arise from the distinct nature of the intestinal immune system in children.

There are conflicting results related to the association between PAB and CD phenotype in adult cohorts. Increased prevalence of PAB was observed in patients with early onset of disease, and stricturing or penetrating phenotypes[39,40,42,43,64]. Lakatos *et al*[42] reported an association between PAB positivity, perianal disease, and EIMs. However, in our pediatric study, we found that the presence of PAB was not associated with disease phenotype in CD[44]. It is difficult to compare the data of these studies, since age may affect localization and behavior as well.

In some studies, the relation between anti-GP2 and CD phenotype was also evaluated. In mixed pediatric and adult cohort with CD (n = 169), humoral autoreactivity to GP2 and ASCA applying ELISA has been reported to be associated with ileocolonic location, suggesting a

Table 3 Association of the new serologic markers with phenotype in pediatric Crohn's disease

Marker	CD phenotype	Ref.
Anti-OmpC	Complicated disease behavior	[10,26]
Anti-CBir1	CD-related surgery	
Anti-I2		
ASCA		
gASCA	Early disease onset	[30[1],31]
	Ileal disease location	
	Complicated disease behavior	
	Perianal disease	
	CD-related surgery	
ACCA	Complicated disease behavior CD-related surgery	[30][1]
ALCA	Ileal disease location	[30[1],31]
	Complicated disease behavior CD-related surgery	
AMCA	Complicated disease behavior	[30[1],31]
	perianal disease	
Anti-L	Ileal disease location	[30[1],31]
	Complicated disease behavior	
	Perianal disease	
	CD-related surgery	
Anti-C	Complicated disease behavior	[30][1]
	Perianal disease	
	CD-related surgery	
Anti-GP2 with ASCA	Early disease onset	[45][1]
	Ileal location	
	Complicated behavior	
	Perianal disease	

[1]Mixed pediatric and adult cohort. Anti-OmpC: Antibodies against outer membrane porin C of *Escherichia coli*; Anti-CBir1: Antibodies against bacterial flagellin CBir1; Anti-I2: Antibodies against the *Pseudomonas*-associated sequence; ASCA: Antibodies against *Saccharomyces cerevisiae*; AMCA: Anti-mannobioside carbohydrate antibodies; ALCA: Anti-laminaribioside carbohydrate antibodies; ACCA: Anti-chitobioside carbohydrate antibodies; Anti-L: Anti-laminarin carbohydrate antibodies; Anti-C: Anti-chitin carbohydrate antibodies; Anti-GP2: Antibodies against glycoprotein 2.

role for GP2 as a receptor on M cells in intestinal Peyer's patches[45]. Moreover, in this cohort, the presence of anti-GP-2 was associated with younger age at the onset of the disease (< 16 years), stricturing behavior, and perianal disease in CD[45]. Similarly, Pavlidis *et al*[46] demonstrated that patients with colonic CD do not show significant antibody reactivity against GP2 compared to those who had ileal localization; the site of GP2-rich M cells. However, a Belgian study by Op De Beéck *et al*[65] did not find any association between anti-GP2 seropositivity and clinical phenotype in CD (n = 164) using the same ELISA.

In patients with UC, both anti-CBir1 and pANCA positivity correlated with the development of pouchitis after ileal pouch-anal anastomosis. In a study by Fleshner *et al*[66], diverse patterns of reactivity to microbial antigens were manifested as different forms of pouchitis (n = 238, age range: 8-81 years). Anti-CBir1 positivity indicated acute pouchitis only in patients who have low-level pANCA expression, with increased incidence of chronic pouchitis only in patients who had high-level pANCA expression. In a meta-analysis by Singh *et al*[67], the risk of chronic pouchitis after IPAA was higher in ANCA-positive patients, but the risk of acute pouchitis was unaffected by ANCA status. These data had a significant influence on the patients' treatment in post-operative course. The studies could not demonstrate any association between the presence of GAB and clinical presentation, medical therapy, or need for surgery in patients with UC.

ASSOCIATION WITH THE RESPONSE TO THERAPY AND DISEASE ACTIVITY

Recent studies have highlighted the connection of serologic markers with biologic therapies. Previous studies demonstrated that ASCA signals do not predict response to anti-tumor necrosis factor (TNF)-α therapies in CD[4,68]. Comparative findings were reported regarding the effect of biological agents in the behavior of anti-GP2 antibodies. Belgian investigators did not find a robust effect of infliximab and adalimumab in patients followed up for 6-44 mo[65].

No association was detected between anti-glycan markers and the response to corticosteroids and disease activity in children with CD[31]. Similarly, in our study, we could not find any association between serum antibodies of PAB, ASCA, and ANCA and response to therapy[44].

Dubinsky *et al*[69] reported that a combination of phenotype, serotype, and genotype is the best predictive model of non-response to anti-TNFα agents in pediatric patients. In this study, anti-OmpC, anti-CBir1, anti-I2, ASCA, and pANCA serum markers were analyzed. The most predictive model included the presence of three novel "pharmacogenetic" loci, the previously identified BRWD1, pANCA, and UC diagnosis ($P < 0.05$). The relative risk of non-response increased 15-fold when the number of risk factors increased from 0-2 to ⩾ 3 ($P < 0.0001$)[69].

Based on longitudinal analysis, the presence of antibodies in IBD is relatively constant during the disease course[62,70]. However, the prevalence of ASCA, anti-OmpC, and anti-I2 has been found to be more frequent when the disease persists for a long time[12,60]. Furthermore, disease activity, CRP levels, or response to corticosteroids does not appear to influence marker levels in longitudinal studies. Therefore, serial measurement of antibodies may not provide additional information for the evaluation of IBD[31,70].

CONCLUSION

The correct diagnosis and classification of IBD as either CD or UC is essential for choosing the appropriate therapy. Combined application of the novel antibodies (PAB/GP2) with conventional serology markers (ASCA/pANCA) increased sensitivity. Therefore, the use of combinations may be advisable in the diagnostic work-up of selected cases. Moreover, childhood-onset CD often leads to complicated disease (stricturing or penetrating) with increasing prevalence in parallel to disease duration.

In CD, information gained from a serologic profile, both qualitatively and quantitatively, may help to determine the likelihood of a more severe phenotype. In addition, pediatric UC is associated with pancolitis and a higher risk of colectomy. In patients with UC, serologic markers are associated with the development of pouchitis after ileal pouch-anal anastomosis. With this knowledge, clinicians will be able to stratify patients regarding the risk of disease progression, create a personalized treatment strategy, and try to modify disease course, thus improving long-term prognosis. Further simultaneous prospective multicentric studies are needed to evaluate the exact prognostic role of serologic markers which may help in the individual therapeutic management of pediatric and adult IBD.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (15): Laparoscopic resection of gastrointestinal

Laparoscopic surgery for benign and malign diseases of the digestive system: Indications, limitations, and evidence

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Abstract

The laparoscopic technique was introduced in gastrointestinal surgery in the mid 1980s. Since then, the development of this technique has been extraordinary. Triggered by technical innovations (stapling devices or coagulation/dissecting devices), nowadays any type of gastrointestinal resection has been successfully performed laparoscopically and can be performed laparoscopically dependent on the patient's condition. This summary gives an overview over 30 years of laparoscopic surgery with focus on today's indications and evidence. Main indications remain the more common procedures, *e.g.*, appendectomy, cholecystectomy, bariatric procedures or colorectal resections. For all these indications, the laparoscopic approach has become the gold standard with less perioperative morbidity. Regarding oncological outcome there have been several high-quality randomized controlled trials which demonstrated equivalency between laparoscopic and open colorectal resections. Less common procedures like esophagectomy, oncological gastrectomy, liver and pancreatic resections can be performed successfully as well by an experienced surgeon. However, the evidence for these special indications is poor and a general recommendation cannot be given. In conclusion, laparoscopic surgery has revolutionized the field of gastrointestinal surgery by reducing perioperative morbidity without disregarding surgical principles especially in oncological surgery.

Key words: Laparoscopy; Gastrointestinal surgery; Esophagus; Stomach; Cholecystectomy, Colorectal surgery; Liver resection; Pancreas resection; Laparoscopic resection of gastrointestinal

Core tip: Laparoscopy is known for more than 100 years. In the last three decades there have been significant innovations in laparoscopic surgery that have revolutionized the field of digestive surgery so that by now every surgical procedure for any benign or malign digestive disease has been performed laparoscopically. This article gives an overview over the development of laparoscopic surgery as well as presents the most recent evidence for laparoscopic surgery with special focus on morbidity and equivalency regarding oncological results compared to the open approach.

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INTRODUCTION

Laparoscopy is known for more than 100 years. The Swedish internist Hans Christian Jacobaeus performed

the first laparoscopy in a human being in 1910[1]. Over the following decades, laparoscopy became an important diagnostic tool in the field of internal medicine until non-invasive imaging techniques like sonography or computed tomography (CT) scans developed rapidly. And despite of the visionary potential of the diagnostic and therapeutic use of laparoscopy it took more than 70 years until the first laparoscopic intervention was performed. Again, it was not a general surgeon but the German gynecologist Semm[2] who performed the first laparoscopic appendectomy in 1982. While most surgeons took little notice, the German surgeon Erich Mühe was fascinated by this new technique and developed the idea to remove gallstones via laparoscopy in the following years. However, his motives were also the fear of losing parts of surgical competence to other specialties[3]. Therefore he developed the "Galloscope" and performed the first laparoscopic cholecystectomy on September 12th in 1985 in the Community Hospital in Böblingen, Germany[4].

Initially, there was a strong opposition for this new technique in the surgical community but the success in the following years was overwhelming. It only took about ten years until any gastrointestinal operation was performed laparoscopically although the initial results were not always promising due to limitations in technical aspects and instruments. However, in the past three decades the field of laparoscopic gastrointestinal surgery was one of the most highly developing fields in surgery. Supported by the industrial lobby, surgeons developed new instruments and devices to overcome surgical challenges like bleeding control or gastrointestinal resections. Especially the development of stapling devices for the laparoscopic use and the evolution of coagulation techniques by ultrasound or bipolar coagulation revolutionized the laparoscopic gastrointestinal surgery.

Until now, every gastrointestinal resection has been performed through laparoscopy and there is no intraabdominal organ which cannot be approached laparoscopically. Therefore it is not surprising that the further development of the laparoscopic surgical technique focuses rather on technical details than on medical indications. Besides the introduction of High-Definition or 3D optical systems, the further minimization or even the complete avoidance of surgical accesses by accessing through natural orifices natural orifice transluminal endoscopic surgery are hot topics at the present time. In the future there will be assisting systems based on robotic platforms combined with intraoperative navigation systems which shall reduce one of the biggest disadvantages of laparoscopic gastrointestinal surgery namely the missing tactile perception by more precise preoperative imaging which can be used for intraoperative navigation.

This summary gives an overview over the development as well as today's indications and limitations of laparoscopic surgery with main focus on high-quality evidence regarding perioperative morbidity and especially oncological outcome for the single intraabdominal organ systems.

GENERAL CONSIDERATIONS

Advantages of laparoscopic gastrointestinal surgery

Minimally invasive abdominal (laparoscopic) surgery shows some significant advantages over open abdominal surgery. It has been proven, that the patient's recovery is significantly shorter after laparoscopic surgery than after open surgery[5-8]. In general, surgeries performed laparoscopically have a lower surgical trauma, less blood loss and less postoperative pain and a reduced incisional hernia rate. The pulmonary function is better, the times until the first bowel movement, full recovery and return to work are shorter and finally the patients report a better cosmetic result due to smaller incisions.

Disadvantages of laparoscopic gastrointestinal surgery

On the other hand, laparoscopic gastrointestinal surgery has some major disadvantages compared to open surgery. First of all and evaluated most critically is the missing haptic perception, a surgical tool which is of major relevance in some fields of gastrointestinal surgery. Especially the exploration of the abdominal cavity in patients with malignant diseases is compromised as liver or small bowel cannot be palpated during laparoscopy. In elective patients, an exact (and extended, *e.g.,* intraoperative laparoscopic ultrasound of the liver) staging is necessary to avoid missing out on metastases. Secondly, another disadvantage is the limited field of vision and the handling of intraoperative complications (especially bleeding) which is more difficult in laparoscopic than in open surgery due to the limited intraabdominal space. Another disadvantage-especially in the context of the present discussion about economic aspects of medical treatment-is the fact that the procedural costs of laparoscopic surgery are higher compared to open abdominal surgery[9,10].

UPPER GI TRACT

Laparoscopic upper GI surgery has been performed since the early 1990s, when benign esophageal disorders like gastroesophageal reflux, achalasia or hiatal and/or paraesophageal hernias[11-15] as well as any bariatric procedure[16-18] became indications for the laparoscopic approach. About 20 years later, today the laparoscopic approach is gold standard for these indications and also most revisions after failed primary surgery are performed laparoscopically. Moreover, in the past few years more and more gastrointestinal resections due to esophageal, esophagogastric junction or gastric malignancies have been performed minimally invasive.

Esophagus

The treatment for esophageal cancer includes thoracoabdominal esophagectomy with the reconstruction through a gastric pull-up and intrathoracic or cervical esophagogastrostomy. Depending on the preoperative staging a neo-adjuvant therapy should be performed (either radiochemotherapy or chemotherapy alone).

The first minimally invasive (thoracoscopic) esophagectomy for esophageal cancer was reported by Law *et al*[19] in 1997. In the following years there have been several reports from separate centers which performed laparoscopic or combined thoraco-/laparoscopic esophagectomy for esophageal cancer[20-23]. However, these reports mainly focused on technical feasibility of this complex procedure. In 2003, Luketich *et al*[24] reported the first larger series with 222 patients who underwent minimally invasive esophagectomy (MIE). They performed a thoraco-/laparoscopic esophagectomy with a conversion rate of 7.2%. At first glance the morbidity rate seems high with a major complication rate of 32% and an anastomotic leakage rate of 11.7%. However both rates do not appear increased compared to the open surgical approach. On the other hand, the mortality rate (1.4%) is very low compared to open esophagectomy (8%-22%)[25]. In 2012, the authors reported results of over 1000 patients who underwent MIE with similar results. Comparable to open esophagectomy patients with intrathoracic anastomosis (Ivor-Lewis-Esophagectomy) showed lower complication rates than patients with neck anastomosis[26]. These results indicate that MIE is feasible with very good perioperative outcomes and they were confirmed by Biere *et al*[27] in 2012 when they presented data from a randomized controlled trial (RCT) comparing open and minimally invasive esophagectomy regarding short-term postoperative complications and found benefits in the minimally invasive group especially regarding pulmonary infections. However, the now widely spread use of MIE is limited by the lack of high-quality studies on the oncological outcome comparing open and laparoscopic approach[28] as well as a lack of high-quality clinical studies comparing the gold standard (open Ivor-Lewis-Esophagectomy) to MIE[29].

Therefore, due to missing evidence, today's MIE should only be performed by surgeons in specialized centers who are experienced in minimally invasive esophageal surgery.

Stomach

Laparoscopic gastric resections have been performed since the early 1990s mainly as bariatric procedures. The advantages of laparoscopic surgery are evident especially in critically ill patients and also revolutionized the bariatric surgery. However, laparoscopic gastric surgery in general benefits from the experience of the bariatric surgeon. By now, every type of gastric resection has been performed laparoscopically. However, the evidence for laparoscopic gastric surgery is still poor-except for bariatric surgery which may be the surgical field with the best overall evidence.

It has been proven in many high-quality-studies that bariatric surgery per se is superior to conservative weight reduction programs in morbidly obese patients[30-33]. Especially regarding the long-term weight-loss[30,31] and the relief of co-morbidities there is no such sufficient therapy like bariatric surgery[32,33]. Whether it is better to perform restrictive procedures like the sleeve gastrectomy, or malabsorptive procedures like the gastric bypass is currently under investigation in good-quality prospective trials[34]. However, laparoscopic bariatric surgery is still the most frequently performed laparoscopic surgery in the upper GI tract.

Other indications for laparoscopic gastric resections are benign or malign lesions of the stomach or the esophagogastric junction. The first report of a laparoscopic distal gastric resection was presented by Goh *et al*[35]. In 1995 Uyama *et al*[36] first reported a laparoscopic resection of the proximal part of the stomach and the esophagogastric junction. One year later the first case series of laparoscopic total gastrectomy was reported by Fowler and White[37]. In the beginning, laparoscopic gastric resections were performed due to benign diseases, mainly peptic ulcer disease. Later on, the range of indications extended to resections for early gastric cancer[38-40]. However, the hope for a renaissance of gastric surgery[41] did not last for long, as with the introduction of the proton-pump-inhibitors in the late 1980s and the further development of endoscopic interventions (*e.g.,* endoscopic submucosal dissection or full-thickness-resection-techniques) today most of these diseases can be treated endoscopically. Therefore, beside bariatric surgery, modern gastric surgery is mainly performed due to gastric cancer. Subtotal or total gastrectomy is the surgical procedure of choice. From the technical point of view there are no limitations for the laparoscopic gastrectomy (LG) as it had been proven since the mid 1990s[37]. So the remaining question is the analysis of oncological results of the laparoscopical approach compared to the standard open gastrectomy (OG) as surgery is the only curative option in patients with gastric cancer. There are a few meta-analyses of LG *vs* OG for advanced gastric cancer[42-44]. Beside the known advantages of laparoscopic surgery (less blood loss, shorter time until first bowel movement, shorter hospital stay and fewer complications) they found no difference regarding oncological parameters: harvested lymph nodes, surgical radicalness, recurrence rates and overall survival were idem in the laparoscopic and the open surgery groups. However, all authors state that LG should be performed by experienced minimally invasive surgeons.

There is clear evidence that LG for gastric cancer shows similar oncological results to the OG with fewer surgical complications in the hands of skilled endoscopic surgeons.

HEPATOPANCREATOBILIARY SYSTEM

As described above, the era of laparoscopic gastrointestinal surgery was started in 1985 by the first laparoscopic cholecystectomy performed by Erich Mühe[3,4]. While today the gallbladder is resected laparoscopically in about 95% of the cases, laparoscopic surgery for liver and pancreatic diseases are not that widespread in the surgical community. This might be due to the fact that these types

of operations by far are less common. However, by now it has been demonstrated that nearly any type of hepatopancreatobiliary resection can be performed laparoscopically.

Liver/gallbladder

Since its introduction in 1985[4], it took only a few years until laparoscopic cholecystectomy became the standard technique for cholecystectomy. Indications were widened over the time and today there are nearly no specific contraindications for laparoscopic cholecystectomy. Initially there were reports of tumor cell seeding and surgical site metastasis in cases of laparoscopic removal of gallbladder carcinomas[45-47]. With the introduction of removal bags, these problems were eliminated and today gallbladders with masses of unknown dignity should also be removed laparoscopically.

The first reported laparoscopic liver operation was a laparoscopic drainage of an amebic liver abscess in 1985[48]. Several reports of laparoscopic fenestration of symptomatic liver cysts and laparoscopic atypic liver resection followed[49-56], but it was not until 1996 that the first laparoscopic major liver resections were performed[56,57]. The disadvantages of laparoscopic surgery (the missing haptic perception and coagulation/dissecting techniques) had to be overcome especially in laparoscopic liver surgery. The development of laparoscopic ultrasound devices, as well as water jet or ultrasonic dissectors and laparoscopic stapling devices, were necessary to take the next step in laparoscopic liver surgery[58-64]. In the following decade, every type of major hepatic resection was performed laparoscopically[65-69] which had previously been the same indications for open surgery. By now, minor atypical resections as well as left-lateral hepatectomies are performed laparoscopically by default. Extended liver resections like right-sided hepatectomy or even trisegmentectomy should only be performed by an experienced hepatobiliary and laparoscopic surgeon due to the complexity of this procedures. Another indication for laparoscopic liver resection is the living-donor hepatectomy for liver transplantation[70,71], either left-sided hepatectomy or right-sided hepatectomy can be performed.

There is increasing evidence in high-quality meta-analyses that laparoscopic liver resection is equivalent to open liver resection regarding mid-term and long-term oncological outcomes in cases of malignant diseases (primary liver tumors as well as liver metastases). However, the perioperative and short-term advantages of laparoscopic surgery are also present in laparoscopic liver surgery[72-74].

Pancreas

The first laparoscopic pancreatic resection was reported in 1994 by the Canadian surgeon Michael Gagner, who performed a laparoscopic pylorus-preserving pancreatoduodenectomy in a patient with chronic pancreatitis[75]. The complex reconstruction required gastrojejunostomy, hepaticojejunostomy and pancreaticojejunostomy. With a hospital stay of 30 d, Gagner concluded that laparoscopic pancreatic head resection is feasible but the known advantages of laparoscopic surgery did not seem evident in pancreatic surgery. In the same year, Cuschieri[76] reported about his experience with laparoscopic pancreas surgery and came to same conclusion. Interestingly, both authors independently revised their opinion two years later, when after a small series of laparoscopic distal pancreatectomies they concluded that the advantages of laparoscopic surgery (less postoperative pain, shorter hospital-stay, fewer perioperative complications) are also shown in pancreatic surgery, with a restriction to distal pancreatectomies for benign indications (mainly insulinoma or chronic pancreatitis)[77-79]. However, during the following years, laparoscopic pancreatic surgery concentrated on laparoscopic staging of pancreatic cancer and a few reports with small numbers of drainage-procedures in cases of post-pancreatitis pancreatic pseudocysts[80-87] were also published. In 2001 the first larger series of 19 patients with laparoscopic pancreatic resection was reported by Patterson *et al*[88], again raising the question of the usefulness of laparoscopic pancreatic surgery[89]. A question which still seems to be current in 2013 as the total number of laparoscopic resections is still quite small[90-95]. So by now, there is no evidence for the practicability of laparoscopic pancreatic surgery as good-quality comparative studies are still lacking and thus laparoscopic pancreatic surgery should only be performed within the context of clinical studies.

LOWER GI TRACT

During the last 25 years colorectal surgery has profited from two relevant innovations: The adoption of the laparoscopic technique which was introduced in the mid 1980s[2-4] and the implementation of the concept of fast-track postoperative rehabilitation by Henrik Kehlet in the late 1990s[96,97]. Due to the fact that colorectal resections for both benign and malign indications are-aside from cholecystectomy-the most frequently performed visceral operations, laparoscopic colorectal surgery has been widely accepted in the surgical community and has been investigated in many high-quality studies. Thus today the laparoscopic technique should be preferred in colorectal resections for most indications.

The first laparoscopic colorectal resections were reported in 1991 with various indications[98-102]. The surgical community was skeptic and so numerous articles raising questions about the effectiveness and safety of laparoscopic colorectal surgery followed only one year later[103,104]. Especially the question of oncological adequateness aroused over the following years[105,106], and it was the group of Steven Wexner of the Cleveland Clinic who claimed patience, since reliable oncological results can only be achieved after five years at the earliest[107]. Also, there were the same problems in laparoscopic colorectal surgery for colorectal cancer, similar to those after cholecystectomy for gallbladder carcinomas[45-47], namely surgical site metastasis at the trocar sites[108-110], a topic which is still up to date[111]. However, the number

of patients who underwent the laparoscopic surgery rose quickly and in 1995 Ballantyne[112] presented a review of 752 patients who had undergone laparoscopic colorectal resections. He came to the conclusion that patients who undergo laparoscopic colorectal resections have fewer complications, less pain and shorter hospital stay-which is all the important advantages of laparoscopic gastrointestinal surgery-compared to open colorectal resections. Interestingly, this was an accepted opinion during the following years, probably due to an appropriation of the results from other laparoscopic fields to colorectal surgery. However, a recent review on the same topic concluded that the impact of the laparoscopic technique on the early postoperative outcome is not as high as expected[113] but factors like patient co-morbidities or the severity of a disease play a greater roll. This must be further evaluated as there is still a lack of high-quality studies on this topic[114]. In the 2000s, indications for laparoscopic colorectal surgery were further extended and today also include surgery for IBD[115] or acute diverticulitis[116] with promising results of the postoperative outcome. Therefore today, patients with any benign indication for colorectal surgery should be offered the laparoscopic approach.

But what about the malign indications? There are the same aforementioned problems of oncological adequateness and safety of the laparoscopic technique, compared to the standard open surgical approach which seems to be more relevant than in other organ systems due to the frequency of this type of surgery. It took quite longer than expected by Wexner, until in 2004 the question concerning colon cancer was answered by the Clinical Outcomes of Surgical Therapy Study Group[117]. Their multicenter RCT included 872 patients at 48 institutions and randomized them into two groups to either open or laparoscopic resection for colon cancer. They found no difference between both groups regarding the primary endpoint time of tumor recurrence with a median of 4.4 years. The Colon Cancer Laparoscopic or Open Resection (COLOR) Study Group came to the same conclusion[118]. They investigated the oncological adequateness of laparoscopic colonic resection for colon cancer in 1076 patients with a median follow-up of 53 mo. Despite a slightly higher 3-year-overall survival in the open surgery group (84.2% *vs* 81.8%) the authors justify the implementation of laparoscopic colonic resection for colon cancer into clinical practice. The third large RCT regarding laparoscopic colorectal resections for colorectal cancer is the United Kingdom MRC CLASICC Trial[119]. The researcher investigated 794 patients who were undergoing surgery for colorectal cancer and randomized them to either the laparoscopic group or the open surgical approach group. The overall survival, as well as the disease-free survival showed equivalent numbers in both groups for colonic resections and rectal resections. However, the circumferential resection in rectal cancer did not show equally satisfying results in the laparoscopic group, leaving some concerns regarding the recommendation to perform rectal resections laparoscopically[120].

However, this final issue regarding the oncological equivalency of laparoscopic rectal resections will soon be answered. In 2013, preliminary results (*e.g.*, perioperative data like morbidity or histopathological findings) of the COLOR-Ⅱ-trial were presented. This multicenter RCT which compares the oncological safety of laparoscopic rectal surgery to open surgery for rectal cancer investigated 1044 patients[121]. The authors found no difference regarding oncological radicalness between both groups but found the "typical" laparoscopic advantages in the secondary endpoints such as less blood loss, shorter time until bowel function or shorter hospital stay. Long-term oncological data can be expected at the end of 2013.

So far, by now laparoscopic surgery for colon cancer has been proven to be equivalent to open surgery regarding oncological outcomes with the additional general advantages of laparoscopic surgery and therefore should be offered to the patients. Laparoscopic rectal surgery can be offered by experienced surgeons but always with the awareness that there is still a lack of a final prove that oncological safety is given in this subgroup of patients with colorectal cancer.

CONCLUSION

In conclusion, laparoscopic gastrointestinal surgery has evolved dramatically over the last three decades and provided an important improvement in the patient-centered care. The perioperative morbidity and mortality could be cut down significantly by reducing the surgical trauma. From the technical point of view, any gastrointestinal resection can be performed laparoscopically today. For the most frequent operations like appendectomy, cholecystectomy, bariatric procedures and colorectal resections it exists high-quality evidence of the benefits or at least evidence of the equivalency of laparoscopy versus open surgical approach. Laparoscopic resection has become the gold standard approach to these procedures no matter if the indication results from a benign or malign disease in the case of colorectal cancer. In the hands of experienced minimally invasive surgeons, those more complex and less frequently performed procedures (esophagectomy, oncological gastrectomy, liver and pancreatic resections) can be carried out safely. However, the question whether there is an equivalent oncological outcome compared to the open approach in these indications still is unanswered by now and have to be proven by future studies.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (15): Laparoscopic resection of gastrointestinal

Laparoscopic liver resections for hepatocellular carcinoma: Current role and limitations

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Abstract

Liver resection for hepatocellular carcinoma (HCC) is currently known to be a safer procedure than it was before because of technical advances and improvement in postoperative patient management and remains the first-line treatment for HCC in compensated cirrhosis. The aim of this review is to assess current indications, advantages and limits of laparoscopic surgery for HCC resections. We also discussed the possible evolution of this surgical approach in parallel with new technologies.

Key words: Hepatocellular carcinoma; Laparoscopic liver resection; Hepatectomy; Minimally invasive; Review; Laparoscopic resection of gastrointestinal

Core tip: We assess in this review current indications, advantages and limits of laparoscopic surgery for hepatocellular carcinoma. We also discuss the possible evolution of this surgical approach in parallel with new technologies.

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INTRODUCTION

Hepatocellular carcinoma (HCC) represents the fifth most frequent cancer[1], and the third most widespread cause of cancer-related deaths in the world[2]. Most HCCs develop within chronic liver disease, mainly chronic hepatitis and cirrhosis. Screening for HCC among patients with chronic liver disease facilitates the early detection of small tumors, increasing the number of curative therapeutic options available. Liver transplantation appears most attractive since it treats both the cancer and the underlying disease. However, the scarcity of donors does not permit transplantation in all patients with early HCC[3]. Liver resection for HCC is currently known to be a safer procedure than it was before because of technical advances and improvement in postoperative patient management[4-7] and remains the first-line treatment for HCC in compensated cirrhosis in many centers.

Many types of liver resections, including major hepatectomies, are now performed by laparoscopy in specialized centers[8]. Since 2000, more than 600 cases of laparoscopic resection for HCC have been reported. Recent studies have confirmed that laparoscopic HCC resection is safe and seems to additionally improve the postoperative course particularly for cirrhotic patients[9-11]. Follow-up data from a few study groups have suggested that the long-term oncologic outcome has not been compromised by the laparoscopic approach compared with open resection[9,11-13].

The aim of this review is to assess current indications, advantages and limits of laparoscopic surgery for HCC resections. We also discussed the possible evolution of

this surgical approach in parallel with new technologies. The information in this review was extracted from the literature after a Medline search.

INDICATIONS AND CURRENT ROLE OF LAPAROSCOPY

There are presently no formalized indications for laparoscopic liver resection (LLR) and selection criteria may be varied among institutes. A consensus of experts who met in Louisville, Kentucky, United States in 2008, said the best indications for laparoscopy were solitary lesions, less than 5 cm, located in the anterior segments, at a distance from the line of transection, the hepatic hilum, and the vena cava[14]. Ever since this consensus conference took place, surgical indications have continued to evolve: tumor size on its own is no longer a contraindication to laparoscopic surgery[15] and experienced centers perform LLR for tumors in the posterior segments or central liver[16,17]. Although tumors should be located at a safe distance from the potential transection line on preoperative imaging, close proximity to the portal pedicle has also become a debatable limit as laparoscopic magnification allows very precise extrahepatic portal dissection[18]. Furthermore, a recent study by Yoon[19] show that LLR can be safely performed in selected patients with centrally located tumors close to the major hepatic veins, or the inferior vena cava. Of course, open approach is still required for some patients necessitating complex liver resection or additional procedures such as vascular or biliary reconstruction.

Most surgical teams consider non-compensated cirrhosis as a contraindication for liver resection and thereby for LLR[20]. An uncontrolled portal hypertension (esophageal varices > grade 1, platelet count < 8×10^{10} /L) is usually considered as an exclusion criterion for the laparoscopic approach[8]. Patients presenting an American Society of Anesthesiologists (ASA) score $\geqslant$ 3 and/or a major vascular invasion are also generally excluded[8]. Moreover, neither previous upper abdominal surgery[21] nor overweight is a contraindication to laparoscopic resection.

Although parenchymal-sparing resection is required by the presence of underlying liver disease, anatomic resection is preferred when liver surgery is performed with a curative intent for hepatocarcinoma due to the tumor's high propensity to invade the portal vein branches[22,23]. Indeed, anatomic resection independently improves long-term survival for patients with HCC, particularly in the case of tumors more than 2 cm in diameter[24]. Laparoscopy allows anatomical resections for HCC[20] with a previous control of portal pedicles including major hepatectomies[8]. Our team even advocated that atypical resections are paradoxically the most difficult and require previous experience in minor anatomic LLR (left lateral sectionectomy)[18].

Laparoscopy is well known for making subsequent surgical procedures easier since intra-abdominal adhesions are reduced after laparoscopic surgery compared with open surgery[20]. Although liver transplantation is considered the best treatment for patients with early HCC, resection may be the initial treatment in some patients, allowing salvage transplantation in case of delayed and limited recurrences within the Milan criteria, or being a selection tool providing complete information about the pathology and prognostic characteristics of the resected tumour, and thus facilitating the selection of the best candidates for further liver transplantation[25,26]. Performing the initial HCC resection by laparoscopy could facilitate a subsequent liver transplantation. In a study by Laurent *et al*[27], it has been shown that when the initial liver resection is done by laparoscopy, the subsequent salvage transplantation is associated with reduced operative time, blood loss, and transfusion requirements.

Screening and treatment of recurrence is another major issue. Repeated hepatectomy is a potentially curative therapy, and offers patients the possibility of long-term survival[28]. In a study by Dagher *et al*[8], re-resection after initial LLR was possible in 18.7% of recurrences. The difficulty of reintervention is increased by modifications of the anatomy and the formation of adhesions that can make a new surgical procedure more difficult and less safe. Two studies comparing laparoscopic and open redo surgery for recurrent HCC are available in the literature[29,30]. Laparoscopic redo procedure was associated with lower intraoperative blood loss, transfusion rates, postoperative morbidity, ascites and shorter hospital stay compared with open redo procedures whatever the initial approach. Moreover, the operating time for patients who underwent laparoscopic primary approach was significantly shorter compared to patients who were treated by primary open liver resection. With a minimally invasive approach, the formation of postoperative adhesions seems to be minimized, and the adhesiolysis procedure seems to be faster and safer in terms of blood loss and risk of visceral injuries, as previously reported[31]. Therefore, the laparoscopic approach for the treatment of HCC in cases of cirrhosis seems to be advisable as the first procedure whenever feasible.

OUTCOMES

Nineteen retrospective comparative studies of patients undergoing liver resection for HCC are available in the literature (Table 1), and five published meta-analysis have investigated the advantages and disadvantages of the LLR for HCC[32-36]. No randomized controlled trials comparing open to LLR have been reported.

Operative outcomes

LLR is associated with significantly less intraoperative blood loss and blood transfusion requirement, which can partly be explained by the hemostatic effect of pneumoperitoneum[18] and the magnified vision afforded by laparoscopy[9,18,34]. Besides, transfusion rates have been identi-

Table 1 Intraoperative outcomes in retrospective comparative studies concerning liver resection for hepatocellular carcinoma *via* laparoscopy or open approach

Ref.	Laparoscopies (*n*)	Median blood loss, mL			Blood transfusion			Median operative time, min		
		L	O	*P* value	L	O	*P* value	L	O	*P* value
Cheung *et al*[11], 2013	32	150	300	0.001	0%	4.7%	NS	204	232	NS
Kanazawa *et al*[10], 2013	28	88	505	0.0003	0%	4%	0.0379	228	236	NS
Lee *et al*[12], 2011	33	150	240	NS	6.1%	10%	NS	225	195	0.019
Ker *et al*[46], 2011	116	139	1147	< 0.001	6.9%	50.9%	< 0.001	156	190	NS
Hu *et al*[69], 2011	30	520 g	480 g	NS	-	-	-	180	170	NS
Kim *et al*[70], 2011	26	-	-	-	19.2%	24.1%	NS	147	220	0.031
Truant *et al*[13], 2011	36	452	447	NS	2.8%	3.8%	NS	193	215	NS
Tranchart *et al*[9], 2010	42	364	723	< 0.0001	9.5%	16.7%	NS	233	221	NS
Aldrighetti *et al*[71], 2010	16	258	617	0.008	4%	6%	NS	150	240	0.044
Belli *et al*[30], 2009	54	297	580	< 0.01	11%	25.6%	0.03	167	185	0.012
Lai *et al*[72], 2009	25	-	-	-	-	-	-	150	135	NS
Endo *et al*[73], 2009	10	-	-	NS	-	-	-	-	-	-
Sarpel *et al*[74], 2009	20	-	-	-	-	-	NS	161	165	NS
Belli *et al*[48], 2007	23	260	377	NS	0%	17.3%	0.036	148	125	0.016
Lee *et al*[75], 2007	25	100	250	0.012	4%	0%	NS	220	195	NS
Kaneko *et al*[76], 2005	30	350	505	NS	-	-	-	182	210	NS
Laurent *et al*[77], 2003	13	620	720	NS	4%	28.6%	NS	267	182	0.006
Shimada *et al*[78], 2001	17	400 g	800 g	NS	5.9%	10.5%	NS	325	280	NS

L: Laparoscopy; O: Open; NS: Non significant.

fied as an independent prognostic factor for disease-free survival in HCC[37,38], and blood loss was shown to be independently associated with recurrence and decreased survival rates after resection of HCC[39]. By decreasing the risk of transfusion, laparoscopy should improve the prognosis of patients undergoing liver resection for HCC[8].

Several transection devices, developed specifically for LLR, were reported[40-44]. However, the superiority of new hemostatic devices for parenchymal transection over the conventional clamp crushing method[45] has yet to be demonstrated. In a multicentric international retrospective study, Buell *et al*[43] advocated that stapler laparoscopic hepatectomy might provides several advantages including diminished blood loss and transfusion requirements however concerns existed regarding smaller surgical margins in the stapler hepatectomy group

In a recent study by Soubrane *et al*[20], conversion to laparotomy was necessary in 13% of 351 LLR performed for HCC in nine French tertiary centers. An underlying liver disease was observed in 85% of patients. The main cause for conversion was bleeding. It is important to note that conversion rates are not significantly different in patients with cirrhosis compared with non-cirrhotic patients, as reported by Dagher *et al*[8].

There was no significant difference in operative time in most studies[35], as observed in other indications.

Postoperative outcomes

Of all advantages of laparoscopy, the decrease of perioperative morbidity is paramount (Table 2). In a recent meta-analysis by Yin *et al*[35], postoperative morbidity rates after laparoscopic resections of HCC were significantly decreased compared with open surgery. Ker *et al*[46] have published the largest series and found a similar postoperative morbidity result. Postoperative outcomes identified in the recent French survey[20] found a 30-d postoperative mortality rate of 2% and an overall morbidity rate of 22%.

The main clinical advantage of laparoscopy for cirrhotic patients is probably the significantly lower rate of postoperative ascitic decompensation which was reported in four comparative studies[9,10,46-48] and three meta-analysis[32-34]. This finding could be explained by the preservation of portosystemic venous collateral circulation around the liver and parietal abdominal wall, limited mobilization and manipulation of the liver, restricted fluid requirements and decreased blood loss[34]. Cannon *et al*[49] advocated that the positive pressure of the pneumoperitoneum might exert a tamponade effect on bleeding from intra-abdominal varices which are a low pressure system, decreasing blood loss. Lower blood transfusion requirement is also an advantage of the laparoscopic approach in this very risky group of patients[9].

The incidence of liver failure was also reported as lower after LLR when compared to open liver resection[9,32,34]. No significant difference was reported regarding the incidence of other specific complications such as bile leakage and postoperative hemorrhage[10,32]. Concerning general complications, the rate of pulmonary complications appears significantly lower after laparoscopic resection[33].

Meta-analysis reporting on length of hospital stay are consistently favorable for LLR[32-36], consequently shortened by lower overall morbidity and incidence of intractable ascites.

Oncologic outcomes

The main concern about the use of laparoscopic procedure for malignancies is the risk of inadequate tumor resection. A positive histologic margin was associated with

Table 2 Oncologic outcomes in retrospective comparative studies concerning liver resection for hepatocellular carcinoma *via* laparoscopy or open approach

Ref.	Laparoscopies (*n*)	Positive surgical margin			Overall survival			Recurrence-free survival		
		L	O	*P* value	L	O	*P* value	L	O	*P* value
Cheung *et al*[11], 2013	32	-	-	-	76.6%[5]	57%[5]	NS	54.5%[7]	44.3%[7]	NS
Kanazawa *et al*[10], 2013	28	-	-	-	-	-	-	-	-	-
Lee *et al*[12], 2011	33	3.0%[1]	2.0%[1]	NS	76%[5]	76.1%[5]	NS	45.3%[7]	55.9%[7]	NS
Ker *et al*[46], 2011	116				62.2%[5]	71.8%[5]	NS	-	-	-
Hu *et al*[69], 2011	30	-	-	-	50%[5]	53.3%[5]	NS	-	-	-
Kim *et al*[70], 2011	26	3.8%[1]	3.4%	NS	-	-	-	84.6%	82.8%	NS
Truant *et al*[13], 2011	36	-	-	-	70%[5]	46%[5]	NS	35.5%[7]	33.6%[7]	NS
Tranchart *et al*[9], 2010	42	-	-	-	59.5%[5]	47.4%[5]	NS	60.9%[6]	54.3%[6]	NS
Aldrighetti *et al*[71], 2010	16	0%[1]	18.7%[1]	NS	-	-	NS	-	-	NS
Belli *et al*[30], 2009	54	0%[1]	40.8%[1]	NS	67%[4]	-	NS	52%[6]	-	NS
Lai *et al*[72], 2009	25	-	-	-	60%[4]	-	-	52%[6]	-	-
Endo *et al*[73], 2009	10	-	-	-	-	-	NS	-	-	NS
Sarpel *et al*[74], 2009	20	10%[1]	26.8%[1]	NS	-	-	NS	-	-	NS
Belli *et al*[48], 2007	23	8.6%[3]	0%[3]	NS	86.9%	82.6%	NS	-	-	-
Lee *et al*[75], 2007	25	-	-	-	-	-	NS	-	-	NS
Kaneko *et al*[76], 2005	30	-	-	-	61%[5]	62%[5]	NS	31%[7]	29%[7]	NS
Laurent *et al*[77], 2003	13	15.4%[1]	14.3%[1]	NS	89%[4]	55%[4]	0.04	46%[6]	44%[6]	NS
Shimada *et al*[78], 2001	17	41.2%[2]	50%[2]	NS	-	-	NS	-	-	-

[1]Tumor at surgical surface; [2]Tumor invasion < 5 mm; [3]Tumor invasion < 1 cm; Overall survival at 2, 3[4] or 5[5] years; Recurrence-free survival at 2, 3[6] or 5[7] years. L: Laparoscopy; O: Open; NS: Non significant.

a higher incidence of postoperative HCC recurrence[50], and a wide margin (2 cm) was reported to be preferable to the conventional 1-cm margin[51]. Most authors found that rates of positive margins after LLR were lower or similar to those after open hepatectomy, supporting that a good surgical margin is provided by the appropriate preoperative choice of the type of resection[9].

Laparoscopic surgery also was feared to increase the risk of peritoneal carcinomatosis and trocar-site deposits[52,53]. In the series reviewed in this study, we observed neither peritoneal carcinomatosis nor port-site recurrence.

Furthermore, in all studies comparing laparoscopic and open liver resection for HCC, there was no significant difference in recurrence-free or overall survival, suggesting that laparoscopic surgery does not compromise oncological principles (Table 3).

LIMITATIONS

Patient selection for laparoscopic hepatic resection is challenging but the most important prerequisite of good outcomes. Stringent selection criteria based on surgeon experience, patient clinical characteristics, lesion size and location must be employed. Thus, only 27% of patients with HCC who are candidates for resection could be operated *via* laparoscopy in an experienced team[9].

It was initially feared that laparoscopic liver resection would decrease the surgical margin due to the lack of palpation, except in the case of intra-abdominal hand-assisted LLR, though the haptic feedback transmitted through laparoscopic graspers is limited. However, manual exploration of cirrhotic liver can be difficult even during open surgery, and palpation probably is less important when an anatomic resection is planned. Intraoperative ultrasound should be systematically used to locate the tumor, making it possible to keep the intended margin. Therefore, a recent comparative study showed that sensitivity and specificity of intra-operative sonography were equivalent, whether performed *via* laparoscopy or laparotomy[54].

Fear of uncontrollable major bleeding or gas embolism explains the initial slow development of the laparoscopic approach. Nonetheless, there have not been any intra-operative deaths reported in the largest study on major LLR[55] and though frequent embolisms of CO_2 have been shown to occur[56], it is without any clinical repercussion, as shown in animal[57] as well as human studies[58].

The main criticism to LLR is its low reproducibility and its confinement in few expert centers. The reasons for such a limitation are 2-fold: the need of expertise in both laparoscopic and hepatic surgery and the expected long learning curve[14,59]. Vigano *et al*[60] observed a clear "learning curve" effect over a 13-year period of 60 procedures before a significant improvement in terms of operative time, conversion rate, blood loss, morbidity, and hospital stay, suggesting that LLR is reproducible in centers regularly performing liver surgery, but requires specific training in advanced laparoscopy.

PERSPECTIVES

Minimally invasive surgery

In an effort to make laparoscopy less and less invasive, surgeons have tried to decrease the number of trocars necessary to perform an operation. Several teams have reported their experience with single incision LLR for HCC[61,62]. The resections reported so far have mainly been small, atypical resections or left lobectomies, al-

Table 3 Postoperative outcomes in retrospective comparative studies concerning liver resection for hepatocellular carcinoma *via* laparoscopy or open approach

Ref.	Laparoscopies (*n*)	Postoperative morbidity			Ascites			Mean postoperative hospital stay, d		
		L	O	*P* value	L	O	*P* value	L	O	*P* value
Cheung *et al*[11], 2013	32	6.3%	18.8%	NS	31.1%	6.2%	0.871	4	7	< 0.0001
Kanazawa *et al*[10], 2013	28	10.7%	71.4%	< 0.0001	3%	18%	< 0.0001	10	19	< 0.0001
Lee *et al*[12], 2011	33	6.1%	24%	0.033	-	-	-	5	7	< 0.0005
Ker *et al*[46], 2011	116	6%	30.2%	< 0.001	1.7%	12.5%	0.002	6.2	12.4	0.001
Hu *et al*[69], 2011	30	13.3%	10%	NS	-	-	-	13	20	< 0.01
Kim *et al*[70], 2011	26	3.8%	24.1%	NS	-	-	-	11	16	0.034
Truant *et al*[13], 2011	36	25%	35.8%	NS	13.9%	22.6%	0.3	6.5	9.5	0.003
Tranchart *et al*[9], 2010	42	21.4%	40.5%	NS	7.1%	26.1%	0.03	6.7	9.6	< 0.0001
Aldrighetti *et al*[71], 2010	16	25%	43.7%	NS	0%	1%	NS	6.3	9	0.039
Belli *et al*[30], 2009	54	19%	36%	0.02	-	-	-	8.4	9.2	NS
Lai *et al*[72], 2009	25	16%	15%	NS	-	-	-	7	9	0.008
Endo *et al*[73], 2009	10	-	-	-	-	-	-	-	-	< 0.05
Sarpel *et al*[74], 2009	20	-	-	-	-	-	-	-	-	-
Belli *et al*[48], 2007	23	13%	47.8%	0.01	13%	39.1%	0.043	8.2	12	0.048
Lee *et al*[75], 2007	25	4%	4%	NS				4	7	< 0.001
Kaneko *et al*[76], 2005	30	10%	18%	NS	-	-	-	14.9	21.6	< 0.005
Laurent *et al*[77], 2003	13	36%	50%	NS	8%	36%	0.15	15.3	17.3	NS
Shimada *et al*[78], 2001	17	5.9%	10.5%	NS	-	-	NS	12	22	< 0.001

L: Laparoscopy; O: Open; NS: Non significant.

though in a recent case series, single port laparoscopic major hepatectomy was performed in 2 patients with HCC[62].

A few cases of Natural Orifice Translumenal Endoscopic Surgery LLR have also been reported[63]. However, this approach is still anecdotal.

The use of these minimally invasive approaches for more complex operations is certainly conceivable and will probably be extended with robotic assistance.

Robotic surgery

Robots are an inevitable part of the future of surgery and liver laparoscopy will undoubtedly follow along this road. Lai *et al*[64] reported the largest series of robot-assisted laparoscopic liver resection for HCC. In a subgroup analysis of minor liver resection, the robotic group had similar blood loss, morbidity rate, mortality rate, and R0 resection rate when compared with the conventional laparoscopic approach. However a significantly longer operative time was observed. Robotic assistance in liver surgery could clearly allow simplification of certain complex procedures such as major LLR and could facilitate additional procedures such as vascular or biliary reconstruction. It is possible that the learning curve for robotic resections may be shorter than that of conventional laparoscopic liver surgery, because the three dimensional imaging camera, wristed instruments, and better ergonomics will help already experienced laparoscopic surgeons to quickly familiarize themselves with the robotic procedure[65]. Most of the series reporting on robotic liver resection have focused on short-term perioperative outcomes. Long-term results and cost-effectiveness are necessary before the advantages and disadvantages of robotic liver resection can be conclusively stated.

Intra-operative guidance

LLR for small HCC remains a challenge because of limited tactile sensation, unidentifiable tumors, and complex liver anatomy. Therefore, intra-operative guidance during LLR is a future topic of interest. Intra-operative sonography has already been alluded to, but newer ingenious procedures to guide the surgeon are under investigation.

Innovations in this direction have actually been attempted. Chopra *et al*[66] reported five case of LLR under intra-operative MRI guidance in a porcine model without employing ferromagnetic material, and Kenngott *et al*[67] recently used robotic C-arm cone-beam computed tomography in a hybrid operating room for computer-assisted guidance during laparoscopic resection for HCC in man. Aside from some interferences between the camera and the magnetic field in the first study and additional radiation exposure for the patient and the need for the surgical team to leave the operating room while performing imaging in the second, the operations were performed under good conditions.

The use of fluorescent imaging technique for detecting HCC has also been reported. Ishizawa *et al*[68] have developed the use of indocyanine green (ICG), which has been intravenously injected for a preoperative liver function test, as a fluorescent source that enables the realtime identification of HCC during surgery. Since well or moderately differentiated HCC tissues retain portal uptake of ICG despite the lack of biliary excretion, small HCC located just beneath the liver surface were detected using a near-infrared light camera system, with high sensibility.

CONCLUSION

Review of the literature shows that in highly selected patients LLR for HCC seems to be superior to the open

liver resection in terms of perioperative results without compromising the oncological outcomes, especially in cirrhotic patients, in whom postoperative ascites is decreased by the use of laparoscopy. A prospective comparative study should be designed to confirm the advantages of laparoscopy for the management of HCC. The development of new technologies and robotics will certainly expand the use of laparoscopy in the multimodal management of hepatocarcinoma.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (15): Laparoscopic resection of gastrointestinal

Role of laparoscopy in rectal cancer: A review

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Abstract

Despite established evidence on the advantages of laparoscopy in colon cancer resection, the use of laparoscopy for rectal cancer resection is still controversial. The initial concern was mainly regarding the feasibility of laparoscopy to achieve an adequate total mesorectal excision specimen. These concerns have been raised following early studies demonstrating higher rates of circumferential margins positivity following laparoscopic resection, as compared to open surgery. Similar to colon resection, patients undergoing laparoscopic rectal cancer resection are expected to benefit from a shorter length of hospital stay, less analgesic requirements, and a faster recovery of bowel function. In the past decade there have been an increasing number of large scale clinical trials investigating the oncological and perioperative outcomes of laparoscopic rectal cancer resection. In this review we summarize the current literature available on laparoscopic rectal cancer surgery.

Key words: Rectal cancer; Laparoscopy; Open resection; Review; Comparison; Outcomes; Laparoscopic resection of gastrointestinal

Core tip: Despite its endorsement for colon cancer resection, laparoscopy for rectal cancer resection is still considered investigational. This is mainly due to initial concerns regarding the feasibility of laparoscopy to achieve an adequate total mesorectal excision specimen. These concerns have been raised following early studies demonstrating higher rates of circumferential margins positivity following laparoscopic resection, as compared to open surgery. In this review, we explore the current relevant literature regarding laparoscopic resection for rectal cancer, with respect to oncologic efficacy and short and long term benefits.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in males and the second most common in females, with 1.2 million annual new cases worldwide. Over 143000 new cases of CRC are diagnosed annually in the United States, and approximately 52000 Americans die of the disease every year. These deaths account for approximately 9% of all cancer mortality[1].

Since its original implementation as a diagnostic tool, laparoscopy has become widely accepted as the favored approach for many procedures (*e.g.*, appendectomy, cholecystectomy, adrenalectomy, bariatric surgery). Not surprisingly, laparoscopy was also utilized for colon and rectal surgery. Laparoscopic colon resection was first reported in 1991[2,3]. Initial reports raised the concern for port site recurrence in up to 21% of the patients, as well as concerns regarding the adequacy of disease clearance by the laparoscopic approach[4-6]. These reports prompted the initiation of several major comparative studies, and

Table 1 Phase Ⅲ randomized controlled trials showing oncological outcomes

Study and design	Recurrence lap *vs* open		Survival lap *vs* open		Tumor location (cm from AV)	Patient enrollment (*n*)		LN harvested (*n*) lap *vs* open	CRM positivity lap *vs* open
	DR	LR	DFS	OS		Open	Lap		
Liang *et al*[17] Single center	N/A	N/A	N/A	76% *vs* 82.8% *P* = 0.46, (44 mo)	N/A	174	169	7.1 *vs* 7.4 *P* = 0.47	N/A
Kang *et al*[18] "COREAN" Multicenter	N/A	N/A	N/A	N/A	Lap-5.6 Open-5.3	170	170	17 *vs* 18 *P* = 0.08	5% *vs* 7% *P* = 0.77
Jayne *et al*[19] "CLASSIC" update Multicenter	21.9% *vs* 21.9% *P* = 0.86, (5 yr)	9.4% *vs* 7.6% *P* = 0.74, (5 yr)	53.2% *vs* 52.1% *P* = 0.95, (5 yr)	60.3% *vs* 52.9% *P* = 0.13, (5 yr)	N/A	128	253	N/A	N/A
Lujan *et al*[20] Single center	N/A	4.8% *vs* 5.3% *P* = 0.78, (5 yr)	84.8% *vs* 81% *P* = 0.9, (5 yr)	72.1% *vs* 75.3% *P* = 0.98, (5 yr)	Lap-5.5 Open-6.2	103	101	13.6 *vs* 11.6 *P* = 0.02	4% *vs* 3% *P* = 0.4
Ng *et al*[21] Single center	12.3% *vs* 18.1% *P* = 0.37, (10 yr)	7.1% *vs* 4.9% *P* = 0.68, (10 yr)	82.9% *vs* 80.4% *P* = 0.69, (10 yr)	83.5% *vs* 78% *P* = 0.59, (10 yr)	12-15	77	76	11.5 *vs* 12 *P* = 0.7	2.6% *vs* 1.3% *P* = 0.62
Ng *et al*[22] Single center	15% *vs* 25% *P* = 0.6, (5 yr)	5% *vs* 11% *P* = 0.6, (5 yr)	78.1% *vs* 73.6% *P* = 0.55, (5 yr)	75.2% *vs* 76.5% *P* = 0.2, (5 yr)	⩽ 5	48	51	12.4 *vs* 13 *P* = 0.72	5.8% *vs* 4.1% *P* = NS
Pechlivanides *et al*[23] Multicenter	N/A	N/A	N/A	N/A	Lap-6 Open-8	39	34	19.2 *vs* 19.2 *P* = 0.2	N/A
Braga *et al*[24] Single center	N/A	4% *vs* 5.3% *P* = 0.97 (5 yr)	N/A	No difference (5 yr)	Lap-9.1 Open-8.6	85	83	12.7 *vs* 13.6 *P* = NA	1.3% *vs* 2.4% *P* = NA

AV: Anal verge; N/A: Not applicable; OS: Overall survival; DFS: Disease free survival; LR: Local recurrence; DR: Distant recurrence; LN: Lymph nodes; CRM: Circumferential margins; NS: Non-significant; NA: Not available.

randomized controlled trials (RCT) comparing laparoscopic and open colon resection[7-15]. Results from these studies clearly showed no difference in resection margin, number of lymph nodes harvested, tumor recurrence rates, and long term overall survival between the two surgical approaches. Additionally, laparoscopy benefited the patients with earlier recovery of bowel function, reduced blood loss, decreased post-operative pain and analgesic use, and a shortened length of stay[7-11].

Despite its endorsement for colon resection, laparoscopy for rectal cancer resection is still considered investigational. As for laparoscopic colon resection, patients undergoing laparoscopic rectal resection are expected to benefit from a faster recovery. Nevertheless, it is of paramount concern whether laparoscopy can achieve an adequate oncological outcome, with total mesorectal excision (TME) being the gold standard, ever since presented by Heald *et al*[16] in 1982. This concern is further strengthened when considering the technical difficulties in rectal surgery, derived from the narrow confines of the bony pelvis, angling limitations of the stapling devices, high body mass index, and the need for autonomic nerve preservation.

The United Kingdom Medical Research Council Conventional versus Laparoscopic Assisted surgery in Colorectal Cancer (MRC CLASSIC) trial, was the first RCT to include rectal cancer patients. In this RCT, the rate of positive circumferential margins (CRM), was non-significantly higher in patients undergoing laparoscopic anterior resection when compared to open resection (12 % *vs* 6%, respectively, *P* = 0.19)[8]. This observation raised concern about the standards of laparoscopic TME when it is practiced by less experienced surgeons. Interestingly the higher CRM positivity rate did not translate to an increase in the 3 year follow-up local recurrence rate[12]. Many other clinical trials investigating the feasibility and efficacy of laparoscopy for rectal cancer resection have been published since.

In this review, we explore the current relevant literature regarding laparoscopic resection for rectal cancer, with respect to oncologic efficacy and short and long term benefits.

ONCOLOGICAL OUTCOMES

Randomized controlled trials

Following the concern raised by the MRC-CLASSIC trial regarding the relatively higher rate of CRM positivity following laparoscopic rectal surgery, several randomized controlled trials have been conducted in recent years investigating the oncological efficacy of the laparoscopic approach. Naturally, special attention was given to the TME specimen, focusing on proximal, distal, and circumferential margin positivity, as well as, the number of lymph nodes harvested.

Oncological outcomes of major phase Ⅲ randomized controlled trials comparing laparoscopic and open rectal resection are shown in Table 1[17-24]. Parameters investigated were overall (OS) and disease free survival (DFS), ocal recurrence (LR) and distant recurrence (DR) rates, number of lymph nodes (LN) harvested and circumferential margin (CRM) positivity.

Table 2 Meta-analyses showing oncological outcomes

Ref.	Trials (*n*)	Patients (*n*)	OS lap *vs* open	DFS lap *vs* open	LR lap *vs* open	LN harvested lap *vs* open (*n*)	CRM positivity lap *vs* open
Huang *et al*[25]	6	1033	HR = 0.76, P = 0.11, 4 trials (3 yr)	HR = 1.13, *P* = 0.64, 3 trials (3 yr)	RR = 0.55, *P* = 0.21, 4 trials (3 yr)	*P* = 0.43, 5 trials	7.94% *vs* 5.37%, *P* = 0.63, 5 trials (3 yr)
Ohtani *et al*[26]	12	2095	N/A	OR = 1.17, *P* = 0.35 (5 yr)	OR = 0.93, *P* = 0.61 (5 yr)	*P* = NS	*P* = NS
Anderson *et al*[27]	24	3158	72% *vs* 65%, *P* = NS, 13 trials (3 yr)	N/A	7% *vs* 8%, *P* = NS, 16 trials (3 yr)	10 *vs* 11, *P* = 0.001 17 trials	5% *vs* 8%, *P* = NS, 10 trials (3 yr)
Aziz *et al*[28]	20	2071	N/A	N/A	N/A	*P* = NS	9.5% *vs* 10.8%, OR = 0.93, *P* = 0.38

OS: Overall survival; DFS: Disease free survival; LR: Local recurrence; LN: Lymph nodes; CRM: Circumferential margins; HR: Hazard ratio; N/A: Not applicable; NS: Non-significant; OR: Odds ratio.

Six trials presented data comparing OS after laparoscopic and open rectal resection. One trial identified comparable 4 years OS (76% *vs* 82.8%, *P* = 0.46)[17], four trials presented 5 years OS [range: 60.3%-76.0% *vs* 52.5%-82.8%, *P* = non-significant (NS)][19,20,22,24], and one trial demonstrated comparable 10 years OS (83.5% *vs* 78%, *P* = 0.59)[21] for the laparoscopic and open groups, respectively. Data regarding 5 years DFS was presented in three trials (range: 53.2%-84.8% *vs* 52.1%-81%, *P* = NS)[19,20,22], and one trial demonstrated no difference in 10 years DFS (82.9% *vs* 80.4%, *P* = 0.69), for laparoscopic versus open resection[21].

Local recurrence rates after 5 years were presented in four studies (range: 4.0%-9.4% *vs* 5.3%-11.0%, *P* = NS)[19,20,22,24], and after 10 years in one study (7.1% *vs* 4.9%, *P* = 0.68)[21]. Similar distant recurrence rates after 5 years were presented in two studies (range: 15.0%-21.9% *vs* 21.9-25.0%, *P* = NS)[19,22], and after 10 years in one study (12.3% *vs* 18.1%, *P* = 0.37), for the laparoscopic and open groups, respectively[21].

Seven trials showed comparable results regarding the number of lymph nodes harvested after laparoscopic and open resection (range: 7.1-19.2 *vs* 7.4-19.2, *P* = NS)[17,18,20-24]. Circumferential margin positivity was investigated in 5 trials, and no difference was shown between the laparoscopic and open groups (range: 1.3%-5.8% *vs* 1.3%-7.0%, *P* = NS)[18,20-22,24].

To note, only two RCTs[20,21] described the relativity of patients by tumor stage. As expected, a larger number of patients in stage Ⅰ-Ⅲ than in stage Ⅳ, were observed in these studies. Hypothetically, in the other RCTs presented above, the number of patients with a lower stage could have been larger in the laparoscopic group, hence causing selection bias and skewing of results.

It is of extreme importance to acknowledge that surgical outcomes presented by all the RCTs above, except for the CLASSIC trial, are a product of an experienced and dedicated colorectal surgical team with experience in the field of laparoscopic colorectal surgery. In the CLASSIC trial, surgeons needed to have performed more than 20 laparoscopic colon or rectal surgery. This number is truly insufficient when considering the complexity of rectal surgery, and might explain the relatively higher rates of local and distant recurrence, as well as lower rates of OS and DFS.

Meta-analyses

Four, large scale meta-analyses were published in recent years comparing oncological outcomes between laparoscopic and open resection for rectal cancer[25-28]. No difference was found between the groups in regards to OS[25,27], DFS[25,26], LR rates[25-27], number of LN harvested[25-28], or the CRM positivity rate[25-28]. Data is shown Table 2.

Perioperative outcomes

Over the past two decades, the true benefits of laparoscopy, such as, lower postoperative morbidity rates, specifically wound infection rates, shorter time to recovery and discharge, and less pain and analgesic use, have turned it in to the preferred surgical approach in many surgical disciplines. This is true for rectal surgery as well, especially when considering the potential advantage for a faster recovery of the intestinal tract, the ability to surgically dissect deep down in a narrow pelvis, and the magnifying capabilities of the laparoscope, helping in nerve preservation. Although less focused on, laparoscopy has also a clear cosmetic advantage over the open approach. This may become an important issue, as more patients are diagnosed at a younger age[29].

Morbidity and mortality

Morbidity rates were presented by seven large scale clinical trials[8,18,20-22,24,30]. Intraoperative complications analyzed, included injury to the bowel or adjacent organs, hemorrhage, and anesthesia related complications. Postoperative complications included anastomotic leak, wound infection, and various cardiac, renal, pulmonary or vascular complications. Intraoperative complication rates ranged from 6.1%-21.2%, and 12.4%-23.5% for the laparoscopic and open groups, respectively (*P* = 0.01, *P* = 0.60). Postoperative complication rates ranged from 2.4%-45.1% and from 10.6%-52.1%, respectively (*P* = 0.01, *P* = 0.96). A recent meta-analysis published in 2013 by Arezzo *et al*[31] included 23 studies, representing 4539 patients, demonstrated a lower overall complication rate in the laparoscopic group (31.8%) compared to the open

Table 3 Operative time for laparoscopic and open rectal resection data presented as mean ± SD, min

Ref.	Laparoscopy	Open	*P* value
Liang *et al*[17]	138 ± 24	119 ± 22	< 0.001
Kang *et al*[18]	245 ± 75	197 ± 63	< 0.001
Lujan *et al*[20]	194 ± 45	173 ± 59	0.02
Ng *et al*[21]	213 ± 59	154 ± 70	< 0.001
Ng *et al*[22]	214 ± 46	164 ± 43	< 0.001
Braga *et al*[24]	262 ± 72	209 ± 70	< 0.001
Zhou *et al*[30]	120	106	0.05

SD: Standard deviation.

Table 4 Estimated intraoperative blood loss and transfusion rate for laparoscopic and open rectal cancer resection

Ref.	EBL (mL)			Blood transfusion rate		
	Lap	Open	*P* value	Lap	Open	*P* value
Liang *et al*[17]	N/A	N/A	N/A	2.4%	4.6%	0.38
Kang *et al*[18]	200	217.5	0.006	0%	0.005%	*P* > 0.99
Lujan *et al*[20]	127.8	234.2	*P* < 0.001	N/A	N/A	N/A
Ng *et al*[22]	321.7	555.6	*P* = 0.09	N/A	N/A	N/A
Braga *et al*[24]	150	350	*P* < 0.001	7.2%	26.8%	*P* = 0.002
Zhou *et al*[30]	20	92	*P* = 0.05	N/A	N/A	N/A

EBL: Estimated blood loss; N/A: Not applicable.

group (35.4%), RR = 0.83 (95%CI: 0.76-0.91, *P* < 0.001). Importantly, this meta-analysis uniquely showed no difference in the leak rates between the two approaches. A possible explanation may be the advent of new technologies, such as the ultrasonic scalpel, and articulated staplers as well as improved surgical experience. In 2006, Gao *et al*[32] published a meta-analysis demonstrating a lower morbidity rate for patients assigned to laparoscopy than for those assigned to open resection (OR = 0.63, 95%CI: 0.41-0.96, *P* = 0.96).

Short term postoperative mortality was reported by six trials comparing laparoscopic and open resection[8,17,20-22,24]. No significant difference was detected between the groups in either study. The "CLASSIC" trial reported the highest mortality rates (laparoscopy -4% *vs* open -5%, *P* = 0.57)[8]. The meta-analysis by Arezzo *et al*[31] presented above, showed a mortality rate of 1% following laparoscopy and of 2.4 % following open resection, (RR = 0.46, 95%CI: 0.21-0.99, *P* = 0.048).

Conversion rate

Eight randomized controlled trials presented the rate of conversion from a laparoscopic to an open rectal resection. Conversion rates ranged between < 1% to 34%[17-24]. A recent large scale meta-analysis showed that overall, 13% (260 of 2005) of laparoscopic procedures were converted to open surgery, 12.5% in the RCTs and 13.3% in the prospective controlled trials[31]. Conversion was not uniformly defined, but the main reasons for conversion were obesity[33], narrow pelvic anatomy, uncontrollable bleeding, ureteral injury, and advanced disease. To note, that mobilization of the rectum can be performed with a total laparoscopic approach or with a hybrid procedure. In this hybrid approach, inferior mesenteric vessels division, mobilization of splenic flexure, and left-side colon are performed laparoscopically, but TME of the rectum is performed partially by technique of open dissection through a Pfannenstiel wound, which is also used for specimen extraction. In our opinion this approach is not to be considered as a converted procedure, although a mini-laparotomy is considered by some as conversion. Since conversion is associated in several trials with increased morbidity and poorer oncological results[8,34], patients should be routinely pre-operatively evaluated for the potential risk of conversion, using radiological and clinical parameters.

Operative time

Data from seven RCTs[17,18,20-22,24,30] comparing operative time for laparoscopic and open rectal cancer surgery, clearly show a significantly longer operative time for the laparoscopic approach. Data from the RCTs is presented in Table 3. In a meta-analysis published recently, including 11 non-RCTs and 7 RCTs, the mean operative time was 219 *vs* 175 min for laparoscopy and open surgery, respectively, with an overall mean difference of 42.8 min (95%CI: 31.4-54.2, *P* < 0.001). Other trials evaluating the impact of surgeon experience on surgical outcome, showed that operative time decreased significantly with number of operations performed (range: 40-90)[35-37].

Estimated intraoperative blood loss and transfusion rate

Five RCTs compared the estimated intraoperative blood loss (EBL) in laparoscopic and open rectal cancer surgery[18,20,22,24,30]. All trials showed a significantly lower EBL in the laparoscopic group (range: 20.0-321.7 *vs* 92.0-555.6, *P* = 0.05 to *P* < 0.001). The blood transfusion rate was non-significantly higher for the open group in two RCTs[17,18], and significantly higher for the open group in one study[24]. Data is shown in Table 4.

Length of hospital stay

Seven RCTs reported data comparing length of hospital stay (LOS) after laparoscopic and open surgery for rectal cancer[8,18,20-22,24,30]. Three trials showed a significantly shorter LOS following laparoscopy[22,24,30], and the other four RCTs presented a similar trend. This was supported by two meta-analyses showing a shorter LOS by 2.67 d [95%CI: -3.8-(-1.54), *P* = 0.06][28], and by 2.7 d [95%CI: -3.6-(-1.7), *P* < 0.001] after laparoscopic rectal resection[31]. Data is shown in Table 5.

Bowel function recovery

Bowel function recovery after laparoscopic and open surgery for rectal cancer was assessed by six RCTs[8,17,18,21,22,30], and two meta-analyses[28,31]. Variable parameters were assessed such as, time to peristalsis, time to 1st flatus or stool, and time to initiating of oral feeding. Time to peristalsis was significantly shorter after laparoscopy in 3 RCTs[17,22,30], and in one meta-analysis[28]. Time to 1st fla-

Table 5 Length of hospital stay

Ref.	Design	Measure	LOS		
			Lap	Open	*P* value
Guillou *et al*[8] "CLASSIC"	RCT	Median (range)	13 (9-18)	11 (9-15)	N/A
Kang *et al*[18] "COREAN"	RCT	Median (range)	8 (7-12)	9 (8-12)	0.06
Lujan *et al*[20]	RCT	mean ± SD	8.2 ± 7.3	9.9 ± 6.8	0.11
Ng *et al*[21]	RCT	Median (range)	10.8 (5-27)	11.5 (3-38)	0.55
Ng *et al*[22]	RCT	Median (range)	8.4 (2-32)	10 (3-39)	0.013
Braga *et al*[24]	RCT	mean ± SD	10 ± 4.9	13 ± 10	0.004
Zhou *et al*[30]	RCT	mean ± SD	8.1 ± 3.1	13.3 ± 3.4	0.001
Aziz *et al*[28]	MA	MD (d)	-2.67 d, 95%CI: -3.8-(-1.54)		0.06
Arezzo *et al*[31]	MA	MD (d)	-2.7 d, 95%CI: -3.6-(-1.7)		0.001

RCT: Randomized controlled trial; MA: Meta-analysis; LOS: Length of stay; SD: Standard deviation; MD: Mean difference; CI: Confidence intervall; N/A: Not applicable.

Table 6 Bowel function recovery

Ref.	Design	Measurement	Lap	Open	*P* value
Time to peristalsis					
Liang *et al*[17]	RCT	d	3.9	4.2	0.001
Ng *et al*[21]	RCT	d	4.1	4.7	0.06
Ng *et al*[22]	RCT	d	4.3	6.3	0.001
Guillou *et al*[8]	RCT	d	5	6	N/A
Zhou *et al*[30]	RCT	d	1.5	2.7	0.009
Aziz *et al*[28]	MA	d	MD -1.52 d [95%CI: -2.2-(-1.01), *P* = significant]		
Time to 1st flatus					
Ng *et al*[22]	RCT	d	3.1	4.6	0.001
Kang *et al*[18]	RCT	h	38.5	60	0.001
Time to 1st stool					
Kang *et al*[18]	RCT	h	96.5	123	0.001
Liang *et al*[17]	RCT	d	3	3.3	0.001
Arezzo *et al*[31]	MA	d	3.3 *vs* 4.4 MD -0.96 d [95% CI -1.3-(-0.6), *P* < 0.001]		
Time to oral feeding initiation					
Kang *et al*[18]	RCT	h	85	93	0.001
Guillou *et al*[8]	RCT	d	6	6	N/A
Ng *et al*[21]	RCT	d	4.3	4.9	0.001
Ng *et al*[22]	RCT	d	4.3	6.3	0.001
Aziz *et al*[28]	MA	d	MD -0.92 d [95%CI: -1.35-(-0.5), *P* = significant]		
Arezzo *et al*[31]	MA	d	3.8 *vs* 4.8 MD -1 d [95%CI: -1.4-(-0.7), *P* < 0.001]		

RCT: Randomized controlled trial; MA: Meta-analysis; MD: Mean difference; N/A: Not applicable; CI: Confidence interval.

tus was significantly shorter as well in 2 RCTs[18,22]. Arezzo *et al*[31] showed an approximate one day shorter hospital stay after laparoscopic surgery in their meta-analysis [lap 3.3 *vs* open 4.4, median difference -0.96 d, 95%CI: -1.3-(-0.6), $P < 0.001$][31]. Similar results were shown by 2 RCTs[17,18]. Time to initiation of oral feeding was shorter by approximately one day in 2 meta-analyses[28,31]. A Similar trend was observed in three RCTs[18,21,22]. Data is shown in Table 6.

Postoperative pain and analgesic use

In a meta-analysis published in 2006 by Aziz *et al*[28] there was no difference with regards to the analgesic use after laparoscopic or open rectal cancer surgery[28]. However, several RCTs published later showed that patients that underwent laparoscopic resections, required fewer injections of analgesics (6 *vs* 11.4, $P = 0.007$ and 4.9 *vs* 8.3, $P = 0.001$)[21,22], and lower doses of morphine (107.2 mg *vs* 156.9 mg, $P < 0.001$). Through less analgesic use, pulmonary complications maybe reduced and a faster bowel recovery may further benefit the patient. Future studies should make use of monitored patient controlled analgesia, and strict drug documentation, led in specialized centers, to accurately measure and compare the true effect of laparoscopy on postoperative pain.

Bladder and sexual function

Jayne *et al*[38] published data regarding bladder and sexual function from the MRC-CLASSIS trials' patient database[8]. Overall questionnaire response rate was above 50%. No difference was observed in bladder function between the laparoscopic and open groups. Approximately 30% of patients reported moderate to severe urinary symptoms in each group. With regards to sexual function, more than 50% of men and women reported being sexually inactive in the questionnaires. In men, overall sexual function and erectile function tended to be worse after laparoscopic than open rectal surgery [overall function: score difference -11.18, 95%CI: -22.9-0.63, $P = 0.063$; erectile function: score difference -5.84, 95%CI: -10.94-(-0.74), $P = 0.068$]. In women, there was no difference in sexual function. In this trial it was shown that oncological requirement for TME (OR = 6.38; $P = 0.054$) and conversion to open surgery (OR = 2.86; $P = 0.041$) were independent predictors of postoperative sexual dysfunction in men. Kang *et al*[18] demonstrated a higher number of urinary problems after laparoscopy than open surgery ($P < 0.001$), but no difference between the laparoscopic and open groups in regards to sexual function.

Adhesion formation and incisional hernia

Adhesion formation is an increasing problem after colorectal surgery[39-41]. Laparoscopic colorectal surgery may result in fewer adhesions because of reduced tissue handling, and less environmental exposure of the bowel. In a recently published study by Burns *et al*[41], patients undergoing laparoscopic colorectal resection were found to have a lower risk of developing clinically significant adhesions. Interestingly, a retrospective study, supplementary to the CLASICC trial[8], showed that more patients undergoing colonic resection were admitted for adhesive intestinal obstruction (AIO) in the open arm than in the laparoscopic arm (4% *vs* 1.3%); however, this was reversed when considering patients with rectal cancer (2% *vs* 3.9%). Furthermore, more patients with rectal cancer who underwent conversion to open surgery were admitted for AIO than those who had open surgery or completed laparoscopic surgery (8%, 2% and 2% for converted, open

and laparoscopic surgery respectively). Surprisingly, this trend was seen for incisional hernia as well. Although not statistically significant ($P = 0.78$), more patients undergoing colonic resection developed incisional hernia in the open arm than in the laparoscopic arm (10% *vs* 6.6%); however, this was reversed when considering patients with rectal cancer (9% *vs* 10.9%). This may partially be explained by the relatively less experienced surgical team (surgeons were required to perform only 20 laparoscopic colorectal procedures for the trial eligibility), or by the relatively small cohort of the rectal cancer subgroup. In our opinion, laparoscopy has a clear advantage in these aspects, however further randomized trials are needed to clarify the impact of laparoscopy on adhesion formation and incisional hernia in rectal surgery.

Current trials

At present, three large scale randomized controlled trials are being conducted. The European Colon Cancer Laparoscopic or Open Resection (COLOR) Ⅱ trial is a randomized, international, multicenter study comparing the outcomes of laparoscopic and open resection of rectal carcinoma, with primary endpoint being locoregional recurrence at 3 years. Secondary endpoints are recurrence-free and overall survival at 3, 5 and 7 years, rate of distant metastases, port site and wound site recurrences, microscopic evaluation of the resected specimen, 8-wk morbidity and mortality, quality of life, and cost[42]. In the United States, the American College of Surgeons Oncology Group (ACOSOG)-Z6051 trial, opened in 2008, and is a phase Ⅲ randomized controlled trial with a non-inferiority design and a 1:1 randomization of laparoscopic and open rectal resection. Primary endpoints include circumferential and distal resection margins, number of lymph nodes harvested, and integrity of the TME specimen. Secondary endpoints include disease free survival and local recurrence at 2 years[43]. Finally, the Japanese Clinical Oncology Group trial JCOG 0404, is a RCT comparing laparoscopic and open surgery for colorectal cancer, with overall survival and relapse free survival as primary endpoints[44].

CONCLUSION

Current evidence suggests that laparoscopic rectal cancer resection results in similar oncological outcomes when compared with the conventional open approach. Initial concern regarding circumferential margin positivity, has not been demonstrated in other large scale randomized controlled trials or meta-analyses presented in this review. Morbidity and mortality rates are at least comparable, with some meta-analyses even showing reduced morbidity and mortality after the laparoscopic approach. Furthermore, the laparoscopic approach benefits patients with a reduced need for analgesics, faster recovery of bowel function, shorter length of stay, and less blood loss. The impact of laparoscopy on bladder and sexual function as well as clinically significant adhesion formation and incisional hernia rates, is still inconclusive, and needs further investigation.

Undoubtedly, surgeon experience and competence in laparoscopic colorectal surgery have a major impact on oncological and other perioperative outcomes. This has led both the American Society of Colon and Rectal Surgeons and the Society of Gastrointestinal and Endoscopic Surgeons to recommend that laparoscopy for rectal cancer resection should be practiced by expert, trained surgeons in institutions where the outcomes can be meaningfully evaluated.

Current large scale randomized controlled trial are conducted worldwide, further investigating the oncological and clinical efficacy of laparoscopic rectal cancer resection.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (15): Laparoscopic resection of gastrointestinal

Laparoscopic resection of pancreatic neuroendocrine tumors

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Abstract

Pancreatic neuroendocrine tumors (PNETs) are a rare heterogeneous group of endocrine neoplasms. Surgery remains the best curative option for this type of tumor. Over the past two decades, with the development of laparoscopic pancreatic surgery, an increasingly larger number of PNET resections are being performed by these minimally-invasive techniques. In this review article, the various laparoscopic surgical options for the excision of PNETs are discussed. In addition, a summary of the literature describing the outcome of these treatment modalities is presented.

Key words: Pancreatic neuroendocrine tumor; Laparoscopy; Surgery; Laparoscopic resection of gastrointestinal

Core tip: Pancreatic neuroendocrine tumors (PNETs) are a rare clinical entity, with surgery being the treatment modality of choice. Over the past several years, laparoscopic techniques have gained popularity in the surgical management of these tumors. This article reviews the available literature on laparoscopic resection of PNETs, with an overview of the commonly-practiced surgical procedures.

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INTRODUCTION

Pancreatic neuroendocrine tumors (PNETs), also known as islet cell tumors, are a rare form of endocrine neoplasms, accounting for a reported 1%-4% of all pancreatic tumors[1-3]. These tumors are associated with an annual incidence of one per 100000 population, and their diagnosis has increased over the past 40 years, most likely due to advances in imaging and histopathological methods[4-6]. PNETs can manifest at any age, however, most present during the 4th to 6th decades of life. When considered as a general entity, no gender predilection is demonstrated, but the various subtypes when observed separately do show slight gender predilection[7]. Although the majority of cases are sporadic, 10%-30% have been shown to be associated with multiple endocrine neoplasia (MEN) 1 syndrome, and < 1% with (Von Hippel-Lindau) VHL disease[8,9]. Other genetic syndromes in which PNETs may present include neurofibromatosis type 1 and tuberous sclerosis[4]. PNETs can be classified as functional and nonfunctional, the latter being far more common and

typically presenting late during disease evolution, with symptoms related to mass effect, invasion into surrounding structures, or metastasis[3].

The most common form of functional PNET is insulinoma, accounting for 70%-80% of cases. Ninety percent are benign and solitary, and they are predominantly located in the body and the tail of the pancreas (65%-80%). Due to the fact that symptoms of hypoglycemia dominate the clinical picture early in the course of the disease, the majority are small in size (< 2 cm) at the time of presentation, and compatible with surgical resection[10-12]. This contrasts with gastrinomas, another type of functional PNET, which in more than 50% are extrapancreatic, tend to be larger in size, and present with metastasis in 60%-70% of cases. Other rare types include glucagonomas and vasoactive intestinal peptide-producing tumors (VIPomas), the majority of which are also malignant (80% and 60%, respectively). Somatostatinomas are typically large neoplasms, causing mass effect around the pancreatic head or periampullary region. The majority of these tumors are malignant (70%)[13].

It is due to the above-mentioned characteristics that the "non-insulinoma PNETs" are less suitable for surgical resection. That said, surgery remains the only curative modality for neuroendocrine neoplasms of the pancreas, and is the treatment of choice when technically feasible, even in the presence of malignancy and occasionally locally advanced or metastatic disease[14-19].

With advances in minimally invasive surgery, laparoscopic resection has become a well-accepted modality in the management of pancreatic tumors, with an increasing number of surgeons utilizing these techniques[18]. The use of laparoscopy in pancreatic surgery was initially introduced in 1994 by Gagner *et al*[20] and Cuschieri[21], and two years later, Gagner *et al*[22] reported on their early experience with laparoscopic resection of islet cell tumors. Since then, several publications have described laparoscopic pancreatic surgery, however, only a small number of large series have described laparoscopic surgery in the setting of PNETs[13,18,23,24]. The purpose of this article is to review the available literature on laparoscopic resection of PNETs, with a focus on the various surgical techniques, and compare laparoscopic surgery to open pancreatic surgery in terms of results and complications.

PREOPERATIVE LOCALIZATION

Preoperative localization of the neuroendocrine tumor is of utmost importance in the management of these neoplasms. Prior to considering laparoscopy, an expected surgical strategy must be contemplated in accordance with the findings on imaging studies.

Imaging studies provide information regarding the location of the tumor, the extent of local invasion, and the presence of metastatic lesions[7]. Localization studies commonly used include ultrasonography, computed tomography (CT) scanning, and magnetic resonance imaging (MRI). CT is generally the initial test used by clinicians to localize PNETs. On CT scans, these tumors typically appear hyperdense on arterial phase. Although there is great variation in the literature regarding the reported usefulness of this modality for the detection of PNETs, it is generally accepted that it has a sensitivity of less than 50%-60%[25-27]. Nevertheless, one study reported a sensitivity of 94% for CT in the detection of PNETs[28]. MRI has the advantage of decreased radiation when compared with CT, and is commonly used to detect small PNETs and to assess local invasion[29]. A sensitivity ranging from 30% up to 95% has been reported in the literature for the detection of PNETs[30,31].

A study that has gained popularity due to increased accuracy is endoscopic ultrasound (EUS), however, the disadvantage of this technique is operator dependence[32-35]. It has a higher success rate in localizing tumors of the head and body than those of the tail. This modality has been associated with a sensitivity of 80% to 88% and a specificity of 95%[13]. One study reported a sensitivity of 82% and a specificity of 95% for EUS in the localization of PNETs not identified by CT or angiography[36]. It is also worth noting that the combination of EUS with biphasic helical CT scanning has been demonstrated to increase the diagnostic accuracy to 97%[13]. Although EUS has been shown to be effective in the detection of regional lymph node involvement, its usefulness in the diagnosis of liver metastasis is largely limited[37].

Angiographic techniques with portal vein sampling are invasive methods, and are typically reserved for patients in whom other less invasive diagnostic tests have failed to localize the pathology. These methods have been shown to provide accurate regionalization (but not exact localization) in up to 90% of cases[38].

A functional study commonly utilized is somatostatin receptor scintigraphy (octreotide scan)[39]. A relatively new modality shown to be superior to the octreotide scan in the diagnosis of neuroendocrine tumors is gallium-68 somatostatin receptor PET scan, which utilizes radio-labeled tracers with affinity to somatostatic receptors to localize these tumors[40]. A recently published meta-analysis demonstrated that this imaging modality has a sensitivity of 93% (when ^{68}Ga-DOTATOC is utilized) and 96% (when ^{68}Ga-DOTATATE is utilized). The specificity was shown to be 85% and 100%, respectively[41]. It should be noted that the diagnostic yield of these tests is reduced in insulinomas, which may not express somatostatin receptors. The use of FDG-PET CT for the diagnosis of PNETs is limited, mainly due to the slow-growing nature of these tumors[42]. However, the ability of this test to detect more aggressive tumors (due to the fact that less differentiated tumors consume more glucose) has been proposed[43].

It appears that after establishing the localization of a lesion by more than one noninvasive study (for example, CT scan and MRI), it is reasonable to explore the patient laparoscopically, and to perform an intraoperative ultrasound (discussed below)[44]. Due to the relative safety and diagnostic accuracy of modern laparoscopic techniques along with the use of intraoperative ultrasound, many

recommend that the utilization of more invasive preoperative diagnostic methods, such as angiography, be reserved for equivocal cases only[13].

DETERMINATION OF SURGICAL TECHNIQUE

Surgery remains the cornerstone of management of PNETs, with increased utilization of the laparoscopic approach demonstrated over the past two decades[45]. The planned surgical approach is governed largely by the findings in preoperative localization studies, but may commonly change in accordance with intraoperative findings.

There is no general consensus regarding the indications for and limitations of laparoscopic surgery for PNETs. Although some have claimed that the presence of a malignant PNET is a contraindication for laparoscopic resection[46], others have shown the feasibility and safety of laparoscopic surgery in these malignant tumors[24].

Laparoscopic enucleation is utilized in lesions less than 3 cm in size which are noninvasive and located peripherally and thereby do not involve the main pancreatic duct. When the above criteria are fulfilled, this procedure may be applicable for tumors located in the pancreatic head, body, or tail[47]. When the tumor is in proximity to the Wirsung duct, enucleation is not suitable due to the elevated risk of pancreatic fistula development[48]. Due to the fact that insulinomas are typically small, single and benign lesions, the use of laparoscopic enucleation for surgical management of these tumors has been widely described in the literature. The use of intraoperative ultrasound, however, is essential in order to rule out the presence of other lesions before the decision to perform enucleation is made, and to assess the proximity of these tumors to the pancreatic duct and vascular structures[48].

When the PNET is not compatible with enucleation, pancreatic resection is necessary[47]. In tumors involving the head of the pancreas, pancreaticoduodenectomy (Whipple Operation) is indicated. This procedure is not widely performed laparoscopically worldwide, due to the associated technical difficulties. However, many studies have shown the effectiveness and safety of this procedure, when performed by sufficiently-trained hepatobiliary or laparoscopic surgeons[49-52].

In tumors that are located in the body or the tail and are not suitable for enucleation, laparoscopic distal pancreatectomy is the treatment of choice. This surgery can be further divided into three different entities: spleen-preserving distal pancreatectomy with splenic vessel preservation, spleen-preserving distal pancreatectomy without splenic vessel preservation, and distal pancreatectomy with splenectomy[53]. The main factors which dictate the procedure chosen are the location of the tumor within the pancreatic body or tail, and its relation to the splenic vessels and splenic hilum. In addition, the presence or suspicion of malignant neuroendocrine tumors generally favors more radical approaches, with the resection of splenic vessels in order to enable adequate lymph node sampling. Ligation of splenic vessels is also advocated when uncontrolled bleeding from the vessels at the upper border of the pancreas is demonstrated intraoperatively[13]. This procedure is less technically demanding and is associated with shorter operation time. Ligation and transection of the splenic vessels is performed at the level of the pancreatic resection and at the splenic hilum. Postoperatively, the spleen receives its vascular supply from the short gastric vessels and left gastroepiploic vessels[13].

When possible, spleen-preserving distal pancreatectomy with splenic vessel preservation is performed; however, this procedure requires higher technical expertise, with separation of the splenic vessels from the pancreatic parenchyma, and the dissection and ligation of the branching vessels supplying the pancreas. As a result, this procedure is associated with a longer operating time[54-57]. Splenic preservation in these PNETs is generally encouraged when it is technically feasible; however, the occasional presence of hilar fibrosis due to previous inflammation can make splenic preservation difficult, and in these cases, *en bloc* pancreaticosplenectomy appears to be the safest option[58]. This is also true in malignant PNETs that involve or are adjacent to the hilum of the spleen and in these cases, the need for a complete oncologic resection supersedes the benefits of splenic preservation. That said, the avoidance of splenectomy can be achieved in the majority of cases[54-56]. In Assalia and Gagner' s publication, the rate of successful splenic salvage in laparoscopic distal pancreatectomy for islet cell tumors approached 85%[13].

It is worth mentioning that in the presence of a functioning PNET, medical control of the patient's symptoms prior to surgical intervention is of utmost importance. Although a detailed discussion of these treatments is beyond the scope of this review, this generally entails strict regulation of blood glucose levels in insulinomas, proton pump inhibitor treatment in gastrinomas, *etc*. In addition, a multidisciplinary approach involving the endocrinologist, surgeon, and anesthesiologist is essential in order to ensure safe resection.

TECHNICAL ASPECTS OF LAPAROSCOPIC SURGERY FOR PNETS

Various surgical techniques for laparoscopic pancreatic surgery have been described in the literature with some modifications that are based on surgeons' experience and preferences[13,22,48,53,55,59]. The following descriptions outline the important aspects and steps that are the basis for laparoscopic resections of PNETs. It is to be noted that the procedural description of laparoscopic pancreaticoduodenectomy (Whipple Operation) will not be described in this review.

Enucleation of tumors of the pancreatic head

After appropriate exposure of the pancreas, intraoperative laparoscopic ultrasonography is performed. Due to

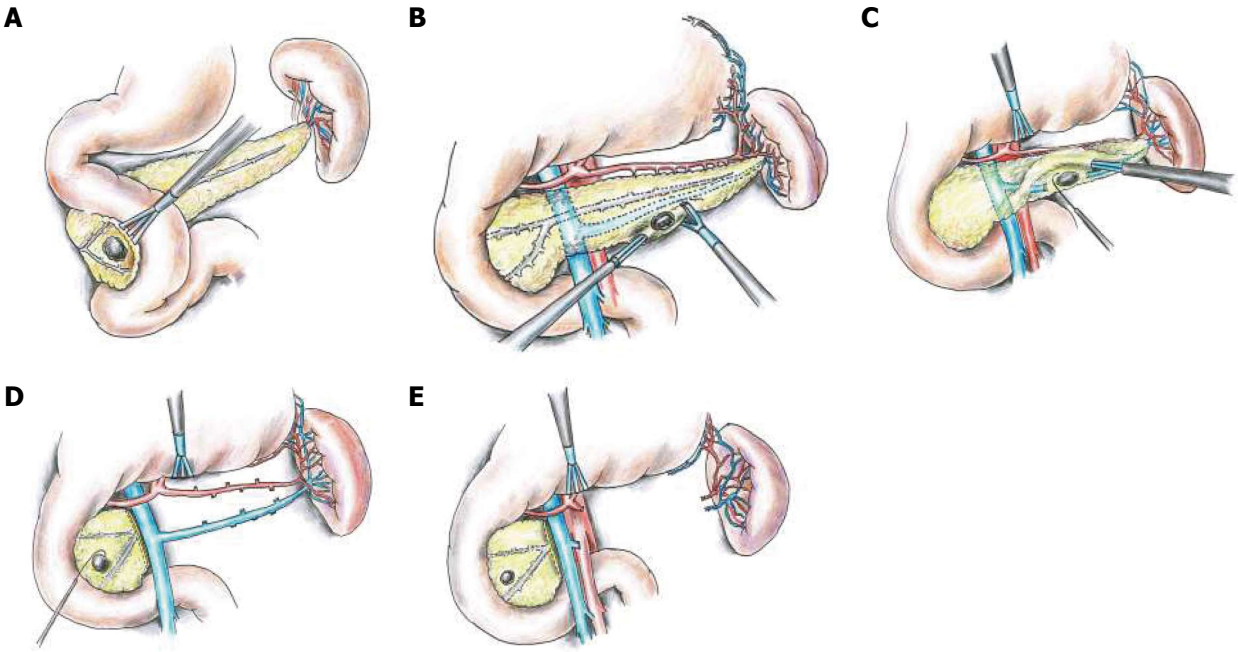

Figure 1 Tumors of the pancreatic head. A: A pancreatic neuroendocrine tumor (PNET) involving the posterior aspect of the pancreatic head, after adequate exposure by extensive kocherization and medial retraction of the pancreatic head, prior to enucleation; B: A PNET involving the inferior border of the body/tail of the pancreas. Resection is being performed using the LigaSure device; C: PNETs located in the posterior aspect of the body/tail occasionally require partial resection of the splenic vein in order to perform successful enucleation; D: The intraoperative appearance after performance of spleen-preserving distal pancreatectomy with splenic vessel preservation; E: The intraoperative appearance after performance of spleen-preserving distal pancreatectomy without splenic vessel preservation. Figure 1D and E represent patients with multiple endocrine neoplasia-1, with an additional PNET located in the head. This synchronous tumor will be excised by enucleation.

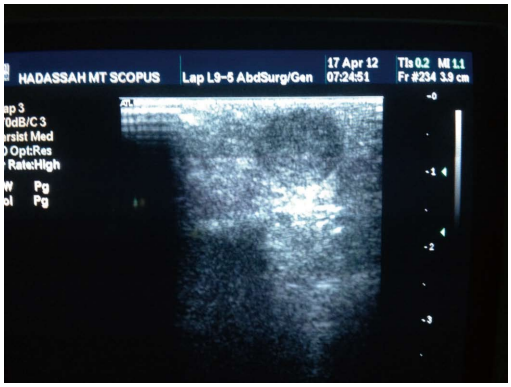

Figure 2 An image from intraoperative ultrasound demonstrating an insulinoma involving the pancreatic tail.

the lack of tactile sensation in laparoscopic surgery, this imaging tool is of utmost importance. It helps localize the tumor, rules out the presence of multiple lesions, and identifies the tumor's relation to and distance from surrounding structures[60]. Depending on the location of the tumor, focal dissection is continued in order to provide maximal exposure prior to enucleation (Figure 1A shows a PNET involving the posterior aspect of the pancreatic head, following appropriate exposure). Laparoscopic ultrasound is used to again identify the exact location of the tumor and its relation to the Wirsung duct and the superior mesenteric vein (SMV). Under extensive care not to damage these structures (which would lead to postoperative leak in the case of damage of the pancreatic duct), electrocautery with the hook coagulator is utilized to dissect the parenchyma surrounding the tumor and perform enucleation. In tumors located at the inferior surface of the pancreatic head, the LigaSure device (Tyco, United States Surgical Volleylab, Boulder, Co. United States) may be used to incise the plane between the pancreas and the tumor. A surgical drain is left at the excision bed[48].

Enucleation of tumors of the pancreatic body and tail

After sufficient surgical exposure and mobilization of the pancreatic body and tail, laparoscopic ultrasonography is performed for tumor localization and to identify the relationship with surrounding structures (the pancreatic duct and splenic vessels). Figure 2 demonstrates the intraoperative sonographic appearance of an insulinoma involving the pancreatic tail.

When the tumor is located anteriorly, an incision is made in the pancreatic capsule using electrocautery, and delicate dissection is carried out between the tumor and the normal pancreatic parenchyma until successful enucleation of the mass is achieved. Bleeding is controlled by clips and cautery.

Tumors located at the inferior pancreatic border are commonly resected by hemostatic dissection using the LigaSure device (Figure 1B).

Posteriorly-located tumors are commonly partially covered by the splenic vein. The inferior border of the pancreas is lifted up, allowing exposure of the posterior pancreatic surface. Occasionally enucleation is only possible after local resection of the adjacent portion of the

vein (Figure 1C). In this process, injury to the splenic artery must be avoided. After enucleation, the tumor bed must be examined for evidence of pancreatic duct injury.

Tumors located in the distal portion of the tail of the pancreas are in very close proximity to the Wirsung Duct, and therefore enucleation of these tumors is commonly not recommended[48].

Spleen-preserving distal pancreatectomy with splenic vessel preservation

Exposure and mobilization of the body and tail of the pancreas is performed, as is mobilization of the splenic flexure. Adhesions are divided between the posterior surface of the stomach and the pancreas; however, care should be taken not to divide the short gastric and the left gastroepiploic vessels. After detaching the inferior pancreatic margin from the retroperitoneum, visualization of the posterior aspect of the pancreas is now feasible, as is identification of the SMV and the splenic vein forming the portal vein. Blunt dissection around the splenic vein is performed, with ligation of the small bridging vessels that reach the pancreas. After identification and preservation of the splenic artery, the pancreas is divided using an endoscopic stapler device. The body/tail of the pancreas is then anteriorly retracted, allowing further separation of small bridging vessels reaching the pancreas from the splenic artery and vein. The resection is completed after reaching the splenic hilum, and a surgical drain is left in proximity to the pancreatic stump[48] (Figure 1D).

Spleen-preserving distal pancreatectomy without splenic vessel preservation

This procedure follows the same course mentioned above until visualization of the posterior aspect of the pancreas and the splenic vein entering the SMV to form the portal vein. At this stage, clips are applied to the splenic vein and it is divided. The pancreas is then divided by endoscopic stapler, followed by ligation and division of the splenic artery. The pancreatic body and tail are retracted upwards (along with the attached splenic artery and vein), and these vessels are clipped and divided between the pancreatic tail and the splenic hilum. After this procedure, the sole remaining blood supply to the spleen is from the short gastric vessels and left gastroepiploic vessels, indicating the importance of their preservation in earlier steps[48] (Figure 1E).

Distal pancreatectomy with splenectomy

This procedure is similar to the previous technique (Spleen-preserving distal pancreatectomy without splenic vessel preservation) with a few exceptions. Unlike in spleen-preserving procedures, the short gastric and left gastroepiploic vessels can be ligated. In addition to mobilizing the splenic flexure (thereby exposing the inferior splenic border), the spleen's lateral aspect is also mobilized, up to the left crus of the diaphragm. The splenic vessels can be divided along with the pancreas or separately. The specimens are typically extracted from the abdomen in two separate specimen bags. As in the previous procedure, a surgical drain is left *in situ*[13]. Note that some surgeons use different methods to seal the bed of tumor enucleation or the margins of resection, including adhesive biologic materials or sutures.

SURGERY IN PANCREATIC NEUROENDOCRINE CARCINOMA

According to the WHO 2010 classification, neuroendocrine carcinomas (NECs) are defined histopathologically as neuroendocrine tumors with a Ki-67 index above 20%[45]. These tumors are extremely invasive and aggressive, and fortunately they are rare, accounting for only 2%-3% of PNETs[45,61,62]. Radical surgery is generally indicated for locally advanced disease, followed by adjuvant chemotherapy. When there is evidence or suspicion of malignant disease, or when the tumor size is greater than 5 cm, it is recommended that a modified strasberg operation be performed[24]. This entails radical lymph node dissection of the peri-pancreatic, portal, hepatic, and superior mesenteric areas.

Not only does the literature support surgical excision of locally invasive disease, but a survival benefit has also been demonstrated after excision of metastases (in addition to the primary tumor) when technically feasible. Therefore, in selected patients with localized liver metastasis, it is recommended that a synchronous resection of the primary tumor and liver metastases should be attempted[14-18,63-68]. Nevertheless, the role of laparoscopy for these complicated procedures is yet to be clarified. Despite case reports of successful laparoscopic synchronous excision of the primary tumor and metastases this issue remains controversial as opponents claim that laparoscopic surgery may jeopardize the oncologic outcome and a planned open procedure must be carried out. As randomized controlled studies are unlikely this controversy will remain a matter of debate and it is reasonable to limit these procedures to highly experienced laparoscopic pancreatic/hepatobiliary surgeons.

SURGERY IN PNETS ASSOCIATED WITH MEN-1

More than 75% of patients with PNETs and MEN-1 have multiple pancreatic tumors, therefore enucleation alone in this clinical setting is likely to be inadequate[24,48]. Generally accepted indications for surgery in MEN-1 include the presence of a functioning PNET, in addition to nonfunctioning tumors of more than 2 cm in size[9,69]. However, some authors consider the diameter of 1 cm to be a safer cutoff, and advocate the surgical resection of nonfunctioning PNETs of more than 1 cm in diameter[69,70]. Smaller nonfunctioning tumors must be closely observed, and their rate of growth may subsequently provide an indication for surgical resection. The recommended surgical procedure for these patients seems to be

intraoperative laparoscopic ultrasonography, followed by subtotal distal pancreatectomy (usually with splenic preservation), along with enucleation of any lesions in the pancreatic head (Figures 1D and E).

OUTCOMES OF LAPAROSCOPIC PANCREATIC SURGERY FOR PNETS

As previously mentioned, surgery is the curative modality of choice for PNETs, improving survival across all stages of the disease. Recent years have shown a significant increase in the laparoscopic approach in these surgeries[18]. In several centers worldwide, almost all patients with suspicious PNET of the pancreatic body or tail undergo laparoscopic surgery[24,48,71,72].

In a recently published series, 75 laparoscopic procedures for PNETs were documented, of which 65 pancreatic resections or enucleations were performed[47]. The most common operation performed was distal pancreatectomy with splenectomy (n = 28), and this was followed by distal pancreatectomy without splenectomy (n = 23). The status of splenic vessel preservation was not clarified. Enucleation of a PNET of the head was performed in 7 cases, and of the body or tail in another 7 patients. The most common surgical complication was found to be post-operative pancreatic fistula (POPF), occurring in 21% of patients. This complication was more common in patients undergoing enucleation (50%) a finding that has been repeatedly shown in the literature, with a reported incidence of 13%-50% of POPF following enucleation[23,73-75]. Other "non-fistula" surgical complications had an incidence of 21%, and no perioperative mortality was demonstrated. In this study, a 5-year disease-specific survival of 90% was demonstrated, which can be compared to another series of 125 patients who underwent open surgical treatment of PNET and were found to have a 5-year survival of 65%[76]. However, issues of selection bias in these two different retrospective studies must be considered. DiNorcia *et al*[18] published a retrospective series in which 45 laparoscopic procedures for PNETs were compared to 85 open surgeries that were performed at the same institution. The two groups were similar with respect to gender, age, and race; however, as expected, a statistically significant difference was observed with regard to pathological characteristics of the tumors, with the laparoscopically operated group having smaller, lower-grade tumors, with less local and lymph node invasion. This study showed no statistically significant difference in overall morbidity rate between the two groups (48.9% *vs* 57.6%, P = 0.34, laparoscopic *vs* open operations, respectively); however, major complications were more prevalent in the open surgery group (11.1% *vs* 28.2%, P = 0.03). No perioperative mortality was seen in the laparoscopic group, while in patients who underwent open surgery, the perioperative mortality rate was 3.5% (P = 0.55). Median length of hospital stay was found to be significantly shorter in the laparoscopy group (6 d *vs* 9 d). Within the 25.4 mo follow-up period of the laparoscopic group, a 4.4% recurrence rate was demonstrated, compared to a 15.3% recurrence after a median follow-up of 42.7 mo in the open surgery group. In the study by Fernández-Cruz *et al*[24] which included 49 patients undergoing laparoscopic surgery for PNETs, a higher perioperative complication rate among patients undergoing laparoscopic enucleation compared to those who underwent laparoscopic distal pancreatectomy (42.8% *vs* 22%, respectively, $P < 0.001$) was observed[24]. The main complication was POPF which also occurred more frequently in the enucleation group (38% *vs* 8.7%, $P < 0.001$). However, all fistulas following enucleation were successfully managed conservatively. No perioperative mortality was demonstrated.

Assalia *et al*[13] reported their experience with 17 cases of laparoscopically treated PNETs, and demonstrated a perioperative complication rate of 23%, and a POPF rate of 15.3%. No mortality or recurrence was shown, although their series did not include patients with malignant neuroendocrine tumors. In the same publication, a review of an additional 93 reported cases of laparoscopically managed PNETs from the literature was also presented. These cases demonstrated a perioperative complication rate of 28%, and a POPF rate of 17.9%. Following enucleation, the fistula rate was higher than that following distal pancreatectomy (30.7% *vs* 5.1%, respectively). Fistulas were managed mainly by drainage alone (11/14), with a combination of drainage and ERCP with stenting (1/14), and two cases required reoperation. No mortality was observed.

In the literature, the reported rate of "conversion to open" in laparoscopic pancreatic surgery ranges from 8%-33%[24,77-82]. Reasons for conversion include intraoperative complications such as bleeding, inability to localize the tumor, or location of the tumor in close proximity to vital structures (such as the main pancreatic duct or portal vein) in which continuation of laparoscopic resection would either jeopardize those structures or would prevent an appropriate oncologic resection. However, as previously mentioned, the presence or suspicion of a malignant lesion in itself is not an indication for conversion or open surgery. A multi-center study compared open distal pancreatectomy with laparoscopic distal pancreatectomy for adenocarcinoma, and similar short- and long-term oncologic outcomes were demonstrated between the two groups[83]. Unfortunately, similar studies are not yet available for pancreatic neuroendocrine carcinomas.

CONCLUSION

Laparoscopy is a safe modality for the surgical treatment of PNETs. Retrospective studies demonstrated similar overall complication rates in comparison with open pancreatic surgery for these tumors; however, there is evidence that the rate of major complications is higher in those undergoing open surgery. Laparoscopy, although considered to be more technically demanding, is not associated with a compromise in terms of oncologic

outcome, and provides the benefits of decreased postoperative pain, better cosmetic results, shorter hospital stay, and a shorter postoperative recovery period. Further prospective, multi-center, and randomized trials are required for the analysis of these minimally invasive surgical techniques for the treatment of PNETs and their comparison to traditional open pancreatic surgery.

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TOPIC HIGHLIGHT

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Peritoneal adhesions after laparoscopic gastrointestinal surgery

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Abstract

Although laparoscopy has the potential to reduce peritoneal trauma and post-operative peritoneal adhesion formation, only one randomized controlled trial and a few comparative retrospective clinical studies have addressed this issue. Laparoscopy reduces de novo adhesion formation but has no efficacy in reducing adhesion reformation after adhesiolysis. Moreover, several studies have suggested that the reduction of de novo post-operative adhesions does not seem to have a significant clinical impact. Experimental data in animal models have suggested that CO_2 pneumoperitoneum can cause acute peritoneal inflammation during laparoscopy depending on the insufflation pressure and the surgery duration. Broad peritoneal cavity protection by the insufflation of a low-temperature humidified gas mixture of CO_2, N_2O and O_2 seems to represent the best approach for reducing peritoneal inflammation due to pneumoperitoneum. However, these experimental data have not had a significant impact on the modification of laparoscopic instrumentation. In contrast, surgeons should train themselves to perform laparoscopy quickly, and they should complete their learning curves before testing chemical anti-adhesive agents and anti-adhesion barriers. Chemical anti-adhesive agents have the potential to exert broad peritoneal cavity protection against adhesion formation, but when these agents are used alone, the concentrations needed to prevent adhesions are too high and could cause major post-operative side effects. Anti-adhesion barriers have been used mainly in open surgery, but some clinical data from laparoscopic surgeries are already available. Sprays, gels, and fluid barriers are easier to apply in laparoscopic surgery than solid barriers. Results have been encouraging with solid barriers, spray barriers, and gel barriers, but they have been ambiguous with fluid barriers. Moreover, when barriers have been used alone, the maximum protection against adhesion formation has been no greater than 60%. A recent small, randomized clinical trial suggested that the combination of broad peritoneal cavity protection with local application of a barrier could be almost 100% effective in preventing post-operative adhesion formation. Future studies should confirm the efficacy of this global strategy in preventing adhesion formation after laparoscopy by focusing on clinical end points, such as reduced incidences of bowel obstruction and abdominal pain and increased fertility.

Key words: Peritoneal adhesions; Laparoscopy; Abdomen; Gastrointestinal surgery; Inflammation; Learning curve; Anti-adhesion; Animal models; Clinical studies; Laparoscopic resection of gastrointestinal

Core tip: Laparoscopy reduces de novo adhesion formation but does not reduce adhesion reformation. Adhesion reduction does not necessarily impact clinical outcomes. CO_2 pneumoperitoneum causes peritoneal inflammation depending on the insufflation pressure and surgery duration. Broad peritoneal cavity protec-

tion by insufflating a low-temperature, humidified gas mixture of CO_2, N_2O, and O_2 seems to represent the best approach for reducing peritoneal inflammation due to CO_2 pneumoperitoneum. A global strategy to prevent adhesion formation following laparoscopy should combine broad peritoneal cavity protection with the local application of a barrier.

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INTRODUCTION

Peritoneal adhesion formation is the most prevalent complication of abdominal and pelvic surgery[1-4]. Peritoneal adhesions can cause small-bowel obstruction, infertility, chronic abdominal pain, and increases in surgical time and in the risk of bowel perforation during preoperative surgery[1-4]. Peritoneal adhesions are among the leading causes of abdominal reoperations up to 10 years following abdominal or pelvic surgery[1,2,4]. Despite the high healthcare costs associated with peritoneal adhesions and the medico-legal consequences of bowel damage due to adhesions complicating preoperative surgery[5,6], the clinical and social problem of adhesions remains underestimated among patients and surgeons[7,8]. In addition, informed consent before surgery is very often inadequate regarding the risk of post-operative adhesion formation[7,8].

The majority of papers dealing with the healthcare and patient burdens of complications related to peritoneal adhesions have focused on the consequences of laparotomy[1-4,9]. However, since the early 1990s[10-13] laparoscopy has offered increasing advantages compared to open surgery for a number of pelvic[12-14], abdominal[10,11,15,16], and cancer procedures[17-19]. The present review was aimed at retrieving all of the data available from the experimental and clinical surgical literature to clarify whether the progressive shift from open to laparoscopic access for many abdominal and pelvic surgical procedures has already had or, in the near future, could have the major impact of reducing post-operative peritoneal adhesion formation.

PATHOGENESIS OF ADHESION FORMATION

Peritoneal adhesion formation is the consequence of abnormal repair of the peritoneum following different peritoneal injuries[20,21]. Surgical trauma, endometriosis, peritoneal infections, and peritoneal inflammation can cause peritoneal mesothelial defects and/or increased vessel permeability, which in turn produces inflammatory exudate[20,21]. Inflammatory exudate results in the presence of a fibrin mass in the peritoneal cavity[20,21]. The fibrin mass is entirely removed from the peritoneal cavity when peritoneal fibrinolytic activity is normal, and complete mesothelial regeneration occurs within 8 d[20,21].

Table 1 Adverse factors causing peritoneal adhesions and proposed preventive factors

Adverse factors	Proposed preventive factors
Surgical trauma	Minimal incisions (laparoscopy)
Infections	Minimal infection risk
Mesothelial defects	Minimal tissue handling (good surgery)
Increased vessel permeability	Corticosteroids and antihistamines
Inflammatory exudate	Corticosteroids and NSAIDs
Blood	Achieve hemostasis (good surgery)
Fibrin mass/fibrin bands	Fibrinolytic agents
Ischemia	Maintenance of vascularity (good surgery)
Thermal injury	Avoidance of thermal injury (good surgery)
Foreign bodies (starch powder)	Good surgery/laparoscopy (starch-free gloves)
Desiccation	Moistening of tissues (irrigation/humidified pneumoperitoneum)
Inflammation	Corticosteroids and NSAIDs/"peritoneum-friendly" pneumoperitoneum
Over-expression of PAI-1 and PAI-2	Reduction of inflammation/"peritoneum-friendly" pneumoperitoneum
Suppression of fibrinolytic activity	Fibrinolytic agents/"peritoneum-friendly" pneumoperitoneum
High-pressure CO_2 pneumoperitoneum	Reduction of pneumoperitoneum pressure
Long-duration CO_2 pneumoperitoneum	Rapid surgery
High intra-peritoneal temperature	Cooling of the peritoneal cavity
100% CO_2 pneumoperitoneum	Lower CO_2 concentration (gas mixture)

NSAID: Non-steroidal anti-inflammatory drug; PAI: Plasminogen activator inhibitor.

The main cause of incomplete removal of the fibrin mass from the peritoneal cavity is the suppression of peritoneal fibrinolytic activity due to ischemia or the inflammation-induced over-expression of plasminogen activator inhibitors 1 and 2[20,21]. When fibrin persists in the peritoneal cavity, fibroblasts proliferate into fibrin bands, and these fibrin bands organize into adhesions (pathological bonds) between organ surfaces[20,21]. Therefore, many adverse factors can cause peritoneal adhesions, and many preventive factors have been proposed (Table 1).

ATRAUMATIC SURGICAL TECHNIQUE FOR PREVENTING PERITONEAL ADHESIONS

Although intraperitoneal adhesions can be due to several injuries, such as cancer, peritoneal infections, or endometriosis, the trauma associated with surgery is the leading cause of peritoneal adhesion formation[20,21]. The formation of post-operative adhesions depends primarily on

impaired fibrinolysis and inadequate blood supply[3,9,20-22]. The operative factors that potentiate intraperitoneal post-operative adhesion formation after open surgery include ischemia, thermal injury, infections, residual blood clots left in the peritoneal cavity at the end of surgery, residual macroscopic or microscopic foreign bodies in the peritoneal cavity and overly vigorous manipulation of structures distal to the operative sites[3,9,20-22].

To prevent post-operative adhesion formation after standard open surgery, in 1980, Gomel[23] described an open microsurgery technique for reconstructive tubal surgery. To reduce the operative factors that could potentiate peritoneal post-operative adhesion formation after standard open surgery, the key points of microsurgery include minimizing tissue handling and being gentle when handling tissue, minimizing foreign bodies and using very small sutures, avoiding the use of dry sponges and moistening tissues with constant irrigation, and achieving hemostasis while maintaining vascularity[23]. Since 1980, any surgical procedure that conforms to microsurgery principles has been considered a "good" atraumatic surgery that is able to reduce peritoneal trauma and post-operative peritoneal adhesion formation[24]. However, randomized clinical trials have not been performed in humans to compare adhesion formation after standard open surgery with adhesion formation after microsurgery[24].

LAPAROSCOPY AS A THEORETICALLY ATRAUMATIC SURGICAL TECHNIQUE

Theoretically, laparoscopy has the potential to be a "good surgery" for reducing peritoneal trauma and post-operative peritoneal adhesion formation[25]. In fact, laparoscopic access to the peritoneal cavity allows the surgeon to perform only minimal incisions of the parietal peritoneum, to minimize tissue handling and to handle tissues gently with atraumatic instruments, to minimize the risk that foreign bodies might be introduced into and left in the peritoneal cavity, and to avoid the use of dry sponges. However, other key points of microsurgery depend on the surgeon's attitude and/or the surgical procedure to be performed through laparoscopy.

Constant irrigation to moisten tissues, accuracy of hemostasis, the suturing technique, and attention to vascularity mainly depend on the surgeon's attitude. Surgeons very often underestimate the clinical problems of adhesions and their complications and only surgeons with the proper perception and knowledge of the problem of adhesions are likely to undertake adhesion prevention during surgery[7,8].

Bleeding during surgery and residual blood left in the peritoneal cavity at the end of surgery depend on the surgical procedure to be performed through the laparoscopic access more than on the access itself. Some procedures, such as myomectomy, liver resection, or rectal resection, are often complicated by excessive bleeding although laparoscopic procedures have been reported to decrease the need for blood transfusions compared to open surgery[13,15,26,27].

PERITONEAL POST-OPERATIVE ADHESION FORMATION AFTER LAPAROSCOPY OR OPEN SURGERY IN ANIMAL MODELS

Very few comparative experimental studies are available, and the data have been equivocal, likely because of the different animal models and experimental settings. In 1994, Marana *et al*[28] were unable to identify significant differences in post-operative adhesion formation in female rabbits undergoing conservative ovarian surgery *via* laparoscopy or laparotomy.

In 1998, Chen *et al*[29] reported fewer post-operative adhesions in female pigs after pelvic and paraaortic lymphadenectomy performed *via* transperitoneal laparoscopy than after the same procedure performed *via* transperitoneal laparotomy. Krähenbühl *et al*[30] evaluated post-operative adhesion formation after laparoscopic or open fundoplication in male Sprague-Dawley rats and reported a reduction in the number and severity of adhesions with laparoscopy. Schippers *et al*[31] compared post-operative adhesion formation after cecal resection and deserosation of the abdominal wall *via* laparoscopy or laparotomy in dogs and found a significant reduction in adhesions to the abdominal incision but no reduction in adhesions at the site of cecal resection following laparoscopy. In 2009, Dubcenco *et al*[32] reported a significant decrease in adhesion formation in female pigs undergoing liver biopsy *via* laparoscopy compared to laparotomy. In 2013, Shimomura *et al*[33] observed a decrease in post-operative adhesion scores associated with the preservation of peritoneal fibrinolysis in male rats undergoing cecal cauterization *via* laparoscopy with CO_2 pneumoperitoneum at 5 mmHg compared to rats undergoing the same procedure through open surgery.

A possible interpretation of the above equivocal results could come from the observations made by Jacobi *et al*[34] in 2001 and by Arung *et al*[35] in 2012. The first authors reported a significant decrease in adhesion formation in rats with peritoneal infections undergoing resection of the cecum *via* laparoscopy with helium pneumoperitoneum compared to rats with peritoneal infections undergoing the same procedure through open surgery or laparoscopy with CO_2 pneumoperitoneum[34]. Arung *et al*[35] reported a decrease in adhesion formation in male rats undergoing peritoneal injury *via* laparoscopy with air pneumoperitoneum compared to rats undergoing peritoneal injury through open surgery or laparoscopy with CO_2 pneumoperitoneum. Thus, depending on the insufflation pressure, pneumoperitoneum with CO_2 seems to induce peritoneal inflammation and ischemia of the intra-peritoneal viscera[33-35]. In fact, high intra-peritoneal CO_2 pressure decreased the fibrinolytic activity of peritoneal tissue in mice[36]. Severe alterations in the circulation of the intra-peritoneal viscera with resulting tissue ischemia have been

observed in dogs during 14 mmHg pneumoperitoneum for 60 min using either CO_2 or helium[37]. An experimental study in pigs demonstrated that the effects of intra-peritoneal pressure on the blood circulation of the intra-peritoneal viscera were independent on the gas (air or CO_2) when the intra-peritoneal pressure was greater than 12 mmHg[38].

PERITONEAL POST-OPERATIVE ADHESION FORMATION AFTER LAPAROSCOPIC OR OPEN SURGERY IN CLINICAL TRIALS

A few comparative clinical studies have addressed this issue, but they have all agreed in suggesting that laparoscopic surgery results in a decrease in post-operative peritoneal adhesion formation. In 2000, Audebert and Gomel[39] showed that women undergoing laparotomy *via* a midline incision had a 50% incidence of umbilical adhesions, whereas women undergoing laparoscopy had only a 1.6% incidence of umbilical adhesions.

Polymeneas *et al*[40] retrospectively compared post-operative peritoneal adhesion formation following laparoscopic or laparotomic cholecystectomy and showed that patients treated *via* laparoscopy had significantly fewer adhesions than patients treated *via* laparotomy. Dowson *et al*[41] performed an observational study to compare adhesion formation after laparoscopic colectomy with that after open colectomy and reported that laparoscopic colectomy resulted in a lower incidence of post-operative peritoneal adhesions compared to open colectomy.

CLINICAL IMPACT OF LAPAROSCOPY ON READMISSION RATES FOR SMALL-BOWEL OBSTRUCTION

A recent meta-analysis of randomized clinical trials compared laparoscopic versus open resection for rectal cancer and showed that laparoscopic rectal resection resulted in a lower rate of readmissions for adhesion-related bowel obstruction[26]. In contrast, several previous studies have suggested that the reduction in peritoneal post-operative adhesions in patients undergoing laparoscopy was not associated with a significant clinical impact[42-44].

Lower *et al*[42] performed a very large epidemiological study that demonstrated similar adhesion-related readmission rates following gynecological laparoscopy or laparotomy. Taylor *et al*[43] reported similar readmission rates for adhesion-related small-bowel obstruction in patients who previously underwent laparoscopically assisted or open surgical procedures for colorectal cancer. Schölin *et al*[44], in a randomized trial comparing laparoscopic versus open resection for colon cancer, reported that the incidence of bowel obstruction episodes in patients treated *via* laparoscopy did not differ from the incidence in patients treated *via* laparotomy.

GAS INSUFFLATION CHARACTERISTICS DURING PNEUMOPERITONEUM

The above contradictory data obtained in clinical studies can be explained by observations made in animal models. Experimental data have suggested that CO_2 pneumoperitoneum can cause peritoneal inflammation during laparoscopy depending on the insufflation pressure and the duration of surgery[33-36].

Over the last ten years, using a laparoscopic mouse model, Koninckx and coworkers have obtained an enormous amount of information aimed at understanding the process by which CO_2 pneumoperitoneum enhances adhesion formation. In 2004, Binda *et al*[45] demonstrated that reductions in body and intra-peritoneal temperatures, the addition of 3% of O_2 to CO_2, and peritoneum humidification were all factors that were able to reduce adhesion formation caused by CO_2 pneumoperitoneum. In the same year, Elkelani *et al*[46] reported that the ideal concentration of O_2 to be added to CO_2 was 3% because higher O_2 concentrations added to CO_2 were unable to reduce adhesion formation caused by pneumoperitoneum. In 2006, Binda *et al*[47] confirmed that intra-peritoneal cooling and the prevention of desiccation were important, mutually dependent factors for preventing adhesions during CO_2 pneumoperitoneum.

In 2011, Corona *et al*[48] demonstrated that inflammation of the peritoneal cavity not only resulted in adhesions of the entire cavity but also enhanced adhesion formation at specific intra-peritoneal sites. The same authors also reported that desiccation depended on CO_2 flow rates and intra-peritoneal relative humidity, whereas intra-peritoneal temperature depended on desiccation[49]. Therefore, with a specific CO_2 humidifier, desiccation could be prevented while maintaining a low intra-peritoneal temperature[49].

Very recently, using a laparoscopic mouse model, Corona *et al*[50] investigated the effects of adding different concentrations of nitrous oxide (N_2O) to CO_2 pneumoperitoneum on peritoneal adhesion formation and the effects of adding different amounts of whole blood, plasma, or red blood cells to the peritoneum. N_2O at a 10% concentration was the most effective in preventing adhesion formation, while the presence of plasma in the peritoneum increased adhesion formation in an amount-dependent manner[50]. N_2O at a 10% concentration was able to reduce plasma-induced adhesions[50].

From all of the above data, we can conclude that the combination of hemostasis accuracy and surgery with broad peritoneal cavity protection, obtained by insufflating a low-temperature, humidified gas mixture of CO_2, N_2O, and O_2 should represent the best approach for the prevention of peritoneal adhesion formation during laparoscopic surgery.

MULTI-PORT *VS* SINGLE-PORT LAPAROSCOPY

The extension of the midline incision has been suggest-

ed to be the key factor in inducing peritoneal adhesion formation in open surgery[51]. Therefore, single-port laparoscopy should have the potential to further reduce peritoneal trauma compared to multi-port laparoscopy. Unfortunately, no studies have investigated the differences between multi-port and single-port laparoscopy with regard to post-operative peritoneal adhesion formation. Thus far, studies evaluating differences between single-port and multi-port laparoscopic procedures have compared operative outcomes, short-term post-operative parameters, complications, and cosmetic results without addressing the issue of post-operative adhesion formation[52].

GENETICS AND SURGEONS' LEARNING CURVES FOR ADHESION PREVENTION

Technical factors play key roles in adhesion prevention during laparoscopic surgery, but one should always bear in mind that surgeons operate on living human beings, and individuals can have different genetic constitutions. In fact, Molinas *et al*[53] demonstrated that the extent of adhesion formation in mice after laparoscopic surgery depended on the different animal strains. These data strongly suggest that genetics are a cofactor in peritoneal adhesion formation.

In contrast, surgeons perform surgery and surgeons are also human beings. Everyone knows that every surgical procedure must be learned well before the best possible results can be achieved. Therefore, the concept of the learning curve should also be applied to "the good surgery" aimed at preventing peritoneal adhesion formation. The impact of the learning curve on peritoneal adhesion formation has already been demonstrated in a laparoscopic mouse model[54]. In this model, peritoneal adhesion formation decreased with the decreasing duration of surgery[54]. Training allowed both senior surgeons and junior surgeons to decrease the duration of laparoscopy and, consequently, adhesion formation, although the senior surgeons had shorter surgery durations compared to the junior surgeons when starting training[54].

The concept that the effects of a laparoscopic learning curve also have an impact in clinical settings can be inferred from the results of previous randomized, controlled clinical trials performed to test the efficacy of different anti-adhesion barriers in the prevention of peritoneal adhesion formation after laparoscopic myomectomy[55,56]. In 1995, women included in a control group undergoing only "good surgery" had an 88% incidence of adhesions, whereas in 2006, the control group undergoing only "good surgery" had a 62% incidence of adhesions, a decrease of 26%[55,56].

The clinical importance of this observation is obvious. The impact of the surgeon's learning curve in reducing adhesion formation in different laparoscopic clinical settings should be always considered when planning future studies to test new anti-adhesion products.

CHEMICAL ANTI-ADHESIVE AGENTS

Theoretically, fibrinolytic agents, anticoagulants, and anti-inflammatory agents (corticosteroids and non-steroidal anti-inflammatory drugs) have the potential to be efficacious in reducing peritoneal adhesion formation[20,25]. However, systemic administration of fibrinolytic agents is not safe because the concentration needed to prevent adhesions is too high and can cause post-operative hemorrhage and delayed healing[20,25]. In contrast, intraperitoneal administration of fibrinolytic agents results in absorption that is too rapid to be effective[20,25]. Similarly, intra-peritoneal administration of heparin at low doses is ineffective in reducing peritoneal adhesion formation whereas high doses are effective in reducing adhesions, but they can induce post-operative hemorrhage and delayed healing[20,25].

Non-steroidal anti-inflammatory drugs have been used in experimental animal models, but no clinical trials have been published[20,25]. Corticosteroids are effective in the majority of animal models, but only limited clinical studies have reported contrasting results on adhesion scores and the occurrence of some adverse events, such as immunosuppression and delayed healing[20,25].

ANTI-ADHESION BARRIERS

A recent systematic review and meta-analysis of randomized, controlled trials evaluated the efficacy of and side effects experienced with the intra-peritoneal application of four anti-adhesion barriers (an oxidized regenerated cellulose absorbable barrier, a sodium hyaluronate/carboxymethylcellulose absorbable barrier, a polyethylene glycol sprayable barrier, and icodextrin 4% solution) after abdominal surgery[57]. None of the barriers showed serious side effects compared to controls[57]. Both the oxidized regenerated cellulose absorbable barrier and the sodium hyaluronate/carboxymethylcellulose absorbable barrier significantly reduced peritoneal adhesion formation[57]. The sodium hyaluronate/carboxymethylcellulose absorbable barrier reduced the incidence of small-bowel obstruction[57].

The sodium hyaluronate/carboxymethylcellulose film was not produced for laparoscopic application. In a recent experimental study in two animal models, its efficacy was compared with that of a sodium hyaluronate/carboxymethylcellulose powder having the same composition as the film but developed for laparoscopic application[58]. Both the film and the powder reduced adhesions to the same extent after local application to peritoneal defects, but only the powder was able to reduce adhesions after application at sites distant from the peritoneal defects[58].

New barrier gels have been produced in the last few years. Some of these gels have already been evaluated in clinical settings, such as an auto-cross-linked hyaluronan gel[56,59-61]. Other gels, such as an ultrapure alginate-based gel, have been efficacious in reducing peritoneal adhesion formation only in experimental animal models[62].

ANTI-ADHESION BARRIERS AND LAPAROSCOPIC SURGERY

Data obtained in a laparoscopic mouse model by Binda *et al*[62,63] demonstrated that further reductions in peritoneal adhesion formation could be achieved in the model by adding the intra-peritoneal use of anti-adhesion barriers to the broad protection of the peritoneal cavity (obtained by insufflating a low-temperature, humidified gas mixture). However, very few human clinical trials have evaluated the effects of the intra-peritoneal use of anti-adhesion barriers during laparoscopic surgery.

An oxidized regenerated cellulose absorbable barrier has been evaluated in four gynecological clinical trials dealing with laparoscopic surgery for myomectomy, severe pelvic endometriosis, or electrosurgical treatment for polycystic ovarian syndrome[55,64-66]. The barrier reduced peritoneal adhesion formation after laparoscopic myomectomy and laparoscopic surgery for severe pelvic endometriosis[55,64,66], but it was apparently unable to reduce ovarian adhesions after laparoscopic electrosurgical treatment for polycystic ovarian syndrome[65].

A modified hyaluronic acid/carboxymethylcellulose powder was recently evaluated in a multicenter, randomized, reviewer-blinded trial performed in women undergoing laparoscopic myomectomy[67]. The laparoscopic application of this sprayable adhesion barrier was associated with a reduction in post-operative peritoneal adhesion development[67].

Polyethylene glycol has been evaluated in three clinical trials investigating laparoscopic myomectomy[68-70]. This sprayable barrier seems to be efficacious in reducing de novo peritoneal adhesion development[68-70].

Auto-cross-linked hyaluronan gel has been evaluated in women undergoing laparoscopic myomectomy[56,59-61]. The barrier gel not only significantly reduces post-operative peritoneal adhesion development[56,59,61] but also increases the pregnancy rates of women undergoing laparoscopic myomectomy and gel application compared to women undergoing laparoscopic myomectomy alone[60].

The use of icodextrin 4% solution following gastrointestinal surgery has been investigated in 269 laparoscopies performed for adhesiolysis, cholecystectomy, hernia repair, or bowel resection with anastomosis[71]. General surgeons from five European countries considered icodextrin 4% solution to be easy to use and acceptable in patients, demonstrating that this fluid barrier could be safely used in a broad range of gastrointestinal surgical procedures[71]. However, this study was only a safety study. The results of multicenter, randomized trials designed to assess the efficacy of icodextrin 4% solution have been ambiguous and are difficult to understand[72,73]. In 2007, Brown *et al*[72] reported a significant reduction in reformed adhesions after laparoscopic surgery for adhesiolysis using the solution, whereas in 2011, Trew *et al*[73] reported no evidence of a clinical effect of the solution in reducing de novo adhesion formation. Apparently, icodextrin 4% solution had the same efficacy as Ringer's solution in reducing de novo adhesions after laparoscopic surgery[73].

A GLOBAL STRATEGY FOR PREVENTING ADHESIONS IN LAPAROSCOPY

To translate the concept of broad peritoneal cavity protection by insufflating "peritoneum-friendly" pneumoperitoneum from animal models to humans, in December 2013, Koninckx *et al*[74] published the first randomized, controlled trial aimed at evaluating the anti-adhesive efficacy of insufflation of a low-temperature (31°C), humidified gas mixture of 86% CO_2, 10% N_2O and 4% O_2 at a pressure of 15 mmHg in a clinical setting (laparoscopic surgery for deep endometriosis).

To obtain the best protection of the entire peritoneal cavity, the gas mixture was humidified by sprinkling Ringer's solution with heparin 1000 IU/L, and the patients also received dexamethasone (5 mg) intramuscularly at the end of surgery[74].

Because site-specific, local treatment with barriers was previously demonstrated to be synergistic with broad peritoneal cavity protection in preventing adhesion formation in an animal model[63], auto-cross-linked hyaluronan gel was applied at the end of surgery in the same patients undergoing broad peritoneal cavity protection[74].

The combination of broad peritoneal cavity protection with local application of a gel barrier was highly effective in preventing post-operative adhesion formation compared to standard laparoscopic surgery with humidified CO_2 pneumoperitoneum. Adhesions were completely absent in 12 of 16 patients, and the other 4 patients who received broad peritoneal cavity protection and local gel barrier application had very low adhesion score[74].

The trial included only a small number of patients (16 treated and 11 controls), but the results were very promising because the patients underwent laparoscopic surgery for deep endometriosis, and endometriosis is one of the primary adverse factors causing peritoneal adhesion formation and reformation[20,25,64].

CONCLUSION

The state of the art regarding the prevention of peritoneal adhesions after laparoscopic gastrointestinal surgery is quite disappointing. Although laparoscopy has the potential to be "the good surgery" for reducing peritoneal trauma and post-operative peritoneal adhesion formation, only a few comparative clinical studies have addressed this issue. Moreover, several studies have suggested that the reduction of de novo post-operative adhesions obtainable by operating *via* laparoscopy has no clinical impact[42-44].

Experimental data in animal models have suggested that CO_2 pneumoperitoneum can cause peritoneal inflammation during laparoscopy depending on the insufflation pressure and duration of surgery[33-35]. Broad peritoneal cavity protection, obtained by insufflating a low-

temperature, humidified gas mixture of CO_2, N_2O, and O_2, represents the best approach for reducing peritoneal inflammation due to CO_2 pneumoperitoneum[45-50]. However, as of December 2013, these experimental data have had no impact in modifying laparoscopic instrumentation[74].

Anti-adhesion barriers have been used in laparoscopy. Spray, gel, and fluid barriers are easier to apply in laparoscopic surgery than solid barriers. The results have been encouraging for solid, spray and gel barriers, but have been ambiguous for fluid barriers[55-57,59-61,67,68,71-73].

Future studies should evaluate the efficacy of a global strategy to prevent adhesion formation during laparoscopy in larger numbers of patients. This global strategy should combine broad peritoneal cavity protection, obtained by insufflating a low-temperature, humidified gas mixture of CO_2, N_2O and O_2, with low-dose intra-peritoneal heparin and low-dose intramuscular dexamethasone and local treatment with barriers of proven efficacy.

These future studies should focus on specific clinical end points, such as reduced instances of bowel obstruction and abdominal and pelvic pain and increased fertility, rather than simply investigating adhesion reduction.

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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (15): Laparoscopic resection of gastrointestinal

Evolution of laparoscopy in colorectal surgery: An evidence-based review

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Abstract

Open surgery for colorectal disease has progressed significantly over the past century from humble beginnings to form the mainstay of treatment for colorectal cancer and a number of benign conditions. Following the introduction of laparoscopic abdominal surgery, the next stage in the evolution of the specialty began in the 1990s with the first laparoscopic colonic resection. Following some early concerns regarding its safety and oncological efficacy during the latter part of that decade, laparoscopic colorectal surgery rapidly came into mainstream use in the early part of the current century with evidence supporting its use being made available from large scale randomised controlled trials. This article provides an evidence-based summary of this evolutionary process as it relates to both benign and malignant colorectal disease, as well as discussion of the next phase of new technologies such as robotic surgery.

Key words: Colorectal surgery; Colorectal cancer; Pelvic floor; Laparoscopy; Robotics; Laparoscopic resection of gastrointestinal

Core tip: This article provides a historical perspective on the development of minimally invasive surgery for colorectal disease, as well as a summary of the key evidence supporting its use for treating both benign and malignant disease. We further discuss new minimally-invasive technologies which represent the next step in the evolution of colorectal surgery.

Blackmore AE, Wong MTC, Tang CL. Evolution of laparoscopy in colorectal surgery: An evidence-based review. *World J Gastroenterol* 2014; 20(17): 4926-4933 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/4926.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.4926

INTRODUCTION

The term "laparoscopy" is derived from the Greek words "lapara", meaning "the soft parts of the body between the rib margins and hips", and "skopein", meaning, "to see, view or examine". Laparoscopy has therefore come to describe the process of viewing the contents of the abdominal cavity indirectly, *i.e.*, using specially designed instruments and a camera system controlled by the surgeon from outside the abdomen.

Following the work of early pioneers of open colonic resection, such as Sir William Arbuthnot-Lane at Guy's Hospital in London during the early part of the 20th century[1], open surgery to resect the colon and rectum for a wide range of diseases developed rapidly during the last century. Although Lane was ridiculed in 1913 for performing total colectomy for patients with chronic constipation, the technique soon became widely accepted for the management of a wide range of elective and emergency conditions, both benign and malignant.

DEVELOPMENT OF LAPAROSCOPIC ABDOMINAL SURGERY

The 1980s heralded the development of laparoscopic general surgical procedures, with the first laparoscopic cholecystectomy being performed by Mühe in Germany on September 12th 1985[2]. This was followed in 1991 by the first reports of colonic resection performed with laparoscopic assistance by Jacobs in Miami, Florida[3], and separately by Fowler *et al*[4] in Kansas. The subsequent development of laparoscopic surgery resulted in the development of a variety of new instruments that have allowed increasingly complex procedures to be performed in a safe and efficient manner. Laparoscopic camera equipment is now available which can focus automatically, and even produce three-dimensional images if required. A wide variety of instruments are now available for retraction and dissection of tissues, as well as laparoscopic stapling devices to efficiently and safely divide both bowel and vascular pedicles, even deep in the pelvis. Several different types of energy devices have been developed or adapted from equipment used in open surgery to dissect tissues and seal vessels, including monopolar electrocautery scissors, ultrasonic coagulating shears and electrothermal bipolar vessel sealers. The precise type of device employed is largely determined by cost and surgeon preference, with no clear evidence suggesting the superiority of any particular energy device[5].

EARLY DEVELOPMENT OF LAPAROSCOPIC COLORECTAL TECHNIQUES

Throughout the 1990s concerns regarding the oncological safety and efficacy of laparoscopic colorectal resection, with little robust evidence as to its advantages over open surgery, limited uptake of the procedure. These concerns included high rates of abdominal port-site metastases reported in small case series and various theories were proposed to explain this phenomenon, including direct implantation of tumour cells either through close contact of instruments coated with tumour cells and the port, during the release of the pneumoperitoneum, or during extraction of the specimen through a small incision. It was also thought to be a possibility that manipulation of the bowel using laparoscopic instruments may lead to increased exfoliation of tumour cells in comparison with open techniques, or even that the gas used to create the pneumoperitoneum could somehow be stimulating tumour growth[6,7]. Further data from larger series in the latter part of the decade, showed that the incidence of abdominal wall metastases could be reduced to an acceptable rate which was similar to that observed for open surgery with the use of wound protection devices at the extraction site[8]. Despite these concerns surrounding surgery for colorectal cancer, the development of laparoscopic procedures for benign conditions continued, particularly for rectal prolapse in the form of rectopexy. There is no doubt that colorectal disease associated with a significant degree of inflammation, such as complicated diverticular or inflammatory bowel disease, can present a formidable challenge for the laparoscopic surgeon, which has been reflected in evidence from large scale trials supporting laparoscopic resection for these indications lagging behind those relating to surgical oncology. This evidence, however, does support the view that this surgery can be performed safely and effectively[9].

One might wonder if the early development of new surgical techniques such as laparoscopic resection might more appropriately be applied to benign conditions rather than malignancy due to concerns over possible inadequacy of oncological clearance, and it would appear that this paradox is being replicated in the development of robotic colorectal surgery. Perhaps this situation has arisen due to concerns over the technical difficulties of resecting inflammatory disorders such as diverticular disease or inflammatory bowel disease, which frequently involves adjacent structures, as opposed to relatively early colorectal tumours, or a desire to pioneer new techniques on the colon as opposed to in the relatively inaccessible pelvis, for example to treat pelvic floor disorders. Another theory is that this situation reflects caseload mix, with a larger number of resections being performed for malignant disease.

BRIDGING THE DIVIDE

Several variations to the technique of laparoscopic colorectal surgery have developed in an effort to bridge the gap between conventional open surgery and minimally invasive approaches.

Laparoscopic-assisted techniques

Most surgeons would consider a laparoscopic colorectal resection to imply intracorporeal division and control of the major vascular pedicle involved, with bowel reanastomosis being performed either intra- or extracorporeally *via* a small extraction site made in the abdominal wall. It is important to bear in mind that there is no universally accepted definition of what actually constitutes "laparoscopic assistance" or even "conversion" from a laparoscopic to an open procedure, resulting in significant differences in reporting of the rates that they occur and are compared[10]. Various degrees of "laparoscopic assistance" can be employed either due to complication or expediency, such as laparoscopic mobilisation of the left colon and division of the inferior mesenteric pedicle for anterior resection, with subsequent rectal dissection being performed open *via* a low midline or pfannenstiel incision, avoiding a high midline wound which would potentially be more painful and reduce cosmesis.

Hand-assisted techniques

A hybrid technique, which attempts to provide the advantages of laparoscopic surgery while reducing the tech-

Table 1 Summary of key papers on laparoscopic resection for colorectal cancer

Trial	Year of publication	Type of study	Numbers of patients	Key findings
Barcelona trial[14] Lap-assisted *vs* open colectomy	2002	RCT Single centre	219	Improved perioperative outcomes and hospital stay in lap group Survival benefit in stage Ⅲ disease for lap group
COST study[15] Lap *vs* open colectomy	2004	RCT Multicentre	872	Longer operating time but quicker recovery for lap No difference in morbidity, mortality, recurrence or survival
COLOR trial[16] Lap *vs* open colectomy	2009	RCT Multicentre	1248	Supported findings of COST
CLASICC trial[17,19] Lap *vs* open colon and rectal cancers	2005 2010 (5 yr follow-up)	RCT Multicentre	794 (2:1 lap: open)	Equivalent perioperative and oncological outcomes 29% conversion rate Higher CRM involvement for rectal cancers with lap
Abraham *et al*[48] Short-term outcomes of lap *vs* open	2004	Meta-analysis of RCTs	2521 12 RCTs	Longer operative times, less morbidity and quicker recovery for lap Mortality and oncological outcomes equivalent
Cochrane review[18] Short-term outcomes after lap	2005	Systematic review		Less morbidity and quicker recovery for lap
Cochrane review[20] Long-term results after lap	2008	Systematic review		Equivalent oncological outcomes for lap *vs* open

RCT: Randomized controlled trial.

nical difficulty and increased operative time, is the hand-assisted approach. The authors believe that this technique can be particularly useful for surgeons who are relatively new to laparoscopic surgery as a useful adjunct to becoming proficient in fully laparoscopic colorectal surgery. This technique involves the insertion of a bespoke port into the abdominal wall that allows the surgeons' hand to enter the abdominal cavity to assist in the operation while maintaining a pneumoperitoneum and therefore continued visualisation of the abdominal contents with the laparoscope. Although data comparing hand assisted and laparoscopic colorectal surgery is limited in comparison to that comparing laparoscopic and open procedures, a Cochrane review of randomised controlled trials concluded that there was a significant decrease in conversion rates in the hand assisted group, although there was no difference in complications or operating times[11].

EVIDENCE FOR LAPAROSCOPIC COLORECTAL CANCER RESECTION

Although the concern regarding port site metastases had been addressed by the turn of the century, and uptake of laparoscopic colorectal surgery began to increase as a niche interest, a lack of long term data evaluating oncological outcomes following cancer resection prevented its use as a mainstream technique in the majority of units. At this time, data from large, multicentre randomised controlled trials across the world was published which suggested that short term outcomes were at least equivalent to open surgery and may have some advantages on perioperative outcomes. When patients were then surveyed on their quality of life following both forms of surgery using validated questionnaires, the authors of a recent systematic review of all available randomised controlled trials on this subject involving 2263 patients concluded that: "based on presently available high-level evidence, this systematic review showed no clinically relevant differences in postoperative quality of life between laparoscopic and open colorectal surgery"[12] (Table 1).

What, therefore, are the advantages of laparoscopic compared to open colorectal surgery? The smaller incisions required for insertion of laparoscopic ports obviously result in less surgical trauma to the abdominal wall, and studies have demonstrated a reduced inflammatory response, possibly as a result of less manipulation of the small intestine during surgery[13].

Several landmark trials then emerged to mark the turning point in laparoscopic colorectal cancer surgery. In 2002, the Barcelona group published a randomised trial of 219 patients in the Lancet comparing laparoscopically-assisted with open colectomy for colon cancer, in terms of both short term perioperative outcomes as well as, for the first time in a large scale randomised trial, tumour recurrence and disease-specific survival[14]. The results of this study suggested that there was a significant benefit for the laparoscopic group in terms of perioperative morbidity and hospital stay, with superior rates of tumour recurrence and disease-specific survival for patients with stage Ⅲ disease. However, the trial was criticised for a 14% increased recurrence rate in the open group and a poor lymph node harvest in both groups. Soon after, the larger multicentre randomised COST trial reported from the United States on the results of 872 patients treated at 48 institutions for colon cancer by surgeons who had completed at least 20 laparoscopic resections[15]. This trial supported the findings of the Barcelona group that hospital stay was shorter in the laparoscopic group, but there was no significant difference in morbidity. There was also equivalence in terms of recurrence and overall survival at three years. This trial also largely answered the concern over port-site recurrence, with the rate for both groups being less than 1%. Further long-term data on the equivalence of laparoscopic and open surgery for colon

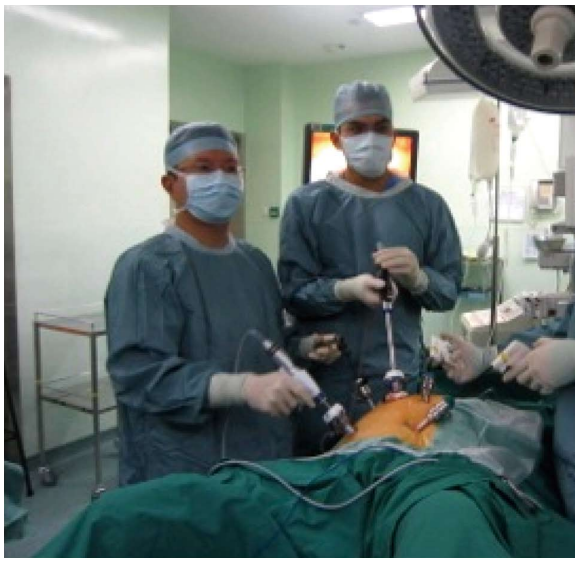

Figure 1 The author performing a laparoscopic rectopexy for rectal prolapse.

cancer in terms of disease-free survival has since been provided by the European Colon Cancer Laparoscopic or Open Resection (COLOR) trial of 1248 patients published in 2009[16]. The large, multicentre, randomised CLASICC trial was the first major trial including rectal resections as well as colectomy to report that short-term outcomes for laparoscopic compared to open colorectal cancer resection were at least equivalent, but was limited by the relative inexperience in laparoscopic surgery of many participating surgeons. This probably accounted for the high conversion rate to open surgery of 29% in the laparoscopic group and the non-significantly higher rate of circumferential resection margin involvement for rectal cancers[17]. These concerns of increased rates of CRM involvement did not, however, translate to a difference in local recurrence at three years. Meta-analysis of randomised controlled trials evaluating the differences in short term outcomes have shown that laparoscopic surgery is associated with less intraoperative blood loss, reduced postoperative pain and ileus, and improved pulmonary function, resulting in reduced postoperative stay in hospital[18]. Further results from the CLASICC trial reported in 2010, as well as a Cochrane review, have demonstrated that oncological outcomes following laparoscopic surgery are not inferior to those in patients undergoing open resection[19,20].

Concerns regarding the safety of laparoscopic surgery for rectal cancers are further being addressed by the COLOR Ⅱ trial, which has randomised 1103 patients with rectal cancer to laparoscopic or open resection with a 2:1 ratio at 30 centres in 8 countries between 2004 and 2010. Initial results published at the beginning of this year showed improved perioperative outcomes in the laparoscopic group in terms of blood loss and hospital stay, with longer operative times, and there was equivalence in terms of completeness of excision and perioperative morbidity and mortality[21]. Data on rates of locoregional recurrence are expected soon.

LAPAROSCOPIC SURGERY FOR BENIGN COLORECTAL DISEASES

Pelvic floor dysfunction

Much of the early experience of laparoscopy for functional colorectal disorders has been focussed on rectal prolapse syndromes, including rectocele, intussusception and procidentia. A case series of 84 patients undergoing laparoscopic ventral mesh rectopexy for symptomatic complex rectocele published in 2011 by this author showed a significant decrease in vaginal discomfort and obstructed defaecation symptoms, with 88% of patients reporting an improvement in overall well-being (Figure 1). There was an acceptable conversion rate of 3.6% and a perioperative morbidity rate comparable to open rectopexy at 4.8% with no mortality, suggesting that the laparoscopic technique is safe and effective for treating symptomatic rectocele[22]. The advantages of laparoscopic compared to open surgery in terms of short term perioperative outcomes, such as reduced blood loss, pain and postoperative stay in hospital, demonstrated in trials comparing the two techniques for colorectal cancer resection, have also been demonstrated in randomised trials comparing laparoscopic and open surgery for benign indications such as rectopexy for rectal prolapse[23].

Inflammatory bowel disease

The available evidence would suggest that laparoscopic surgery for small bowel Crohn's disease is at least as safe as open surgery, although there may be less of an advantage in terms of short term outcomes than for other indications[24]. One of the areas in which the benefits of a laparoscopic approach may be most obvious is in colectomy for ulcerative colitis necessitating ileostomy formation, whereby the colectomy specimen may be extracted *via* the ileostomy site prior to formation of the stoma, avoiding the need to make a separate abdominal incision for extraction. The results of a meta-analysis of trials comparing laparoscopic and open surgery for this indication published in 2007 seemed to support this view, with reduced morbidity and hospital stay in the laparoscopic group[25].

EMERGENCY LAPAROSCOPIC COLORECTAL PROCEDURES

The initial experience with laparoscopic colorectal surgery was almost exclusively restricted to elective procedures, and there is little robust data to evaluate the role of laparoscopy for colorectal emergencies. As colorectal surgeons become increasingly experienced in laparoscopic techniques, many are turning towards laparoscopy as a tool for managing acute conditions such as complicated diverticular disease and inflammatory bowel disease as one of the new frontiers in our specialty. In 2008, a study was published of 100 patients who had undergone laparoscopy for perforated diverticulitis. The authors proceeded to convert to standard open surgery if faecal peritonitis was

revealed, with the remaining 92 receiving laparoscopic lavage and drainage without bowel resection. The results were encouraging, with low mortality and morbidity, and only 2 patients being readmitted with recurrent diverticulitis at a median follow-up of three years[26]. These results have subsequently been replicated in meta-analysis[27], and the results of ongoing randomised trials are awaited[28].

TRAINING IN LAPAROSCOPIC COLORECTAL SURGERY

There is no doubt that the surgical techniques required to perform laparoscopic colorectal surgery are demanding, both for surgeons in training and for those experienced in open colorectal surgery. A recent systematic review and international multicentre analysis of 4852 cases performed by surgeons on this learning curve suggests that it is indeed steep, at between 88 cases for blood loss and 152 cases for conversion to an open procedure[29]. The results of this review also suggest that body mass index and pelvic dissection, particularly in male patients, increased the risk of complications and conversion, and that increasing T stage of tumours and complicated inflammatory disease increased the complexity of the case.

NEW TECHNOLOGIES

In recent years, further techniques utilising minimally invasive techniques have begun to be employed by colorectal surgeons, and technologies involving robotic, single port and natural orifice instrumentation are now available in many units across the world, although the paucity of robust evidence on the effectiveness of these procedures means that their role remains unclear[30].

Robotic colorectal surgery

The use of robotic systems for performing minimally invasive colectomy was first reported in 2002 by Weber *et al*[31], following earlier work in the fields of urological and cardiac surgery. Indeed, over 50000 robotic prostatectomies were performed in the United States in 2007[32]. There is no doubt that these robotic systems are significantly more expensive than conventional laparoscopic or indeed open colorectal procedures, so it is important that the evidence base for these procedures is strengthened in the future. To date, only one randomised study from South Korea comparing robotic with conventional laparoscopic surgery has been published, which focused on total mesorectal excision for rectal cancers and consisted of only 18 patients in each group[33]. This limited study did suggest that short-term outcomes for robotic surgery were at least equivalent, with acceptable specimen quality on pathological analysis for oncological status. Further data from the international, multicentre randomised ROLARR trial comparing robotic-assisted versus standard laparoscopic surgery for rectal cancer in terms of both short term perioperative and longer term outcomes is awaited in the coming years[34]. Meta-analysis of the available non-randomized studies comparing robotic and laparoscopic rectal resection including a total of 854 patients suggests a lower conversion rate to open surgery for robotic procedures, with similar operative times and other short-term outcomes[35]. It may be that the maximum benefit of robotic surgery for colorectal resection may be in dissection of the rectum within the bony pelvis, where the more stable platform provided by the robot to eliminate tremor of the surgeons hand, improved imaging in three dimensions controlled by the surgeon rather than assistant and wrist movement of robotic instruments allows for more precise dissection of tissue planes[36]. A number of authors had reported reduced rates of circumferential resection margin involvement and autonomic nerve dysfunction in patients undergoing robotic total mesorectal excision[33,37,38]. Although most of the published literature on robotic colorectal resection is focused on rectal resection, presumably due to the perceived advantages being maximal in this area, data from meta-analysis of 39 case series or comparative non-randomised studies combining both rectal resections and abdominal colectomies also concluded that conversion rates and perioperative morbidity was similar for the colectomy group, and considerably lower for robotic anterior resection of the rectum, with an adequate lymph node harvest[39].

In more recent years the range of procedures and indications relating to prolapse of the pelvic floor has expanded and begun to involve robotic technology. A recent prospective analysis of 63 consecutive patients undergoing robotic-assisted or laparoscopic ventral mesh rectopexy for symptomatic complex rectocele showed a significantly longer operating time in the robotic group, but slightly less blood loss, with similar conversion rates and hospital stay[40].

Single incision laparoscopic colorectal surgery

Other devices have been developed to allow colorectal procedures to be performed endoscopically *via* a single incision as opposed to multiple ports, even in some centres utilising robotic systems to access the abdomen *via* a single-access port. Evidence as to the efficacy of these procedures from randomised trials is lacking, but several reports including an early feasibility study by the authors shown the safety and feasibility of such an approach in right hemicolectomy[41]. The authors followed-up their experience with a case cohort comparison of short-term outcomes in 144 consecutive cases of laparoscopic and single-incision right hemicolectomy performed at our unit showed that there was at least no disadvantage of the single-incision technique, with no significant difference in operative time, lymph node clearance or recovery parameters (pain score, length of stay and complications[42]). These findings have been replicated in much larger meta-analyses, with a study including 1075 procedures from 15 studies comparing single-incision approach with conventional laparoscopy finding no difference in conversion rates or operation times between the two groups, with a significantly shorter length of postoperative stay in hos-

Figure 2 The author at the Da Vinci Si robotic console performing a robotic-assisted ultra-low anterior resection for rectal cancer.

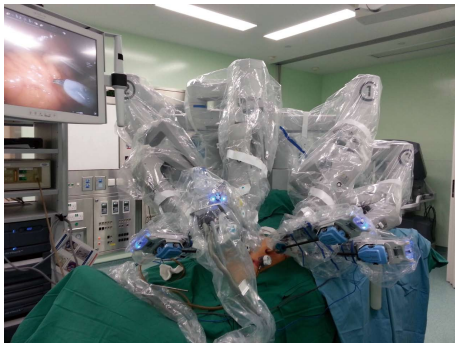

Figure 3 The 4-arm Da-Vinci Si robotic setup for low rectal cancer resection.

pital in the single-incision group[43]. However, the authors believe that the case for single-incision approach has not yet been conclusively made, with evidence from a large-scale randomised trial being needed.

DISCUSSION

In the United Kingdom and United States, the National Institute of Clinical Excellence and the Society of American Gastrointestinal and Endoscopic Surgeons respectively, now support laparoscopic resection for colorectal cancer performed by suitably experienced surgeons[44,45]. An audit of the proportion of colectomies performed laparoscopically in the United States for the years 2008 and 2009 showed that of 9075 patients identified retrospectively from administrative data, 50% were performed laparoscopically[46]. In the United Kingdom, data from the 2013 National Bowel Cancer Audit suggest that this figure has improved from 25% in 2008 to over 40% of resections for both colon and rectal cancer in 2012.

In conclusion, laparoscopic surgery for colorectal disease has moved from being an experimental procedure performed by a small number of pioneers in the early 1990s, to today being firmly established in the mainstream around the world. This has occurred despite the fact that laparoscopic surgery is more expensive and requires a longer operating time than the equivalent open colorectal procedure[47]. Large-scale international multicentre randomised trial data has established that laparoscopic colorectal surgery is safe both in terms of short-term perioperative outcomes and long-term oncological efficacy, and we are now into the robotic era as perhaps the next stage of minimally invasive colorectal procedures (Figures 2 and 3).

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REVIEW

Vitamin D improves inflammatory bowel disease outcomes: Basic science and clinical review

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Abstract

Vitamin D deficiency is commonly diagnosed among patients with inflammatory bowel disease (IBD). Patients with IBD are at risk of low bone density and increased fractures due to low vitamin D levels, long standing disease, and frequent steroid exposures; as a result, it is well established that vitamin D supplementation in this population is important. There is increasing support for the role of vitamin D in strengthening the innate immune system by acting as an immunomodulator and reducing inflammation in experimental and human IBD. The active form of vitamin D, 1,25(OH)D3, acts on T cells to promote T helper (Th)2/regulatory T responses over Th1/Th17 responses; suppresses dendritic cell inflammatory activity; induces antibacterial activity; and regulates cytokine production in favor of an anti-inflammatory response. Murine and human IBD studies support a therapeutic role of vitamin D in IBD. Risk factors for vitamin D deficiency in this population include decreased sunlight exposure, disease duration, smoking, and genetics. Vitamin D normalization is associated with reduced risk of relapse, reduced risk of IBD-related surgeries, and improvement in quality of life. Vitamin D is an inexpensive supplement which has been shown to improve IBD outcomes. However, further research is required to determine optimal serum vitamin D levels which will achieve beneficial immune effects, and stronger evidence is needed to support the role of vitamin D in inducing disease response and remission, as well as maintaining this improvement in patients' disease states.

Key words: Vitamin D; Inflammatory bowel disease; Immune response; Inflammation; Cytokines; Supplementation

Core tip: There is support for the importance of maintaining normal vitamin D levels in inflammatory bowel disease (IBD) patients, demonstrated by its anti-inflammatory actions in the gut. A randomized controlled trial examined the impact of vitamin D supplementation on IBD outcomes and demonstrated a reduced risk of relapse in vitamin D-treated Crohn's disease patients. Furthermore, vitamin D3 and active vitamin D have been shown to reduce clinical disease activity and improve quality of life in IBD patients. Normalization of vitamin D levels is also associated with a decreased risk for IBD-related surgery. This vitamin has therapeutic benefit in IBD patients.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the intestine that causes abdominal pain, diarrhea, and weight loss and includes two forms, Crohn's disease and ulcerative colitis[1]. IBD reduces quality of life and may cause financial stress by increasing disability and decreasing the capacity for work[1,2]. The disease is complex and etiology is not completely understood; however, IBD is associated with abnormal immune responses to the body's natural intestinal bacteria[1,2], which activate the gastrointestinal immune system. It is expected that genetic and environmental interactions play a role in the susceptibility of IBD[1,2]. As there is currently no cure for IBD, medical therapy remains the mainstay treatment for achieving and maintaining remission[2].

It is well-established that vitamin D plays a critical role in improving bone health and is specifically important for patients who are at risk of low bone density. Low bone mineral density is more prevalent among patients with Crohn's disease and ulcerative colitis compared to healthy controls[3]. Long standing disease, multiple steroid exposures, and low serum 25-hydroxycholecalciferol [25(OH)D3] levels are contributing factors to decreased bone mineral density and increased fractures in patients with IBD[3-5]. Therefore, vitamin D supplementation is essential in this high-risk group. There is, however, growing support for non-traditional actions of vitamin D including anti-inflammatory, anti-proliferative, cell differentiation, and apoptotic effects[6]. These effects have led to examination of vitamin D in the pathogenesis of autoimmune diseases such as IBD[6]. This review will address the regulatory role of vitamin D on immune responses and describe its relevance in regards to inflammatory bowel disease.

VITAMIN D PHYSIOLOGY

Vitamin D metabolism

Vitamin D is present in two major forms. Vitamin D2 (ergocalciferol) is present in plants, yeast, and fungi[6,7], while vitamin D3 (cholecalciferol) can be obtained from animal sources such as oily fish and egg yolk[6-8]. Vitamin D3 is also synthesized endogenously in the skin upon ultraviolet light exposure. Sun light exposure to the skin results in a photochemical conversion of 7-dehydrocholesterol to pre-vitamin D, which then rapidly converts to cholecalciferol. This process is self-limiting to prevent toxicity[6,8].

Vitamin D is fat soluble and absorbed in the small intestine along with dietary fat. After incorporation into chylomicrons, it is rapidly delivered into the venous circulation[3,4]. It is then transported by vitamin D binding proteins (DBPs) to the liver where it is converted to 25(OH)D3 by hepatic 25-hydroxylase[6,7,9]. 25(OH)D3 is inactive, however, it is the main circulating form of vitamin D and the best indicator of vitamin D status[6,9]. Vitamin D is primarily stored in the liver as well as in adipose tissue. Once saturation of these tissues occur, 25(OH)D3 is released to circulate in the blood[4], where it is predominantly bound by DBPs and albumin, leaving little in the free form[3,7]. DBPs transport 25(OH)D3 to the kidney, where it is converted to its active hormonal form, 1α,25-dihydroxycholecalciferol [1,25(OH)2D3], by the enzyme 25-hydroxyvitamin D3-1α-hydroxylase[6-9]. It can now act on its receptor, the vitamin D receptor, in many target tissues, including the intestine, kidney, and bone, thereby altering transcription of target genes[10,11]. In the target tissue, 24-hydroxylase catabolizes 1,25(OH)2D3 and 25(OH)D3 into their inactive metabolites, which are then excreted as calcitroic acid in the urine[3,8,10].

The rate limiting enzyme in the metabolism of vitamin D is 1α-hydroxylase. This enzyme is tightly regulated by plasma parathyroid hormone (PTH) and 1,25(OH)2D3. Active vitamin D production in the kidneys is directed by PTH[4,6,8,9], which upregulates transcription of CYP27B1, the gene encoding for 1α-hydroxylase[9]. This results in an increased production of 1,25(OH)2D3 in the kidney. In turn, 1,25(OH)2D3 takes part in a negative feedback loop to suppress the transcription of PTH and CYP27B1, thereby decreasing production of 1,25(OH)2D3[9]. Simultaneously, 1,25(OH)2D3 induces 24-hydroxylase production[8,9]. This is an autoregulatory mechanism to suppress the actions of 1,25(OH)2D3[9]. An overview of vitamin D sources and metabolism is outlined in Figure 1.

Vitamin D receptor

The vitamin D receptor (VDR) plays an important role in how vitamin D exerts its biological effects. It belongs to a superfamily of nuclear hormone receptors and is specifically activated by 1,25(OH)2D3[11,12]. In response to 1,25(OH)2D3 binding, VDRs regulate gene transcription, thereby producing specific proteins to carry out vitamin D3 biological activity. The DNA binding domain of the zinc finger recognizes vitamin D response elements (VDREs), which are specific DNA sequences on cell-targeted genes[11]. 1,25(OH)2D3 binding results in the formation of the VDR and retinoid X receptor (RXR) heterodimer[12]. This complex binds to VDREs and recruits large coregulatory complexes to these specific genes through the VDR transactivation domain[11,12]. The activities of coregulatory complexes may include nucleosomal remodeling, selective chromatic histone modification, or RNA polymerase Ⅱ recruitment and initiation. All of these activities work to enhance or suppress gene expression[11].

Multiple tissues and immune cells express VDRs and the enzymes needed to produce local 1,25(OH)2D3[6,13-15]. This enzyme activity is regulated in a different manner compared to the enzymatic renal production of 1,25(OH)2D3; it is no longer under an endocrine feedback mechanism, but is induced by other factors[6]. These findings have led to the examination of multiple roles of vitamin D in the pathogenesis of autoimmune diseases such as IBD[6].

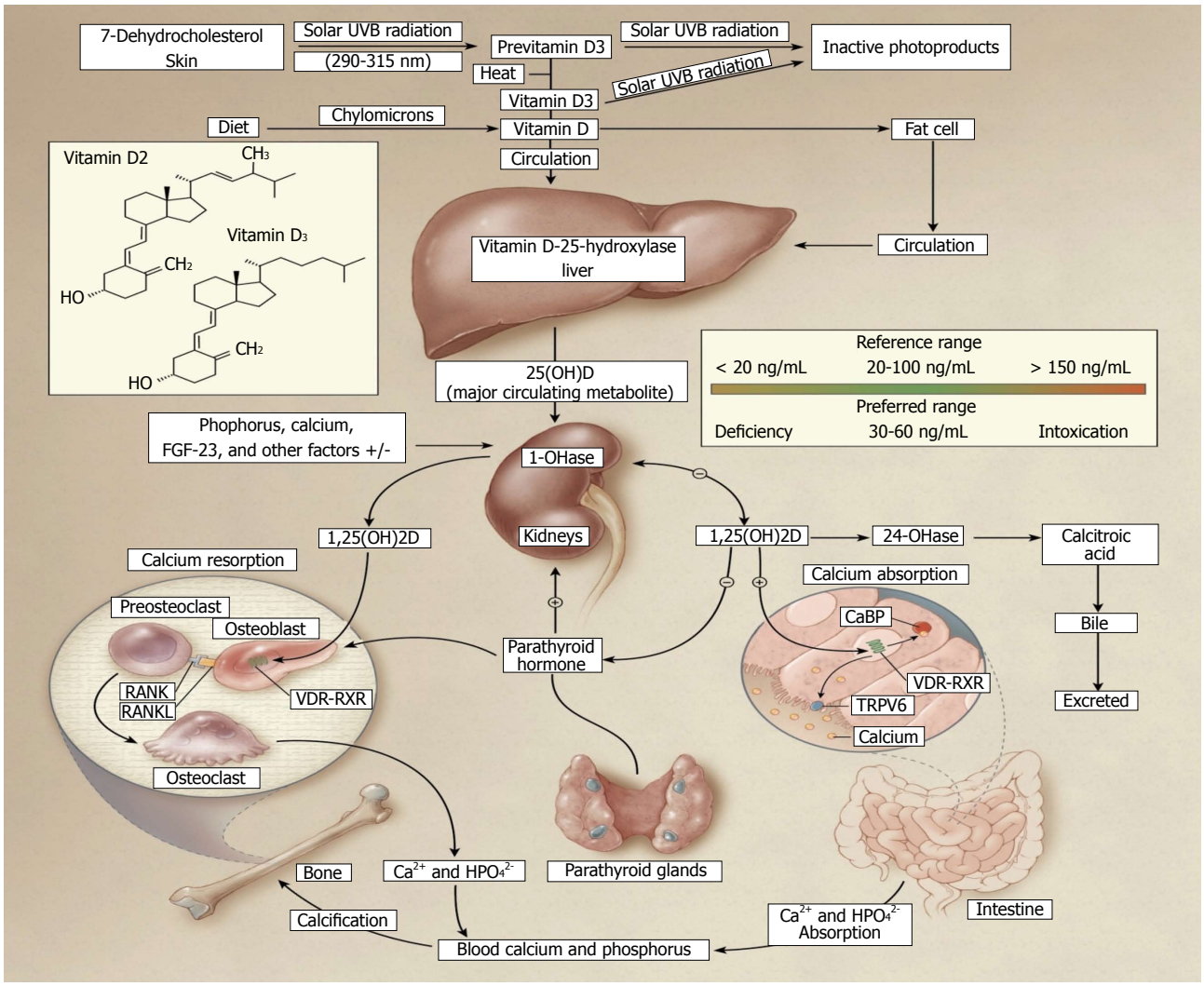

Figure 1 Vitamin D sources and metabolism[8]. Vitamin D can be obtained either from the diet or synthesized in the skin. Under solar ultraviolet (UVB) radiation, 7-dehydrocholesterol in the skin is converted into cholecalciferol (vitamin D3). Vitamin D from the diet enters chylomicrons, which transport it into circulation. Vitamin D is stored in adipose tissue, but when released into circulation, vitamin D binding proteins direct it to the liver where it is converted into its major circulating form, 25-hydroxyvitamin D3 [25(OH)D3] by 25-hydroxylase. In the kidneys, 25(OH)D3 is converted into 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3], the active form, by 1α-hydroxylase. It can now exert its biological effects including calcium absorption, resorption, and bone development. Parathyroid hormone released from the parathyroid glands upregulates hepatic conversion of 1,25(OH)2D3 by stimulating 1α-hydroxylase production; however, autoregulatory mechanisms suppress these actions through negative feedback loops. 1,25(OH)2D3 suppresses parathyroid hormone and 1α-hydroxylase production. Vitamin D is then catabolized by 24-hydroxylase and excreted as calcitroic acid. The recommended optimal range for vitamin D levels is 30-60 ng/mL (75-150 nmol/L). Copyright© 2007 Massachusetts Medical Society. All rights reserved; VDR: Vitamin D receptor; RXR: Retinoid X receptor.

EFFECTS OF VITAMIN D ON THE IMMUNE SYSTEM

Vitamin D is important in both the innate and adaptive immune systems[13]. Immune cells express VDRs and the enzymes necessary to convert vitamin D3 and 25(OH)D3 into 1,25(OH)2D3, wherein locally produced 1,25(OH)2D3 can exert specific autocrine and paracrine effects without producing unnecessary systemic effects[16,17]. 1,25(OH)2D3 can modulate the adaptive immune responses by altering the actions of activated T and B cells, and it can modulate the innate immune responses by regulating macrophages and dendritic cells[16].

Vitamin D and T-cell differentiation

It has been established that vitamin D is an immune system regulator through its role in targeting $CD4^+$ cells of T lymphocytes to suppress T helper type 1 (Th1) cell driven immune responses[17-19]. Th1 cells produce pro-inflammatory cytokines including IFN-γ, interleukin (IL)-2, and tumour necrosis factor-alpha (TNF-α), which are important for reducing intracellular infections. 1,25(OH)2D3 works to inhibit the over production of these pro-inflammatory cytokines[19].

Overall, the main action of 1,25(OH)2D3 on T-cells is mediating T helper type 1 (Th1)/T helper type 2 (Th2) development and differentiation[17,18]. Vitamin D3 affects

the Th1-Th2 balance in favour of Th2 cell development. The development into either Th1 or Th2 from $CD4^+$ T cells is directed by cytokines[18]. Cytokine IL-12 induces Th1 cell development whereas IL-4 induces Th2 cell development. The effects these cytokines have on the Th1-Th2 balance determines which cytokines will be produced and therefore determine the type of immune response. Th1 cells produce pro-inflammatory IFN-γ and lymphotoxin, and Th2 produces anti-inflammatory IL-4, IL-5, and IL-13[17,18]. The increased development of Th2 is a result of direct action of vitamin D3 on $CD4^+$ cells[18], whereas the reduction in Th1 cell development is due to the effects of vitamin D3 on dendritic cells[17,18]. Using mice Ag-specific T cells, Boonstra *et al*[18] demonstrate that vitamin D3 increases the frequency of IL-4 producing cells *in vitro* from 8.0%-55.8%, thereby supporting a vitamin D induced Th2 profile. This study also shows that vitamin D3 increases IL-10 and IL-5 producing cells and decreases IFN-γ producing cells[18]. The role of vitamin D in regulating cytokine production has been suggested to limit inflammatory tissue damage, but it also reduces bacterial killing. IFN-γ augments toll-like receptor (TLR) 2/1 induced expression of CYP27B1 and bacterial killing, and IL-4 depresses TLR 2/1 activation of bacterial killing[13]. Therefore, a balanced response is important.

At the transcription level, c-maf and GATA-3 are the transcription factors relating to Th2 development[18]. Vitamin D3 can directly target $CD4^+$ cells to promote Th2 development at the level of transcription[17,18]. *In vitro* studies show a correlation between increased expression of GATA-3 and c-maf and Th2 cytokine levels, IL-5, IL-10, and IL-4, after vitamin D3 treatment. In addition, there is a reduction in IFN-γ[18]. Boonstra *et al*[18] conclude that vitamin D likely functions to diminish cell-mediated immune responses by skewing toward a Th2 phenotype, which fights extracellular infections.

Th17 cells are another vitamin D target because of their ability to produce IL-17, a pro-inflammatory cytokine. Vitamin D reduces inflammatory tissue damage by suppressing Th17 development and in doing so, reduces IL-17 production[13]. Furthermore, 1,25(OH)2D3 increases the development of T regulatory cells by acting on naïve $CD4^+$ cells. Regulatory T cells are a group of $CD4^+$ T cells that have immunosuppressive properties by depressing the proliferation of other $CD4^+$ T cells[13]. Vitamin D in combination with another immunosuppressive drug, dexamethasone, has been shown to increase IL-10 producing regulatory T cells. This supports the idea that regulatory T cells located at sites of inflammation down-regulate the immune response[19].

Research supports the immunosuppressive effect of vitamin D due to its involvement in T-cell differentiation. The evidence shows that vitamin D plays a role in maintaining a balance between the inflammatory response of Th1/Th17 cells and the immunosuppressive response from Th2/Treg cells.

Vitamin D and dendritic cells

Dendritic cells (DCs) are antigen presenting cells (APCs) and are important in initiating $CD4^+$ T cells responses[20]. Vitamin D inhibits differentiation and maturation of human DCs *in vitro*[20] by suppressing the IL-12 production from DCs and increasing IL-10 production[17,20]. This is an important immunosuppressive activity as IL-12 is an important cytokine in inducing Th1 development[17,18]. 1,25(OH)2D3 inhibits the differentiation, maturation, and immunostimulatory capacity of DCs[21]. Canning *et al*[21] confirm that 1,25(OH)2D3 suppresses monocyte differentiation into DCs, thereby generating immature DCs. This suppresses DC ability to stimulate T-cell proliferation.

Lipopolysaccharide (LPS) is a component of the Gram-negative bacterial wall, which induces monocytes/macrophages to produce cytokines. After LPS stimulation, DCs and monocyte-derived macrophages (MACs) have a high local 1-α-hydroxylase production of 1,25(OH)2D3, which positively modulates MAC differentiation and depresses actions of DCs and lymphocytes[14]. This mechanism facilitates a normal immune response as some DCs still mature; vitamin D prevents over stress of this immune response that may lead to pathological effects[13]. Vitamin D skews the development of the immune response towards a non-specific innate immune response and away from an antigen-specific immune response.

Antibacterial activity of vitamin D

As a fundamental part of the innate immune response, human monocytes have been shown to locally produce 1,25(OH)2D3, which triggers increased autophagy, an important mechanism for eliminating pathogens by antibacterial proteins[15]. TLR expressed on monocytes recognize pathogens, and under TLR 2/1 stimulation, CYP27B1, the gene encoding for 1α-hydroxylase, and VDR expression is upregulated[13,15]. 1,25(OH)2D3 acts on monocytes to induce expression of the cathelicidin antimicrobial peptide (CAMP) gene (LL-37), producing a protein that enhances intracellular killing of bacteria[13,15,22]. The CAMP gene is a direct target of the vitamin D receptor[22]. Furthermore, nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor, has an important role in inducing antibacterial activity. NOD2 activation by muramyl dipeptide, a product of Gram-negative and Gram-positive bacteria, stimulates transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which induces gene expression for antimicrobial peptide defensin β2 (DEFB2)[13,23]. Interestingly, 1,25(OH)2D3 induces NOD2 expression in many cell types, thereby increasing cell sensitivity to these bacteria products; as a result 1,25(OH)2D3 enhances NOD2 mediated DEFB2 transcription[13,23].

Anti-inflammatory role of vitamin D

As a part of the innate immune system, macrophages release proinflammatory cytokines and chemokines upon stimulation. This leads to an inflammatory response to protect the body from pathogenic microorganisms[24]. TNF-α is a proinflammatory cytokine that is produced early in the course of inflammation by macrophages and lymphocytes. It is involved in autoimmune diseases, including IBD[25]. Proinflammatory cytokines are positive for host defense, but overproduction leads to unresolved inflammation[26]. Muller *et al*[27] examine the effect of 1,25(OH)2D3 on LPS-stimulated human blood monocytes, and find it inhibits the release of IL-1α, IL-6, and TNF-α. LPS binds to TLR4 on monocytes to mediate activation of mitogen-activated protein kinase (MAPK)[26]. MAPKs are critical regulators of proinflammatory cytokine production, including IL-6 and TNF-α. 25(OH)D3 treatment has been shown to inhibit LPS-induced IL-6 and TNF-α production in peripheral blood mononuclear cells (PBMC) of healthy donors *via* upregulation of MKP-1 (MAPK phosphatase-1). MKP-1 switches off cytokine production in monocytes/macrophages after inflammatory stimuli. Interestingly, doses of 25(OH)D3 that relate to vitamin D sufficiency (> 30 ng/mL or 75 nmol/L) significantly inhibited mRNA production of these cytokines[26].

Vitamin D analogues have also been proven to have immunomodulatory effects. Stio *et al*[28] demonstrate the vitamin D analogue KH 1060 and a monoclonal anti-TNF-α antibody to work synergistically in significantly inhibiting PBMC proliferation and TNF-α production in healthy subjects. *In vitro* experiments using PBMCs from healthy volunteers were also able to demonstrate the effect of paricalcitol, a vitamin D analogue, in reducing both basal TNF-α and LPS-induced TNF-α[25].

The importance of VDRs in the inflammatory response has also been demonstrated. Chen *et al*[24] report a more robust and prolonged production of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β in bone marrow derived macrophages (BMDMs) from VDR-/- mice compared to wild type (WT) mice after LPS exposure. This suggests a dysregulated and over sustained innate immune response in macrophages under attenuated VDR signalling. Interestingly, 1,25(OH)2D3 and its analogue, paricalcitol, have been shown to reduce LPS-induced TNF-α and IL-6 cytokines in WT BMDMs[24].

Chen *et al*[24] have also examined novel anti-inflammatory effects of vitamin D by investigating its microRNA-155-SOCS1 target. MicroRNAs (miRNAs) are small noncoding RNAs that control gene expression. Recently, miRNA-155 has been shown to regulate innate immune responses and TLR signaling. It targets the "suppressor of cytokine signalling" (SOCS) family of proteins, specifically SOCS1. miRNA profiling in mice cells was examined after the treatment with LPS, with or without 1,25(OH)2D3. As a result, miR-155 increased the most with LPS and was suppressed the most by vitamin D; miR-155 were elevated in VDR -/- bone marrow derived macrophages (BMDMs) after exposure to LPS compared to the WT BMDMs; and 1,25(OH)2D3 suppressed the induction of miR-155 by LPS in these cells. Additionally, 1,25(OH)2D3 blocked TNF-α, IL-6, and miR-155 induction in human PBMCs. Overall, 1,25(OH)2D3 was found to upregulate SOCS1 through its suppression of miR-155. SOCS1 is important in the negative feedback regulation of LPS-induced inflammation and inhibits TNF-α, IL-6, and IFN-γ pathways. In the absence of VDRs, the negative feedback loop is dysregulated[24].

Seasonal variations in serum vitamin D levels have an effect on the innate immune response. The increase in serum vitamin D levels during the summer months is associated with a significant drop in LPS-induced TNF-α (64%), IL-6 (33%), IL-1β (59%), and IFN-γ (46%) from PBMCs in healthy individuals *in vivo* compared to LPS-induced levels during the winter months[29]. Caution does need to be taken when considering the difference in physiological up-regulation of vitamin D levels by solar radiation and the doses of vitamin D employed *in vitro* and *in vivo*; however, this data does support how the innate immune response can be regulated by the physiological variation of serum vitamin D3 levels during the four seasons of the year[29].

Role of vitamin D in gastrointestinal inflammation

There is support for the role of vitamin D in the immune system, particularly in reducing inflammatory responses. Recently, more studies have demonstrated the role for vitamin D specifically in gastrointestinal inflammation and suggest deficiency is associated with IBD.

The importance of vitamin D in reducing the proinflammatory profile of IBD patients is demonstrated by the impact of cytokine-induced apoptosis and cytokine disruption of epithelial barrier function. Epithelial apoptosis occurs as a normal physiological event in the gastrointestinal tract and the mucosal barrier function is still maintained; however, cytokine-induced apoptosis may disrupt the barrier, leading to abnormal mucosal permeability, a common occurrence among IBD patients[30]. Bruewer *et al*[30] address the mechanisms by which pro-inflammatory cytokines disrupt the barrier through non-apoptotic mechanisms. IFN-γ was demonstrated to reduce epithelial gate function, and the effects were increased when combined with TNF-α, resulting in intestinal epithelium paracellular permeability. The authors suggest this allows for the barrier function to quickly normalize when inflammatory cytokines are reduced[30], stressing the importance of medical therapy in maintaining disease remission.

There is evidence to suggest that there are distinct cytokine profiles in Crohn's disease and ulcerative colitis. Papadakis *et al*[31] report Crohn's disease to show a Th1 type of immune response with elevated IL-12, TNF-α, and IFN-γ cytokines, whereas ulcerative colitis presents with increased IL-5 secretion. Specific pro-inflammatory cytokines have been identified in the inflamed mucosa of Crohn's disease and ulcerative colitis patients such as

IL-1, IL-6, IL-8, and TNF-α[31]. Each of these cytokines upregulate the inflammatory cascade leading to more inflammation and tissue damage in the inflamed mucosa[31]. Vitamin D has been shown to target these inflammatory pathways.

TNF-α is a central cytokine in the pathogenesis of inflammatory bowel disease[31]. In the review by Papadakis *et al*[31], they report three lines of evidence to support the importance of TNF-α in IBD. They explain that anti-TNF therapy has been very successful in treatment of IBD in humans and in animal models, and that a "Crohn's like" phenotype is expressed in mice that over express TNF-α[31]. Interestingly, a vitamin D analogue has recently been shown to work synergistically with infliximab, an anti-TNF therapy used in the treatment of IBD, to reduce the cytokine TNF-α in human peripheral blood monocytes[28]. Treatment with 1,25(OH)2D3 in the colonic tissue of IL-10 knock-out (KO) mice has also been shown to down-regulate TNF-α-associated genes in these mice[32]. Furthermore, *in vitro* studies of $CD4^+$ T-cells of healthy controls and patients with Crohn's disease have shown that 1,25(OH)2D3 increases the production of anti-inflammatory cytokine IL-10 and decreases the production of pro-inflammatory IFN-γ, supporting a therapeutic role of vitamin D in IBD[33].

Stio *et al*[34] examine the effects of vitamin D on PBMCs from patients with active Crohn's disease on infliximab, an anti-TNF therapy. After vitamin D treatment, PMBC proliferation decreased in both responders and non-responders, but to a greater extent in responders[34]. Interestingly, vitamin D analogue treatment increased VDR expression in unresponsive patients and VDR levels did not change in responsive patients. The authors suggest infliximab induces VDR expression in the presence of vitamin D in unresponsive patients; as a result, there are differences in sensitivity to vitamin D between responders and non-responders. This suggests that VDR expression and PMBC proliferation may be useful indicators to predict response of Crohn's disease patients to infliximab therapy[34].

Studies have gone on to suggest that vitamin D deficiency and deficiency in its signaling pathways are contributing factors in the pathogenesis of IBD. Wu *et al*[35] propose a mechanism by which vitamin D deficiency may cause IBD through the changes in vitamin D receptor signaling in autophagy homeostasis, including increases in TNF-α-induced autophagy. Another study by Wang *et al*[23] examine the role of vitamin D in NOD2 expression, as NOD2 deficiency, due to mutations in its gene, has been linked to the pathogenesis of Crohn's disease[22]. These authors show that 1,25(OH)2D3 signaling induces NOD2 expression in human intestinal epithelial cells, thus supporting the idea that vitamin D deficiency plays a causative role in Crohn's disease.

As discussed previously, local production of vitamin D is an important part of regulating microenvironment inflammatory profiles. DCs from healthy subjects convert 25(OH)D3 to the active form of vitamin D, 1,25(OH)2D3, which could be important in local control of inflammation in Crohn's disease patients[36]. Bartels *et al*[36] isolate peripheral monocytes from 20 Crohn's disease patients who were vitamin D deficient, which were then matured into DCs *in vitro*. Addition of the active [1,25(OH)2D3] and inactive [25(OH)D3] vitamin D3 metabolites inhibited LPS-induced DC maturation, and supplementation changed the cytokine profile of LPS-matured DC cultures compared to those not supplemented. The production of TNF-α and IL-12 decreased, while unexpectedly, there was a trend towards reduced IL-10 and IL-6 significantly increased[36]. DCs of Crohn's disease patients could be exposed to LPS in the gut resulting in similar findings[36]. Furthermore, vitamin D supplementation reduced proliferation of the entire lymphocyte population and the $CD4^+$ lymphocytes; therefore, vitamin D could be protective against Crohn's disease as inflammation in this disease can be characterized as uncontrolled lymphocyte development[36].

While the addition of vitamin D *in vitro* changes cytokine profiles, there may be differences in the effects of vitamin D in *in vivo* studies. A study was conducted on Crohn's disease patients who were treated with 1200 IU of vitamin D3 per day or placebo for 26 wk to assess differences in induced T-cell mediated immune function between the treatment and placebo groups during *in vivo*[37]. PBMCs were cultured from patients of each group, and there was a significant increase in production of IL-6 in T cells from vitamin D treated patients. IL-6 significantly correlated with serum vitamin D [25(OH)D3] levels ($P = 0.02$). In the vitamin D treated patients, there was also a trend to increased IL-4 and no effect on TNF-α and IFN-γ production[37]. There was no change in IL-10 production nor in the amount of T regulatory cells $CD4^+$, $CD25^+$, $FoxP3^+$ T cells; however, the amount of proliferating $CD4^+$ cells significantly increased. IL-6 can induce T cell IL-4 production, which may explain the increase in IL-4[37].

There is strong evidence for a protective effect of vitamin D against IBD related inflammatory responses; however, there are mixed findings on the production of IL-6 with the treatment of vitamin D. IL-6 has been shown to increase in certain situations and decrease in others under vitamin D treatment. Interestingly, *in vitro* and *in vivo* studies examining vitamin D treatment of LPS-matured DCs and PBMCs from Crohn's disease patients show increased IL-6 production, whereas these studies examining healthy volunteers demonstrate a decrease in IL-6 after vitamin D treatment. IL-6 has been reported to support Th17 development, which have important anti-microbial roles[36]. Additionally, IL-6 may have anti-inflammatory roles by reducing DC maturation and increasing T cell IL-4 production[36,37]. Therefore, vitamin D does have immunosuppressive properties; however, instead of isolating vitamin D to this effect, it may be better to suggest it is an immunomodulator that strengthens the innate immune response and depresses the adaptive immune reaction[36], which may explain the

different actions in healthy volunteers and IBD patients who have intestinal mucosal injury.

EFFECT OF VITAMIN D IN ANIMAL MODELS OF IBD

VDR KO mice models

Vitamin D and the vitamin D receptor have been shown to have an important role in animal models of colitis. Vitamin D receptor KO mice treated with dextran sodium sulfate (DSS) to induce colitis demonstrate markedly elevated levels of a number of tissue pro-inflammatory cytokines including TNF-α, IL-12p70, and IFN-γ, and have significantly more intestinal injury, fewer intact crypts, more epithelium loss, and more inflammation. This indicates that the absence of VDRs increases the sensitivity of the intestinal mucosa to very low doses of DSS[38], thereby increasing the susceptibility to chemical injury in the gut. This confirms a prominent role of VDR signalling in the regulation of gut inflammation[38].

Oral intake of 1,25(OH)2D3 improves DSS induced colitis in WT mice, and there is an upregulation of anti-inflammatory cytokine IL-10 production, which may help to reduce other cytokine responses[38]. Interestingly, rectal administration of 1,25(OH)2D3 in the WT mice is more effective than oral in regards to decreasing DSS colitis. This is justified by obtaining the same results but only injecting half the dose of vitamin D in the rectum (site of inflammation)[38].

Vitamin D and its receptors play important roles in maintaining gut integrity and protecting the intestine from pathogenic enteric bacterial infection. Wu *et al*[39] support the role of vitamin D in suppressing bacterial-induced NF-κB activity in the intestine. NF-κB is a regulator of inflammatory cytokines such as IL-6, and after bacterial invasion, VDRs inhibit its actions. Intestinal VDRs protect against bacterial infection, demonstrated by a 6-fold increase in IL-6 and more severe inflammation after a *Salmonella* in VDR-/- mice compared to VDR+/+ mice[39]. Furthermore, after exposure to Salmonella, mice lacking VDRs had more Salmonella invasion of the intestine compared to WT[39]. Interestingly, VDR expression increased in WT mice as a direct result of an enteric bacterial infection, indicating a role of VDR signalling pathway in responding to specific pathogenic bacteria. VDRs relocated from the surface of colonic epithelial cells to the surface of crypts as well as in the middle and bottom of the crypts after infection[39]. Furthermore, IL-6 was undetectable in VDR+/+ mice and present in VDR-/- mice; as a result, this suggests that VDR-/- mice start with a pre-inflammatory state even before being infected[39].

The intestinal epithelial layer contains a highly specialized immune system and physical barrier to protect against the invasion of pathogens. Intraepithelial lymphocytes (IELs) are found in the intestinal epithelial layer, and they are an important part of maintaining intestinal integrity[40]. Specific IELs, CD8 $\alpha\alpha^+$ T cells, aid in the maintenance of gut health. VDR KO mice have fewer CD8 $\alpha\alpha^+$ T cells in the gut, implying VDRs are important in regulating the growth and maintenance of CD8 $\alpha\alpha^+$ T cells[40]. Moreover, VDR-/- mice have significantly reduced intestinal transepithelial resistance (TER) compared to VDR+/+ mice. TER is reduced when epithelial barrier function is compromised, making it a good indicator of this malfunction. Additionally, tight junctions and desmosomes are severely disrupted in VDR-/- colonic mucosa[41]. The breakdown of the physical barrier that separates the host from its gastrointestinal microorganisms results in intestinal permeability and the development of IBD. These studies demonstrate prominent role of VDR signalling in maintaining the integrity of the epithelial barrier in the intestine and in its protection against IBD[42].

Increased IL-17 production has also been reported in VDR null mice. Th17 cells are pro-inflammatory and have been associated with increased severity of IBD in animal models. VDR KO mice have significantly higher induced Th17 cell and IL-17 production compared to WT mice[43]. Additionally, 1,25(OH)2D3-deficient T cells overproduce IL-17 compared with WT cells[43]. Recombinase-activated gene 2 knockout mice lack mature T and B cells. When WT $CD4^+$ T cells are transferred to VDR/Rag double KO mice, the colonic sections of these recipients show significantly more hyperplasia and inflammation compared to Rag KO recipients[43]. There is an increased expression of IL-17 secreting cells in the gut and periphery of these double KO mice, suggesting VDRs on accessory cells direct Th17 development[43].

Conversely, there are some limitations of vitamin D on host susceptibility to pathogens. While increased production of Th1/Th17 cytokines is related to the pathogenesis of Crohn's disease, Th1/Th17 responses are critical for the removal of many infectious bacteria[44]. Examining the role of 1,25(OH)2D3 in *Citrobacter rodentium* (*C. rodentium*) infected mice, Ryz *et al*[44] demonstrate that mice treated with 1,25(OH)2D3 have significantly increased scores for edema, goblet cell depletion, hyperplasia and infiltrating inflammatory cells compared to vehicle-treated mice[44]. These mice also have a reduced number of Th17 T cells in their colons and decreased production of Th17 associated antimicrobial peptide TEG3γ, which works as a host defense against infection with *C. rodentium*[44]. This study demonstrates the potential negative consequences of vitamin D treatment. It can protect against Th17 cell driven damage, but it is important to note these responses are key in host defense against pathogens[44].

IL-10 KO mice models

VDR/IL-10 double KO mice develop severe IBD involving all areas of the small intestine and colon and have the largest change in colon anatomy and inflammation compared to single VDR KO and IL-10 KO mice[45]. WT mice do not express any cytokines and VDR/IL-10 double KO mice express two-three fold higher levels of IL-1β, IL-2, IFN-γ, and TNF-α mRNA in their colons

compared to the single KO mice colons[45]. IL-10 KO mice with normal VDR function experience a milder form of disease; thus, VDR deficiency seems to intensify IBD severity. Single VDR KO mice also express a milder form of disease; therefore, IL-10 and VDRs have a collective effect on disease severity[45]. Furthermore, vitamin D deficiency in IL-10 KO mice enhances inflammation in the small intestine in comparison to vitamin D sufficient and vitamin D supplemented IL-10 KO mice[46]. Evidently, vitamin D deficiency augments disease severity; however, dietary intake of vitamin D can be problematic, as few foods are high in vitamin D and weight loss is common in patients with IBD[46]. Therefore, vitamin D supplementation to achieve and maintain sufficient levels is critical.

Trinitrobenze sulfonic acid and DSS induced colitis mice models

Colitis can be induced in mice with treatment of trinitrobenzene sulfonic acid (TNBS). While treatment alone with calcitriol, a vitamin D analogue, significantly reduces colitis in acute TNBS colitis, treatment with both calcitriol and dexamethasone shows the best improvement in health[47]. This is demonstrated by the study by Daniel *et al*[47], which showed weight gain and improvement in macroscopic, microscopic, and immunological parameters of colitis after treatment of TNBS-induced colitis with the combined therapies. Calcitriol treatment reduced Th1 mediators and increased IL-4, thereby promoting the Th2 subset[47]. Additionally, calcitriol upregulated IL-10 and enhanced regulator T cell function, specifically transforming growth factor beta, and FoxP3 levels. Calcitriol also decreased IL-12p70 and IL-23p19 expression, which are DC mediators. As a result, calcitriol downregulates the pro-inflammatory response of intestinal DCs and counteracts Th1 action[47].

A vitamin D analogue has been shown to provide similar results. ZK156979 is a new low calcemic vitamin D analogue, and its use in treating TNBS-induced colitis in mice results in remarkable disease improvement. Daniel *et al*[48] show a reduction in colitis-associated hypercalcemia and inflammation as a result of treatment of TNBS-induced colitis with this vitamin D analogue. Infiltration of inflammatory cells, neutrophils and lymphocytes, ulcerations, loss of goblet cells, and fibrosis in the colon were restored after vitamin D treatment[48]. TNF-α and IFN-γ levels decreased, and the Th2 profile was induced with increased production of IL-4 and IL-10[48]. This analogue effectively treats Th1 colitis.

DSS treated vitamin D deficient and sufficient mice both show increased expression of mRNAs for cytokines IL-1, IL-10, IL-17, and IFN-γ[49]. Both types of mice show severe ulceration, granulation, and inflammation; however, symptoms are exacerbated in vitamin D deficient mice[49]. Vitamin D acts to protect DSS treated mice, as there is an increased expression of vitamin D activating enzyme CYP27B1 in these mice[49] and CYP27B1 KO mice are more susceptible to colitis after DSS treatment[50]. Lagishetty *et al*[49] examine the impact of vitamin D status on antibacterial activity in DSS-induced experimental colitis. The expression of an antimicrobial protein, angiogenin-4 (Ang4), decreased in vitamin D deficient mice[49]. Ang4 has bactericidal activity in the intestine and a decreased expression resulted in a 50-fold increase of bacterial infiltration in vitamin D deficient mice[49]. It is worthy to note dietary restrictions in these mice resulted in 25(OH)D3 concentrations less than 50 nmol/L, which is consistent with human parameters of deficiency. The authors note that the consequences of vitamin D deficiency resulted after treatment with DSS; therefore, vitamin D deficiency may be important after inflammation has occurred[49]. On the other hand, the expression of Ang4 was associated with vitamin D status; therefore, the authors hypothesize impaired vitamin D status may be a predisposing factor for IBD due to the regulation of enteric bacteria[49].

Although these animal results are limited in their application to humans as chemical induced colitis in mice may not fully be representative of human IBD forms, they are important in examining treatment and disease activity outcomes. Investigation into determining whether other mouse models better replicate human IBD will be important[50]. The differences in results between models with impaired vitamin D status as opposed to deletion of VDRs or the *CYP27B1* gene expression are also important in determining IBD susceptibility[50].

EFFECT OF VITAMIN D IN HUMAN IBD

Geographical distribution of vitamin D deficiency in IBD

Epidemiological evidence for a role of vitamin D in IBD is seen in the geographical distribution of disease, with higher incidences and prevalence in temperate climates and lower risks in persons living near the equator[6,51,52]. Additional environmental, lifestyle, or genetic factors that have similar associations with geographical location may play a part in the association between sun exposure and IBD[51]; however, this "north-south" gradient in the risk of Crohn's disease and ulcerative colitis is likely explained by the variation in sun exposure, a major determinant of vitamin D levels[51,52], thereby strengthening the role of vitamin D in IBD.

Vitamin D deficiency has been well described in IBD patients from all over the world with varying prevalence. In Ireland, 63% of Crohn's disease patients had 25-OH vitamin D levels < 50 nmol/L; however, using the higher cut-off to define vitamin D deficiency (< 80 nmol/L), 90% of this Crohn's disease cohort was vitamin D deficient[53]. The frequency of vitamin D deficiency in Canada is approximately 8% (< 25 nmol/L) with an additional 22% having insufficiency (< 40 nmol/L)[54]. A Japanese study found that 27.3% of Crohn's patients were vitamin D deficient (< 10 ng/mL or < 25 nmol/L) compared to only 6.7% of controls[55]. In an IBD cohort of children, adolescents, and young adults from Philadelphia and Pennsylvania, 16% of the Crohn's disease patients enrolled were vitamin

D deficient (< 38 nmol/L)[56]. In a cohort study of IBD patients in Wisconsin, 49.8% of patients had levels < 50 nmol/L with 10.9% having levels < 25 nmol/L[57]. Lastly, in London, United Kingdom, 68% of the IBD cohort was vitamin D deficient (< 50 nmol/L)[58].

Higher 25-OH vitamin D plasma levels, predicted on the basis of a validate regression model in the Nurses' Health Study, have been shown to be associated with a lower incidence of Crohn's disease and ulcerative colitis[59]; therefore, obtaining and maintaining optimal vitamin D levels are important, and yet, cut-off values used to define vitamin D deficiency among studies are variable. It is, however, generally accepted that vitamin D deficiency is defined as a serum 25-OH vitamin D level < 75 nmol/L[8,60].

Risk factors of vitamin D deficiency in IBD

Many risk factors for vitamin D deficiency in IBD have been reported. Seasonal variation is evident in most studies with lower levels seen in winter months[53,54,56,58]. Lower vitamin D levels have also been associated with longer disease duration and smoking[53,57]. Poor health outcomes such as the need for intestinal resection, a structuring disease phenotype, the need for oral corticosteroids within 3 mo of diagnosis, and a diagnosis of pancolitis in ulcerative colitis are more prevalent in severely vitamin D deficient patients (< 25 nmol/L) compared to those with normal levels (> 50 nmol/L)[58]. Intestinal resection may be a risk factor as the prevalence of at least one intestinal resection is significantly higher in those with vitamin D deficiency than those with adequate levels ($P < 0.05$)[58].

The role of ethnicity as a risk factor for vitamin D deficiency in IBD has also been examined. A one year prospective study was conducted to examine the association between vitamin D levels, ethnicity, and human IBD. A significantly higher percentage of South Asian IBD patients were vitamin D deficient (< 50 nmol/L) compared to Caucasians[61]. For both Caucasians and South Asians with Crohn's disease, an inverse relationship was found between clinical disease severity and vitamin D levels. For all IBD patients, CRP levels were inversely related to vitamin D levels; however, none of these results reached a significant association[61]. Chatu *et al*[58] also examine ethnicity as a predictor of vitamin D deficiency in an IBD cohort. No differences were found in median vitamin D levels among Crohn's disease and ulcerative colitis patients; however, the median vitamin D level was significantly lower in non-Caucasians (Asian and Black) compared to Caucasians. The multivariate regression analysis showed a history of IBD related surgery and ethnicity to be independently associated with vitamin D deficiency in Crohn's disease and ethnicity alone to be independently associated with vitamin D deficiency in ulcerative colitis[58]. In an IBD cohort of children, adolescents, and young adults from Philadelphia and Pennsylvania, deficiency was more prevalent among African American subjects, Crohn's disease patients with upper gastrointestinal tract involvement, and patients with a significantly greater lifetime exposure to glucocorticoid therapy[56]. Other risk factors may include decreased nutrition intake due to Crohn's associated anorexia[56,62], fear of GI discomfort from dairy due to lactose intolerance, and active disease associated with decreased physical activity resulting in reduced sun exposure[56].

Genetic variants in the VDR and DBP have been shown to be associated with increased risk of IBD[63,64]. Analysis of the frequency of common genetic variants in the DBP have shown the DBP 420 variant Lys to be less common in IBD patients as compared to healthy controls ($P = 0.034$)[63]. A meta-analysis found that *VDR* gene polymorphisms are associated with the susceptibility to IBD. The TT genotype of TaqI was associated with Crohn's disease in Europeans (OR = 1.23; 95%CI: 1.02-1.49)[64]. This polymorphism is a base substitution resulting in two encodings of isoleucine instead of an amino acid change. It has been suggested to result in lower VDR mRNA levels and less vitamin D/VDR inhibition on immune activation[64]. Additionally, this variant was significantly associated with IBD in males. Furthermore, an increased risk of ulcerative colitis in Asians was significantly associated with the ff genotype of FokI on the promoter region of the VDR (OR = 1.65; 95%CI: 1.11-2.45)[64]. This was compared to the FF genotype in Asians. The fourth finding was associated with decreased Crohn's disease susceptibility if one was a carrier of the "a" allele (Aa + aa genotypes) of ApAI (OR = 0.81; 95%CI: 0.67-0.97)[64]. These studies demonstrate a genetic role explaining the high prevalence of vitamin D deficiency among IBD patients.

Vitamin D and disease activity

There is strong evidence to support a high prevalence of significantly lower serum 25-OH vitamin D levels among the IBD population, which have been shown to correlate with increased disease activity. Vitamin D levels have been shown to correlate negatively with disease activity assessed by the Harvey Bradshaw score[57,61,62,64] or Crohn's disease activity index (CDAI) score[65]. Joseph *et al*[66] show that Crohn's disease patients have significantly lower vitamin D levels than their age- and sex-matched controls who were patients diagnosed with irritable bowel syndrome[66]. Predictors of vitamin D status in this study were disease activity and sun exposure. In regards to severity of disease, patients with mild disease had vitamin D levels similar to the controls, but vitamin D levels were significantly lower in patients with moderate-severe Crohn's disease[66]. As expected, lower vitamin D levels were observed in patients who had jejunal involvement of their disease[66]. This association has also been reported in patients with ulcerative colitis. Blanck *et al*[67] conducted a cross-sectional study and reported a larger number of patients with clinically active disease, using the six-point partial Mayo index, in the vitamin D deficient group compared to the vitamin D sufficient group ($P = 0.04$). Patients were stratified based on their vitamin D levels as either vitamin D suf-

ficient, insufficient, and deficient, and there continued to be a trend towards more active disease as vitamin D levels decreased[67]. Furthermore, in a retrospective review of South Asian patients with IBD, patients with vitamin D deficiency appeared to have a more aggressive disease course with 14% of deficient patients requiring surgical management[68]; therefore, optimizing vitamin D status and assessing the relationship between vitamin D treatment and disease activity is important[68].

Despite a high prevalence of vitamin D deficiency among IBD patients, serum vitamin D levels may not always be associated with disease activity. Hassan *et al*[69] found no association between low vitamin D levels and increased disease activity in IBD patients. Vitamin D deficiency may also be explained by the increased risk of intestinal malabsorption among the IBD population, particularly if patients have undergone small bowel resections or use cholestyramine for postresectional diarrhea. Cholestryamine reduces bile acids, which are required for vitamin D absorption[6]. It has been demonstrated that Crohn's disease patients with quiescent disease have on average a 30% decrease in their ability to absorb vitamin D in comparison to normal subjects after supplementation with 50000 IU of vitamin D2[70]. Furthermore, Suibhne *et al*[53] report vitamin D deficiency to be common among Crohn's disease patients in clinical remission. Even in the summer, vitamin D deficiency among these patients continued to remain high (50%). About 40% of these patients were supplementing with vitamin D (200-400 IU), but it was not enough to maintain optimal vitamin D levels[53]. The location of disease, disease activity, or prior resection may not be the only factors affecting vitamin D bioavailability[70].

According to the previous studies, vitamin D status has been reported to be inversely correlated with disease activity[57,61,62,64,67]. However, Abreu *et al*[71] demonstrate a significant positive correlation between modified Harvey Bradshaw indices and 1,25(OH)2D3 levels in Crohn's disease patients taking corticosteroids. This correlation, however, did not exist in the patients who were not taking this drug[71]. The increase in systemic 1,25(OH)2D3 may be a result of increased inflammation as a result of Crohn's disease[71]. This can be explained by the expression of 1α-hydroxylase in the intestine and increased expression of 1α-hydroxylase in inflamed biopsies of Crohn's patients[71]. The authors conclude that elevated 1,25(OH)2D3 is an additional risk factor for osteoporosis in this study population as they determine glucocorticoid use and high 1,25(OH)2D3 levels to be independent risk factors for low bone mineral density. They also suggest that 1,25(OH)2D3 may be a direct cause of bone loss or a surrogate marker for the type of intestinal inflammation leading to osteoporosis[71]. The evidence from this review would support the latter. It should be noted that the previous studies measured 25(OH)D3 to determine vitamin D status, not 1,25(OH)2D3 levels.

Vitamin D supplementation in IBD

Vitamin D supplementation has traditionally been recommended in patients with IBD for management of bone disease. There is now increasing evidence for the potential immunomodulatory effects of supplementation. To date, the optimal level of 25-OH vitamin D for immunomodulatory effects is not known[6].

Ananthakrishnan *et al*[72] demonstrate an increased risk for IBD-related surgery in patients who have low plasma 25(OH)D levels. This association was found to be similar in both patients with Crohn's disease and ulcerative colitis. Furthermore, Crohn's disease patients who initially had a low level, which then was normalized, were significantly less likely to undergo surgery in comparison to patients who continued to maintain a low vitamin D level[72]. A significantly lower C-reactive protein was also seen in these "normalized" patients. There was no association seen in ulcerative colitis patients, which the authors suggest may be due to a higher plasma 25(OH)D threshold in these patients to obtain any immune effects. Another explanation may be that vitamin D has a stronger interaction in Crohn's disease *vs* ulcerative colitis[72]. Furthermore, Zator *et al*[73] report a significant association between earlier cessation of anti-TNF therapy in IBD patients who had insufficient vitamin D levels prior to initiation of anti-TNF therapy, suggesting vitamin D may be an important adjuvant treatment aiding in the maintenance of response to this therapy. These studies denote the importance of repleting and maintaining sufficient vitamin D levels in patients who have IBD, specifically above 30 ng/mL (75 nmol/L), to reduce the risk of flares and to maintain response to IBD-therapies[72,73].

One randomized placebo-controlled study has assessed the effectiveness of vitamin D supplementation in improving Crohn's disease activity. In comparison to the placebo, oral vitamin D supplementation of 1200 IU in adult patients with Crohn's disease in remission was shown to increase the 25-OH vitamin D levels and reduce the risk of relapse from 29% to 13% at 1 year (P = 0.06)[74]. Although this difference in relapse was not statistically significant, the difference is clinically meaningful and does warrant further study. The authors did discuss the risk of type II error as an explanation for not reaching statistical significance[74,75].

A prospective study completed by Miheller *et al*[76] compares supplementation with active vitamin D (alfacalcidiol) to non-active vitamin D (cholecalciferol) in Crohn's patients. Looking at the clinical course of Crohn's disease at 6 wk, disease activity significantly decreased in the active vitamin D group; however, there was no difference by the end of the trial at 12 mo[76]. Active vitamin D treatment resulted in a significant decrease in CDAI scores and CRP levels, as well as improvement in quality of life scores. It has prominent short-term effects and may be due to improved immune responses[76].

A systematic review[75] was completed to examine the efficacy of vitamin D supplementation for treating colitis

Table 1 Vitamin D supplementation improves disease activity and outcomes in human inflammatory bowel disease

Ref.	*n*	Methodology	Aims	Intervention	Definition of improvement	Conclusions
Miheller *et al*[76]	37	Prospective single cohort study	Compare the effects of active vitamin D and plain vitamin D on bone health and disease status in Crohn's disease patients	Group A: 2 μg × 0.25 μg alfacalcidiol (active vitamin D) daily Group B: 1000 IU cholecalciferol daily (plain vitamin D)	Assessment: Osteocalcin (OC) and beta-CrossLaps (βCL) concentrations CDAI, CRP, IBD-questionnaire (IBD-Q) Improvement: Significant decrease in OC and βCL concentrations Significant decrease in CDAI and IBDQ scores and CRP concentrations	Significant reduction in βCL and OC concentrations showed decelerated bone resorption and bone turnover in the active vitamin D group compared to the plain vitamin D group at 6 wk and 3 mo ($P < 0.05$) Significant reduction in disease activity and improved quality of life in the active vitamin D group at 6 wk ($P < 0.05$) No difference at 12 mo
Jørgensen *et al*[74]	94	A multi-centre randomized double blinded placebo controlled trial	Assess the efficacy of vitamin D supplementation in reducing the risk of relapse in Crohn's disease patients compared to placebo	Treatment group: 1200 IU vitamin D3 + 1200 mg calcium/d Placebo group: placebo + 1200 mg calcium/d	Assessment: CDAI Improvement: Decreased proportion of patients who achieve a CDAI score of 150+ and a 70 point increase in CDAI compared to baseline	Decreased risk of relapse (29% to 13%) at 1 yr with vitamin D treatment, but did not reach significance ($P = 0.06$)
Ananthakrishnan *et al*[72]	3217	Retrospective study	Assess the association between plasma 25(OH)D3 levels and IBD related surgeries and hospitalizations Assess changes in these outcomes after normalization of IBD patients' vitamin D levels	None	Assessment: Risk of IBD-related surgery or hospitalization (OR); Improvement: Reduction in risk (OR < 1) for surgery or hospitalization	Low levels of vitamin D significantly increased risk for IBD-related surgery and hospital admissions (OR = 2.05; 95%CI: 1.53-2.75 for Crohn's disease and OR = 1.75; 95%CI: 1.21-2.52 for ulcerative colitis) Achieving normal vitamin D levels decreased risk of Crohn's disease-related surgery (OR = 0.56; 95%CI: 0.32-0.98)
Yang *et al*[77]	18	Prospective clinical pilot study	Establish the oral dose of vitamin D3 required to achieve serum 25(OH)D3 concentrations above 40 ng/mL (100 nmol/L) in mild-moderate Crohn's disease patients Assess improvement in disease activity and quality of life after vitamin D supplementation in Crohn's disease patients	Initiated on 1000 IU per day of vitamin D3 for 2 wk. Increased dose every two wk by 1000 IU/d until achievement of serum 25(OH)D level of 40 ng/mL occurred or patients were taking a total of 5000 IU/d	Assessment: Dose of vitamin D3 and serum 25(OH)D3 levels CDAI and IBDQ Improvement: Increase in serum 25(OH)D3 levels Reduction in CDAI scores of > 70 points or achievement of CDAI score < 150, and increase in IBDQ scores	Vitamin D supplementation improved vitamin D status at 24 wk ($P < 0.001$). 78% of patients required 5000 IU/d of vitamin D3, suggesting this is an effective dose in raising serum 25(OH)D3 levels in mild-moderate Crohn's patients Vitamin D treatment significantly improved disease activity and quality of life at 24 wk ($P < 0.001$)

CDAI: Crohn's disease activity index; CRP: C-reactive protein; IBD: Inflammatory bowel disease.

in humans and animals. With a primary outcome of inducing or maintaining remission of the disease, the above study by Miheller *et al*[76] was included. The authors conclude that the inability to sustain differences in clinical outcomes with active vitamin D does not undermine the efficacy of vitamin D[75]. It shows the additional improvement of the active form to the plain form. In comparison, Yang *et al*[77] assessed the improvement in vitamin D status, clinical disease activity, and quality of life scores in 18 mild-moderate Crohn's disease patients who underwent vitamin D3 supplementation for 24 wk. Patients were started with 1000 IU/d of vitamin D3, and the dose was increased every two wk by 1000 IU until serum 25(OH)D3 levels were above 40 ng/mL (100 nmol/L) or the patients were taking 5000 IU/d. After 24 wk, the maximum dose of 5000 IU/d was required by 78% of patients and effectively raised serum 25(OH)D levels. The assessment of CDAI scores at 24 wk showed that 78% of patients achieved clinical response defined by a decreased CDAI score of 70 points or more. Additionally, 67% of patients were in remission and disease-specific quality of life significantly improved[77]. Table 1 outlines the four studies examining vitamin D supplementation on IBD outcomes.

In a randomized clinical trial of children and adolescents with IBD, weekly high dose vitamin D2 (50000 IU) or daily vitamin D3 (2000 IU) were superior to daily vitamin D2 (2000 IU) in raising the serum 25-OH vitamin D level at 6 wk (25.4 ± 2.5, 16.4 ± 2.0, 9.3 ± 1.8 ng/mL, respectively)[78]. Adherence may be improved with large doses, and dosing according to individual levels may achieve target levels most effectively[3]. Recent studies have suggested that optimal targets for serum 25-OH vitamin D levels are greater than 30 ng/mL (75-80 nmol/L), with levels between 21-29 ng/mL (51-74 nmol/L) being defined as insufficient and levels < 20 ng/mL (< 50 nmol/L) being defined as deficient[60].

CONCLUSION

Recent advances in the understanding of the effects and mechanism of action of vitamin D on the mucosal and systemic immune system and subsequently on intestinal inflammation suggests it has a role to play in the therapeutic management of IBD. Furthermore, both epidemiologic and emerging retrospective and prospective clinical evidence supports a significant beneficial role of vitamin D supplementation in patients with IBD. While the precise level of 25(OH)D3 that needs to be achieved for these therapeutic effects is unknown, it has been established that levels of 75 nmol/L or higher are generally adequate[3,8,26,60,72]. Given the safety profile and low cost of vitamin D, its addition to the therapeutic armamentarium as a supplement to induction and maintenance therapy should be strongly considered.

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MINIREVIEWS

Participation of microbiota in the development of gastric cancer

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Abstract

There are a large number of bacteria inhabiting the human body, which provide benefits for the health. Alterations of microbiota participate in the pathogenesis of diseases. The gastric microbiota consists of bacteria from seven to eleven phyla, predominantly *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*. Intrusion by *Helicobacter pylori* (*H. pylori*) does not remarkably interrupt the composition and structure of the gastric microbiota. Absence of bacterial commensal from the stomach delays the onset of *H. pylori*-induced gastric cancer, while presence of artificial microbiota accelerates the carcinogenesis. Altered gastric microbiota may increase the production of N-nitroso compounds, promoting the development of gastric cancer. Further investigation of the carcinogenic mechanisms of microbiota would benefit for the prevention and management of gastric cancer.

Key words: Microbiota; *Helicobacter pylori*; Gastric cancer; Nitrite; Metagenomics

Core tip: The gastric microbiota consists of bacteria from seven to eleven phyla, predominant with *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*. Absence of bacterial commensal from the stomach delays the onset of *Helicobacter pylori*-induced gastric cancer, while presence of artificial microbiota accelerates the carcinogenesis. Altered gastric microbiota may increase the production of N-nitroso compounds, promoting the development of gastric cancer. Further investigations of the carcinogenic mechanisms of microbiota would benefit for the prevention and management of gastric cancer.

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INTRODUCTION

The surface of the human gastrointestinal mucosa is inhabited by a huge number of microbes of diverse species[1,2]. They interact with each other, constituting an integrated and functional ecosystem, the gastrointestinal microbiota. It provides immune, nutritional and energetic benefits for its host[3]. Disruption of the microbiota may lead to the development of diabetes mellitus, asthma, colorectal cancer and inflammatory bowel disease[4-7].

Gastric cancer is the fourth most common malignant carcinoma and the second leading cause of cancer-related death[8,9]. It is estimated that 989000 new cases of gastric

cancer occur each year[10]. In East Asia, the incidence of gastric cancer is much higher than that in the other regions[11]. The gastric microbiota has long been considered an important factor contributing to the development of cancer[12,13]. Secretion of gastric acid drops in patients with mucosal atrophy. This reduces the acid inhibition of bacterial growth, resulting in the overgrowth of bacteria in the stomach. Under the influence of bacterial enzymes, the production of N-nitroso compounds in the stomach is increased[14]. The latter causes DNA damages and methylation of epithelial cells, promoting the carcinogenesis of gastric mucosa[15-17]. With the advance of the sequencing technique, it is possible to examine the microbiota in details. The role played by microbiota in the gastric carcinogenesis has been re-evaluated. We searched for publications related to gastric cancer and microbiota in PubMed using key words including gastric cancer, microbiota, pH and nitrite. Publications pertinent to carcinogenesis associated with microbiota were selected. The current knowledge of the gastric microbiota and its carcinogenic potentials is reviewed in this paper.

COMPOSITION AND BIODIVERSITY OF THE GASTRIC MICROBIOTA

The median pH of the stomach is 1.4. The high acidity inhibits the survival and proliferation of bacteria in the stomach. However, the gastric mucus forms a pH gradient, thus providing protection of bacteria from acid attack[18]. The presence of non-*Helicobacter pylori* (*H. pylori*) bacteria in the gastric mucosa has been demonstrated using histological methods[19]. A number of bacteria have been isolated from gastric juice[20-22]. The bacterial counts, however, appear to be lower in the stomach than in the other parts of the gastrointestinal tract[23]. It is estimated that there are 10^{2-4} cfu/mL of bacteria in the gastric juice, but 10^{10-12} cfu/mL in the colon. The results using bacterial culture methods show that gastric microbiota is mainly composed of bacteria present in the upper respiratory tract, oropharyngeal and intestinal microbiota. In healthy individuals, *Veillonella* sp., *Lactobacillus* sp. and *Clostridium* sp. are most frequently isolated bacteria from the gastric mucosa[24]. However, the compositions of gastric microbiota vary remarkably between individuals and studies. A study from Spain found that the most abundant bacteria isolated from stomach were *Propionibacterium*, *Lactobacillus*, *Streptococcus* and *Staphylococcus*[23]. Considering the limitations of the bacterial culture method, it is unattainable to thoroughly examine the compositions of the gastric microbiota.

With the advance of the sequencing technology, it is achievable to analyze the gastric microbiota in detail by sequencing the bacterial 16S rRNA gene. Molecular analyses reveal much more diverse microbial communities in the stomach. It harbors more than 130 phylotypes representing seven to thirteen bacterial phyla[25,26]. *Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria* and *Fusobacteria* are the major phyla in the gastric microbiota. The most abundant phyla was *Proteobacteria*, *Streptococcus* and *Prevotella* are the most abundant genus found in the stomach in *H. pylori*-negative subjects[26]. The compositions of gastric microbiota from gastric antrum and corpus are nearly identical. In preterm neonates, bacteria from gastric juice were mainly composed of *Firmicutes*, *Tenericutes*, *Actinobacteria* and *Proteobacteria* in the first week of life, but the abundance of *Proteobacteria* increased steadily, becoming the predominant bacteria by the fourth week of life[27]. Roles of the diverse and abundant gastric microbiota in the pathogenesis of gastric diseases have been explored in recent years.

INFLUENCE OF GASTRIC MICROBIOTA BY *H. PYLORI*

H. pylori is a Gram-negative carcinogenic bacterium colonizing the human stomach[28]. In addition, overgrowth of bacteria in the stomach has been considered to be another risk factor for gastric cancer[12,13]. It is, therefore, a great concern of whether there is an interaction between *H. pylori* and gastric microbiota.

Using a high density 16S rRNA gene microarray, the compositions of gastric microbiota have been analyzed from eight *H. pylori* infected patients and four *H. pylori* negative patients[29]. The relative abundance of *Proteobacteria* (excluding *H. pylori*) and *Acidobacteria* was higher in *H. pylori* infected patients, whereas, greater relative abundance of *Actinobacteria* and *Firmicutes* was found in the gastric microbiota from *H. pylori* negative patients[29]. In experimentally *H. pylori* infected BALB/c mice, the biodiversity of gastric microbiota was increased[30]. Vaccination against *H. pylori* prevented the alteration of gastric microbiota[30]. Therefore, it appears that *H. pylori* infection may alter the composition and biodiversity of the gastric microbiota.

Contradicting findings, however, have been reported. Acute or chronic infection of *H. pylori* did not alter the compositions of murine gastric microbiota[31]. There is no difference in the gastric microbiota between gerbils persistently infected with *H. pylori* and those uninfected[32]. A culture-based analysis of 29 healthy volunteers found that the composition of gastric microbiota was similar regardless of *H. pylori* status[23]. The composition and biodiversity of gastric microbiota were explored by analyzing 1833 sequences of 16S rDNA generated by broad-range bacterial PCR from the gastric mucosa of 23 individuals[26]. Double principal coordinate analysis and redundancy analysis revealed no significant association between phylotype distribution and *H. pylori* status. Hierarchical clustering found no distinct cluster between *H. pylori*-negative and -positive subjects. These findings suggest that the presence of *H. pylori* in the gastric mucosa does not affect the composition of the gastric community. Thus, it appears that *H. pylori* acts more like a commensal bacteria, rather than an intruder, to the gastric microbiota. Further studies are required to clarify the interactions between *H. pylori* infection and the gastric microbiota. This would

substantially enhance our understanding of the development of gastric pathologies, especially gastric cancer.

ROLES OF MICROBIOTA IN THE DEVELOPMENT OF GASTRIC CANCER

It has been proposed that gastric microbiota plays a role in the development of gastric cancer[12,13]. Lowered acid secretion due to gastric atrophy favors overgrowth of bacteria in the gastric fluid, enhancing the production of carcinogenic N-nitrosamine compounds. Recent studies on animal models strongly support the fundamental role of microbiota in the development of gastric cancer. Transgenic INS-GAS mice over-expressing human gastrin may spontaneously develop intramucosal carcinoma[33]. Gastric intraepithelial neoplasia developed in all specific pathogen-free male INS-GAS mice with a complex microbiota 7 mo after *H. pylori* infection[34]. For germ free male INS-GAS mice which were absent of microbiota, however, the incidence of gastric intraepithelial neoplasia was only 10.0%. The incidence merely increased to 44.4% 11 mo after *H. pylori* infection[34]. These results suggest a role of microbiota in the carcinogenesis of the stomach. Furthermore, colonization of the stomach by an artificial intestinal microbiota (Altered Schaedler's Flora, including ASF356 *Clostridium* species, ASF361 *Lactobacillus murinus* and ASF519 B*acteroids* species) increased the incidence of gastric intraepithelial neoplasia to 69.0% in male INS-GAS mice 7 mo after *H. pylori* infection[35]. Antibiotic treatments significantly delayed onset of gastric neoplasia in helicobacter-free and specific pathogen-free INS-GAS mice[36]. These findings indicate the involvement of microbiota in the development of gastric cancer.

Elevation of pH dramatically influences the bacterial growth. Treatments with acid inhibition drugs increase the luminal pH, and the total bacterial count is increased[19,37,38]. It returns to normal after discontinuation of the treatment. The increased pH and bacterial count correlate with the enhanced production of nitrite in the stomach[39]. This could be attributed to the increased abundance of nitrate-reducing bacteria[40], which catalyse the nitrite production from the nitrate reduction. *Haemophilia* and *Veillonella* reduce nitrate more rapidly than nitrite. They could be responsible for the accumulation of nitrite in the stomach[41,42]. An increased luminal pH is common in precancerous conditions and gastric cancer. This may lead to alterations of the compositions of gastric microbiota. In gastric cancer patients, gastric microbiota was predominated by *Veillonella*, *Haemophilus* along with *Streptococci*, *Lactobacillus*, *Prevotella* and *Neisseria*[43]. These studies suggest that alterations of gastric microbiota occur under the influence of pH. Further studies are required to investigate roles and mechanisms of these alterations in the development of gastric cancer. A recent study on *H. pylori* infected mice suggests that gastric microbiota promotes the carcinogenesis, but its composition does not influence the incidence of gastric cancer[34]. For the *H. pylori* infected INS-GAS mice, colonization of the stomach with an artificial Altered Schaedler's Flora including ASF356 *Clostridium* Species, ASF361 *Lactobacillus murinus* and ASF519 *Bacteroides* Species promoted the development of cancer[34]. However, the incidence of gastric cancer did not significantly differ from *H. pylori* infected INS-GAS mice fed under specific pathogen free conditions.

N-nitroso compounds, consisting of N-nitrosamines and N-nitrosamides, are potent carcinogens[35,44]. Humans are exposed to N-nitroso compounds from diet, tobacco smoke and other environmental sources. Increased exposure to these exogenous N-nitroso compounds has been linked to an increased incidence of gastric cancer[45]. The amount of endogenous formation of N-nitroso compounds, however, is much higher than that of exogenous formation[46]. The study on a population of more than a half million individuals revealed that endogenous N-nitroso compounds are significantly associated with gastric cancer[46]. Nitrite is a precursor of the endogenous N-nitroso compounds. Bacterial cytochrom-cd1-nitrite reductase catalyzes the conversion of nitrite to nitrosamines in the presence of secondary amines[47]. In gastric cancer patients, the concentration of nitrite in gastric juice may increase up to 107.6 μmol/L[48]. When the acid output reduces, bacterial overgrowth occurs in the stomach. These bacteria contain both nitrate reductase and nitrite reductase, which catalyze the reduction of nitrate and nitrite, respectively. However, some bacteria have a differential rate in nitrate reduction and nitrite reduction. *Veillonella parvula* and *Haemophilus parainfluenzae* have a higher capacity in nitrate reduction than nitrite reduction, thus increasing nitrite accumulation in the gastric juice[42]. In nature, many bacteria produce enzymes influencing the production of nitrite. Ammonia oxidizing bacteria possess ammonia monooxygenase and hydroxylamine oxidoreductase which catalyze the production of nitrite from ammonia under aerobic conditions[49,50]. Ammonia oxidizing bacteria mainly include species from the phylum of *Planctomycetes*[50]. The phylum of *Nitrospirae* is a group of nitrite oxidizing bacteria. They encode nitrite oxidoreductase which oxidizes the formation of nitrate from nitrite[51,52]. Thus, they tend to decrease the production of nitrite. These bacteria involved in the production of nitrite are widely present in soil, water and marine, where humans are frequently exposed to. Molecular analyses of the gastric microbiota suggest their potential presence in the stomach. Their participation in the accumulation of nitrite in the stomach remains to be studied in the future.

Findings from current studies support a role of microbiota in the development of gastric cancer. However, techniques used in many studies have limited powers in examination of composition, richness and biodiversity of gastric microbiota. With the application of the metagenomics and single cell genomics[53-55], we could further understand the properties of carcinogenic microbiota and mechanisms by which they participate in the genesis of gastric cancer.

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ORIGINAL ARTICLE

Inhibition of autophagy significantly enhances combination therapy with sorafenib and HDAC inhibitors for human hepatoma cells

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Abstract

AIM: To clarify whether histone deacetylase inhibitors histone deacetylase inhibitors (HDACIs) can sensitize hepatocellular carcinoma (HCC) cells to sorafenib treatment.

METHODS: Bax, Bcl-2, ATG5-ATG12, p21, and p27 protein levels in Hep3B, HepG2, and PLC/PRF/5 cells were examined by Western blot. CCK8 and a fluorometric caspase-3 assay were used to examine cellular viability and apoptosis levels. The effect of Beclin-1 on sensitization of HCC cells to sorafenib was examined by transfecting Beclin-1 siRNA into Hep3B, HepG2, and PLC/PRF/5 cells.

RESULTS: Autophagy inhibition enhances the inhibitory effects of vorinostat and sorafenib alone or in combination on HCC cell growth. Vorinostat and sorafenib synergistically induced apoptosis and cell cycle alterations. Western blot data indicated that HDACIs and Beclin-1 knockdown increased the p53 acetylation level. The knockdown of Beclin-1 enhanced the synergistic effect of the combination of vorinostat with sorafenib.

CONCLUSION: HDACIs can sensitize HCC cells to sorafenib treatment by regulating the acetylation level of Beclin-1.

Key words: Hepatocellular carcinoma; Histone deacetylase inhibitors; Autophagy; Sorafenib; Chemoresistance

Core tip: In this study, we investigated the antiproliferative effect of sorafenib in combination with the histone deacetylase inhibitor vorinostatin in human hepatoma cell lines (Hep3B, HepG2, and PLC/PRF/5). We also examined whether the combination therapy was enhanced by the inhibition of autophagy. Our results showed that the combination of vorinostat with sorafenib synergistically reduced cell proliferation in hepatocellular carcinoma cells by inducing apoptosis and cell cycle arrest. Synergistic changes in cell cycle and cell survival regulators were also observed.

Yuan H, Li AJ, Ma SL, Cui LJ, Wu B, Yin L, Wu MC. Inhibition of autophagy significantly enhances combination therapy with sorafenib and HDAC inhibitors for human hepatoma cells. *World J Gastroenterol* 2014; 20(17): 4953-4962 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/4953.htm

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common cause of cancer-associated mortality in China and one of the leading causes of death in the world[1]. Due to the lack of effective biomarkers for early detection, most patients diagnosed with HCC die within one year because radical resection of the tumors is performed late in the disease process, meaning that the options for these patients are chemotherapy, radiotherapy, or interventional treatment[2]. Importantly, previous clinical trials have shown that patients with HCC do not benefit from traditional systemic chemotherapy[3].

One area of cancer research interest concentrates on epigenetic changes caused by modifications of histone proteins. Acetylation of histones reduces the affinity of histones for DNA, producing an open DNA structure that facilitates gene expression[4]. Histone deacetylases (HDACs) are overexpressed in many types of tumor cells, including human hepatoma cells, and suppress the expression of genes involved in tumor suppression and differentiation[5]. HDACs, along with histone acetyltransferases, reciprocally regulate the acetylation status of the positively charged NH2-terminal histone tails of nucleosomes[6]. HDAC inhibitors (HDACIs) are interesting as cancer therapeutics due to their ability to induce cell differentiation, growth arrest and apoptosis[7]. HDACIs represent a variety of agents that block histone deacetylation genes, thereby modifying chromatin structure and gene transcription[8].

Sorafenib, a multi-target biological agent that targets cancer cells and that was jointly developed by Bayer and Onyx, has shown significant inhibitory effects on tumor cell proliferation and angiogenesis and has become the first clinical drug for HCC approved by the US FDA[9]. By inhibiting the activity of the Raf serine and threonine kinase in the ERK 1/2 signaling pathway, sorafenib effectively controls tumor cell proliferation. In addition, sorafenib also inhibits VEGFR and PDGFR, thus blocking tumor angiogenesis[10]. Recent studies also have found that sorafenib can activate tumor cell autophagy[11]. Numerous drug studies have found that the stimulation of the autophagy pathway can inhibit apoptosis signals; on the contrary, inhibited autophagy can promote apoptosis signals. For example, increased p53 expression can induce rapid apoptosis in lymphoma cells in a Myc-induced lymphoma mouse model, but tumor recurrence occurs soon after. However, inhibition of the autophagy lysosomal pathway by clioquinol (CQ) or Atg5 siRNA can enhance tumor cell apoptosis and reduce recurrence[12]. Klappan *et al*[13] found that the genetic interference of Beclin-l, Atg5, Atg10, and Atg12 by siRNAs could lead to the inhibition of autophagy, which enhances nutritional deficiencies and causes HeLa cell death. These studies show that autophagy and apoptosis signaling share common signaling pathways (such as PI3K/Akt/mTOR, NF-κB, and ERK) and effector proteins (such as Bcl-2, Bcl-xL, Mcl-1, Atg5, and p53), suggesting that regulating the autophagy pathway should improve HCC chemotherapy[14].

To overcome drug resistance, the combination of HDACIs with existing chemotherapeutic agents has been identified as a potential approach, due to the effect of reducing the dose of other anti-neoplastic drugs. However, whether HDACIs can sensitize HCC cells to sorafenib treatment remains largely unexplored, and very few studies have investigated the activation of the autophagy signaling pathway and its related effects during the combined treatment with sorafenib and HDACIs.

The objective of the present study was to determine the synergistic antiproliferative effect of sorafenib in combination with HDACIs and examine the mechanisms underlying the synergistic antiproliferative effects. In particular, we also explored the possibility that the inhibition of autophagy can enhance the synergistic effect of the combination of vorinostat with sorafenib.

MATERIALS AND METHODS

Materials and cell culture

Sorafenib (Nexavar) was purchased from Bayer Pharmaceuticals. Vorinostat and 3-methyladenine (3-MA) were purchased from Sigma. For *in vitro* studies, various concentrations of sorafenib and vorinostat were dissolved in DMSO. In all experiments, the maximal concentration of DMSO in the medium was 0.02% (v/v), which does not affect cell growth. Hep3B, HepG2, and PLC/PRF/5 human cell lines were obtained from the American Type Culture Collection (ATCC). For cell culture, the following media were used: MEM for Hep3B and HepG2 cells and DMEM for PLC/PRF/5 cells. The ATCC cell bank performed cell line characterizations, and cells were passaged in the laboratory for fewer than 6 mo after thawing. Cells were treated with vorinostat or sorafenib in 5% (v/v) FBS-containing RPMI 1640 medium. For sequential combination treatment with HDACI and sorafenib, the cells were exposed to the former drug for 24 h and then to the next drug for additional 48 h. The single treatment time was consistent with the combined treatment group.

Antibodies for immunoblotting, such as Bax, Bcl-2, ATG5-ATG12, p21, and p27, were obtained from Cell Signaling. Commercially available validated short hairpin RNA molecules to knock down RNA/protein levels were obtained from Qiagen. All other chemicals were purchased from Sigma if not stated otherwise.

Measurement of growth inhibition and cell viability assay

Hep3B, HepG2, or PLC/PRF/5 cells were seeded at a density of 10000 cells/well in 96-well plates and incubated with various concentrations of HDACI, sorafenib, or the combination of the two. The cell number was evalu-

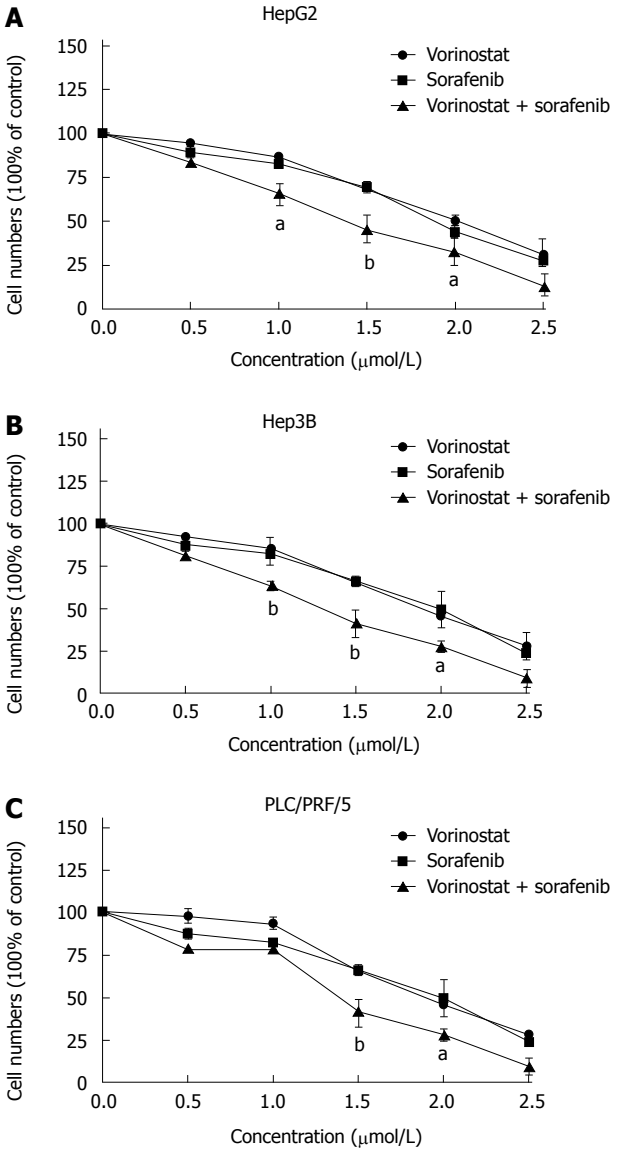

Figure 1 Antiproliferative effects of sorafenib, vorinostat, or the drug combination on human hepatoma cell lines. HepG2 (A), Hep3B (B), and PLC/PRF/5 (C) cells were treated with various concentrations of sorafenib, vorinostat, or the drug combination. The results are expressed as the percentage of viable cells in the control group. The results are expressed as mean ± SD from three independent experiments. $^{a}P < 0.05$ and $^{b}P < 0.01$ *vs* control as evaluated by Student's *t* test.

ated by crystal violet staining. For sequential combination treatment with HDACI and sorafenib, the cells were exposed to the former drug for 24 h and then to the next drug for an additional 48 h. Following the method previously described[15], 10 g/L glutaraldehyde was added to the cells in 96-well plates. Then, the cells were stained with 1 g/L crystal violet in phosphate buffered saline (PBS). The excess dye was removed by washing with sterile water. Bound crystal violet was solubilized with 2 mL/L Triton X-100 in PBS. Light extinction, which has a linear dependence on cell number, was read at 570 nm by a microplate reader. The number of cells was determined from the absorbance of each well relative to the average absorbance of the control wells (defined as 100%).

Detection of apoptosis

The activity of caspase-3 was determined using the Fluorometric Caspase 3 Assay Kit (Sigma, United States), and the cell lysates were prepared as described previously[15]. According to the manufacturer's instructions, the activity of caspase-3 was calculated from the cleavage of the fluorogenic substrate, Ac-DEVD-AMC. The cell lysates were incubated with substrate solution (caspase-3 substrate Ac-DEVD-AMC 20 mg/L, HEPES 20 mmol/L, glycerol 100 mL/L, and DTT 2 mmol/L, pH 7.5) for 1 h at 37 ℃, and substrate cleavage was measured with a VersaFluor fluorometer (excitation: 360 nm, emission: 460 nm).

Cell cycle analysis

The cells were fixed with 70% ethanol overnight at 4 ℃, and DNA was stained with 60 μg/mL propidium iodide (Sigma, United States) containing 10 U/mL RNaseA for 30 min according to the manufacturer's instructions. The percentage of cells in the sub-G_1 phase (apoptotic cells) was measured by counting 10000 cells using a FACS Calibur flow cytometer (Becton Dickinson, United States) with the ModFit LT 3.0 software.

Western blot analysis

For SDS-PAGE and immunoblotting, the cells were plated at 10^5 cells/mL in 6-well plates, treated with various types of drugs at the indicated concentrations, and then lysed in whole cell lysis buffer (0.5 mol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, and 0.02% bromophenol blue). The samples were boiled at 100 ℃ for 5 min. The boiled samples containing 30 μg of protein were subjected to gel electrophoresis. The proteins were then transferred onto PVDF membranes by electroblotting for 90 min. The blots were blocked with 50 g/L non-fat dry milk in a TBS-Tween solution for 1 h at room temperature and then incubated at 4 ℃ overnight with primary antibodies against different proteins. Anti-β-actin (1:5000) from Sigma served as a loading control. After incubation with horseradish peroxidase-coupled anti-IgG antibodies at room temperature for at least 1 h, the blot was developed using enhanced chemiluminescent detection (GE Healthcare) and subsequently exposed to Hyperfilm ECL film.

Transfection of cells with siRNA

Cells were seeded in 60-mm dishes and transfected 24 h after plating. For transfection, 10 nmol/L of the annealed siRNA, the positive sense control double-stranded siRNA targeting GAPDH, or the negative control were used. SiRNA (10 nmol/L; scrambled or experimental for knockdown) was diluted in serum-free medium. Five microliters of HiPerFect Reagent (Qiagen, Valencia, CA) was added to this mixture, and the solution was mixed by pipetting up and down several times, followed by incubation at room temperature for 10 min.

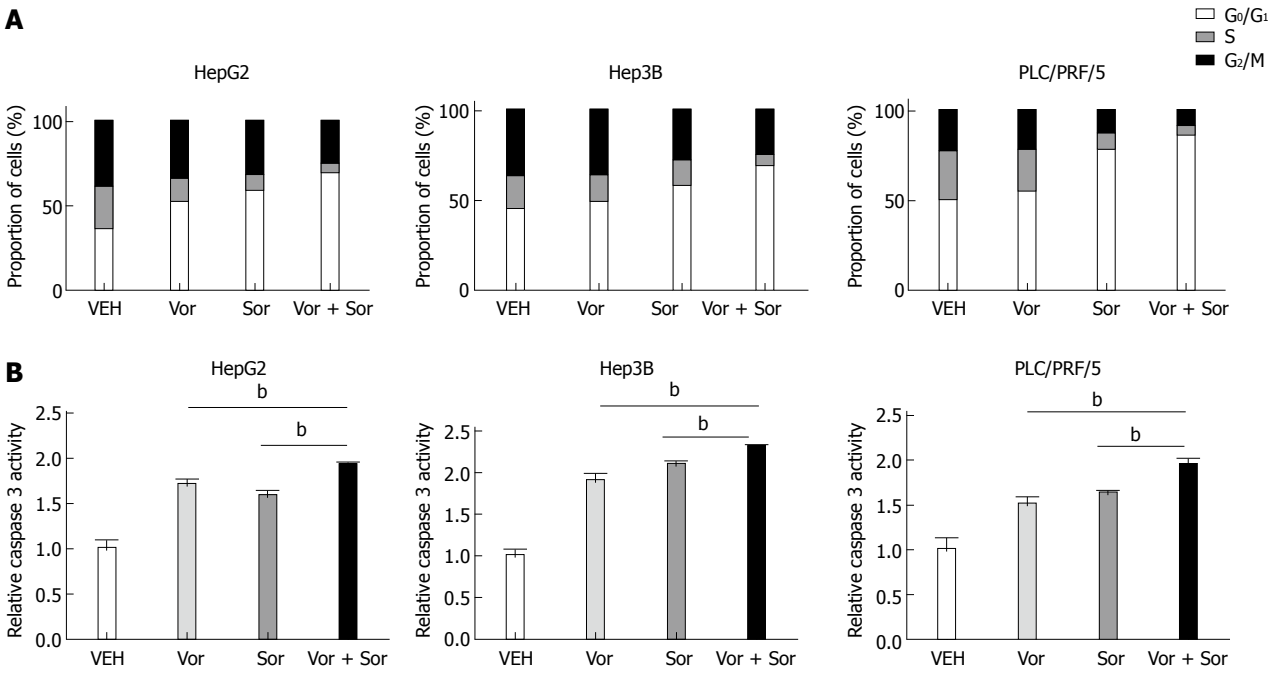

Figure 2 Sorafenib, vorinostat, or the drug combination induces cell cycle arrest and apoptosis in human hepatoma cell lines. HepG2, Hep3B and PLC/PRF/5 cells were treated with various concentrations of sorafenib (Sor), vorinostat (Vor), or the drug combination. A: Cell cycle distributions were analyzed by flow cytometry, and the percentage of cells in the G_0/G_1, S, or G_2/M phases of the cell cycle is indicated; B: The apoptosis-specific caspase-3 activity induced by various concentrations of sorafenib, vorinostat, or the drug combination. The results are expressed as mean ± SD from three independent experiments. $^bP < 0.01$ using Student's *t* test.

The medium in each dish was swirled gently to mix and then incubated at 37 ℃ for 2 h. Then, 1 mL of 10% (v/v) serum-containing medium was added to each plate, and the cells were incubated at 37 ℃ for 36 h before treatment with vorinostat or sorafenib. Flow cytometry assays and Western blot analyses were performed at the time points indicated in each figure.

Statistical analysis

All data are presented as mean ± SD of more than three individual experiments. Statistical significance was determined by the Student's *t*-test.

RESULTS

Growth inhibitory effects of vorinostat and sorafenib alone or in combination on HCC cells

Using the crystal violet staining test, we first determined the growth inhibitory effects of sorafenib and the HDAC inhibitor, vorinostat (Vor), on human hepatoma cell lines (Hep3B, HepG2, and PLC/PRF/5). As shown in Figure 1, treating different types of hepatoma cells with 0.5-2.5 μmol/L vorinostat or sorafenib for 48 h reduced cell growth in a dose-dependent manner by up to 90%. Moreover, the combination of vorinostat with sorafenib significantly increased growth inhibitory effects in a dose-dependent manner compared to the agents alone. These results showed that the combination of vorinostat with sorafenib synergistically reduces cell proliferation in HCC cells (0.5-2.5 μmol/L vorinostat or sorafenib, $P < 0.05$, $P < 0.01$).

Synergistic induction of apoptosis and cell cycle alterations by vorinostat and sorafenib

To determine the mechanism responsible for the antiproliferative effects of the combination of vorinostat with sorafenib, we next examined the effect of vorinostat and sorafenib individually or in combination on the cell cycle and apoptosis. As shown in Figure 2A, incubating HCC cells with 2.5 μmol/L vorinostat or sorafenib for 24 h resulted in a significant arrest in the G_0/G_1 phase of the cell cycle, whereas the proportion of cells in the S phase and G_2/M phase decreased. Notably, a significant increase in G_0/G_1 phase arrest and decrease in the S phase and G_2/M phase were observed in the vorinostat and sorafenib combination treatment groups for all three HCC cell lines. In addition, the vorinostat and sorafenib combination treatment significantly increased the apoptosis rate, as determined by caspase-3 enzyme activity in HCC cells compared to treatment with either agent alone (Figure 2B).

Synergistic changes in cell cycle and cell survival regulators

To identify the potential mechanisms of the combined action of vorinostat and sorafenib, their effects on the regulatory proteins that govern cell cycle and cell survival were investigated using Western blot. The HCC cells treated for 48 h with vorinostat or sorafenib showed a significant decrease in anti-apoptotic Bcl-2, whereas the expression of pro-apoptotic Bax was increased in all cell lines (Figure 3A). Furthermore, the changes induced by the combination treatment were much more profound.

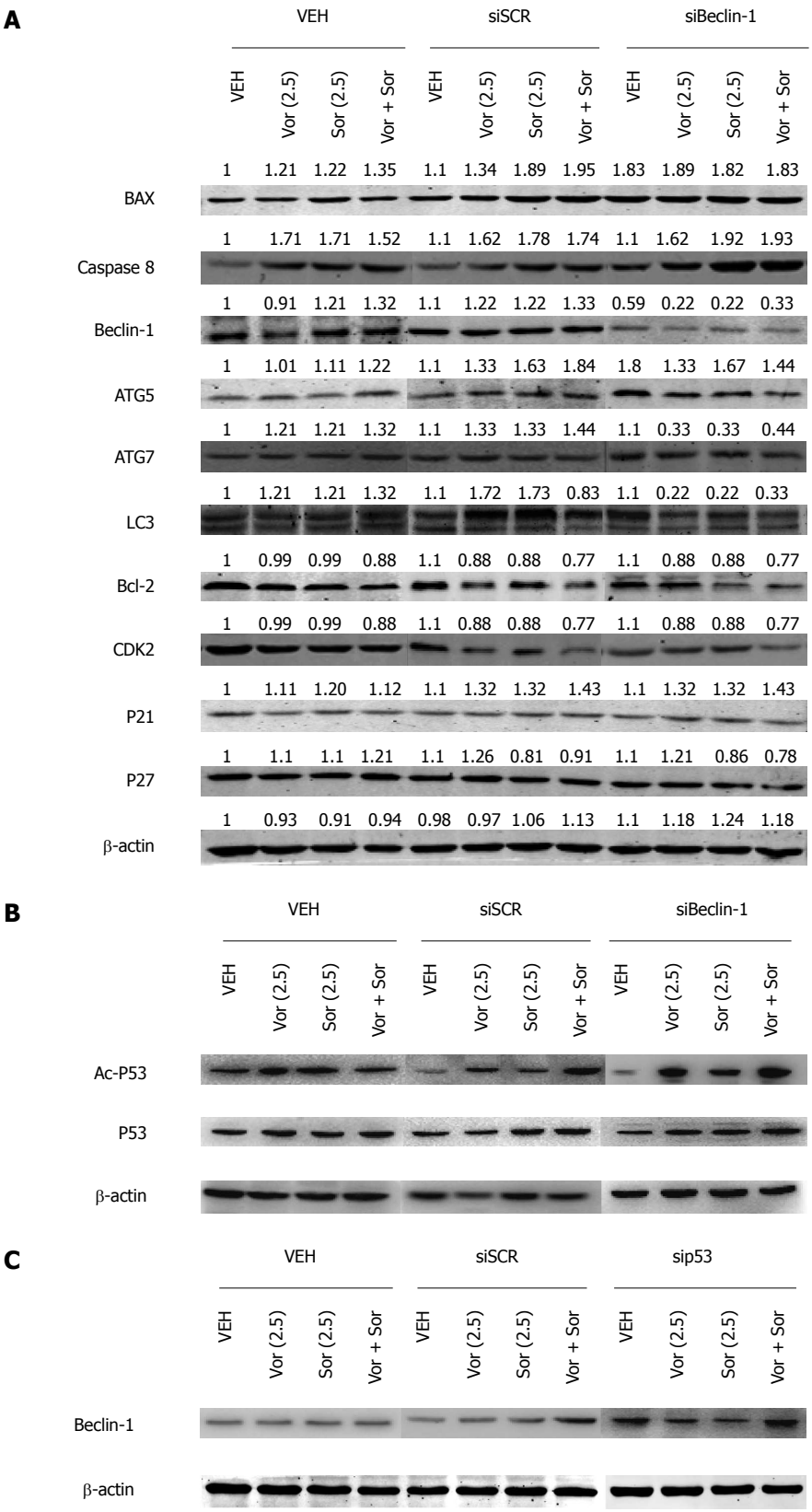

Figure 3 Sorafenib, vorinostat, or the drug combination induces the modulation of apoptosis-, cell cycle- and autophagy-related proteins. A: Representative images of Western blot showing the effect of treatment with siBeclin-1 and vorinostat/sorafenib on apoptosis-, cell cycle-, and autophagy-related proteins in HepG2 cells. siSCR, siRNA scramble; B: Representative images of Western blot showing the acetylated p53 level in HepG2 cells treated with or without siBeclin-1 or vorinostat/sorafenib. siSCR, siRNA scramble; C: Protein level of Beclin-1 in HepG2 cells infected with sip53 combined with or without vorinostat/sorafenib. All experiments were performed independently in triplicate. siSCR, siRNA scramble.

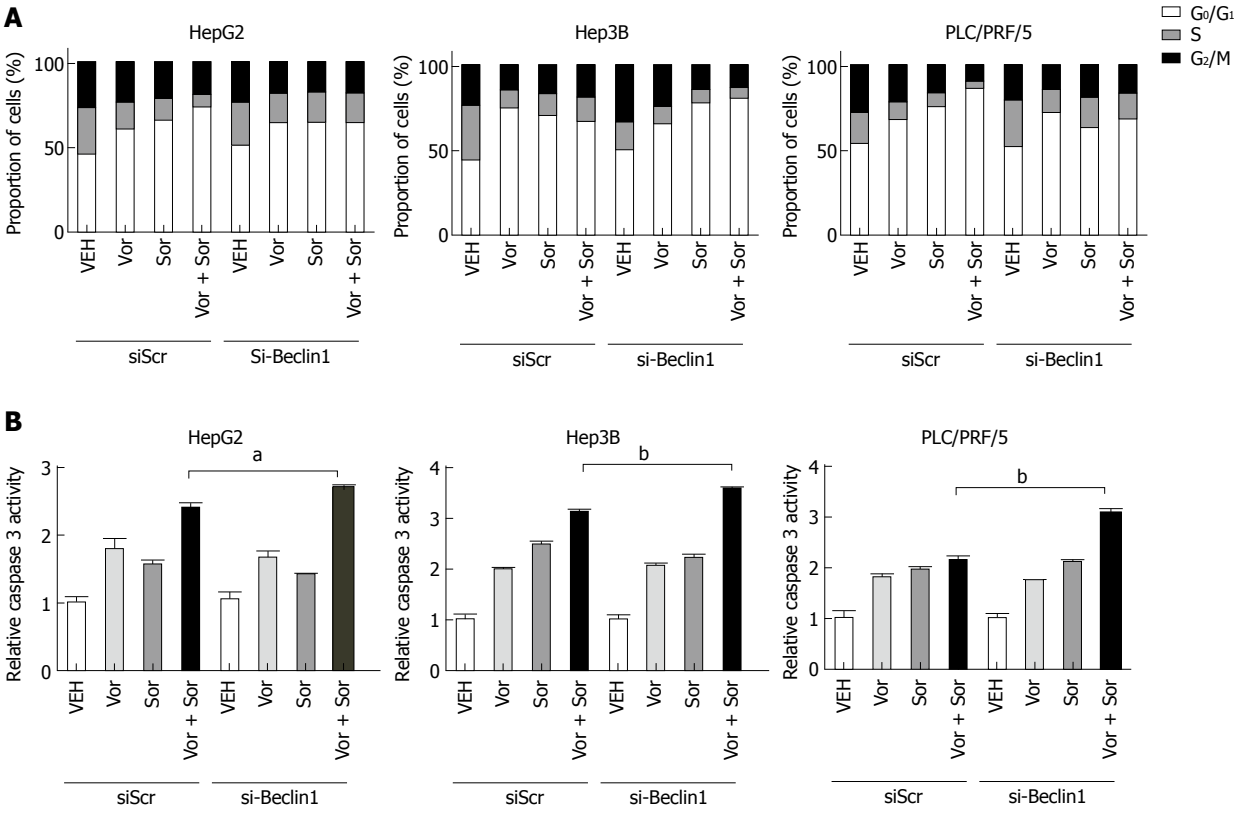

Figure 4 Knockdown of Beclin-1 enhances the synergistic effect of the combination of vorinostat with sorafenib. A: Knockdown of Beclin-1 enhanced the vorinostat/sorafenib drug combination-stimulated cell cycle alterations. siSCR, siRNA scramble; B: Knockdown of Beclin-1 enhanced the vorinostat/sorafenib drug combination-stimulated apoptosis. The results are expressed as mean ± SD from three independent experiments. $^{a}P < 0.05$ and $^{b}P < 0.01$ using Student's *t* test. siSCR, siRNA scramble.

Moreover, upon combination treatment, a more significant increase in the expression of p21 (Waf-1/CIP1) was observed compared with the individual treatments. By contrast, no obvious change in the cyclin-dependent kinase inhibitor p27 was observed in any treatment group.

Activation of autophagy has been reported to mediate drug resistance and promote the survival of cancer cells. Therefore, we examined autophagy marker proteins, such as Beclin-1, ATG5, ATG7, and LC3, after drug treatment. As shown in Figure 3A, a significant increase in Beclin-1, ATG5, ATG7, and LC3 was observed in the cells treated with vorinostat or sorafenib, suggesting that the combined action of vorinostat and sorafenib generate a Beclin1-dependent protective form of autophagy.

HDACIs induce p53 acetylation

HDACIs induced apoptosis of human hepatoma HepG2 cells in a p53-dependent manner[16]; therefore, Western blot was used to further determine the acetylation and protein levels of p53. The HCC cells treated for 48 h with vorinostat or sorafenib or the combination (2.5 μmol/L) showed a significant increase in acetylated p53, but not in the total p53 level. Moreover, the acetylation of p53 increased noticeably after Beclin-1 knockdown, which indicated that Beclin-1 might negatively regulate the p53 acetylation level (Figure 3B). Furthermore, the Beclin-1 level was also increased in sip53 cells (Figure 3C).

Knockdown of Beclin-1 or the autophagy inhibitor 3-MA enhances the synergistic effect of the combination of vorinostat with sorafenib

To observe whether the inhibition of autophagy can enhance the synergistic effect of the combination of vorinostat with sorafenib, the knockdown of Beclin-1 was performed by transient transfection of small interfering RNA (siRNA) oligos. The knockdown efficiency was confirmed by quantitative RT-PCR (data not shown). As shown in Figures 4 and 5, the knockdown of Beclin-1 increased the growth inhibitory effects of the combination of vorinostat with sorafenib, as well as the cell cycle alterations and induction of apoptosis. Furthermore, the knockdown of Beclin-1 also increased the expression of Bax and p21 compared with the control group (Figure 3A). Consistently, the autophagy inhibitor 3-MA also enhanced the synergistic effect of the combination of vorinostat with sorafenib (Figure 5). These data indicate that the inhibition of autophagy enhanced the synergistic effect of the combination of vorinostat with sorafenib.

DISCUSSION

Previous studies have confirmed that the inhibition of

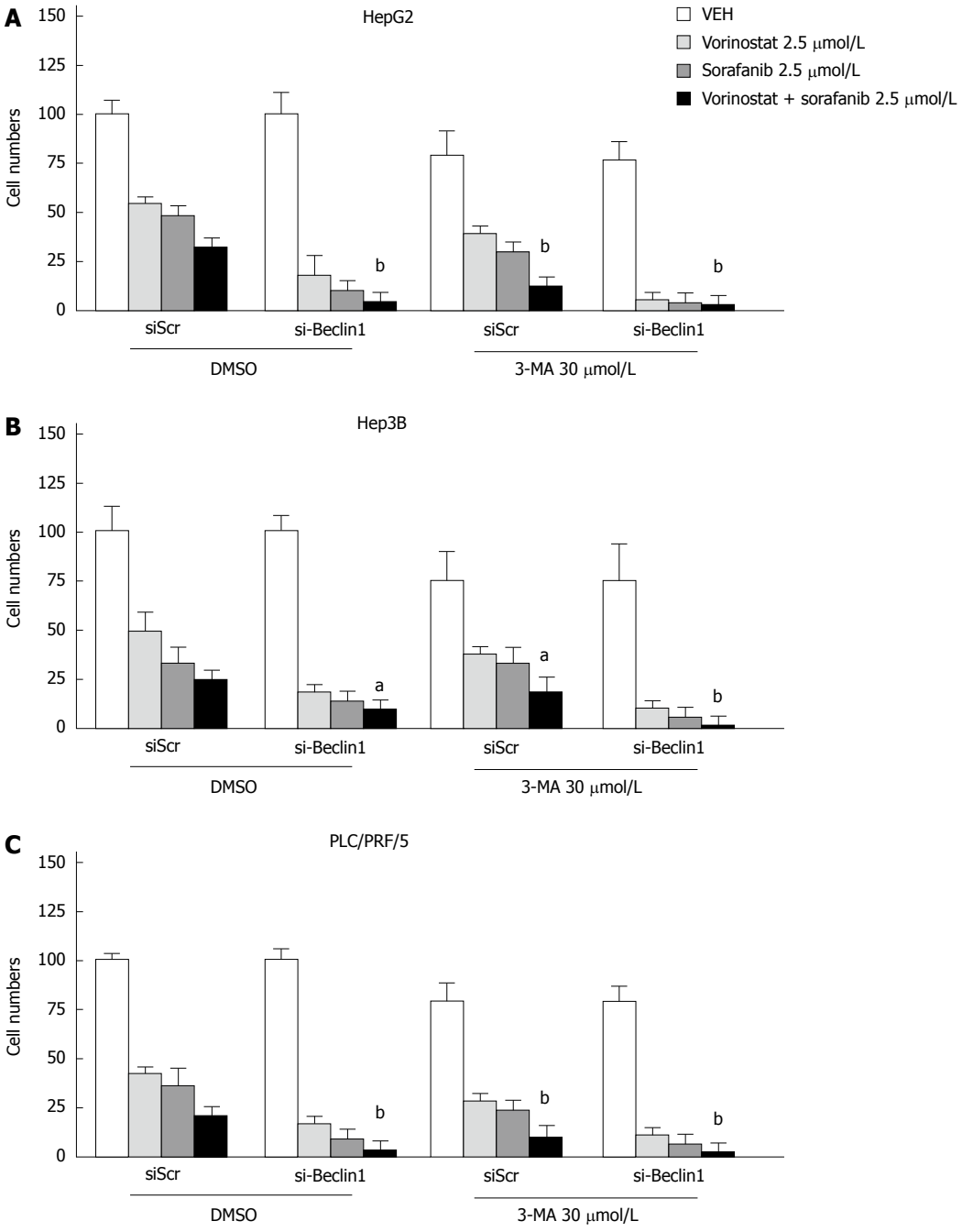

Figure 5 Knockdown of Beclin-1 or the autophagy inhibitor 3-MA enhances the growth inhibitory effects of the combination of vorinostat with sorafenib. A: HepG2; B: Hep3B; C: PLC/PRF/5. The results are expressed as mean ± SD from three independent experiments. $^{a}P < 0.05$ and $^{b}P < 0.01$ *vs* the siSCR DMSO group using Student's *t* test.

HDAC activity stimulates apoptosis in a variety of cancers, including breast and prostate cancer, neuroblastoma, hepatoma, gastrointestinal neuroendocrine tumor cells, and some types of hematologic malignancies[17]. HDACIs have been found to induce apoptosis, reduce tumor growth, and inhibit angiogenesis in hematological malignancies and solid tumors[18]. Defective histone acetylation regulatory enzymes have been identified in malignant cells, and HDAC inhibition may have anticancer properties through the restoration of normal acetylation[19]. Vorinostat has induced histone acetylation, cell cycle arrest, apoptosis, and anti-tumor activity in preclinical cancer models[20].

Sorafenib is the first drug that was approved for the clinical treatment of HCC and exhibits significant inhibitory effects on tumor cell proliferation and angiogenesis[9]. A better understanding of the mechanisms that underlie these effects would allow for an understanding of its efficacy and assist in predicting synergistic effects with other drugs. Recent studies also have found that sorafenib can activate tumor cell autophagy[11], but the precise role of autophagy in survival or death within these studies was not investigated. The present study was designed to explore the effect of combination treatment

with sorafenib and HDACI on HCC cells. Meanwhile, we sought to determine the role of autophagy in the response of tumor cells to sorafenib or vorinostat and to understand how the levels of autophagy caused by HDACI could cause the additional effect of cell death by combined treatment with the multi-RTK inhibitor sorafenib.

Our study found that sorafenib or vorinostat potently inhibited the growth of HCC cells HEPG2, HEP3B, and PLC/PRF/5 in a dose-dependent manner, and the combination treatment exhibited higher antiproliferative activity. Submicromolar concentrations of sorafenib or vorinostat were sufficient to significantly inhibit the proliferation of Hep3B, HepG2, and PLC/PRF/5 cells, and the sorafenib or vorinostat concentration of half-maximal anti-neoplastic effects (IC_{50}) was approximately 1.0 and 1.2 μmol/L, respectively, in all cell lines. The drug combination exhibited elevated anti-tumor effects *in vitro* compared with the individual agents in HCC cells.

The mechanisms involved in the HDACI-induced apoptosis are complex and differ among cell types[21]. HDACIs have been shown to up-regulate pro-apoptotic Fas, a member of the tumor necrosis factor receptor superfamily, and the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors/death receptors DR4 and DR5. Through these factors, HDACIs trigger an extrinsic pathway that is paralleled by the down-regulation of the caspase-8 inhibitor c-FLIP, leading to caspase-8 and subsequently caspase-3 activation[22]. Moreover, HDACIs have been reported to sensitize AML cells to HDACIs *via* ROS-mediated activation of the extrinsic apoptotic pathway[23]. Up-regulation of pro-apoptotic Bak and induction of the pro-apoptotic protein Bax can stimulate the mitochondrial apoptosis pathway[24]. HDACIs can also inhibit the expression of anti-apoptotic proteins, such as Bcl-2, Bcl-xL, XIAP, Mcl-1, and survivin[25]. Here, we demonstrated that the pro-apoptotic effect of HDAC inhibition by vorinostat or sorafenib or the combination in HCC cells is regulated by the activation of caspase-3 and a shift in the balance of pro-apoptotic Bax over anti-apoptotic Bcl-2. The combination of sorafenib with vorinostat significantly increased the expression of caspase-3 compared with the individual drug treatments. However, the knockdown of Beclin-1 enhanced the efficacy of the three treatment groups, indicating that autophagy may participate in the additional increase in tumor cell inhibition caused by the drugs alone or in combination.

Flow cytometry was performed to analyze the cell cycle to further determine the growth inhibitory activity of sorafenib and vorinostat. After treatment with the drugs, we found that cell cycle progression was blocked in both the G_0/G_1 and G_2/M phases. The induction of cell cycle arrest was connected with an increase in the expression of the cyclin-dependent kinase inhibitor (CDKI) p21 Waf-1/Cip1, which is a key component of the cell cycle checkpoints, such as the G_1/S and G_2/M checkpoints. Accordingly, sorafenib and vorinostat were found to inhibit both the G_1/S and G_2/M transition, and the combination exhibited a higher inhibitory effect. With the knockdown of Beclin-1, the percentage of cells in the G_0/G_1 and G_2/M phases increased again, in agreement with the fact that autophagy was involved in the additional levels of tumor cell inhibition caused by the drug combination. However, no cell-specific inhibitory effect was observed during any of the experiments. SiRNA knockdown partially reduced sorafenib HDACI lethality in hepatoma cells. Because sorafenib and vorinostat therapy will soon be explored in a phase I trial in hepatomas, our data suggest that the incorporation of GX15-070 (obatoclax) together with sorafenib-HDACI therapy may provide significant additional value in tumor control, including tumors that lack extrinsic pathway signaling. As previously reported by other groups, autophagy either protects cells from toxic stress or facilitates the toxicity of the stress, all of which seem to be based on the stimulus[26].

In conclusion, our study indicates that HDACIs and sorafenib interact in a highly synergistic manner to enhance the antiproliferative activity in HCC cells *in vitro*. Autophagy is activated and plays a compensatory role during treatment with sorafenib, vorinostat, or the drug combination. Importantly, the knockdown of Beclin-1 enhanced the synergistic effect of the combination of vorinostat with sorafenib, suggesting that the combination of HDAC inhibitors with sorafenib is a promising approach to reduce the dose of other anti-neoplastic drugs and to overcome drug resistance. Future animal studies will be required to fully verify the importance of autophagy in the treatment with sorafenib and vorinostat (or other HDACIs) as a therapeutic in HCC.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the most common cause of cancer-associated mortality in China and one of the leading causes of death in the world. Although sorafenib, a multi-target and multi-kinase inhibitor, currently sets the new standard for advanced HCC, the tumor response rates are actually quite low. Therefore, it is important to improve the response to sorafenib in HCC.

Research frontiers

Histone deacetylases (HDACs) are overexpressed in many types of tumor cells, including human hepatoma cells. Histone deacetylase inhibitors (HDACIs) are interesting as cancer therapeutics, and the combination of HDACIs with existing chemotherapeutic agents has been identified as a potential approach to overcome drug resistance.

Innovations and breakthroughs

Whether HDACIs can sensitize HCC cells to sorafenib treatment remains largely unexplored. The study indicates that HDACIs and sorafenib interact in a highly synergistic manner to enhance the antiproliferative activity in HCC cells *in vitro*. Autophagy is activated and plays a compensatory role during treatment with sorafenib, vorinostat, or the drug combination. Knockdown of Beclin-1 or the autophagy inhibitor 3-MA enhanced the synergistic effect of the combination of vorinostat with sorafenib.

Applications

These findings highlight that the combination of HDAC inhibitors with sorafenib appears to be a promising approach to considerably improve treatment response in HCC patients.

Peer review

The manuscript described the experiments that aimed to clarify the mechanism

of enhancing hepatoma cell death by using the combination of two known anticancer agents: sorafenib and vorinostat. They concluded that inhibition of autophagy could enhance the effect of vorinostat/sorafenib.

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ORIGINAL ARTICLE

Naofen promotes TNF-α-mediated apoptosis of hepatocytes by activating caspase-3 in lipopolysaccharide-treated rats

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Abstract

AIM: To investigate whether naofen is involved in tumor necrosis factor (TNF)-α-mediated apoptosis of hepatocytes induced by lipopolysaccharide (LPS).

METHODS: *In vivo*, rats were treated with LPS or anti-TNF-α antibody, whereas *in vitro*, primary hepatocytes and Kupffer cells (KCs) were separately isolated from rat livers using collagenase perfusion, and primary hepatocytes were cultured in medium containing LPS or TNF-α, or in conditioned medium from LPS-treated KCs (KC-CM)/KC-CM + anti-TNF-α antibody. Naofen and TNF-α mRNA expression was examined by real-time reverse transcription-polymerase chain reaction. Immunoblotting was used to measure protein expression. Hepatocyte apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay.

RESULTS: LPS significantly induced both naofen expression and caspase-3 activity in the rat liver, which coincided with an increase in the number of TUNEL-positive hepatocytes. The increase of TNF-α expression induced by LPS was preceded by increases in naofen and caspase-3 activity. Elevation of naofen expression and caspase-3 activity was abrogated by pretreatment with anti-TNF-α antibody. In KCs, LPS caused an increase in TNF-α that was almost consistent with that in the liver of LPS-treated rats. In hepatocytes, neither LPS nor TNF-α alone affected either naofen expression or caspase-3 activation. The incubation of hepatocytes with KC-CM significantly enhanced both naofen expression and caspase-3 activity. Moreover, the effects of the KC-CM-induced increase in naofen expression and caspase-3 activity were blocked by anti-TNF-α antibody.

CONCLUSION: TNF-α released from KCs treated with LPS may induce hepatic naofen expression, which then stimulates hepatocellular apoptosis through activation of caspase-3.

Key words: Naofen; Tumor necrosis factor-α; Apoptosis; Lipopolysaccharide; Kupffer cells; Caspase-3

Core tip: Naofen, a WD40-repeat protein, is increased in the liver but not in the kidneys, thymus or spleen of rats injected with lipopolysaccharide (LPS). Increased naofen expression is blocked by pretreatment with anti-tumor necrosis factor (TNF)-α antibody. TNF-α has no effect on naofen expression or caspase-3 activation in primary hepatocytes, but conditioned medium from LPS-treated Kupffer cells (KC-CM) significantly enhances both. KC-CM-induced increase in naofen expression and caspase-3 activity is blocked by anti-TNF-α antibody. LPS in the liver may enhance release of TNF-α

from KCs, and induce hepatocyte apoptosis, for which naofen promotes caspase-3 activity through the mitochondrial pathway.

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INTRODUCTION

Lipopolysaccharide (LPS) is a major structural component of the outer membrane of Gram-negative bacteria[1]. Under normal conditions, a small amount of LPS, mainly from the intestine, can periodically be taken up into the liver through the portal vein and then scavenged by Kupffer cells (KCs), the resident macrophages in the liver[2]. The liver functions as the first barrier to LPS entering the circulation and as a detoxification organ, therefore, it is deeply affected by endotoxemia. However, in patients with severe trauma, burns, intestinal ischemia and liver diseases, LPS can spill over into the systemic circulation because of the increased permeability of the intestinal wall and/or the decreased phagocytic ability of liver KCs[3-5]. Under septic conditions, LPS-induced hepatocyte death may have a role in liver dysfunction, possibly associated with apoptosis of hepatocytes[5-7]. It is clear that LPS does not directly have pathogenetic roles, but rather the effects are mainly dependent on the production and release of potent inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-10[8,9]. These mediators, especially TNF-α, can induce apoptotic liver injury[10] and the infiltration of inflammatory cells. The latter, in turn, can further exacerbate liver injury, which continues the vicious circle of infiltration/liver injury[3,6-8]. A number of inflammatory liver diseases in humans, including viral hepatitis, alcoholic liver disease, immune- or drug-induced liver injury and ischemia/reperfusion liver failure, have been shown to be dependent on TNF-α production[5,11,12]. Therefore, to control liver damage under such pathological conditions, it may be important to understand the functions of TNF-α in liver injuries.

Hepatocyte apoptosis, as a general feature, is the most important event in the molecular mechanisms of hepatic failure, because apoptosis is the first cellular response of the liver to a wide range of toxic substances (including LPS), and necrosis in hepatic tissues is often found to follow the appearance of apoptosis[13-15]. It has been well documented that the caspase cascade involved in apoptosis includes both initiator and effector caspases[13-15]. Two main initiator caspases, caspase-8 and caspase-9, mediate distinct sets of death signals. Caspase-8 is activated by death signals that bind to death receptors on the cell surface. In contrast, caspase-9 is activated by cytochrome *c* released from mitochondria. Proapoptotic signals activate an initiator caspase that, in turn, activates effector caspases, for example, caspase-3. Sequential activation of caspases results in cleavage of substrate proteins and breakdown of DNA molecules, leading to apoptosis. So far, although many studies of hepatocyte apoptosis have been conducted, the precise molecular mechanisms remain incompletely defined. Therefore, the identification of signal pathways in LPS-mediated hepatocyte apoptosis would contribute to understanding the pathophysiological roles of apoptosis in liver diseases.

Recently, naofen was found as an intracellular protein reactive to anti-verotoxin Ⅱ antibody and classified in the aspartate-tryptophan (WD) 40-repeat protein family[16]. In deoxycorticosterone-induced renal hypertension in rats, naofen is increased in vascular endothelial cells and suppresses nitric oxide synthesis[16]. Naofen also induces apoptosis in streptozotocin-induced diabetic rat kidney[17] and mediates spontaneous and TNF-α induced apoptosis in human embryonic kidney (HEK) 293 cells[18]. Furthermore, naofen was increased in hepatocytes, causing apoptosis in LPS-treated rat liver[19]. Thus, it was hypothesized that naofen may be involved in TNF-α-induced apoptosis of hepatocytes. The present study was undertaken to examine whether naofen participates in the TNF-α-mediated apoptosis of hepatocytes in LPS-treated rats. Moreover, the correlating mechanisms were evaluated, utilizing primary cultures of KCs and hepatocytes.

MATERIALS AND METHODS

Animal treatment

Male Sprague-Dawley rats (weighing 200-250 g; SLC Inc., Guangxi, China) were maintained in climate-controlled rooms under a 12-h light-dark cycle. All experiments were conducted in accordance with the Institutional Guidelines of Guangxi Medical University for the care and use of laboratory animals.

Rats were injected with LPS (500 μg/kg; Sigma, St. Louis, MO, United States) *via* the femoral vein under ether anesthesia, and saline was used as a control as previously reported[19]. A second set of experiments was performed to determine the influence of anti-TNF-α on the expression of naofen in response to LPS. Rats received femoral vein injection of nonspecific IgG (2 mg/kg; Biosensis, Thebarton, SA, Australia) + LPS (500 μg/kg; Sigma), anti-TNF-α (2 mg/kg; R and D Systems, Minneapolis, MN, United States) + LPS (500 μg/kg), saline + IgG (2 mg/kg), or saline + anti-TNF-α (2 mg/kg). The anti-TNF-α and IgG were administered 24 h before LPS. Ten rats were used for each time point. At 1, 3, 6, 9 and 12 h after injection, animals were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally), and blood samples were collected from the inferior vena cava. The livers were removed, immediately frozen, and stored in liquid nitrogen for RNA and protein extraction.

Preparation of hepatocytes and KCs

Hepatocytes and KCs were separately prepared from the livers of Sprague-Dawley rats using collagenase perfusion[20,21]. Hepatocytes were cultivated in Williams' E medium containing 10% calf serum, 2 mmol L-glutamate and antibiotics (100 U/mL penicillin G and 100 μg/mL streptomycin sulfate). KCs were cultured with RPMI 1640 medium containing 10% calf serum and antibiotics. Hepatocytes (1×10^6) and 5×10^5 KCs per well were plated on a 6-cm plate and incubated at 37 °C under 5% CO_2 and 95% O_2 for 6 and 1 h, respectively. The culture medium was then changed to remove nonviable and unattached cells. The viability of cells tested by trypan blue dye exclusion ranged between 87% and 95%. The purity of hepatocytes examined by light microscopy and of KCs identified by phagocytosis of latex beads (polystyrene beads, mean particle size 1.1 μm; Sigma) ranged between 85% and 95%. Duplicate cultures were prepared for each treatment, and independent experiments were performed at least four times.

Preparation of Kupffer cell-conditioned medium

After 24 h of culture, KCs were incubated in medium containing 100 ng/mL LPS for 1-12 h, and TNF-α expression was measured. In some experiments, KCs were treated with LPS for 6 h, and the culture medium, as Kupffer cell-conditioned medium (KC-CM), was collected and centrifuged at 15000 *g* at 4 ℃ for 10 min to remove cell debris. To confirm the effects of TNF-α, an antibody against TNF-α (500 ng/mL) was added to KC-CM (6 h) and incubated at 37 ℃ for 1 h (6 h KC-CM + anti-TNF-α). Hepatocytes were incubated respectively with LPS (100 ng/mL), TNF-α (10 ng/mL), IgG (500 ng/mL), 6 h KC-CM or 6 h KC-CM + anti-TNF-α for 12 h, and the expression of naofen, TNF-α and caspase-3 activity was analyzed.

Real-time quantitative PCR

Total RNA (1 μg) was extracted from livers or primary cells using TRIzol reagent (Invitrogen, Carlsbad, CA, United States) and reversely transcribed using a ReverTra Ace quantitative PCR (qPCR) RT kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. Target mRNA expression was quantified using qPCR as described previously[19]. The primers and probe for naofen (forward primer 5'-CGATTTCTGCATTTTGGCCACAA-3', reverse primer 5'-TCCAAGGGTGTGCCAATAGAATT-3 and TaqMan MGB probe 5'-CAAACTGAGGGTGATTTT-3') and TaqMan Gene Expression Assays for naofen (ID: Rn01769571_m1), TNF-α (ID: Rn99999017_m1), GAPDH (ID: Rn99999916_s1) and β-actin (ID: Rn00667869_m1) were purchased from Applied Biosystems (Foster City, CA, United States).

Immunoblotting assay

Protein samples (30-50 μg) were prepared from livers and cells and separated by SDS-PAGE, followed by transfer to PVDF membranes (Millipore, Billerica, MA, United States) as reported previously[19]. Blots were incubated with an anti-naofen antibody (anti-NF, 1:500), which was designed and produced by Medical & Biological Laboratories (Nagoya, Japan) or antibodies (1:1000, respectively) against TNF-α and GAPDH (Cell Signaling Technology, Danvers, MA, United States), followed by incubation with a peroxidase-conjugated goat IgG (1:5000; Sigma). Proteins were visualized using ECL Plus Western blotting Reagent (GE Healthcare, Chalfont St Giles, Bucks, United Kingdom). Changes in target protein levels were measured quantitatively using Image J (free software made by NIH initiative).

Assessment of caspase-3 activation

Caspase-3 activation was determined using a Caspase Fluorometric assay kit (Medical and Biological Laboratories) as previously reported[19]. Free AFC cleaved by caspase-3 from the substrate, DEVD (Asp-Glu-Val-Asp)-AFC (7-amino-4-trifluoromethyl coumarin), was quantified by Fluoroskan Ascent FL (Labsystems, Helsinki, Finland) with excitation/emission (Ex/Em) = 400/505 nm.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay

Livers were fixed with 4% paraformaldehyde in PBS and embedded in paraffin. Serial 5-μm sections were made for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, a method for detecting DNA fragmentation in apoptosis, using an ApopTag Plus peroxidase *in situ* apoptosis detection kit (Millipore) according to the manufacturer's instructions[22]. For each sample, five high-power fields (× 200) were randomly selected, each containing an average of 400 cells, and the number of apoptotic cells was counted for each field. Apoptosis index (AI) (%) was calculated as number of positive cells/number of total cells × 100%.

Statistical analysis

Results are expressed as mean ± SE ($n = 10$), unless otherwise indicated. Statistical analyses were performed using Kruskal-Wallis one-way analysis of variance. $P < 0.05$ was considered significant.

RESULTS

Changes of TNF-α and naofen expression in livers of LPS-injected rats

Changes in the time course of TNF-α expression were investigated in the livers of rats injected with 500 μg/kg LPS + 2 mg/kg IgG for 1-12 h. TNF-α mRNA rapidly increased by the greatest amount within 1 h after injection, and then gradually decreased (Figure 1A). In the immunoblotting assay with anti-TNF-α (Figure 1C), compared to the control saline + IgG in which TNF-α was almost undetectable, LPS resulted in the strongest signal intensity for TNF-α protein after 1 h injection, then diminished, and recovered to an undetectable level within 12 h.

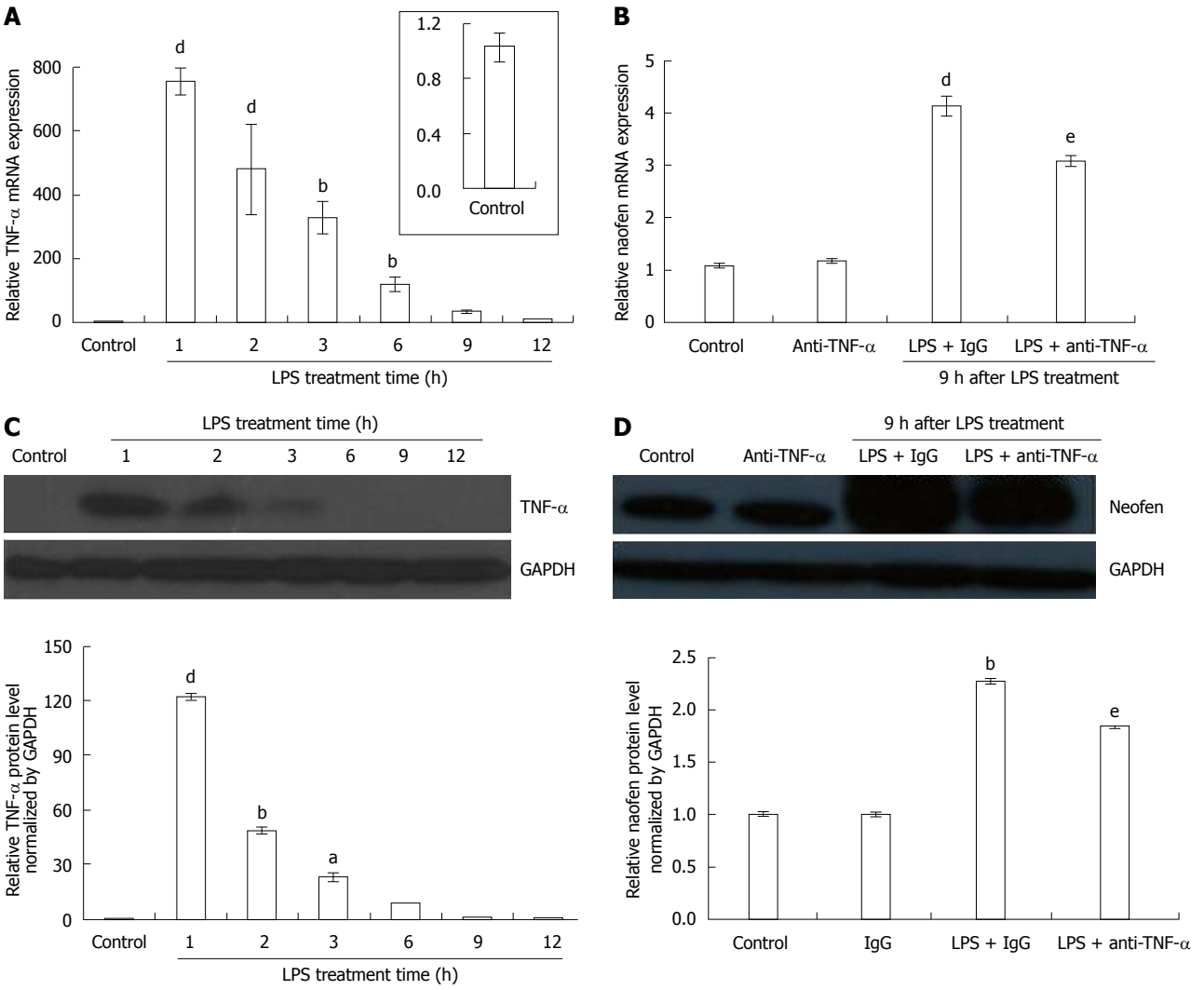

Figure 1 Changes in tumor necrosis factor-α and naofen expression in the liver of lipopolysaccharide-treated rats. Rats were intravenously injected with saline + IgG (control), LPS (500 μg/kg) + IgG (2 mg/kg) or LPS (500 μg/kg) + anti-TNF-α (2 mg/kg), and livers were removed to evaluate expression of TNF-α and naofen. A: Time course of the effects of LPS on TNF-α mRNA expression; B: Effect of anti-TNF-α antibody on naofen mRNA expression at 9 h after LPS injection; total RNA was extracted using TRIzol reagent and relative mRNA was quantified using qPCR (n = 10). Tissue lysates were examined by Western blot; C: TNF-α protein expression; D: Naofen protein expression (n = 6). Results are presented as ratios of target mRNA or protein normalized to internal GAPDH. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{d}P < 0.001$ *vs* control; $^{e}P < 0.05$ *vs* LPS + IgG. TNF-α: Tumor necrosis factor-α; LPS: Lipopolysaccharide.

As previously reported[19], the expression of naofen was increased from 5 μg/kg LPS and peaked at 500 μg/kg. In addition, naofen mRNA increased from 3 h, peaked at 9 h, and then diminished. Thus, changes in naofen expression were investigated using 500 μg/kg LPS + 2 mg/kg IgG. In contrast to the control saline + IgG, naofen expression was obviously increased at 9 h (Figure 1B). Immunoreactivity for naofen also appeared to have a similar pattern with its mRNA expression (Figure 1D).

Gene expression and protein level for naofen were found to be significantly reduced in rats treated with 2 mg/kg anti-TNF-α + LPS compared to LPS + IgG (Figure 1B and D). The expression of TNF-α and naofen were not significantly different between saline and saline + IgG (data not shown).

Liver apoptosis in LPS-injected rats

Liver apoptosis induced by LPS was confirmed by studying caspase-3 activation and TUNEL assay. Caspase-3 activation in LPS-treated rat livers increased 9 h after LPS + IgG injection, while the increased caspase-3 activity was significantly decreased by pretreatment with anti-TNF-α (Figure 2A). Typical TUNEL results are shown in Figure 2B and C. In the livers of LPS + IgG-treated rats, approximately 25% of hepatocytes nuclei were clearly stained 9 h after injection, whereas 2% positive changes were observed in control saline + IgG rat livers. Although IgG did not suppress an increase in the number of apoptotic hepatocytes, the addition of anti-TNF-α significantly inhibited the appearance of hepatocyte apoptosis (Figure 2B and C).

LPS-induced TNF-α production in KCs

In unstimulated KCs (control saline + IgG), TNF-α was hardly detected; however, KCs treated with LPS (100 ng/mL) + IgG (500 ng/mL) showed marked production of

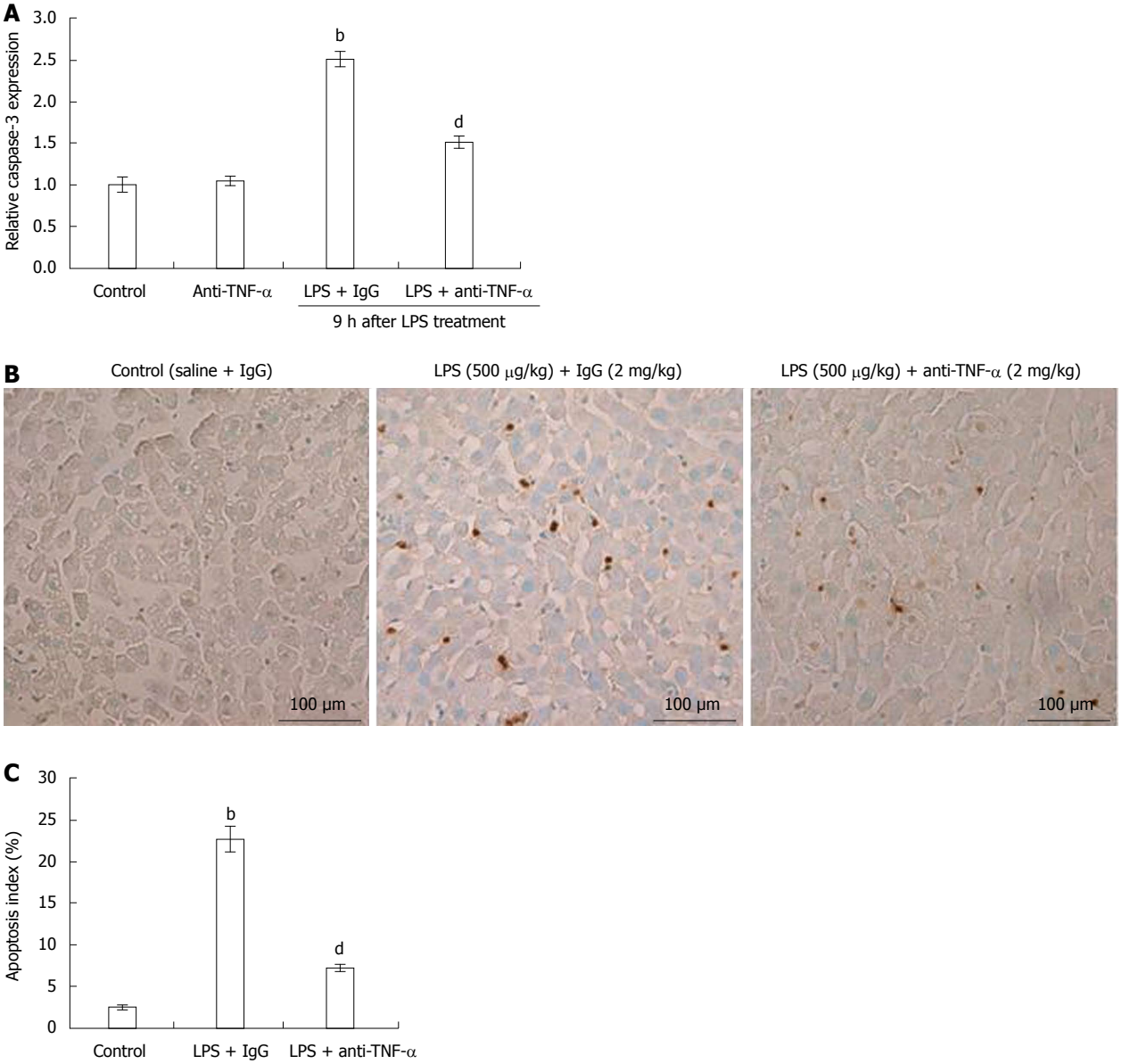

Figure 2 Effects of anti-tumor necrosis factor-α antibody on caspase-3 activation and apoptosis. Rats were treated as described above. A: Caspase-3 activation. Livers were lysed in lysis buffer and caspase-3 activation was measured. $^{b}P < 0.01$ *vs* controls; $^{d}P < 0.01$ *vs* LPS + IgG ($n = 10$); B: Liver apoptosis was examined using dUTP nick end labeling assay in rats 9 h after injection with control (saline + IgG), LPS + IgG or LPS + anti-TNF-α. Bar: 100 μm; C: Apoptosis index in the different groups. $^{b}P < 0.01$ *vs* control; $^{d}P < 0.01$ *vs* LPS + IgG ($n = 10$). TNF-α: Tumor necrosis factor-α; LPS: Lipopolysaccharide.

TNF-α at 1 h after addition, and then a gradual decrease in mRNA and protein level (Figure 3A and B). Although the expression of TNF-α mRNA in LPS-treated KCs was similar to that obtained in LPS-treated rat livers, there was even stronger signal intensity for TNF-α protein in the former, appearing as a clearly detectable band 6 h after LPS administration (Figure 3B).

Effect of TNF-α on expression of naofen and caspase-3 activity in hepatocytes

When LPS alone was added to hepatocytes (Figure 4A) or KCs, no change in naofen was observed (data not shown). As previously reported[19], KC-CM treated with LPS (100 ng/mL) for 3 h significantly increased naofen expression in hepatocytes, and extension of the LPS treatment time to 6 h had a stronger effect. In the following experiments, KC-CM treated with LPS for 6 h was used.

However, it was surprising that TNF-α alone did not enhance naofen expression in hepatocytes (Figure 4A). We have showed that anti-TNF-α antibody inhibits liver apoptosis induced by LPS (Figure 2), therefore, we studied the effect of anti-TNF-α antibody on KC-CM-induced naofen expression. As expected, pretreatment with 500 ng/mL anti-TNF-α antibody almost completely inhibited the increase of naofen induced by KC-CM (Figure 4A and B). An irrelevant antibody conferred no effect, suggesting the possible participation of TNF-α in the induction of naofen.

LPS alone did not affect caspase-3 activity, but in

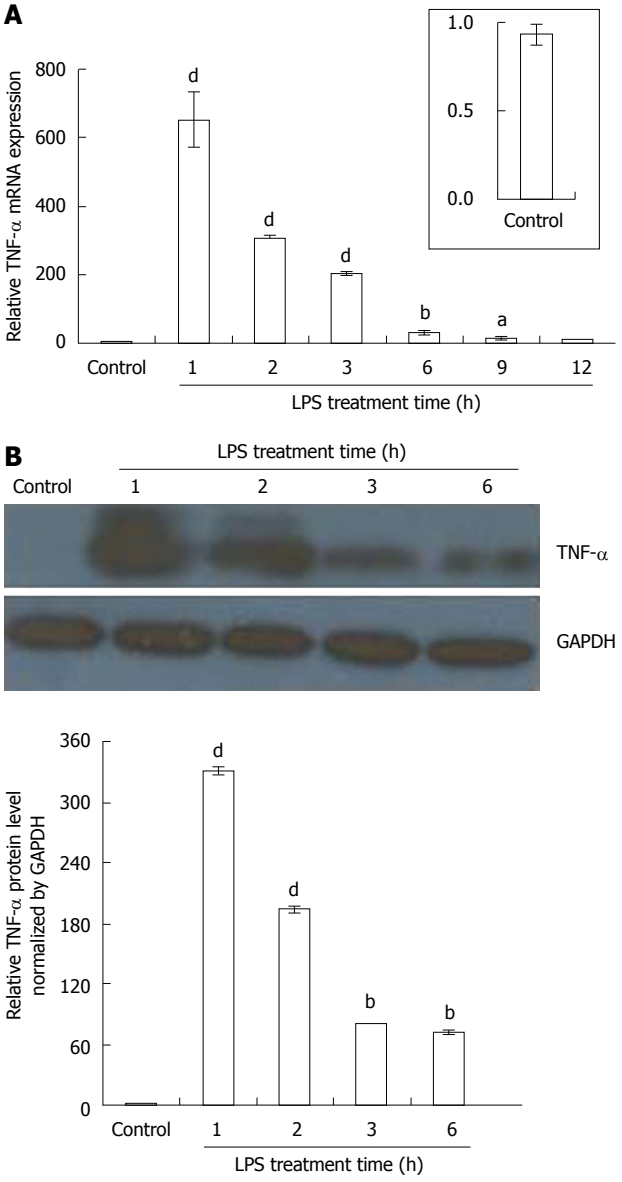

Figure 3 Effects of exposure period of Kupffer cells to lipopolysaccharide on tumor necrosis factor-α production. Primary Kupffer cells (KCs) were separated from rat livers using collagenase perfusion. After 24 h of incubation, KCs were incubated with lipopolysaccharide (LPS) (100 ng/mL). A: Tumor necrosis factor (TNF)-α mRNA was quantified using qPCR (n = 6); B: Immunoblotting assay for TNF-α. KC lysates were analyzed with TNF-α antibody (n = 6). GAPDH was used as an internal control. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{d}P < 0.001$ *vs* controls (without LPS treatment).

hepatocytes incubated with KC-CM for 6 h, caspase-3 activity significantly increased, which was clearly inhibited by pretreatment with 500 ng/mL anti-TNF-α (Figure 4C). These findings suggested that liver injury caused by LPS depended on TNF-α released from activated KC.

DISCUSSION

The results obtained in the present study demonstrated that LPS induced both naofen and TNF-α expression in rat liver. Naofen promotes TNF-α-mediated apoptosis of hepatocytes by activating caspase-3 in LPS-treated rats. *In vitro*, hepatocyte apoptosis caused by LPS was mediated by TNF-α, which was released from KCs in the presence of LPS, induced naofen expression and activated caspase-3. Our data suggested that hepatocyte apoptosis induced by KC-CM was associated with an increase in naofen expression (Figure 4), which was consistent with the results obtained in LPS-treated rats (Figure 1). Furthermore, naofen siRNA inhibited the increase in naofen protein induced by 6 h KC-CM, and naofen-siRNA also prevented KC-CM-induced caspase-3 activation in a previous study[19]. These results coincided with our recent data that naofen overexpression enhanced apoptosis by activating caspase-3 in HEK293 cells and, in contrast, naofen-siRNA inhibited TNF-α-induced caspase-3 activation and apoptosis[18]. Such results suggest that naofen is also involved in hepatocyte apoptosis induced by LPS-activated TNF-α. Previously, Morikawa *et al*[23] demonstrated that the injection of LPS and D-galactosamine into mice caused apoptosis in the kidneys, thymus, spleen, and lymph nodes besides the liver, whereas our findings verified that the increase in naofen induced by LPS was limited to the liver, and was not found in the kidneys, thymus or spleen (data not shown). This indicates that naofen, in LPS treated rats, may only make a limited contribution to liver injury.

Neither LPS nor TNF-α alone affected the expression of naofen in KCs or hepatocytes, whereas KC-CM significantly increased naofen expression in hepatocytes (Figure 4), indicating that the increase in naofen in the liver caused by LPS may be closely associated with KCs. As previously reported, the liver injury caused by LPS was dependent on KC activation, as demonstrated both *in vitro* and *in vivo*[8,9,24]. Intercellular signal transduction between KCs and hepatocytes has now been proposed, possibly mediated by cytokines such as TNF-α and IL, and inflammatory mediators such as eicosanoids, NO, and/or reactive oxygen species[6-9,25]. In particular, TNF-α has been shown to be an important mediator of LPS-induced apoptosis of hepatocytes[10,23,24]. The present study showed that LPS markedly enhanced TNF-α production in KCs in a time-dependent manner (Figure 4). It was noted that the time course of TNF-α expression in LPS-activated KCs accorded with that in LPS-treated rat livers (Figures 1 and 4), suggesting that LPS-induced TNF-α production in the liver may be ascribed to KCs, but not to hepatocytes. Furthermore, the increased naofen expression in LPS-treated rats, as well as the effects of KC-CM on naofen expression in hepatocytes, was clearly blocked by pretreatment with anti-TNF-α antibody (Figures 1 and 4), suggesting that TNF-α may play an important role in naofen expression. Regarding the little effect of TNF-α alone on naofen expression in hepatocytes, other unknown mediators may be associated with TNF-α, such as IL-1β, IL-6, IL-8, platelet-activating factor or NO[6-8,26]. Inhibitors of nuclear factor (NF)-κB may also be involved because blocking TNF-α-induced NF-κB activation in primary hepatocytes[27] or the liver *in vivo*[28] converts the hepatocellular TNF-α response from proliferation to apoptosis. In order to identify the nature

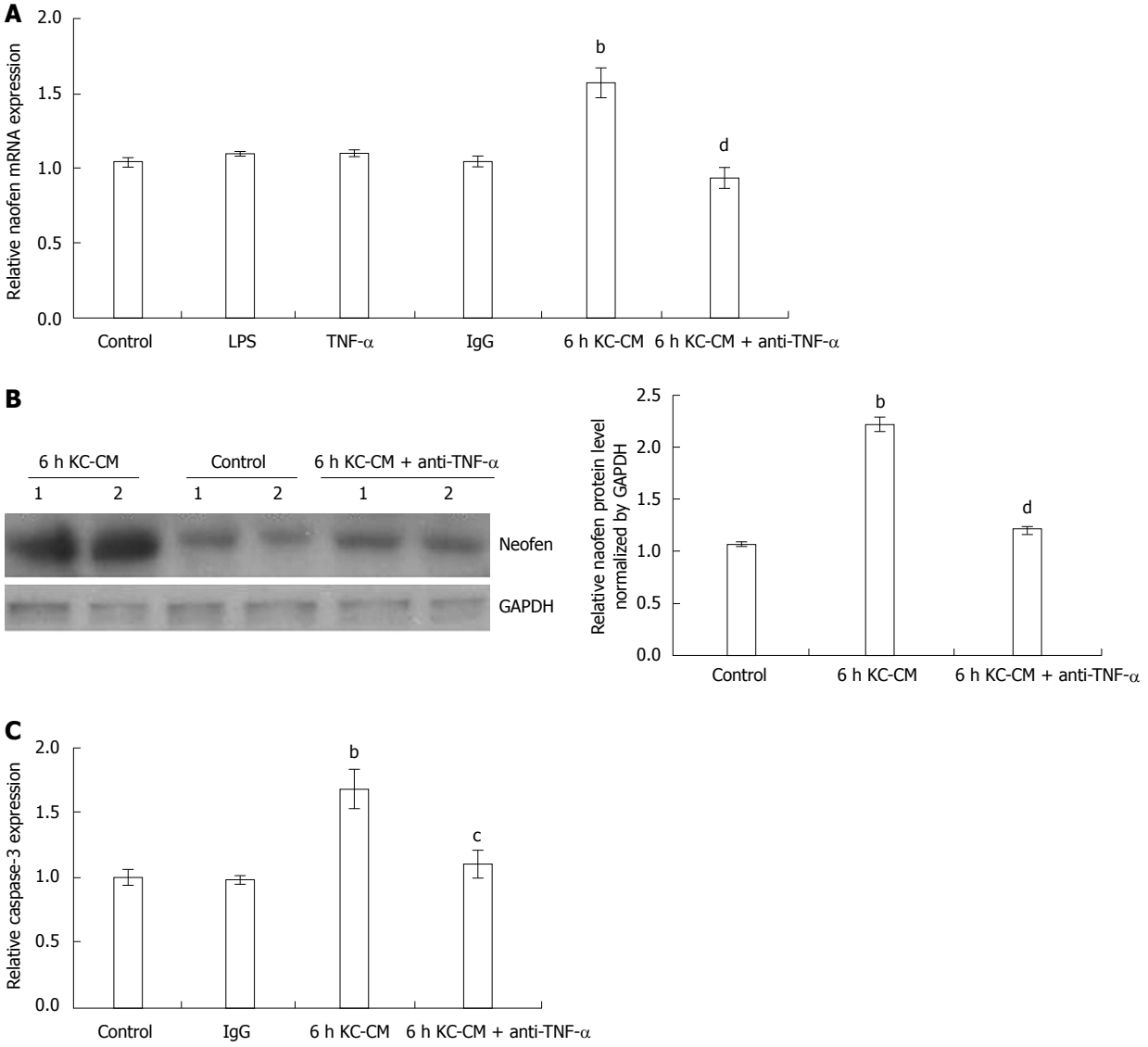

Figure 4 Effect of Kupffer cell-conditioned medium on naofen mRNA expression and caspase-3 activation. Primary Kupffer cell (KCs) and hepatocytes were separated as described above, and KC-CM was obtained by incubating KCs with LPS (100 ng/mL) for 6 h. Anti-TNF-α antibody (500 ng/mL) was added to KC-CM treated with LPS for 6 h and incubated at 37 ℃ for 1 h (anti-TNF-α + 6 h KC-CM). Hepatocytes were incubated with LPS (100 ng/mL), TNF-α (10 ng/mL), IgG (500 ng/mL), 6 h KC-CM and anti-TNF-α + 6 h KC-CM for 12 h, respectively. A: Naofen mRNA in hepatocytes was measured with qPCR and GAPDH was used as an internal control (n = 6); B: Immunoblotting assay for naofen. Hepatocyte lysates were analyzed with naofen antibody (n = 6); C: Caspase-3 activation was also measured (n = 6). $^{b}P < 0.01$ *vs* controls; $^{c}P < 0.05$ and $^{d}P < 0.01$ *vs* 6 h KC-CM. TNF-α: Tumor necrosis factor-α; LPS: Lipopolysaccharide; KC-CM: Kupffer cell-conditioned medium.

of these unknown mediators, the effects on naofen expression of combination of TNF-α with IL-1, IL-6 and interferon-γ (10 ng/mL each) or inhibitors of NF-κB, such as BAY 11-7082 and DHMEQ, have been examined. However, combination with TNF-α or metabolites of TNF-α treated with trypsin failed to enhance naofen expression in primary hepatocytes (data not shown). It has been reported that the trend from TNF-α production to subsequent hepatocyte apoptosis may contribute to the development of several inflammatory liver diseases, including viral hepatitis, alcoholic liver disease, Wilson's disease, drug-induced liver failure, and ischemia/reperfusion liver damage[7-10,24]. Identification of the relationship between TNF-α and naofen in liver injury may contribute to understanding the pathophysiological roles of apoptosis in liver diseases.

As previously reported, naofen is overexpressed in hepatocytes and markedly downregulates the expression of Bcl-2 and Bcl-xL, which is accompanied by the release of cytochrome *c* from mitochondria, resulting in caspase-3 activation[19]. Bcl-2 and Bcl-xL have critical roles in mitochondrial apoptotic signaling, through the controlled release of cytochrome *c* in hepatocytes[8,15]. This suggests that naofen is an upstream signal of Bcl-2 and Bcl-xL, consequently inducing the mitochondrial apoptotic pathway. Translocation of cytochrome *c* from mitochondria to cytosol has already been reported by many investigators, which forms a complex of Apaf-1 and procaspase-9, leading to the activation of caspase-9, followed by activation of downstream caspase-3 and development of

hepatocyte apoptosis[13-15]. Likewise, previous studies have demonstrated that LPS-activated KCs also stimulate the apoptosis of hepatic stellate cells by activating caspases-9, -3 and -8[26,29]. Most importantly, naofen siRNA reverses KC-CM-induced responses, resulting in prevention of the decrease in Bcl-2 and Bcl-xL expression and increase in capase-3 activity[19]. Overall, naofen may act on the mitochondrial pathway in the KC-CM-induced apoptosis of hepatocytes. Therefore, it is possible that naofen is an intracellular mediator involved in TNF-α-mediated apoptosis of hepatocytes, and may be relevant to the investigation on LPS-induced hepatic injury.

In conclusion, naofen may be involved in part in LPS-induced hepatocyte apoptosis, which is mediated by mediators including TNF-α released from KCs. Naofen elicits inhibition of the expression of Bcl-2 and Bcl-xL, releasing cytochrome *c* from mitochondria, and activating caspase-3, finally leading to apoptosis of hepatocytes. Although the precise molecular mechanisms of LPS-mediated hepatocyte apoptosis are still incompletely defined, LPS-induced apoptotic mechanisms in relation to naofen may be relevant to understanding clinical endotoxin or septic shock, and offer a new approach to therapeutic applications.

COMMENTS

Background

Lipopolysaccharide (LPS) has no direct pathogenic effect in hepatocytes, but the apoptosis and inflammatory responses of the liver may be attributed mainly to the effusion of potent inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and/or IL-10, from Kupffer cells (KCs). Among these mediators, TNF-α has been emphasized as a candidate for apoptosis and liver injury, as well as for infiltration of inflammatory cells. However, no clear mechanisms of LPS-induced hepatic damage have been demonstrated. Naofen, a WD40-repeat protein, may reduce NO synthesis or participate in TNF-α-induced apoptosis of HEK293 cells, but bupivacaine induces apoptosis independently of naofen expression. Whether naofen participates in TNF-α-mediated hepatocyte apoptosis in LPS-treated rats has not been investigated to date.

Research frontiers

Naofen was recently found as an intracellular protein reactive to anti-verotoxin Ⅱ antibody and classified in the aspartate-tryptophan (WD) 40-repeat protein family. The research hotspot is whether naofen participates in TNF-α-induced hepatocyte apoptosis in LPS-treated rats.

Innovations and breakthroughs

This study verified that the increase in naofen induced by LPS was limited to the liver, and was not found in the kidneys, thymus or spleen, indicating that naofen, in LPS treated rats, may only have a limited contribution to liver injury. So, the correlating mechanisms were evaluated, utilizing primary cultures of KCs and hepatocytes. Moreover, the action of LPS on apoptosis of hepatocytes was not induced by direct effects, but rather *via* an indirect pathway through the enhanced release of TNF-α from KCs. LPS or TNF-α alone did not elicit apoptosis of primary hepatocytes, or affect naofen expression or caspase-3 activation in primary hepatocytes. To overcome these disadvantages, combination of TNF-α with IL-1, IL-6 and interferon-γ or inhibitors of nuclear factor-κB was investigated. Conditioned medium from LPS-treated KCs (KC-CM) and anti-TNF-α antibody were used for further experiments. In this study, elevation of naofen expression and caspase-3 activity in LPS-treated rats was abrogated by pretreatment with anti-TNF-α antibody. Furthermore, the KC-CM-induced increase in naofen expression and caspase-3 activity was blocked by anti-TNF-α antibody. Naofen may be involved in part in LPS-induced hepatocyte apoptosis, which is mediated by mediators including TNF-α released from KCs.

Applications

The roles of naofen in LPS-induced apoptosis may be relevant to the understanding of clinical endotoxin/septic shock, and offer a new approach to therapeutic applications.

Terminology

LPS is a major structural component of the outer membrane of Gram-negative bacteria, and causes hepatic dysfunction, possibly associated with apoptosis of hepatocytes, which is mediated by inflammatory substances, such as TNF-α released from KCs. Naofen was recently identified as an intracellular protein reactive to anti-verotoxin Ⅱ antibody and classified in the WD 40-repeat protein family.

Peer review

This was a follow-up study of previous studies published by the authors. The study was novel and well designed. The study investigated the role of naofen in TNF-α-mediated apoptosis of hepatocytes induced by LPS. It was concluded that TNF-α released from KCs treated with LPS may induce hepatic naofen expression and then stimulate hepatocellular apoptosis through activation of caspase-3.

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BRIEF ARTICLE

Patient perceptions of stool DNA testing for pan-digestive cancer screening: A survey questionnaire

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Abstract

AIM: To explore patient interest in a potential multi-organ stool-DNA test (MUST) for pan-digestive cancer screening.

METHODS: A questionnaire was designed and mailed to 1200 randomly-selected patients from the Mayo Clinic registry. The 29-item survey questionnaire included items related to demographics, knowledge of digestive cancers, personal and family history of cancer, personal concern of cancer, colorectal cancer (CRC) screening behavior, interest in MUST, importance of test features in a cancer screening tool, and comparison of MUST with available CRC screening tests. All responses were summarized descriptively. χ^2 and Rank Sum Test were used for categorical and continuous variables, respectively.

RESULTS: Completed surveys were returned by 434 (29% aged 50-59, 37% 60-69, 34% 70-79, 52% women). Most participants (98%) responded they would use MUST. In order of importance, respondents rated multi-cancer detection, absence of bowel preparation, safety and noninvasiveness as most attractive characteristics. For CRC screening, MUST was preferred over colorectal-only stool-DNA testing (53%), occult blood testing (75%), colonoscopy (84%), sigmoidoscopy (91%), and barium enema (95%), $P < 0.0001$ for each. Among those not previously screened, most (96%) indicated they would use MUST if available. Respondents were confident in their ability to follow instructions to perform MUST (98%). Only 9% of respondents indicated that fear of finding cancer was a concern with MUST, and only 3% indicated unpleasantness of stool sampling as a potential barrier.

CONCLUSION: Patients are receptive to the concept of MUST, preferred MUST over conventional CRC screening modalities and valued its potential feature of multi-cancer detection.

Key words: Stool-DNA testing; Colorectal cancer screening; Gastrointestinal cancer screening; Patient perceptions

Core tip: The value of stool DNA testing could be expanded beyond colorectal cancer screening by simultaneously targeting gastrointestinal cancers above the colon. Early data suggest technical feasibility for such pan-cancer detection. However, while multi-organ stool DNA testing (MUST) would seem intuitively to have broad appeal; patient perceptions have not been evalu-

ated. In this exploratory study, we demonstrate that patients were interested in using MUST if it was available to them. The potential unique ability to detect multiple cancers was its most distinguishing and attractive feature. General population surveys are warranted to corroborate these early findings.

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INTRODUCTION

In aggregate, malignancies in the digestive track account for roughly 1/4 of all cancer deaths in the United States[1] and worldwide[2]. Although early stage detection and resection lead to a favorable prognosis with tumors at each gastrointestinal site, only colorectal cancer (CRC) is currently screened at the population level in most countries. It is remarkable that the common cancers above the colon remain unscreened despite the reality that their collective mortality substantially exceeds that of CRC alone[1].

Early studies suggest that supra-colonic gastrointestinal cancers can be detected noninvasively by stool DNA testing. In 2009[3], our research group evaluated the feasibility of stool-DNA testing for detection of common neoplasms throughout the gastrointrstinal GI tract. We were able to detect specific mutations (TP53, KRAS, APC, CDH1, CTNNB1, BRAF, SMAD4, and P16) present in primary tumor tissue from matched stools of patients with diverse supra-colonic gastrointestinal malignancies. Target mutations were detected in stools from 71% (36/51) of patients with cancer overall [40% (2/5) with oropharyngeal, 65% (11/17) with esophageal, 100% (4/4) with gastric, 55% (6/11) with pancreatic, 75% (3/4) with biliary or gallbladder, and 100% (4/4) with colorectal], while none were detected in the matched-control groups. In the same year, a group from Japan[4] used a novel fecal DNA methylation assay to detect increased methylation of gene promoters in patients with gastric and colorectal tumors (57%-75%) as opposed to only 10% of subjects without neoplasms. More recently, using a similar approach, we evaluated aberrantly methylated genes as non-invasive markers by stool DNA testing for the detection of pancreatic cancer[5]. The results from this study demonstrated that at 90% specificity, methylated BMP3 detected 51% of pancreatic cancer, while a combined stool assay of methylated BMP3 and mutant KRAS increased pancreatic cancer detection to 67%. Overall, these early findings support the potential and feasibility of a non-invasive multi-organ gastrointestinal stool-DNA test for cancer screening.

Ideally, such multi-organ stool DNA testing (MUST) would have the potential to expand the value of stool screening beyond that of CRC detection alone and address the existing gap in screening for upper gastrointestinal cancers. While the potential availability of MUST would seem intuitively to have broad patient appeal, there are no data on patient acceptability or perceptions of such an approach.

Endorsed by the American Cancer Society, the US Multi-society Task Force, and the American College of Radiology, stool DNA testing has emerged as an approach to CRC screening[6]. Stool DNA testing offers user-friendly features of noninvasiveness, avoidance of unpleasant bowel preparation associated with other approaches[7-12], ease of access *via* off-site sample collection and shipping, single rather than multiple stool sampling per screen, no diet or medication restriction, and possibly reduced screen frequency because of its capacity to detect precursor lesions[13,14]. With advanced next generation technology, stool-DNA testing has proven highly accurate for detection of both CRC and advanced precancer[15,16], and an automated test is currently under review by the FDA following evaluation in a general population[17]. In prior surveys, patients showed interest in using stool-DNA testing for CRC screening and appeared to prefer it over both fecal occult blood testing (FOBT) and colonoscopy[9,18-22]. However, it is not clear if an expanded capacity of stool-DNA testing for multi-cancer detection would enhance or impede participation in a CRC screening application.

Knowledge of patient perceptions and preferences regarding screening tools is important to understand compliance to screening[22-26]. For example, patient concern about pain, potential injury and discomfort with cathartic preparations are recognized barriers to routine screening with colonoscopy, flexible sigmoidoscopy, or barium enema[27,28]. While FOBT is a low risk and noninvasive screening alternative, the variability in cancer detection rates, inconvenient stool sampling, dietary restrictions, and poor sensitivity for precursor lesions, may limit its acceptance by some[29-32]. If MUST is to be further considered for a potential future pan-digestive cancer screening application, an early appraisal of patient attitudes would be instructive.

In this exploratory study, we designed a questionnaire to assess interest in and preferences for using MUST. We examined and compared perceptions and preferences for MUST against available CRC screening options.

MATERIALS AND METHODS

Study population and data collection

A total of 1200 patients were randomly selected within age and gender groups from the Mayo Clinic registry. Questionnaires were mailed to 400 candidates (200 men, 200 women) in each of 3 average-risk sub-groups between 50-79 years of age (50-59, 60-69, and 70-79 years).

Sample size considerations: In this exploratory study, we targeted a sample large enough to provide a 95%

confidence interval within ± 10 percentage points; and 100 respondents would yield such confidence. Based on 1200 candidates, we assumed that 1000 would have a current address, 500 would respond to the survey, and 100 respondents would not have undergone routine CRC screening.

Questionnaire survey

Questionnaire mailing from the Survey Research Center included a cover letter explaining the nature and purpose of the study and inviting the subject to complete the survey and return it in the stamped, pre-addressed envelope provided. A waiver consent form was included with the mailing and required signature for participation. Only one mailing was sent per participant with no follow-ups attempted.

Survey instrument: The questionnaire was designed in collaboration with the Mayo Clinic Survey Research Center (Appendix). Question format was modeled after those developed in the Health Information National Trends Survey (HINTS) 2007 on perceived risk, screening behavior, knowledge and concern about cancer.

The 29-item survey questionnaire included items related to demographics, knowledge of airway and digestive cancers, personal and family history of cancer, personal concern of cancer, CRC screening behavior, interest in MUST, importance of test features in a cancer screening tool, and comparison of MUST with available CRC screening tests.

Respondents' general knowledge of cancer was assessed by their ability to associate common risk factors (*i.e.,* age, smoking, obesity, alcohol consumption) with cancer development. Patients who specified a personal and/or family diagnosis of cancer (lung, breast, prostate, colon or rectal, esophageal, stomach, pancreatic, melanoma, and/or other) were considered to have a positive history of cancer. Personal concern of cancer was evaluated by asking how often (*i.e.,* all the time, often, sometimes, rarely or never) patients worried about developing any of the following cancers: lung, breast, colon or rectal, esophagus, stomach, pancreas, prostate.

Patients were asked about their likelihood of using MUST if it was available to them on a 5-point Likert-like scale with the following response options: very likely, likely, unlikely, and not sure. Seven items were also included describing possible reasons patients might choose MUST. Patients were again asked to rate these test features in terms of importance to them on a 5-point Likert-like scale.

Patients were asked to rank order their preferences for CRC screening tests among the following options: MUST, FOBT, colorectal-only stool-DNA testing, colonoscopy, flexible sigmoidoscopy, and barium enema. Patients were asked to rate the importance of test features (*i.e.,* ability to detect pre-cancerous lesions, accuracy, risk of injury, degree of discomfort, need for bowel preparation, cost) when choosing a regular CRC screening test on a 5-point Likert-like scale.

Statistical analysis

All responses were included for analysis when possible. If there was any confusion over the intent of an answer, the response was not included. All responses to surveys were summarized descriptively. χ^2 tests were performed to test for differences in baseline characteristics for all categorical characteristics. The Rank Sum Test was used to test for differences for all continuous characteristics. Since only a small subset of items were available for non-respondents, we also explored differences between early and later respondents in order to better understand the impact of potential non-response bias. χ^2 tests were used for these comparisons. In addition, the Wilcoxon Sign Rank Test was performed to compare the preference rank for MUST when compared to each of the other 5 colorectal screening tests. A P value of < 0.05 was considered statistically significant.

RESULTS

Sample characteristics

Thirty-six percent (434 of 1200) of mailed out surveys were completed and returned between November 14th, 2008 and January 16th, 2009. When respondents were compared to non-respondents, there was no difference in median age (66.0 years *vs* 64.4 years, $P = 0.64$), or in the number of days since the patient was last seen at the Mayo Clinic (82.5 *vs* 89.0, $P = 0.16$). Women accounted for 52% of respondents compared to 49% of non-respondents ($P = 0.34$).

Demographic and baseline characteristics of the sample population are summarized (Table 1). The majority of respondents were white, from Minnesota, and with the equivalent of a college degree or higher. A personal history of cancer was reported by 44%, with 9% originating from the airway or digestive tract; and 67% indicated a history of cancer in a first-degree relative. Most respondents acknowledged a personal concern with cancer (74%).

Knowledge about digestive and airway cancers

Most subjects correctly identified age over 50 years (85%), smoking (99%), alcohol consumption (74%) and obesity (76%) as factors that can increase a person's risk of developing cancer. Many understood that pain or other symptoms are generally absent at early curative stages of lung (65%), pancreas (60%), colorectal (63%), esophageal (47%) and stomach (50%) cancers.

Perceptions of and interest in MUST

Responses regarding the likelihood of using MUST are summarized (Table 2). Overall, most (98%) were interested in MUST and would likely use it, irrespective of physician recommendation. Subgroup comparisons were performed to assess whether likelihood of using MUST varied by age, gender, prior CRC screening, or personal

Table 1 Demographics and baseline characteristics of sample population *n* (%)

Characteristics	Value
Age (yr)	
50-59	122 (28.6)
60-69	159 (37.2)
70-79	146 (34.2)
Sex	
Female	225 (51.8)
Male	209 (48.6)
Race/ethnicity	
White	424 (98.4)
Non-white	7 (1.6)
Education	
Some high school	11 (2.6)
High school graduate or GED	108 (25.2)
Vocational, technical or business school	40 (9.3)
Some college or associate's degree	98 (22.9)
4-yr college graduate or Bachelor's degree	76 (18.8)
Graduate or professional school	95 (22.2)
Region	
Minnesota	251 (57.8)
Other	183 (42.2)
Positive personal history of cancer	
Aero-digestive cancer[1]	38 (8.8)
Other[2]	152 (35.0)
No	244 (56.2)
Positive familial history of cancer	
Yes	278 (66.8)
No	125 (30.0)
Not sure	13 (3.1)
CRC screening history	
Yes	355 (84.9)
No	50 (12.0)
Not sure	13 (3.1)
Personal concern with cancer[3]	
Yes	311 (73.5)
No	112 (26.5)

[1]Includes responses from subjects who specified they had been diagnosed with cancer from any of the following: lung, esophagus, stomach, pancreas, colon or rectum; [2]Includes responses from subjects who specified they had been diagnosed with cancer from any of the following: breast, prostate, skin (melanoma only), or specified as other. [3]Defined as subjects who responded "all the time" or "often" when asked how often they worry about getting one or all of the following cancers: lung, breast, colon or rectal, esophagus, stomach, pancreas, prostate. GED: General equivalent diploma; CRC: Colorectal cancer.

concern with cancer. "Very likely" and "likely" categories were combined and considered a positive response towards likelihood of using MUST. Interest in using MUST was high across all subgroups, and no statistically significant differences were observed.

MUST features rated as very important included its multi-cancer detection (95%), noninvasiveness (85%), avoidance of bowel preparation (81%), ability to perform the test at home (74%), and other features (Table 3). Subjects were provided with a description of the steps required to complete MUST. Most (98%) were confident in their ability to follow the instructions to complete the test. Reasons for not choosing MUST included uncertainty about physician recommendation (21%), not enough information on MUST (12%), and fear of finding cancer (9%). Only 3% responded that the unpleasantness of stool sampling represented a barrier to using MUST.

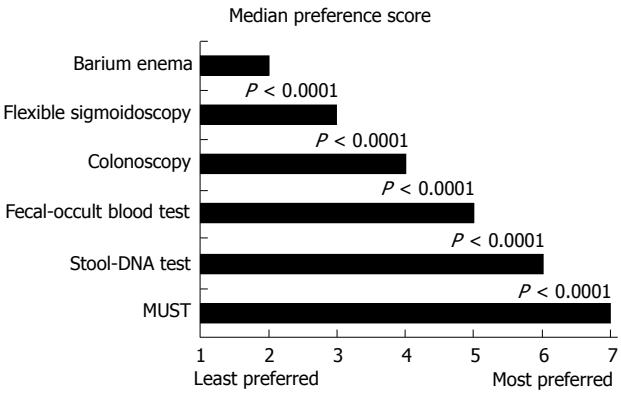

Figure 1 Median preference score for colorectal cancer screening. Scores were assigned from 1 (least preferred) to 7 (most preferred) for currently used screening approaches and for multi-organ stool-DNA test (MUST). $P < 0.0001$ using Wilcoxon sign rank test.

Perceptions and preferences regarding colorectal cancer screening

Most respondents (85%) indicated that they had previously undergone CRC screening, including by colonoscopy (79%), FOBT (41%), flexible sigmoidoscopy (38%), barium enema (28%), and/or stool-DNA testing (3%). Among the respondent subset without prior CRC screening, most (> 95%) indicated that they would likely use MUST if it was available. The most commonly cited barriers against CRC screening by those who had no prior CRC screening and those who had been screened, but did not intend to do so again, included the perceived low risk of cancer in the absence of symptoms (57%), lack of physician recommendation (56%), bowel preparation (38%), unpleasant or embarrassing elements of the test (29%), and concern about complications such as bleeding, perforation, or injury (22%).

Respondents were asked to rank different tests for regular CRC screening, irrespective of cost or insurance coverage in their decision-making process, by assigning a number from 1 to 7 (1 representing the least preferred and 7 the most preferred). Median preference score was highest for MUST (7.0) and lowest for barium enema (2.0), as shown in Figure 1. MUST was preferred over colorectal-only stool-DNA testing by 53% of respondents, over occult blood testing by 75%, over colonoscopy by 84%, over sigmoidoscopy by 91%, and over barium enema by 95%, $P < 0.0001$ for each. Most indicated the ability of a test to detect pre-cancerous lesions (97%), test sensitivity (95%), test specificity (94%), insurance coverage (62%) and risk of injury (56%) as very important test features when choosing the type of screening test (Table 3).

Assessment of potential response bias

To evaluate the potential for response bias, participants were stratified into early respondents (returned the survey in < 3 mo) and late respondents (returned survey > 3 mo). Early respondents were predominantly women (55% *vs* 44%, $P = 0.04$) and from Minnesota (63% *vs* 46%, P

Table 2 Likelihood of using multi-organ stool-DNA test

Characteristic (*n*)	Very likely	Likely	Unlikely	Very unlikely	Not sure	*P* value[1]
Age (yr)						
50-59 (121)	69.4%	25.6%	3.3%	1.7%	0.0%	0.56
60-69 (157)	82.2%	15.3%	1.3%	0.0%	1.2%	
70-79 (145)	75.2%	20.7%	2.1%	0.0%	2.0%	
Sex						
Female (219)	76.2%	20.1%	1.8%	0.5%	1.4%	0.89
Male (204)	76.0%	20.0%	2.5%	0.5%	1.0%	
Prior CRC screening						
Yes (352)	75.6%	20.5%	2.6%	0.6%	0.9%	0.66
No (49)	75.5%	20.4%	0.0%	0.0%	4.1%	
Do not know (13)	76.9%	23.1%	0.0%	0.0%	0.0%	
Personal concern with cancer						
Yes (311)	79.1%	16.7%	2.6%	0.3%	1.3%	0.48
No (112)	67.9%	29.4%	0.9%	0.9%	0.9%	
Respondents[2]						
Early (303)	76.6%	19.1%	3.0%	0.0%	1.3%	0.38
Late (120)	75.0%	22.5%	0.0%	1.7%	0.8%	

CRC: Colorectal cancer. [1]χ^2 test; [2]Comparing the likelihood of using a multi-organ stool-DNA test between early and late respondents.

Table 3 Respondents' rating of test features in multi-organ stool-DNA test and routine screening tool

	Very important	Somewhat important	Not at all important	Not sure
Stool-DNA test				
Detects multiple cancers with single test	95.1%	4.2	0.0	0.7
Safe noninvasive test	85.0%	13.3	0.7	1.0
No need for bowel preparation	80.8%	15.7	3.0	0.5
No need for sedation	77.8%	18.7	3.0	0.5
No need to change diet or medications	75.4%	22.0	1.9	0.7
Performed in the privacy of home	73.8%	21.5	4.2	0.5
No need to take time off from work	56.8%	15.2	27.3	0.7
A routine screening tool				
Ability of test to detect pre-cancer or change in body before it becomes cancer	96.5%	3.0	0.2	0.2
Accuracy of the test to say there is a cancer when there really is a cancer	94.7%	4.6	0.0	0.7
Accuracy of the test to say there really is no cancer when there is no cancer	93.3%	5.3	0.5	0.9
Whether test is covered by insurance	62.2%	27.7	8.5	1.6
Risk of injury with test	55.6%	31.4	10.2	2.8
How often the test has to be done	34.9%	43.1	19.2	2.8
The cost of the test	34.0%	44.4	17.8	3.8
Use of laxatives and/or enemas for bowel preparation	27.8%	46.5	22.9	2.8
Discomfort associated with the test	24.9%	48.2	24.2	2.6

= 0.001) when compared to late respondents (Table 4). There was no difference in race/ethnicity or educational background between early and late respondents. Interest in using MUST was high in both early and late respondents, and no statistical significant difference was observed (Table 2).

DISCUSSION

This study found that most respondents to a survey questionnaire were interested in using MUST if it was available to them. The likelihood of using MUST did not vary significantly on the basis of age, gender, prior history of CRC screening, or personal concern with cancer. The potential to simultaneously screen cancer at multiple organ sites was the most attractive feature of MUST. Our results suggest that the concept of screening for multiple digestive cancers with a stool test is an incentive to its potential use, and stool sampling *per se* was not perceived as a barrier

Of note, MUST was perceived as the preferred test for CRC screening, including a subset of respondents who had not previously undergone routine CRC screening. The concept of a stool test with capacity to detect both supra-colonic cancers and colorectal cancer was highly rated by respondents when asked to choose a CRC screening method. The majority of respondents identified accuracy, low risk of injury, and avoidance of bowel preparation and sedation as very important features when choosing a screening test. In this survey, noninvasive tests (MUST, colorectal-only stool-DNA testing, and FOBT) were preferred over invasive tests (colonoscopy, flexible sigmoidoscopy, and barium enema). However, it was the feature of multi-cancer detection that was most distinguishing in favoring MUST. These results suggest that multi-cancer detection is perceived as a value-add

Table 4 Demographics of early versus late respondents

Characteristics	Early respondents	Late respondents	*P* value[1]
Sex			0.040
Female	55.0%	44.0%	
Male	45.0%	56.0%	
Race/ethnicity			0.400
White	98.7%	97.6%	
Non-white	1.3%	2.4%	
Education			0.340
Some high school	2.9%	1.6%	
High school graduate or GED	25.2%	25.4%	
Vocational, technical or business school	10.8%	5.7%	
Some college or associate's degree	23.5%	21.3%	
4-year college graduate or Bachelor's degree	17.7%	18.0%	
Graduate or professional school	19.9%	27.9%	
Region			0.001
Minnesota	62.8%	45.6%	
Other	37.2%	54.4%	

[1]χ^2 test. GED: General equivalent diploma.

and that implementation of MUST has the potential to enhance patient participation in CRC screening.

Barriers to screening must be considered with the application of any new methods. Previous studies have identified lack of physician recommendation, lack of awareness of cancer, absence of symptoms and fear of finding cancer as common barriers to screening[7,9,18,19,21-28,33-35]. In our study, absence of provider recommendation was cited by some as a potential reason for not choosing MUST, highlighting the influential role of physicians in patient adherence to cancer screening. Whether personal concern with cancer would negatively impact patients' attitudes towards multi-cancer screening has not been previously assessed. In this study, fear of finding cancer did not appear to be an obstacle to using MUST, and the concept of multi-cancer detection was positively perceived. Furthermore, nearly all respondents indicated that stool sampling and collection per se was not a barrier.

In this study, respondents identified other specific test attributes, such as the ability to detect precancerous lesions and accuracy for cancer detection as key features when choosing a CRC screening tool. Recent studies have demonstrated that next-generation stool DNA testing can detect curable stage CRC and large precancerous lesions with high sensitivity, irrespective of neoplasm site in the colorectum[15,16]. In light of these advances in stool DNA technology, recent studies have evaluated the possibility of detecting supra-colonic gastrointestinal cancers in the stool[3-5]. Clearly, more clinical studies will be required to further develop and validate stool DNA testing for pan-digestive cancer detection. The results of our survey suggest that this expanded detection capacity of stool-DNA testing appeals to patients and that there are no obvious perceptual barriers to pan-cancer screening.

This exploratory survey study has several limitations and the findings may not be generalizable. First, as a majority of those contacted did not participate, response bias may have influenced our results. However, the similarity in demographics between respondents and non-respondents as well as the striking similarities in baseline characteristics, perceptions, and preferences between early and late respondents may be evidence against a major response bias. Second, the study population of this exploratory survey questionnaire lacked the demographic diversity reflective of the general population. Third, while this study was adequately powered for its objectives, the small sample size did not allow definitive co-variate analyses by demographic subsets. Fourth, our study population was well-informed. Their educational level and knowledge of cancer characteristics may have contributed to the overall positive response to using MUST. Fifth, this study was designed as an exploratory questionnaire survey and thus, the survey tool was not piloted and reliability analysis was not performed. Last, MUST is a hypothetical rather than an actual product at this point. Further research and development are needed before it can be offered for screening. Our survey can only assess perceptions, attitudes and likelihood of using a hypothetical MUST in comparison to already available CRC screening modalities. As such, respondents' perceptions of MUST may have been affected by its conceptual appeal and the lack of definite information on actual performance on cancer screening. Whether the overall positive response to MUST will translate to utilization once it is available remains to be determined; however, these results encourage further development and testing of MUST.

In conclusion, this study found that our population was interested in using MUST if it was available to them. The potential unique ability to detect multiple cancers was its most distinguishing and attractive feature. Other highly valued test characteristics included its noninvasiveness, absence of bowel preparation and sedation, avoidance of medication or dietary changes, and convenience of performing the test at home. MUST was preferred over conventional screening tools for routine CRC testing. Further studies are needed to determine whether a more diverse ethnic and socioeconomic population would express similar perceptions and preferences for MUST and CRC screening options.

COMMENTS

Background

The value of stool DNA testing could be expanded beyond colorectal cancer (CRC) screening by simultaneously targeting gastrointestinal cancers above the colon. Early data suggest technical feasibility for such pan-cancer detection.

Research frontiers

Knowledge of patient perceptions and preferences regarding screening tools is important to understand compliance to screening. While a multi-organ stool DNA test (MUST) would seem intuitively to have broad appeal; patient perceptions have not been evaluated. In this exploratory study, the authors demonstrate that patients are interested in using MUST if it was available to them.

Innovations and breakthroughs

Prior studies have shown patients' interest and preference in using stool DNA testing over both fecal occult blood testing and colonoscopy for colorectal can-

cer screening. This is the first study to evaluate patients' perceptions and preference for a MUST if it was available to them. The potential to simultaneously screen cancer at multiple organ sites was highly regarded by patients. MUST was preferred over conventional screening tools for routine CRC testing.

Applications

This study highlights the potential ability to detect multiple cancers by MUST as its most distinguishing and attractive feature. Patients valued the noninvasive test characteristics of MUST, and stool sampling was not considered a barrier for screening. Further studies are needed to corroborate these initial findings and to determine receptiveness of such a test in the general population.

Terminology

Stool-DNA testing: biological rationale of targeting DNA alterations (tumor markers, mutations) exfoliated from cancer cells arising in the gastrointestinal tract into stool. A MUST represents a potential noninvasive test that can detect different neoplasms in the GI tract based on multiple target DNA alterations. The concept of a MUST is based on the feasibility of stool-DNA testing for the detection of common supracolonic GI malignancies.

Peer review

This paper evaluates stool DNA testing for pan-digestive cancer screening. This is a well-designed study of survey questionnaire. This manuscript is interesting and most parts of the paper are clearly detailed.

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BRIEF ARTICLE

Histological healing favors lower risk of colon carcinoma in extensive ulcerative colitis

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Abstract

AIM: To search for the answer in extensive ulcerative colitis as to whether histological inflammation persisting despite endoscopic mucosal healing serves to increase the risk of colon cancer (CC) or high grade dysplasia (HGD).

METHODS: This is a single center (Lenox Hill Hospital) retrospective cohort and descriptive study of extensive ulcerative colitis (UC) for 20 years or more with a minimum of 3 surveillance colonoscopies and biopsies performed after the first 10 years of UC diagnosis. Data analyzed included: duration of UC, date of diagnosis of (CC) or (HGD), number of surveillance colonoscopies, and biopsies showing histological inflammation and its severity in each of 6 segments when endoscopic appearance is normal. Two subgroups of patients were compared: group 1 patients who developed CC/HGD and group 2 patients who did not develop CC/HGD.

RESULTS: Of 115 patients with longstanding UC reviewed, 68 patients met the inclusion criteria. Twenty patients were in group 1 and 48 in group 2. We identified the number of times for each patient when the endoscopic appearance was normal but biopsies nevertheless showed inflammation. Overall, histological disease activity in the absence of gross/endoscopic disease was found in 31.2% (95%CI: 28%-35%) of colonoscopies performed on the entire cohort of 68 patients. Histological disease activity when the colonoscopy showed an absence of gross disease activity was more common in group 1 than group 2 patients, 88% (95%CI: 72%-97%) *vs* 59% (95%CI: 53%-64%). Only 3/20 (15%) of patients in group 1 ever had a colonoscopy completely without demonstrated disease activity (*i.e.*, no endoscopic or histological activity) as compared to 37/48 (77%) of patients in group 2, and only 3.3% (95%CI: 0.09%-8.3%) of colonoscopies in group 1 had no histological inflammation compared to 23% (95%CI: 20%-27%) in group 2.

CONCLUSION: Progression to HGD or CC in extensive ulcerative colitis of long standing was more frequently encountered among those patients who demonstrate persistent histological inflammation in the absence of gross mucosal disease. Our findings support including the elimination of histological inflammation in the definition of mucosal healing, and support this endpoint as an appropriate goal of therapy because of its risk of increasing dysplasia and colon cancer.

Key words: Histological inflammation; Risk; Colon cancer; Ulcerative colitis; High grade dysplasia

Core tip: Patients with long standing and extensive ulcerative colitis who develop colon cancer rarely have histological healing despite gross endoscopic healing. The persistence of histological inflammation is common in those who develop colon cancer (CC) or high grade dysplasia (HGD). When surveillance colonoscopies in ulcerative colitis of 20 years duration reveal persistent histological inflammation, patients are at high risk for the development of CC/HGD. Consideration of increasing drug therapy should arise, and the patient is entitled to share in this knowledge and contribute to the decision.

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INTRODUCTION

Ulcerative colitis (UC) is one of the two major chronic inflammatory bowel diseases, almost always involving the rectum and any or all segments of the colon proximally in continuity. It is recognized that long-standing UC carries an increased risk for the development of colorectal carcinoma (CC) and high grade dysplasia (HGD), with estimates of risk as high as 20% following 30 years of diagnosis[1]. This risk appears to be especially prominent in cases of universal UC, which has traditionally been defined by endoscopic evidence of disease proximal to the mid-transverse colon on at least one occasion following diagnosis. Chronic macroscopic disease activity has also been implicated as a risk factor for the development for CC[2]. These observations have led to the practice of surveillance by colonoscopy in cases of universal UC. Current practice guidelines recommend that surveillance be performed every 1-2 years in these patients beginning 8-10 years after the initial UC diagnosis[3]. Surveillance typically involves four-quadrant biopsies of the colon, either every 10 cm during withdrawal from the cecum, or by colonic segments (*e.g.*, cecum, ascending colon, *etc.*) as well as biopsies of specific lesions when encountered.

While it is generally accepted that extent, duration and chronicity of inflammation directly impact cancer risk, less well studied is the role of the PERSISTENCE of microscopic inflammation after gross inflammation has subsided in the pathogenesis of colon cancer[4-6]. Recent case control and cohort studies have shown that the severity of microscopic inflammation is also associated with an increased risk for colorectal neoplasia[7,8]. Rutter *et al*[7] noted that both endoscopic and histological severity of disease impacted cancer risk on univariate analysis, only histological severity continued to show an increased risk for neoplasia following multivariate analysis.

In patients with inflammatory bowel disease (IBD), clinical remission is valuable for quality of life but does not necessarily correlate with "mucosal healing"[9]. Increasingly, disease and treatment outcomes for IBD are being assessed in terms of mucosal healing, which in most trials is defined by the normal appearance of the colonic mucosa as described by the endoscopist at colonoscopy[10-13]. Nevertheless, histological inflammation of varying degrees is common even when the mucosa appears normal. Though some may consider normal appearing mucosa as healed, it is unknown whether histological disease activity even in the absence of gross disease carries an increased risk for the development of CC or HGD.

To help answer this question we conducted a retrospective cohort descriptive study of long-standing universal UC patients. Our primary goal was to determine if there was a relationship between histological disease activity and risk for CC and HGD in the absence of gross inflammation. Our secondary goal was to define the incidence of histological disease activity in the absence of gross disease activity, possibly predisposing to the later development of high grade dysplasia or colon cancer.

MATERIALS AND METHODS

This is a study of UC patients utilizing the inflammatory bowel disease data base of over 3000 patients followed by the senior investigator Korelitz BI at Lenox Hill Hospital over a 50 year period. Inclusion criteria required a diagnosis of universal UC defined by the presence of endoscopically active disease proximal to the mid-transverse colon on at least one colonoscopy following the date of UC diagnosis.

Candidates were only included if they had at least 3 surveillance colonoscopies performed following 10 years of UC diagnosis and a minimum of an additional 10 years (total $\geqslant$ than 20 years of disease). Data recorded included gender, age at diagnosis, year of diagnosis, and disease duration. A Microsoft excel spreadsheet was constructed documenting each surveillance colonoscopy, recording the presence or absence of gross endoscopic disease, and the presence or absence of microscopic inflammation in those macroscopically normal in each colonic segment to include the cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum. No specific index system for histological inflammation was used since none was available during most of the study period. The slides had been read by the institutional pathologists who all had extensive experience with surveillance biopsies in ulcerative colitis. All cases of dysplasia were reviewed by a second pathologist specializing in gastrointestinal diseases. Each subject's follow-up continued until either colectomy, the finding of CC/HGD, or a duration of at least 10 additional years of surveillance after the first 10 years of disease up until

Table 1 Demographics, decades of surveillance, and probable influence of advances in drug therapy

Characteristics	Group 1 CA/HGD	Group 2 Non-CA/HGD
n = 68	20	48
Gender (% female)	50%	46%
Age at diagnosis (yr), range	27.3 (8-51)	25 (6-61)
Disease duration (yr), range	27.3 (12-54)	29.6 (16-48)
Treatment with 6 MP/IFX		
Subjects/decades of diagnosis of UC	Subjects received[1]	Subjects received[2]
1930-1949	1	0
1950-1959	1	0
1960-1969	7	5
1970-1979	9	17
1980-1989	2	16
1990-1999	0	10

[1]1970-1989, 6-mercaptopurine (6MP) = 55%, infliximab (IFX) = 0%; [2]1960-1999, 6MP = 79%; 1980-1999, IFX = 27%. CA: Carcinoma; HGD: High-grade dysplasia; UC: Ulcerative colitis.

the final documented surveillance examination. All colonoscopies were performed by the Senior Author alone or with Fellows in Gastroenterology.

Ethical considerations

This study received approval of the Institutional Review Board of Lenox Hill Hospital on September 15, 2009.

Statistical analysis

This is a retrospective cohort and descriptive study, of greater than 20 year duration, which was undertaken to assess the frequency and extent to which histological inflammation is present in the absence of gross endoscopic findings amongst patients with long-standing ulcerative colitis and whether this observation is more prevalent amongst patients who later develop colon cancer or high grade dysplasia. We report the observed prevalence of histological inflammation and its associated 95%CI in the total cohort and the two groups; the group which later developed colon cancer/dysplasia and the group which did not.

These important observations result from a study the strength of which is its long standing duration and the relative limited variation associated with a single practice. Obviously, its retrospective nature places the usual number of anticipated limitations so that the conclusions must be viewed with caution and taken to generate a hypothesis. Due to the long standing duration required to develop such a study, a prospectively controlled examination of this question in order to confirm these observations is not feasible.

RESULTS

Of 115 patients with longstanding UC reviewed, 68 patients met the inclusion criteria. 47 were excluded either for lack of the minimum of 20 years of surveillance or less than 3 documented colonoscopies during the second 10 years of ulcerative colitis. Patients were subsequently divided into two groups, group 1 which was comprised of 20 patients who developed CC and/or HGD, and group 2 comprised of 48 patients who did not. Demographic data are summarized in Table 1. Overall, groups 1 and 2 were similar in terms of gender, age of UC diagnosis and disease duration. More of the patients in group 2 were diagnosed and treated in recent decades than those in group 1.

Table 1 notes the decade during which the diagnosis of extensive ulcerative colitis was recognized and records the percentage of patients treated with immunosuppressives and/or biological during these decades.

Table 2 charts the number of surveillance colonoscopies done after 10 years of disease for Groups 1 and 2 and identifies the number of times for each when the endoscopic appearance was normal but biopsies nevertheless showed inflammation. The 20 patients in group 1 had 120 surveillance colonoscopies, range 3-14, median 4. Of the 48 patients of group 2, 550 surveillance colonoscopies were performed, range 3-28, median 8.5. Overall, histological disease activity in the absence of gross/endoscopic disease was found in 31.2% (95%CI: 28%-35%) of colonoscopies performed on the entire cohort of 68 patients. Histological disease activity when the colonoscopy showed an absence of gross disease activity was more common in group 1 than group 2 patients, 88% (95%CI: 72%-97%) *vs* 59% (95%CI: 53%-64%). Only 3/20 (15%) of patients in group 1 ever had a colonoscopy completely without demonstrated disease activity (*i.e.,* no endoscopic or histological activity) as compared to 37/48 (77%) of patients in group 2.

Among the 20 patients who developed CC/HGD, 17 (85%)(95%CI: 62%-98%) were found distal to the splenic flexure, including 11 (55%)(95%CI: 31%-37%) which developed in the rectum. In only 2 of the 20 cases (10%) (95%CI: 1%-32%) was carcinoma (CA)/HGD found isolated proximal to the descending colon. In no case was CA/HGD found in a colonic segment without prior histological inflammation. Table 3 shows the segments of the colon involved with histological inflammation when the colonic mucosa appeared normal and the degree of inflammation on a progressive scale of 1-5. The severity of inflammation was much more marked in group 1 than group 2. In these 20 patients of group 1 who did develop neoplasia, both the persistence of histological inflammation and its severity was most marked in the rectum and sigmoid where 12 of the cases of cancer (70.6%) and 10 with severe dysplasia were found. This finding was similar to that reported by Goldstone *et al*[14] In only 2 of the 20 patients was CC/HGD found isolated proximal to the descending colon and in no case was it found without there having been previous inflammation. The severity of inflammation was greater in all segments of group 1 than group 2. Features of the 20 patients with high grade dysplasia and/or colon cancer are shown in Tables 4 and 5.

Table 2 Summary of colonoscopic outcomes with and without neoplasia *n* (%)

Colonoscopic outcome	Group 1 (*n* = 20)	Group 2 (*n* = 48)	Total (*n* = 68)
Colonoscopies after 10 yr of UC	120	550	670
	Range 3-14 median 6	Range 3-28 median 11.5	
Colonoscopies with endoscopically active colitis	87 (72.5)	243 (44.2)	330 (49.2)
Colonoscopies without endoscopically active colitis but with histological inflammation[1]	29 (24.2)	180 (32.7)	209 (31.2)
Colonoscopies without gross/endoscopic or histological inflammation	4 (3.3)	127 (23.1)	131 (19.6)
% of endoscopically negative colonoscopies with histological inflammation[2]	29 (88)	180 (59)	

[1]Prevalence in all colonoscopies; [2]Prevalence in all colonoscopies. UC: Ulcerative colitis.

Table 3 Segments of colon showing histological inflammation and its degree when endoscopically normal

Case-index	Cecum	Asc colon	Trans colon	Desc colon	Sigmoid	Rectum
1N	2	2	2	2	2	2, 4, 5
2N	2, 4	2, 4	2	2, 4	2, 4	2, 4
3N	3	3	3	3	3	3, 4
4N	1	1	1	1	1, 5	1, 5
5N	3	3	3	3, 4	3, 5	3, 4, 5
6N	2	2	3, 5	3	3, 5	2
7N	1	1	1	1	2, 4, 5	2, 4
8N	2	2	3, 5	3	3, 5	2
9N	2	2	2	2	2, 4	2, 4
10N	3	3	3	3, 4	3	3
11N	3	3	3	3	3,5	3
12N	1	1, 5	1, 4, 5	2	2	2
13N	3, 5	3, 5	3	3	3	3
14N	1	2, 5	2, 4	2, 4	1, 4	1
15N	2	2	2	2, 5	2	2
16N	2	2	2	3	3, 4, 5	3, 5
17N	2	2	2	3	3	3, 5
18N	1	1	1	1	2, 4, 5	2
19N	3	3	3	3	3	3, 5
20N	3	3	3	3	3, 4, 5	3, 5
1	1	1	3	2	3	3
2	0	0	0	0	0	1
3	0	0	1	1	0	1
4	2	0	1	1	0	3
5	1	0	2	2	2	2
6	3	3	0	0	1	2
7	0	0	0	0	0	0
8	2	2	0	0	1	1
9	0	0	1	1	1	1
10	1	1	1	1	1	1
11	1	1	1	1	1	1
12	0	1	2	2	2	1
13	1	1	3	2	2	0
14	0	0	0	0	0	0
15	1	0	0	1	1	1
16	0	0	2	1	1	0
17	1	2	2	2	2	2
18	0	0	0	0	0	0
19	1	1	1	1	1	1
20	2	2	0	2	2	0
21	2	0	2	2	1	2
22	0	0	1	1	1	1
23	1	1	1	1	0	0
24	1	1	1	1	1	0
25	0	0	1	1	2	2
26	1	1	1	3	0	2
27	0	0	2	2	2	2
28	1	1	1	2	1	1
29	1	1	2	1	1	3
30	1	1	1	1	1	1
31	0	0	0	1	1	1
32	2	0	2	2	1	1
33	2	2	0	2	2	2
34	0	0	0	1	0	0
35	1	1	1	3	1	1
36	1	1	1	2	3	2
37	1	1	0	0	2	2
38	0	0	1	1	1	0
39	1	1	1	1	1	0
40	1	1	1	1	2	2
41	0	0	3	0	0	0
42	1	0	2	2	2	2
43	1	1	1	0	1	1

Group 1 = 1N→20N (29 colonoscopies); Group 2 = 1→43 (180 colonoscopies). Degree of inflammation: 0, none; 1, mild; 2, moderate; 3, severe; 4, dysplasia; 5, cancer.

DISCUSSION

Our study demonstrates an incidence of CC/HGD of almost 30% following average disease duration of over 27 years. While this finding in a tertiary care/IBD specialty practice may not reflect community norms, it is clearly in line with the incidence in prior observations[1,6]. While others have examined the risk of CC and dysplasia as a function of duration, extent and severity of inflammation, our goal was to examine whether the persistence of microscopic inflammation was itself a risk factor. We found that microscopic disease in the absence of macroscopic disease was a common finding on surveillance in the group who developed CC/HGD as well as the group who did not. However, a finding of both endoscopic and histological healing was a rare event in the CC/HGD group (3.3% of colonoscopies) *vs* the non-CC/HGD group (23% of colonoscopies), and that few patients in group 1 would ever demonstrate microscopic mucosal healing (15%), while a majority of those in group 2 would at some point during their follow up (77%). Furthermore, the severity of inflammation was much greater for all biopsied segments for group 1 than group 2. These findings add to those earlier observations by reinforcing the prognostic benefits of histological mucosal healing in addition to gross mucosal healing.

Additionally, we sought to determine the incidence of histological disease activity when gross mucosal healing was observed. Overall, we found that 31.2% (95%CI: 28%-35%) of all colonoscopies that demonstrated grossly normal appearing colonic mucosa also

Table 4 Features of 20 patients with high grade dysplasia or carcinoma of colon

Case	High grade dysplasia	Multiple sites of dysplasia	Low grade dysplasia	Colon cancer	Location of cancer
1	+	1	0	0	-[1]
2	+	1	0	0	-[1]
3	+	0	0	0	-[1]
4	+	0	0	+	Recto-sigmoid
5	+	1	0	+	Sigmoid
6	+	1	0	+	Sigmoid
7	+	1	0	+	Sigmoid
8	0	0	0	+	Distal transverse
9	0	1	1	+	Ileo-Anal pouch
10	+	1	0	+	Sigmoid
11	0	0	0	+	Sigmoid
12	0	0	0	+	Prox transverse
13	+	1	1	+	Cecum
14	0	0	1	+	Ascending
15	0	0	0	+	Descending
16	+	0	1	+	Recto-sigmoid
17	+	1	1	+	Rectum
18	0	0	0	+	Sigmoid
19	0	0	0	+	Rectum
20	+	0	0	+	Sigmoid
Totals	12	9	5	17	

[1]High grade dysplasia at multiple sites, not cancer.

Table 5 Features of the patients with carcinoma of colon

Features	Colectomies
Cancer discovered at endoscopy led to colectomy	8
Colectomy for high grade dysplasia also disclosed cancer	8
Colectomy for high grade dysplasia did not disclose cancer	3
Cancer discovered by metastases (no colectomy)	1
Most cancers in sigmoid and rectum	12/17
Multiple areas of dysplasia	6
Low grade as well as high grade dysplasia	6
Alive in 2012	9
High grade dysplasia at multiple sites, not cancer	3/20

demonstrated evidence of microscopic inflammation. To the experienced IBD gastroenterologist this finding will come as no surprise. The persistence of histological inflammation in ulcerative colitis without evident clinical activity or abnormal endoscopic appearance was reported over 50 years ago by Truelove and Richards[15], Dick and Grayson[16] and Matts[17]. Later Morson[18] and Dick *et al*[19] further popularized the value of rectal biopsies and Sommers *et al*[20] and Korelitz *et al*[21] introduced the technique of mucosal cell counts for evaluating persistence of inflammation and for response to specific drug therapy. Their findings were based mostly on finding an excess of chronic inflammatory cells including plasma cells and an apparent increase in polymorphonuclear leukocytes. Riley *et al*[22] reported the risk of relapse in UC when biopsies showed any acute inflammatory infiltrate histologicallyand Bitton *et al*[23] showed that the findings of plasmacytes on biopsy specifically increased the likelihood of relapse. Bessissow *et al*[24] suggested optimizing medical therapy when this finding is disclosed.

Discussion on the value of surveillance for ulcerative colitis provides a wide range of opinions. Higgins *et al*[25] and Dhanda *et al*[26] raise the option of eliminating colonoscopy entirely since it contributes little to the degree of ulcerative colitis activity beyond clinical activity as reported by the patient; we feel that this should pertain only to an index of activity but not to surveillance for dysplasia and cancer. Rutgeerts *et al*[27], Regueiro *et al*[9], and Pineton de Chambrun *et al*[13] emphasize the lack of correlation between clinical and endoscopic findings and support endoscopic healing for clinical trials but do not include histological healing as a component of mucosal healing for surveillance purposes. Baars *et al*[28] found an incidence of histological inflammation of 49% when the mucosa appeared endoscopically normal. Rutter *et al*[7], Gupta *et al*[8] and Mathy *et al*[29] have recognized the importance of histological inflammation in providing a risk factor for colon cancer in long-standing ulcerative colitis and propose the inclusion of microscopic inflammation in a grading system for risk stratification. Such histological grading scales have been proposed by Geboes *et al*[30] and Korelitz[31].

The most notable drawback to our own analysis is the discrepancy between the numbers of surveillance colonoscopies performed between the two groups, with a median number of examinations more than double in group 2 who did not develop HGD/CC. While more examinations decreased the chance of missing HGD/CC, it also increased the number of opportunities for the patients in the non-CC/HGD group to show histological mucosal healing. As such, it is likely that the percentage of individuals who had a colonoscopy with complete mucosal healing would have been less if this groups' surveillance frequency was closer to the HGD/CC group.

Also, our comparison of the two groups does not account for possible treatment differences. It is notable that most of the patients in the non-CC/HGD group were diagnosed and received treatment during an era of increasing use of immunosuppressive and then biologics to treat UC, suggesting a beneficial effect of such therapy. We have previously shown a trend toward a reduced risk for colon cancer in IBD patients treated with 6-mercaptopurine (MP)[32] but were unable to confirm this statistically though a more recent analysis of the CESAME cohort has shown a significant decrease in the incidence of colon cancer in patients with extensive colitis[33] treated with thiopurines. In the present study, we show that the decades of treatment with 6 MP alone coincide with reduced risk of neoplasia (6 MP used in 79% of group 2 *vs* 55% in group 1) and similarly with infliximab (27% in group 2 *vs* none in group 1). Though others have observed an association between CC and decade of disease diagnosis, the relationship of treatment with 5-amino salicylic acid preparations and CC/HGD rates in UC remains controversial, as the continuing pro and con debate surrounding their role in CC prevention bears out[34-37].

In conclusion, our findings clearly add to the argument in favor of defining mucosal healing not only by endoscopic findings, but by histological healing as well. Confirmation of gross mucosal healing has been advocated as an appropriate and objective measure of successful treatment in clinical trials[38,39], but this definition of mucosal healing remains controversial[10,11,39,40]. Our findings confirm previously reported high rates of CC/HGD in patients with longstanding extensive colitis. We show that progression to CC/HGD appears to be less common in those patients who demonstrate histological mucosal healing compared to those who persistently show microscopic disease activity. The endoscopist should acknowledge that endoscopic healing and histological healing are not synonymous and surveillance biopsies should be performed even when the endoscopic appearance is normal, and the results should be a part of patient counseling regarding the goals and expected outcomes in this high risk group.

COMMENTS

Background

Ulcerative colitis (UC) is one of the two major chronic inflammatory bowel diseases, almost always involving the rectum and any or all segments of the colon proximally in continuity. It is recognized that long-standing UC carries an increased risk for the development of colorectal carcinoma (CC) and high grade dysplasia (HGD), with estimates of risk as high as 20% following 30 years of diagnosis.

Research frontiers

This risk appears to be especially prominent in cases of universal UC, which has traditionally been defined by endoscopic evidence of disease proximal to the mid-transverse colon on at least one occasion following diagnosis. Chronic macroscopic disease activity has also been implicated as a risk factor for the development for CC. These observations have led to the practice of surveillance by colonoscopy in cases of universal UC. Current practice guidelines recommend that surveillance be performed every 1-2 years in these patients beginning 8-10 years after the initial UC diagnosis.

Innovations and breakthroughs

Progression to HGD or CC in extensive ulcerative colitis of long standing was more frequently encountered among those patients who demonstrate persistent histological inflammation in the absence of gross mucosal disease. Their findings support including the elimination of histological inflammation in the definition of mucosal healing, and support this endpoint as an appropriate goal of therapy because of its risk of increasing dysplasia and colon cancer.

Peer review

This is an interesting manuscript investigating the importance of histologic inflammation in the development of HGD/CRC in UC. The evidence presented here adds support to the fact that persistence of microscopic inflammation increases the risk of colon cancer in ulcerative colitis.

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BRIEF ARTICLE

NAFLD prevalence differs among hispanic subgroups: The multi-ethnic study of atherosclerosis

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Abstract

AIM: To compare prevalence rates of non-alcoholic fatty liver disease (NAFLD) between Hispanics of Mexican origin and Hispanics of Dominican and Puerto Rican origin.

METHODS: We evaluated prevalence rates of NAFLD between the two largest sub-populations of Hispanics in the United States; Hispanics of Mexican origin and Hispanics of Caribbean origin (Dominican and Puerto Rican), in the multi-ethnic study of atherosclerosis (MESA) cohort. MESA is a large, population based, multi-center cohort study comprised of 6814 healthy Caucasian, African-American, Hispanic, and Asian men and women aged 45-84. We utilized the baseline serum, anthropometric and radiographic measurements obtained between 2000 and 2002. NAFLD was measured *via* computed tomography scan and was defined as liver/spleen attenuation ratio < 1.

RESULTS: There were 788 Hispanic participants included in the study after exclusions. The prevalence of NAFLD was 29% (n = 225). Hispanics of Mexican origin had a significantly higher prevalence of NAFLD (33%), compared to Hispanics of Dominican origin (16%), (P < 0.01) and Hispanics of Puerto Rican origin (18%), (P < 0.01). After controlling for age, sex, BMI, waist circumference, hypertension, serum HDL, triglyceride and CRP level and insulin resistance, Hispanics of Mexican origin remained significantly more likely to have NAFLD than those of Dominican and Puerto Rican origin.

CONCLUSION: United States Hispanics of Mexican origin have a significantly higher prevalence of NAFLD when compared to United States Hispanics of Dominican or Puerto Rican origin after controlling for known risk factors. Care should be taken when performing risk assessment in Hispanic populations not to make assumptions of homogeneity.

Key words: Non-alcoholic fatty liver disease; Prevalence; Hispanic subpopulations

Core tip: Hispanics have a significantly higher prevalence of non-alcoholic fatty liver disease (NAFLD) and evidence of more advanced disease when compared to other ethnic groups. As a consequence it has been proposed that clinicians perform biopsies on Hispanics diagnosed with NAFLD given the increased of fibrosis development. Most of the studies examining Hispanics with NAFLD evaluated those of Mexican descent. It is unknown if this increased propensity to develop NAFLD is uniform among all people classified as Hispanics or if certain subpopulations are at higher risk. This study aims to compare the prevalence rates of NAFLD between US Hispanic subgroups.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common etiology of chronic liver disease in the United States as well as worldwide[1-4]. While the true prevalence of NAFLD is unknown, it is thought that up to 30% of the adult United States population may have steatosis due to NAFLD[1,5]. NAFLD is projected to be a major cause of liver-related morbidity and mortality over the next several decades in parallel with the obesity crisis with a prevalence predicted to increase 50% by the year 2030[6]. While not all individuals with NAFLD develop liver-related complications, it is clear that up to 30% will develop non-alcoholic steatohepatitis (NASH) and be at risk for fibrosis, cirrhosis, portal hypertension and hepatocellular carcinoma[7-11].

While central obesity, insulin resistance and dyslipidemia are well-established risk factors for the development of NAFLD, not all individuals with these risk factors develop hepatic steatosis[12]. There also appears to be racial-ethnic variations in the propensity to develop NAFLD with Hispanics being overrepresented in population studies when compared to blacks and whites[13-15]. Furthermore, when compared to blacks and whites, Hispanics with NASH appear to have more advanced histology on liver biopsy[16,17]. There is evidence that the increased prevalence of NAFLD in Hispanics does not appear to be solely attributable to an increased presence of metabolic risk factors associated with NAFLD such as insulin resistance and obesity[15,18] The mechanism by which Hispanics are at a higher risk to develop NAFLD and NASH is unclear but may be attributable to increased intraperitoneal fat distribution[18]. Genetic polymorphisms related to steatosis and hepatocyte injury may also explain such variation in the prevalence of NAFLD among Hispanics[19]. The single amino acid isoleucine to mehtionine polymorphism in the Patatin-like phospholipase domain containing 3 gene (PNPLA3) has been strongly associated with hepatic steatosis across multiple racial-ethnic groups and has a higher frequency among Hispanics when compared to European and African American individuals[20,21].

What is unclear is if the propensity to develop NAFLD is uniform among all subgroups that fall under the umbrella term Hispanic. The term Hispanic is non-specific in that it encompasses a culturally and genetically diverse collection of individuals, illustrated by the poor concordance between genetic based ancestry models and self-reported ethnicity among Hispanics[22-24]. Given such genetic variability and the observed differences in the prevalence of metabolic diseases among Hispanic subgroups, such as type 2 diabetes, we hypothesize that the tendency to develop NAFLD is not uniform among all Hispanics[25]. The aim of this study is to compare prevalence rates of NAFLD between the two largest US Hispanic subgroups: Hispanics of Mexican origin and Hispanics of Caribbean origin (Dominican and Puerto Rican) in the multi-ethnic study of atherosclerosis (MESA) database.

MATERIALS AND METHODS

Study population

The MESA is a large, population based, multi-center cohort study designed to describe risk factors related to the development and progression of subclinical coronary atherosclerotic heart disease. The cohort is comprised of 6814 Caucasian, African-American, Hispanic, and Asian men and women aged 45-84 who were clinically free from cardiovascular disease at baseline and followed for a period of eight years. Study participants were recruited from six communities (Los Angeles County, CA; Manhattan, New York, NY; Baltimore, MD; Chicago, IL; St. Paul, MN and Forsyth County, NC) in the United States. Internal Review Boards at each participating centers approved the studies and each participants gave written informed consent. Participants with active cancer, cognitive impairment, or weight greater than 300 pounds (136 kg) and pregnant individuals were excluded. This study has been described in detail elsewhere[26].

In addition to the MESA exclusion criteria, we additionally excluded for the following: significant alcohol consumption (defined as > 14 drinks on average per week in men and > 7 drinks on average per week in women), a history of Hepatitis C and the absence of a visible liver and/or spleen on cardiac CT (Figure 1). This study focused on comparing Hispanics of Mexican and Caribbean Origin (Dominican and Puerto Rican). Hispanics of Cuban Origin were excluded due to the small number of individuals with NAFLD; $n = 2$ out of total $n = 41$ total participants. Hispanics that were described as of Central American origin ($n = 46$), South American origin ($n = 66$) and "other" were excluded ($n = 12$) in an effort to maintain homogeneity.

Clinical parameters

We utilized the baseline serum, anthropometric and radiographic measurements obtained between 2000 and 2002 of the MESA study. Information on sociodemographic factors (age, sex, and education), lifestyle factors (alcohol consumption and smoking status) and self-reported medical history (hypertension, diabetes, liver disease and cirrhosis) were collected at the baseline examination using standardized questionnaires. A central laboratory (University of Vermont, Burlington) measured levels of total and HDL cholesterol, triglycerides, plasma glucose, and high-sensitivity C-reactive protein in blood samples obtained after a 12 h fast. Waist circumference

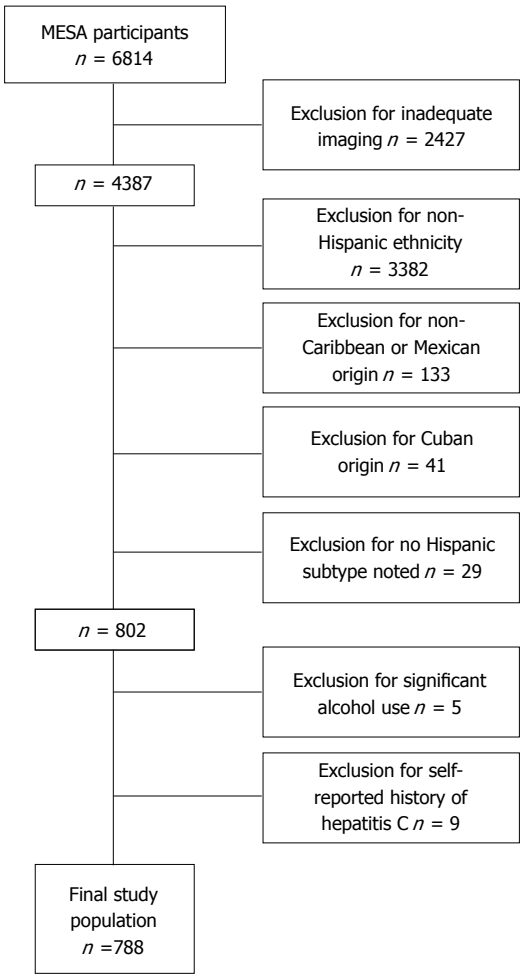

Figure 1 Multi-ethnic study of atherosclerosis participants: Exclusion criteria. Study exclusion criteria. Participants without adequate imaging for liver:spleen ratio calculations were excluded as were individuals with self-reported history of hepatitis C and significant alcohol consumption (> 14 drinks per week in men, > 7 drinks a week in women); Non-Hispanic and Hispanics not of Caribbean origin were excluded; Cubans, due to small number with non-alcoholic fatty liver disease (n = 2) were also excluded. MESA: Multi-ethnic study of atherosclerosis.

was measured horizontally at the level of the umbilicus. Hip girth was measured at the maximum circumference of the buttocks. Body mass index was calculated as weight in kilograms divided by height in meters squared. Homeostatic model assessment was used to measure insulin resistance (HOMA-IR) calculated as fasting glucose (mg/dL) × fasting insulin (μU/mL) /405[27].

NAFLD definition and radiographic method

All participants in the MESA study obtained non-contrast cardiac computed tomography (CT) scans at baseline that included areas of the upper abdomen. Attenuation coefficients in HU were measured in four areas of the liver and two areas of the spleen. Measurements for each area included the minimum, maximum and mean HU for a 2 cm round or ellipse region of interest (ROI) in the right and left lobes of the liver, as well as in the spleen. If sufficient tissue was not available for a 2 cm measure, a 1 cm measurement was obtained. If less than 1 cm of tissue was evident on the cardiac scan, the study was deemed not sufficient for measure. A final HU value for each liver was calculated by averaging the four ROI analyzed. Similarly a final HU value for the spleen was calculated by averaging the two ROI analyzed. A liver/spleen attenuation ratio (LS ratio) was then calculated comparing the final HU measurement between the liver and spleen on each CT scan. NAFLD was defined as a LS ratio of < 1. LS ratio < 1 has an area under the receiver operating curve (AUROC) of 0.991 when measuring hepatic steatosis > 30%, corresponding to moderate to severe steatosis on histology[28-31].

Statistical analysis

Results of summary outcome measures were reported as mean ± standard deviation and proportions. Differences between groups were tested using χ^2 analyses for categorical data and two sample Student's t test for continuous variables. Logistic regression was used to determine predictors of NAFLD using univariate and multivariate analysis. In our analytical models we included a core set of covariates which included age (as a continuous measure), gender and education (measured dichotomously; completed high school yes or no). We included the variables that were significant with a P value of 0.20 or smaller on univariate analysis into our multivariate model of the entire population. All analyses were performed using Stata version 11.0 (StataCorp LP, College Station, Texas).

RESULTS

Baseline characteristics of the study population

After applying our exclusion criteria there were a total of 788 participants available for analysis, baseline descriptive characteristics are reported in Table 1. The mean age (years) of participants was 61 ± 10 and 54% of participants were female. Of the final study population, 67% (n = 524) were of Mexican (MX) origin, 15 % (n = 121) of Dominican (D) origin and 18% (n = 143) of Puerto Rican (PR) origin. Diabetes was reported in 19% of the population. A total of 225 (29%) participants had radiographic NAFLD at baseline as defined by LS ratio < 1. Radiographic NAFLD was found in 34% of participants of Mexican origin (n = 179), 17% (n = 21) of Dominican origin and 17% (n = 25) of Puerto Rican origin (Figure 2).

There were several differences in the descriptive baseline variables between participants of Mexican and Dominican origin. When compared to participants of Dominican origin, Hispanics of Mexican origin were older, more likely to be male, have a higher BMI and have a larger waist circumference. There were also several physiologic and metabolic differences between these two groups. When compared to participants of Dominican origin, Hispanics of Mexican origin had lover HDL levels, higher triglyceride levels and HOMA insulin resistance scores. They were more likely to have metabolic syndrome but less likely to have hypertension (Table 2).

Participants of Puerto Rican and Mexican origin were similar with respect to descriptive baseline variables except that when compared to those of Puerto Rican ori-

Table 1 Descriptive characteristics of the study populations *n* (%)

Variable	Value (*n* = 788)
NAFLD	225 (29)
Age (yr)	61 ± 10.4
Male gender	361 (46)
Puerto Rican	143 (18)
Dominican	121 (15)
Mexican	524 (67)
BMI (kg/m^2)	29.6 ± 5.1
Waist circumference (cm)	100.9 ± 12.9
Hypertension	280 (36)
C-reactive protein (mg/dL)	4.3 ± 5.3
Total cholesterol (mg/dL)	198.5 ± 37.0
Triglyceride (mg/dL)	162.0 ± 103.0
LDL (mg/dL)	119.8 ± 33.0
HDL (mg/dL)	47.2 ± 13.0
Diabetes	148 (19)
HOMA-IR	2.2 ± 2.7
Metabolic syndrome	355 (45)
High school completion	416 (53)

Data are expressed as *n* (%) or mean ± SD. NAFLD: Non-alcoholic fatty liver disease; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

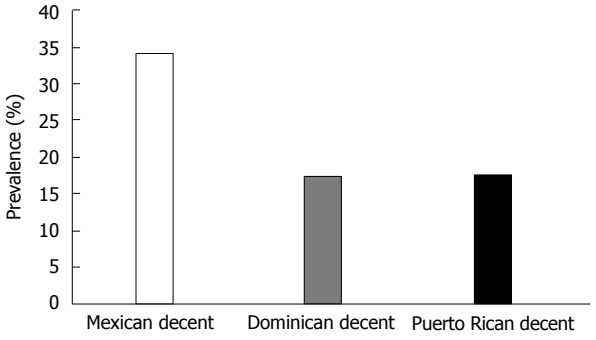

Figure 2 Prevalence of non-alcoholic fatty liver disease by Hispanic subgroup.

gin, Hispanics of Mexican origin had a lower rate of high school completion. There were several differences with respect to physiologic and metabolic parameters. When compared to participants of Puerto Rican origin, Hispanics of Mexican origin had higher triglyceride and HOMA insulin resistance levels, lower HDL levels and higher rates of metabolic syndrome (Table 3).

Hispanics of Dominican and Puerto Rican origin were similar with respect to baseline descriptive values except that participants of Dominican origin had lower BMI (kg/m^2) and smaller waist circumference (cm) than participants of Puerto Rican origin as well as lower rates of high school completion. With respect to physiologic and metabolic parameters, participants of Dominican and participants of Puerto Rican origin were similar except that participants of Puerto Rican origin had higher HOMA insulin resistance scores (Table 4).

Hispanic subgroup as a risk factor for NAFLD

Univariate analysis: Being a Hispanic of Dominican origin was associated with a significantly lower crude odds ratio for the presence of NAFLD when compared to being a Hispanic of Mexican origin. Similarly Hispanics of Puerto Rican origin had significantly lower crude odds ratio of NAFLD prevalence when compared to Hispanics of Mexican origin (Table 5).

We compared the predictors of NAFLD in individuals with NAFLD in each subgroup. There was no statistically significant difference in any predictor across groups (Table 6). When we compared the groups to each other, the only significant difference found was that Hispanics of Mexican origin with NAFLD had a significantly higher BMI than Hispanics of Dominican origin with

Table 2 Baseline demographic, anthropomorphic and physiologic characteristics of the study population: Hispanics of Mexican and Hispanics of Dominican origin *n* (%)

Variable	Mexican (*n* = 524)	Dominican (*n* = 121)	*P* value
NAFLD	179 (34)	21 (17)	< 0.001
Age (yr)	62 ± 10.3	59 ± 10.7	0.005
Male gender	256 (49)	45 (37)	0.02
BMI (kg/m^2)	30.1 ± 5.1	27.8 ± 4.6	< 0.001
Waist circumference (cm)	102.0 ± 12.4	96.5 ± 12.8	< 0.001
Hypertension	172 (33)	54 (45)	0.01
C-reactive protein (mg/dL)	4.5 ± 5.7	3.8 ± 5.6	0.25
Total cholesterol (mg/dL)	198.7 ± 37.8	197.9 ± 34.6	0.84
Triglyceride (mg/dL)	176.7 ± 113.9	124.8 ± 61.3	< 0.001
LDL (mg/dL)	118.4 ± 32.5	124.4 ± 34.1	0.07
HDL (mg/dL)	46.1 ± 12.4	48.8 ± 12.7	0.03
Diabetes	108 (21)	15 (12)	0.10
HOMA insulin resistance	2.4 ± 3.2	1.5 ± 1.0	0.002
Metabolic syndrome	266 (51)	36 (30)	< 0.001
High school completion	266 (51)	59 (49)	0.69

Data are expressed as *n* (%) or mean ± SD. NAFLD: Non-alcoholic fatty liver disease; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

Table 3 Baseline demographic, anthropomorphic and physiologic characteristics of the study population: Hispanics of Mexican and Hispanics of Puerto Rican origin *n* (%)

Variable	Mexican (*n* = 524)	Puerto Rican (*n* = 143)	*P* value
NAFLD	179 (34)	25 (17)	< 0.001
Age (yr)	62 ± 10.3	60 ± 10.5	0.07
Male gender	256 (49)	60 (42)	0.14
BMI (kg/m^2)	30.1 ± 51	29.5 ± 5.2	0.29
Waist circumference (cm)	102.0 ± 12.4	100.3±14.0	0.16
Hypertension	172 (33)	54 (38)	0.27
C-reactive protein (mg/dL)	4.5 ± 5.7	4.2 ± 3.6	0.52
Total cholesterol (mg/dL)	198.7 ± 37.8	198.2 ± 36.6	0.89
Triglyceride (mg/dL)	176.7 ± 113.9	139.5 ± 73.1	< 0.001
LDL (mg/dL)	118.4 ± 32.5	121.0 ± 33.7	0.40
HDL (mg/dL)	46.1 ± 12.4	49.6 ± 14.6	0.01
Diabetes	108 (21)	25 (17)	0.61
HOMA	2.4 ± 3.2	1.9 ± 1.5	0.05
Metabolic syndrome	266 (51)	53 (37)	0.003
High school completion	266 (51)	91 (64)	0.01

Data are expressed as *n* (%) or mean ± SD. NAFLD: Non-alcoholic fatty liver disease; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

Table 4 Baseline demographic, anthropomorphic and physiologic characteristics of the study population: Hispanics of Puerto Rican and Hispanics of Dominican origin *n* (%)

Variable	Dominican (*n* = 121)	Puerto Rican (*n* = 143)	*P* value
NAFLD	21 (17)	25 (17)	0.98
Age (yr)	59 ± 10.7	60 ± 10.5	0.35
Male gender	60 (42)	45 (37)	0.43
BMI (kg/m^2)	27.8 ± 4.6	29.5 ± 5.2	0.006
Waist circumference (cm)	96.5 ± 12.8	100.3 ± 14.0	0.02
Hypertension	54(38)	54 (45)	0.25
C-reactive protein (mg/dL)	3.8 ± 5.6	4.2 ± 3.6	0.56
Total cholesterol (mg/dL)	197.9 ± 34.6	198.2 ± 36.5	0.95
Triglyceride (mg/dL)	124.8 ± 61.3	139.5 ± 73.1	0.08
LDL (mg/dL)	124.4 ± 34.1	121.0 ± 33.7	0.42
HDL (mg/dL)	48.8 ± 12.7	49.6±14.6	0.67
Diabetes	15 (12)	25 (17)	0.25
HOMA	1.5 ± 1.0	1.9 ± 1.5	0.02
Metabolic syndrome	36 (30)	53 (37)	0.21
High school completion	59 (49)	91 (64)	0.02

Data are expressed as *n* (%) or mean ± SD. NAFLD: Non-alcoholic fatty liver disease; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

NAFLD ($P = 0.05$) and Hispanics of Puerto Rican origin with NAFLD had significantly more individuals with hypertension than those of Mexican origin ($P = 0.04$) with NAFLD.

Multivariate analysis: Hispanics of Dominican and Puerto Rican origin had a significantly lower risk for the prevalence of NAFLD compared to Hispanics of Mexican origin on multivariate analysis after controlling for age, sex, BMI, waist circumference, hypertension, level of education, serum HDL, triglyceride and CRP levels and HOMA. Adjusted odds ratios can be seen in Table 5.

DISCUSSION

While several studies have demonstrated a higher frequency of NAFLD among Hispanic individuals, little is known about the distribution of NAFLD among various Hispanic subgroups. Given the genetic and cultural variance among Hispanics, illustrated by poor concordance between genetic ancestry markers and self-reported ethnicity, we hypothesized that the distribution of NAFLD would not be uniform among Hispanic subgroups. Here we demonstrate a significant difference in the frequency of moderate to severe steatosis between the two largest United States subgroups of Hispanics (those of Mexican *vs* Caribbean origin) utilizing a large cohort database.

Hispanics of Mexican origin showed a higher prevalence of NAFLD when compared to both Hispanics of Dominican and Puerto Rican origin, while there was no significant difference in the frequency of NAFLD between Hispanics of Dominican and Puerto Rican origin. At baseline, Hispanics of Mexican origin were more similar to Hispanics of Puerto Rican origin than those of Dominican origin with respect to demographic, metabolic and anthropomorphic features. Despite these similarities, Hispanics of Mexican origin had a much higher frequency of NAFLD than Hispanics of Puerto Rican origin in our study suggesting perhaps that additional factors other than the presence of the traditional risk factors for NAFLD is related to the development of steatosis in Mexican individuals. Although Hispanics of Mexican origin were more likely to be diabetic and have metabolic syndrome than Hispanics of Dominican origin, Hispanics of Mexican origin were still more likely to have NAFLD when these traditional NAFLD risk factors were controlled for on multivariate analysis, again suggesting other factors at play perhaps genetic increasing the predisposition of Hispanics of Mexican origin to develop NAFLD compared to other Hispanic groups. PNPLA3 has been shown to account for up to 72% of the ethnic differences in the prevalence of NAFLD[32]. Further studies are necessary to see if this explains the differences in prevalence we have found among Hispanic subtypes living in the US.

Our study does have some limitations. This study is a cross-sectional analysis of a prospective cohort population and causality cannot be determined. In our study, NAFLD was defined indirectly by using radiographic methods. Although there is a strong concordance between moderate to severe steatosis (> 30%) and LS ratios, the gold standard is liver biopsy and less severe steatosis may have been present in individuals but not seen by radiographic methods. Additionally, due to the lack of histologic data, we cannot comment on the prevalence of NASH in Hispanics of Caribbean origin which has been shown to be more severe in Hispanics of Mexican origin[16,17]. Individuals with a weight greater than 300 lbs were excluded from the MESA database and we were not able to assess the prevalence rates of NAFLD in very obese individuals based on Hispanic subgroups.

As far as we know, this is the first study to compare the frequency of NAFLD between the major Hispanics subgroups living the United States. The majority of studies describing NAFLD in Hispanic populations were performed predominantly on Hispanics of Mexican origin. Given that the current American Association for the Study of Liver Disease guidelines recommend liver biopsy in individuals with NAFLD deemed to be at high risk of NASH and fibrosis, and count a Hispanic ethnic background as a risk factor, it is important to determine which specific Hispanic individuals are in fact are at high risk for NAFLD. Such risk stratification could significantly decrease the number of invasive procedures performed. Our findings help clinicians to better determine and ascribe the risk of NAFLD to the Hispanic patients they see based on region of origin. Further studies are needed to explore if there are similar differences in the prevalence of NAFLD between other Hispanics subgroups not included in this study as well as determine what genetic factors are contributing to these differences seen. For now, care should be taken in generalizing studies related to NAFLD that involves Hispanics, especially

Table 5 Crude and adjusted odds ratios for non-alcoholic fatty liver disease by Hispanic subgroup with Hispanics of Mexican origin as the reference population

Hispanic subgroup	Total (*n*)	With NAFLD (*n*)	Crude			Adjusted		
			OR	95%CI	*P* value	OR	95%CI	*P* value
Mexican	524	179	1 (ref)			1 (ref)		
Dominican	121	21	0.40	0.24-0.67	< 0.001	0.49	0.28-0.86	0.01
Puerto Rican	143	25	0.41	0.26-0.65	< 0.001	0.44	0.29-0.86	0.002

Multivariate regression analysis was performed, controlling for age, sex, BMI, waist circumference, hypertension, level of education (completion of high school), serum HDL levels, serum triglycerides levels, serum C-reactive protein levels and homeostasis model assessment of insulin sensitivity. NAFLD: Non-alcoholic fatty liver disease.

Table 6 Comparison of predictors in Hispanic subgroups with non-alcoholic fatty liver disease *n* (%)

Variable	Mexican (*n* = 179)	Dominican (*n* = 21)	Puerto Rican (*n* = 25)	*P* value
Age (yr)	60 ± 9.9	62 ± 9.9	58 ± 10.5	0.43
Male gender	86 (48)	7 (33)	11 (44)	0.43
BMI (kg/m^2)	32.0 ± 5.4	29.5 ± 5.0	32.2 ± 5.4	
Waist circumference (cm)	105.7 ± 12.5	103.7 ± 14.0	107.0 ± 13.9	0.67
Hypertension	68 (38)	11 (52)	15 (60)	0.07
C-reactive protein (mg/dL)	5.1 ± 5.3	7.1 ± 8.2	5.9 ± 4.2	0.24
Total cholesterol (mg/dL)	196.8 ± 41.4	190.0 ± 31.0	197.3 ± 31.6	0.74
Triglyceride (mg/dL)	202.4 ± 140.6	138.6 ± 54.8	173.5 ± 106.7	0.08
LDL (mg/dL)	114.1 ± 31.1	117.6 ± 26.4	120.0 ± 108.7	0.62
HDL (mg/dL)	43.3 ± 11.4	44.7 ± 9.5	44.4 ± 9.6	0.80
Diabetes	45 (25)	3 (14)	7 (28)	0.80
HOMA	3.1 ± 2.1	2.3 ± 1.4	2.6 ± 1.5	0.14
Metabolic syndrome	117 (66)	12 (57)	16 (64)	0.74
High school completion	87 (49)	8 (38)	16 (64)	0.20

Data are expressed as *n* (%) or mean ± SD. NAFLD: Non-alcoholic fatty liver disease; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

of Mexican origin, to all groups that fall under the umbrella term Hispanic.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is the most common etiology of chronic liver disease and is projected to soon be the leading cause of liver transplants. Hispanics are significantly more likely to have NAFLD when compared to other ethnic groups. Hispanics with NAFLD are also more likely to have advanced disease steatosis and fibrosis on histology compared to other ethnic groups. As a result, it is recommended that liver biopsy should be obtained in Hispanic patients found to have NAFLD according to American Association for the Study of Liver Diseases guidelines. The term "Hispanic" applies to a very diverse group of people and it is unclear if this increased risk and therefore the need for liver biopsy is uniform.

Research frontiers

NAFLD is the leading cause of chronic liver disease. A research hotspot in this field is being able to better define which individuals are at risk.

Innovations and breakthroughs

The majority of studies evaluating NAFLD in United States Hispanics are performed on Mexican Americans. This is the first study to compare Hispanic subgroups in the United States.

Applications

Currently guidelines recommend liver biopsy in those individuals deemed high risk for non-alcoholic steatohepatitis. Their findings allow for appropriate risk stratification among a culturally and genetically diverse group of people.

Terminology

According to the US census bureau The term "Hispanic" is used to describe people who classify themselves as being of "Mexican, Mexican Am., Chicano" or "Puerto Rican" or "Cuban" - as well as those who indicate that they are "another Hispanic, Latino, or Spanish origin". They further state that origin can be viewed as the heritage, nationality group, lineage, or country of birth of the person or the person's ancestors before their arrival in the United States and people who identify their origin as Hispanic, Latino, or Spanish may be of any race.

Peer review

This is a cross-sectional observational study to compare prevalence rates of NAFLD between the two largest subpopulations of Hispanics. NAFLD was diagnosed by computed tomography. The results of this study show that Hispanics of Mexican origin had a significantly higher prevalence of NAFLD than Dominican or Puerto Rican origin.

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BRIEF ARTICLE

Clinical and histopathological correlations of fecal calprotectin release in colorectal carcinoma

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Abstract

AIM: To determine calprotectin release before and after colorectal cancer operation and compare it to tumor and histopathological parameters.

METHODS: The study was performed on patients with diagnosed colorectal cancer admitted for operation. Calprotectin was measured in a single stool sample before and three months after the operation using an enzyme-linked immunosorbent assay (ELISA). Calprotectin levels greater than or equal to 50 μg/g were considered positive. The compliance for collecting stool samples was assessed and the value of calprotectin was correlated to tumor and histopathological parameters of intra- and peri-tumoral inflammation. Surgical specimens were fixed in neutral buffered formalin and stained with hematoxylin and eosin. Staging was performed according to the Dukes classification system and the 7th edition tumor node metastasis classification system. Intra- and peri-tumoral inflammation was graded according to the Klintrup criteria. Immunohistochemical quantification was performed for MPO, CD45R0, TIA-1, CD3, CD4, CD8, CD57, and granzyme B. Statistical significance was measured using Wilcoxon signed rank test, Kruskal Wallis test and Spearman's rank correlation coefficient as appropriate.

RESULTS: Between March 2009 and May 2011, 80 patients with colorectal cancer (46 men and 34 women, with mean age of 71 ± 11.7 years old) were enrolled in the study. Twenty-six patients had rectal carcinoma, 29 had left-side tumors, 23 had right-side tumors, and 2 had bilateral carcinoma. In total, 71.2% of the patients had increased levels of calprotectin before the operation (median 205 μg/g, range 50-2405 μg/g) and experienced a significant decrease three months after

the operation (46 μg/g, range 10-384 μg/g, $P < 0001$). The compliance for collecting stool samples was 89.5%. Patients with T3 and T4 tumors had significantly higher values than those with T1 and T2 cancers ($P = 0.022$). For all other tumor parameters (N, M, G, L, V, Pn) and location, no significant difference in calprotectin concentration was found. Furthermore, the calprotectin levels and histological grading of both peri- and intra-tumoral inflammation was not correlated. Additional testing with specific markers for lymphocytes and neutrophils also revealed no statistically significant correlation.

CONCLUSION: Fecal calprotectin decreases significantly after colorectal cancer operation. Its value depends exclusively on the individual T-stage, but not on other tumor or histopathological parameters.

Key words: Calprotectin; Colorectal cancer; Inflammation; Tumor size; Granulocytes

Core tip: Colorectal cancer (CRC) patients have a significant increase of fecal calprotectin release. The mechanisms for this observation are unclear. In our study, we examined the calprotectin release before and after operation of 46 CRC patients. This is the first study that assessed the correlation of calprotectin with both tumor as well as histopathological parameters. Our study contains the following new information: (1) the release of calprotectin is exclusively correlated to the T-stage, but to no histopathological parameters; and (2) except the T-stage, all other tumor characteristics assessed by the seventh edition of the tumor node metastasis classification are not correlated.

Lehmann FS, Trapani F, Fueglistaler I, Terracciano LM, von Flüe M, Cathomas G, Zettl A, Benkert P, Oertli D, Beglinger C. Clinical and histopathological correlations of fecal calprotectin release in colorectal carcinoma. *World J Gastroenterol* 2014; 20(17): 4994-4999 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/4994.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.4994

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy in the world and accounts for more than 10% of all cancer deaths[1,2]. Recent studies have shown that stool parameters such as calprotectin and lactoferrin are increased in many CRC patients[3-8]. The increase in calprotectin in CRC patients is, however, highly variable with levels ranging from insignificant to 100% sensitivity[9]. In fact, a meta-analysis by von Roon *et al*[10] revealed the increase of calprotectin in CRC patients was not recommended as screening tool for CRC. Calprotectin is a small calcium-binding protein consisting of two heavy and one light polypeptide chains. It is found in abundance in neutrophilic granulocytes, where it accounts for 60% of the cytosolic fraction, as well as in monocytes and macrophages[11,12].

CRC is associated with a local acute inflammatory reaction of variable intensity. The recruitment of neutrophils to the tumor site is hypothesized to be due to the local release of chemotactic factors[4,6,7]. Calprotectin enters the bowel lumen by migration rather than by bleeding or shedding of tumor cells. The neutrophilic infiltrate is variable and might be related to the tumor size, suggesting calprotectin would be a less sensitive marker in smaller tumors[13].

To date, no correlation of calprotectin and tumor parameters including tumor localization, size or stages has been found, as assessed by the Dukes classification or older TNM classifications[6,7]. In our study, we used the seventh edition of the TNM classification introducing additional components (G, L, V and Pn)[14]. In addition, our study contains the first systematic assessment of different histopathological markers to examine the correlation of calprotectin and parameters of peri- as well as intratumoral inflammation. We measured fecal calprotectin concentrations in patients with proven CRC before and after operation and correlated the results to tumor and histopathological parameters. We hypothesized that increased calprotectin levels were related to the T-stage, as well as to the grading of peri- and intratumoral inflammation and specific neutrophil markers.

MATERIALS AND METHODS

Patients

Eighty patients with proven CRC, admitted for treatment to one of the following hospitals: University Hospital of Basel, St. Claraspital Basel and the Bruderholzspital, Switzerland, were included. The study was carried out according to the Principles of the Declaration of Helsinki and the protocol was accepted by the local ethical committee. All patients gave written informed consent before participating in any protocol-specific procedures.

Measurement of fecal calprotectin

Calprotectin was measured in a single stool sample from each patient collected in the hospital 24 h prior to the operation and 3 mo after hospital discharge. Samples were stored at 4 ℃ before transfer to the laboratory (Viollier Laboratories, Basel, Switzerland) within 48 h for analysis. Calprotectin is stable up to seven days at room temperature[3].

Fecal calprotectin levels were determined using an enzyme-linked immunosorbent assay (ELISA) (Viollier Laboratories, Basel, Switzerland). Aliquots of approximately 100 mg feces were homogenized in 5 mL extraction buffer. Two mL of the homogenate was centrifuged for 5 min at 3000 *g* and 100 μL of the diluted supernatant (1:50 with incubation buffer) were incubated at room temperature onto a microtiter plate coated with a monoclonal capture antibody highly specific to the calprotectin heterodimeric and polymeric complexes. After

Table 1 Antibodies used for immunohistochemistry

Antibody	Clone	Dilution	Technique
CD 45R0 (DAKO)	UHCL-1	1:1600	ABC
TIA-1 (IMMUNOTECH)	2G9A10F5	1:1000	ABC
CD3 (VENTANA)	2GV6	Pre-diluted	Benchmark XT
CD4 (VENTANA)	SP-35	Pre-diluted	Benchmark XT
CD8 (VENTANA)	SP-57	Pre-diluted	Benchmark XT
CD57 (VENTANA)	NK-1	Pre-diluted	Benchmark XT
Granzyme B (VENTANA)	Polyclonal	Pre-diluted	Benchmark XT

incubation, washing, a second incubation with a specific detection antibody, and a further washing step, tetramethylbenzidine (blue color formation) followed by a stop solution (change to yellow color) were added. The absorption was determined at an optical density of 450 nm. The measuring range of the test was 10-600 μg calprotectin/g feces with an intra- and inter-assay coefficient of 4.7% and 4.1%, respectively. Calprotectin levels greater than or equal to 50 μg/g were considered positive. All fecal samples were processed within 72 h after collection. The laboratory personnel carrying out the analysis was blinded to the clinical history of the patients.

Pathology

Surgical specimens were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin. Staging was performed according to the Dukes classification and the 7th edition of the TNM classification by the Union for International Cancer Control[14]. Blinded senior pathologists examined all specimens.

Histopathological assessment

Peri- and intratumoral inflammation were graded from 1-3 according to the Klintrup criteria as used by Richards *et al*[15]. Immunohistochemical quantification (score 0-3) was performed for MPO, CD45R0, TIA-1, CD3, CD4, CD8, CD57 and granzyme B. Grading and immunohistochemistry were performed in 49 patients. Sections (4 μm) of paraffin embedded tissue were immunostained for the antibody (Table 1). Staining was carried out according to the manufacturer's protocol (Table 1). Negative controls for all proteins consisted of omission of the primary antibody. Three microscopic images (× 40) from each sample were obtained as representative of tissue type, distinct from lymphoid aggregation and within the area of most positive staining. The number of positive cells was counted in tumor and stromal tissue to give a score of inflammatory cellular infiltrate.

Immunostaining was performed as described previously[16,17]. Briefly, after dewaxing and rehydration in dH_2O, sections for immunostaining were subjected to heat antigen retrieval in a microwave oven (1200 W, 60 min) in 0.01 mol/L citrate buffer pH 7.0 for TIA-1. Endogenous peroxidase activity was blocked using 0.5% H_2O_2. After transfer to a humidified chamber, the sections were incubated with 10% normal goat serum (Dako Cytomation) for 20 min and incubated with primary antibody overnight at 4 ℃ (CD45RO and TIA-1) Sections were then incubated with peroxidase-labeled polymer {K4005, EnVision + System-Horseradish Peroxidase (HRP) [3-amino-9-ethylcarbazole (AEC)]; DakoCytomation} for 30 min at room temperature.

For visualization of the antigen, sections were immersed in AEC + substrate-chromogen [K4005, EnVision + System-HRP (AEC); DakoCytomation] for 30 min and lightly counterstained with Harris's hematoxylin), Ventana BenchMark XT system was used for immunohistochemical analysis.

Statistical analysis

The proportion of patients with pathological calprotectin concentrations was estimated together with the 95%CI. Pre- and post-operative calprotectin concentrations were compared by a Wilcoxon signed rank test. For various descriptors, calprotectin concentrations between patients with different factor levels were compared using Kruskal-Wallis test. The correlation between calprotectin concentration and various histopathological variables was assessed graphically as well as based on Spearman's rank correlation coefficient. All analyses were performed with R (version 2.13.2). A two-sided P value < 0.05 was considered significant.

RESULTS

Patients

Eighty patients with proven CRC (46 men, 34 women, 71 +/- 11.7 years old) were included in the final analysis. A second calprotectin level 3 mo after the operation was only determined if the first concentration was > 50 μg/g. A second assay was not possible in nine patients, six denied a second test, one was not operated on and two could not be asked for ethical reasons. The compliance for collecting stool samples was 89.5%. Baseline characteristics are shown in Table 2.

Calprotectin before and after operation

In 57 of 80 patients (71.2%, 95%CI: 60.1%-80.3%), calprotectin was significantly increased (> 50 μg/g). The median fecal calprotectin concentration was 205 μg/g (range 50-2405 μg/g) before and 46 μg/g (range 10-384 μg/g) three months after the operation (P < 0.001, Figure 1).

Correlation of calprotectin and tumor parameters

Twenty-six patients had rectal carcinoma, 29 had tumors of the left side, 23 had right-side tumors and two had a double carcinoma. No significant difference in calprotectin concentration was found between the three locations. Patients with T3 and T4 tumors had significantly higher calprotectin values than those with T1 and T2 stages (P = 0.022). For all other tumor parameters (N, M, G, L, V, Pn), no significant difference in calprotectin concentration was found between the factor levels of the individual parameters. Further, no difference in calprotectin release was found between Dukes B (n = 39) and Dukes

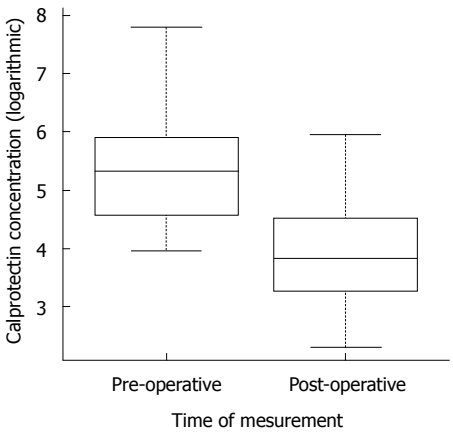

Figure 1 Boxplot of pre- and post-operative calprotectin concentration (log-transformed) for 46 patients. Post-operative calprotectin concentration was only determined if the pre-operative value was > 50 μg/g.

Table 2 Baseline characteristics *n* (%)

Characteristics		Statistics
Calprotectin		(*n* = 80)
Demographic data		
	Males	46 (57.5)
	Age (yr)	71.1 ± 11.7
Tumor location		
	Left	29 (36.2)
	Right	23 (28.7)
	Rectum	26 (32.5)
	Two locations	2 (2.5)
Tumor classification		
	T1	5 (6.2)
	T2	12 (15.0)
	T3	49 (61.3)
	T4	13 (16.2)
	N0	44 (55.0)
	N1	16 (20.0)
	N2	18 (22.5)
	M0	71 (88.8)
	M1	8 (10.0)
	G1	1 (1.2)
	G2	57 (71.2)
	G2-3	3 (3.8)
	G3	13 (16.2)
	V0	62 (77.5)
	V1	14 (17.5)
	Pn0	64 (80.0)
	Pn1	12 (15.0)

Data are presented as number of patients (%) and as mean ± SD.

C (*n* = 29) stages (*P* = 0.132).

Correlation of calprotectin and histopathological parameters

Peri- and intratumoral inflammation was graded from 1 - 3 according to the Klintrup criteria as used by Richards *et al*[15]. Calprotectin levels and histological grading of both peri- and intratumoral inflammation were not correlated. Additional testing with specific markers for lymphocytes and neutrophils such as CD3, CD4, CD8, CD45, TIA-1, granzyme B and myeloperoxidase also revealed no statistically significant correlation (Table 3).

Table 3 Spearman's rank correlation coefficient between calprotectin and histopathological parameters

	Spearman's rho	*P* value
CD45.R.intra	-0.19	0.18
CD45.peri	0.05	0.74
CD3.intra	0.03	0.85
CD3.peri	-0.1	0.47
CD8.intra	-0.04	0.76
CD8.peri	-0.14	0.31
CD4.intra	-0.07	0.64
CD4.peri	-0.06	0.66
TIA1.itra	0.06	0.66
TIR1.peri	0.05	0.71
Granzyma.intra	-0.08	0.57
Granzyma.peri	0.09	0.52
CD57.intra	0.02	0.91
CD57.peri	-0.14	0.31
MPO.intra	0.12	0.4
MPO.peri	0.09	0.55

DISCUSSION

We examined the calprotectin release before and after operation of 46 colorectal cancer patients. This is the first study that assessed the correlation of calprotectin with tumor and histopathological parameters. We found the release of calprotectin is exclusively correlated to the T-stage, but not to histopathological parameters. Further, we found all tumor characteristics assessed by the 7th edition of the TNM classification, with the exception of the T-stage, are not correlated with calprotectin.

Our results are in line with most previous studies showing significantly increased levels of fecal calprotectin in CRC patients[5-7]. While patients with active Crohn's disease (CD) or ulcerative colitis (UC) exhibit more consistent elevated calprotectin levels, those from CRC patients are highly variable. In CRC, the sensitivity of calprotectin varies between 100%[9] and not significant[10], indicating it is not a suitable tool for CRC screening.

The significant fall in fecal calprotectin after surgical tumor removal was first described by the study group of Kristinsson *et al*[5] and Johne *et al*[18], although their participant numbers were smaller than in our study. Interestingly, that there is no corresponding decrease of elevated calprotectin after polypectomy[19].

We found T3 and T4 cancers significantly induce higher levels of calprotectin. This could be explained by the hypothesis that they attract more neutrophils than T1 and T2 tumors[13]. A correlation of fecal calprotectin and tumor size or T-stage has not been shown[7], with Kristinsson *et al*[5] providing the only indication that T1 and T2 tumors may be associated with lower calprotectin concentrations than T3 and T4 cancers. The lack of correlation between calprotectin and tumor localization, grading, as well as clinical stages in our patients is in line with findings from previous studies[5-7]. In our analysis, tumor characteristics have been assessed for the first time

by the 7th edition of the TNM classification[14]. Previous studies used either Dukes or older TNM classifications. Our data revealed no difference in calprotectin release between Dukes B (n = 39) and Dukes C (n = 29) stages (P = 0.132). It would be of interest to correlate the calprotectin release with additional variables such as ESR, plasma CRP, blood platelets, LDH, as well as patient outcomes data (survival, time-to recurrence). However, these analyses were beyond the scope of this study.

Our study contains the first systematic assessment of different histopathological markers to examine the correlation of calprotectin and parameters of peri- as well as intratumoral inflammation. Various inflammatory cells, mainly along the invasive margin, infiltrate human CRC tissue. In colorectal tumors, calprotectin reactivity is found in granulocytes and macrophages, but not in neoplastic cells[4]. Increased amounts of granulocytes have been described in the stool of patients with CRC, possibly due to shedding from the ulcerated tumor[20,21]. It has been postulated that circulating leukocytes may actively migrate through neoplastic tissues in response to intraluminal antigens[4]. Interestingly, the immunohistochemical expression of calprotectin correlates with the degree of neutrophilic infiltration[22]. However, these studies are hampered by lack of a specific tissue marker. Kim *et al*[23] did show significant expression of the two calprotectin subunits S100A8 and S100A9 in tumor infiltrating lymphocytes.

In UC, calprotectin correlates significantly with clinical, endoscopic and histological parameters of disease activity[3,24,25]. The level of calprotectin seems to correlate more closely with the grading of histological than of endoscopic findings[26]. The concentration of calprotectin is directly proportional to the intensity of the neutrophilic infiltrate in the gut mucosa[26]. Active UC is characterized by a 10-fold or more increased migration of neutrophils from the circulation to the inflamed colon mucosa. Røseth *et al*[24] demonstrated the microscopic inflammation was graded 0 (normal mucosa) to 3 (extensive crypt injury with crypt abscesses and ulcerations). The correlation of histological grading and calprotectin concentration was statistically significant (P < 0001). In our study, the Klintrup score was used for histological grading of peri- and intratumoral inflammation. Several clinical studies have clearly shown that the grading of local inflammation as assessed by the Klintrup criteria is an independent predictor of survival in colon and rectal cancers[15,27,28]. In contrast to the findings in patients with UC, grading of tumor-associated inflammation was not correlated with calprotectin concentrations. The lack of correlation applies to the individual markers for lymphocytes and neutrophils as well. There are several explanations for this obvious discrepancy: (1) peri- and intratumoral inflammation are local. This is also expressed by the lower calprotectin concentration in CRC in comparison to active IBD[10]; (2) the local inflammatory reaction in CRC is of variable intensity; and (3) tumor-associated inflammation is not uniformly characterized by a significant amount of neutrophils[9]. This is in line with the observation that leukocyte scintigraphy is only sometimes positive in CRC patients[21].

In a current review of Gisbert *et al*[29], it has been questioned whether the need to collect one or several fecal samples might be a disadvantage for clinical use of calprotectin. This could not be confirmed in our study. The compliance rate in our study was 89.5% with only 6 out of 57 patients with increased calprotectin denying a second stool test. This is in the same range as the compliance rate of 96% in 602 patients referred for colonoscopy described by Tibble *et al*[30]. For a longer follow-up period, the compliance rate might be lower.

In summary, we have shown that most colorectal cancer patients have increased levels of fecal calprotectin, which is followed by a significant fall after the operation. Patients with T3 and T4 tumors have significantly higher calprotectin values than those with T1 and T2 stages.

COMMENTS

Background

Previous studies have shown a significant, but highly variable increase of fecal calprotectin release in patients with colorectal cancer (CRC). The mechanisms for this observation are not fully elucidated. CRC is associated with a significant recruitment of neutrophils to the tumor site. Activated neutrophils, monocytes and macrophages are believed to be the cellular source of calprotectin release.

Research frontiers

The increase of calprotectin in CRC is highly variable. The analysis of fecal calprotectin may have the potential for clinical CRC surveillance. In most previous studies, no correlation of calprotectin and tumor parameters assessed by older classification systems could be found. To better characterize the role of calprotectin in CRC, its correlation with tumor as well as histopathological parameters should be examined.

Innovations

The study contains the following relevant information: (1) calprotectin shows a significant decrease three months after the operation; (2) the release of calprotectin is exclusively correlated to the T-stage, but not to histopathological parameters; and (3) except the T-stage, all other tumor characteristics assessed by the 7th edition of the TNM classification are not correlated.

Applications

The study results clearly indicate that fecal calprotectin cannot be used for colorectal cancer screening. In patients with initially elevated calprotectin, its role could be tested in the clinical follow up of CRC patients.

Terminology

Calprotectin is a small calcium-binding protein consisting of two heavy and one light polypeptide chains. It is found in abundance in neutrophilic granulocytes, in which it accounts for 60% of the cytosolic fraction, as well as in monocytes and macrophages. It is a simple, rapid, sensitive, inexpensive and non-invasive marker to detect and monitor intestinal inflammation, but is not disease-specific.

Peer review

This is a simple study with a clear message that at least in some cases the tumor node metastasis classification may have advantages over the traditional Dukes or Stage classification of CRC which are the dominant systems, even in the reporting of clinical trials. The authors make the link between granulocytes (neutrophils) and degree and extent of inflammation. It would be interesting to learn if the authors measured some other variables in this context, such as the ESR, or plasma CRP.

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BRIEF ARTICLE

Caecal pH is a biomarker of excessive colonic fermentation

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Abstract

AIM: To ascertain whether caecal pH is different in patients with irritable bowel syndrome (IBS), whose primary symptoms are bloating and distension, to healthy controls.

METHODS: Motility and pH data were reviewed from 16 patients with Rome Ⅲ defined IBS and 16 healthy controls, who had undergone a wireless motility capsule (WMC) study using a standardized protocol. Motility measures were anchored around known anatomical landmarks as identified by compartmental pH changes. Sixty-minute epochs were used to quantify antral, duodenal, ileal, caecal and distal colonic contractility. The maximum and minimum pH was measured either side of the ileo-caecal junction.

RESULTS: No differences were seen in motility parameters, compartmental transit times or maximal ileal pH between the two groups. Caecal pH was significantly lower in patients compared to controls (5.12 ± 0.05 *vs* 6.16 ± 0.15, $P < 0.0001$). The ileal:caecal Δchange was greater in patients than controls (-2.63 ± 0.08 *vs* -1.42 ± 0.11, $P < 0.0001$). There was a significant correlation between caecal pH and right colonic contractility ($r = 0.54$, $P = 0.002$).

CONCLUSION: Patients with bloating and distension have a lower caecal pH compared to controls. The measurement of caecal pH using the WMC provides a quantifiable biomarker of fermentation potentially identifying those patients that may preferentially benefit from antibiotic or dietary interventions.

Key words: Caecal pH; Caecoparesis; Bloating; Colonic microbiota; Fermentation

Core tip: Colonic bacterial fermentation has been implicated in the pathogenesis of irritable bowel syndrome. Hitherto, the measurement of fermentation *in vivo* in humans has been invasive and technically challenging. A major by product of colonic bacterial fermentation are short chain fatty acids. These short chain fatty acids act to reduce colonic pH. Herein, we demonstrate that the measurement of caecal ph using the wireless motility capsule provides a quantifiable biomarker of fermentation potentially identifying those patients with irritable bowel syndrome that may preferentially benefit from antibiotic or dietary interventions.

Farmer AD, Mohammed SD, Dukes GE, Scott SM, Hobson AR. Caecal pH is a biomarker of excessive colonic fermentation. *World J Gastroenterol* 2014; 20(17): 5000-5007 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5000.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5000

INTRODUCTION

Bloating and distension are both common and vexatious symptoms with community-based estimates of prevalence of 19% and 8.9% respectively[1]. Bloating is largely regarded as a subjective sensation of abdominal swelling, whereas distension refers to an observable increase in abdominal girth[2]. Bloating is associated with a reduction in quality of life, is a cause for healthcare seeking and represents a considerable challenge to manage effectively[3,4]. Bloating and distension are common complaints in patients with functional gastrointestinal disorders (FGID) such as irritable bowel syndrome (IBS) and functional dyspepsia[5-7].

The pathophysiological mechanisms that account for bloating and distension are poorly understood. They have been proposed to include disturbances in the handling of gas and its elimination from the gastrointestinal (GI) tract[8], psychological factors[9], carbohydrate malabsorption[10], musculoskeletal abnormalities[11], sensorimotor aberrancies[5], small intestinal bacterial overgrowth (SIBO)[12] and alterations within the GI microbiota[13].

The human microbiota is a complex symbiotic ecosystem residing largely in the GI tract. The composition and concentration of the microbiota varies along the length of the GI tract[14]. In humans, the colon receives digested material from the small bowel where it is mixed, stored and eventually excreted as faeces. The anaerobic breakdown of carbohydrates and protein by bacteria, largely occurring within the proximal colon, is through a process known as fermentation, the principal products of which are short chain fatty acids (SCFA)[15,16]. The direct *in vivo* measurement of SCFA concentrations in the human proximal colon is technically difficult and invasive[17,18]. Given that the degree of bacterial fermentation is directly proportional to the concentration of SCFA, the measurement of segmental intra-colonic pH is an inverse surrogate proxy of the degree of fermentation occurring within that territory[19]. It has been over 40 years since the stereotypical pH profile of the GI tract was first investigated using radio-telemetric techniques[20]. Upon entering the acidic environment of the stomach there is an immediate fall in pH, followed by a sharp rise on exiting the stomach, and a further fall in pH some hours later, a fall hypothesized to occur across the ileo-caecal junction (ICJ)[21]. Until recently, controversy remained as to the exact location of this fall in pH, as previous methods directed at validating position of the capsule within the GI tract were subject to limitations, particularly regarding accurate anatomical localization. These concerns were resolved in a study by Zarate *et al*[22], using a dual-scintigraphic technique of direct GI manometric measurements and pH evaluation using a wireless motility capsule (WMC), demonstrating that the drop in pH did indeed occur across the ICJ. The WMC is an ambulatory and relatively non-invasive diagnostic technique that continuously samples intraluminal pH, temperature and pressure as it traverses the GI tract. As changes in GI microbiota and fermentation have been linked to the development of bloating and distension, it is not known whether the measurement of caecal pH and the pH gradient across the ICJ, using WMC, offers a relatively non-invasive objective surrogate biomarker of this process. The WMC also allows examination of the hypothesis that these pH changes influence motility and transit parameters. In this retrospective study we aimed to address these knowledge gaps.

MATERIALS AND METHODS

Subjects

Sixteen consecutive outpatients, whose chief complaint and indication for the WMC investigation was bloating and distension were enrolled in the study between June 2011 and August 2012. Sixteen healthy age and sex-matched participants were recruited as controls. All participants provided written informed consent to undertake the investigations and the retrospective analysis of data was approved by the local institutional committee and East London and The City Research Ethics Committee (reference no. 07/H0703/77, permission granted March 2008).

Patients

All patients underwent a detailed clinical history and physical examination. Standard haematological, biochemical, immunological, upper and lower GI endoscopy with histology were performed in all patients prior to the WMC study by their referring gastroenterologist to rule out structural or biochemical causes for their symptoms. Patients did not undergo direct small bowel visualisation with enteroscopy or wireless capsule endoscopy. Bloating and/or distension were the chief presenting complaint in all patients. All patients fulfilled the Rome Ⅲ criteria for IBS and had alternating bowel habit, characterized by variable stool consistency and frequency[23,24].

Healthy controls

All healthy subjects had a normal bowel habit, defined as between three bowel movements a per day and one bowel movement every three days, with no symptoms of suggestive of SIBO or a rectal evacuatory disorder. No subject had any GI symptoms or history of metabolic, neurogenic, or endocrine disorder known to influence GI motor activity. In addition, no subject had undergone GI surgery other than appendicectomy and none were taking either laxatives or medications known to influence GI motility or pH.

Exclusion criteria

The presence of a positive pregnancy test, "red flag"/alarm symptoms (such as weight loss, anaemia or rectal bleeding), a positive microbiological, immunological or histological investigation suggesting another cause for symptoms, recent antibiotic use in the preceding 4 wk, recent probiotic use in the last 2 wk, concurrent use of promotile or acid-suppressing medications, history of a systemic

disorder with known GI manifestations (such as diabetes mellitus, connective tissue disorders *etc.*) and previous GI tract surgery were treated as criteria for exclusion. Specific contraindications to WMC were dysphagia, recent abdominal surgery, Crohn's disease and diverticulitis.

Wireless motility capsule study

All subjects were kept nil-by mouth except for small amounts of water from 9 pm on the before the study. Prior to ingestion of the WMC, all subjects were given a test meal (SmartBar, SmartPill Corporation, Buffalo, United States), a cereal bar of known calorific and nutritional content (260 kcal, 2% fat, 1 g fibre). The WMC was then swallowed with 60 mL of water. Once the communication was established between the WMC and data receiver, and the capsule was confirmed to be in the stomach (pH < 4), the patient was instructed in receiver care and allowed to leave the unit. No further meals or drinks were allowed for 6-h post capsule ingestion. After this, patients were allowed to eat and drink normally. After each bowel movement, the patient was instructed to wait for 1-min prior to flushing the toilet. Once they had flushed the toilet, they checked the data receiver to see if the signal connection had been lost and this confirmed exit of the capsule. The patient would then call the department, be instructed in how to turn the receiver off on return the box for download of the data. Continuous pH and pressure data were obtained by using the WMC system (SmartPill, Given Imaging Ltd, Yoqneam, Israel). The WMC contains sensors for pH, temperature and pressure and which are transmitting to a data receiver worn by the subject during ambulatory monitoring with data sensed at a frequency of 434 MHz. The pH is accurate to within ± 0.5 units and pressure measurements are accurate to ± 5 mmHg below 100 mmHg. After completion of the study, data was downloaded from the receiver to a compatible computer (Dell, Bracknell, United Kingdom) *via* a USB docking station and was analysed using semi-automated pressure analysis software (MotiliGI; Given Imaging Ltd, Yoqneam, Israel).

Compartmental transit times

The position of the various physiological landmarks within the GI tract, were determined from the physiological traces by two independent experienced investigators (Scott SM, Hobson AR) thus identifying gastric emptying, exit from the ileum into the right colon and excretion of the capsule. Disagreement regarding landmark locations was resolved by further review. The regional transit times were defined according to method proposed by Sarosiek *et al*[25] and anchored around stereotypical pH changes. Briefly, gastric emptying time (GET) was defined as the time between the ingestion of capsule and a sharp abrupt, pH rise (> 3 pH units) from gastric baseline to a pH > 4.0, marking the passage of the capsule from the acidic antrum to the relative alkaline environment of the duodenum. Small bowel transit time (SBTT) was defined as the time from which the WMC left the stomach until it arrived at the cecum as denoted by a pH drop of at least 1 pH unit, observed at least 30 min after GET and persisting for a minimum of 10 min. Colonic transit time (CTT) was defined as the elapsed time from the WMC accession at the ICJ until the capsule's exit from the body. The exit of the capsule from the body was determined either by an abrupt cessation data being recorded in conjunction with a subject's report of passing the capsule coinciding with a bowel movement entry in the subject's activity diary or an abrupt drop in temperature as the WMC exits the body. Thus, whole gut transit time (WGTT) was defined as the time from ingestion to excretion of the WMC.

Motility measures

In addition to measuring transit times, the WMC also measures intraluminal pressure across the GI tract thereby measuring frequency of contractions and amplitude of contractions. Motility measures are presented as area under the curve (AUC), anchored around the pH landmarks. Sixty-minute epochs were used to quantify antral, duodenal, ileal, caecal and distal colonic motility.

Caecal pH, magnitude of pH drop across the ileocaecal valve and caecal contractility

Caecal pH was defined as the fall in pH from the stable ileal peak to its nadir value as the WMC passed from the ileum into the cecum, as per the method defined by Zarate *et al*[22]. The Δchange was derived from the caecal nadir to the stable ileal peak. Caecal contractility was derived from the AUC for 1-h post passage through the ICJ, see Figure 1.

Statistical analysis

Data distribution was analysed using the D'Agostino-Pearson omnibus K2 normality test[26]. Results of quantitative data are presented either as median with interquartile ranges, for non-normally distributed data, or mean ± standard deviation (SD) and range for parametric data. Categorical data were summarised as the percentage of the group total. For quantitative data, differences between the groups were assessed using the Student's *t*-test. Correlational analyses were performed using Pearson's correlation. Two-tailed tests were used throughout. $P < 0.05$ was adopted as the statistical criterion. All analyses were performed using proprietary software (GraphPad Prism 5, CA, United States).

RESULTS

Participant characteristics

Sixteen female patients (median age 31 years, range 24-52 years) and 16 age, sex matched healthy controls (median age 38.5 years, range 21-74) completed the study.

Compartmental transit times

There were no appreciable differences in GET, SBTT,

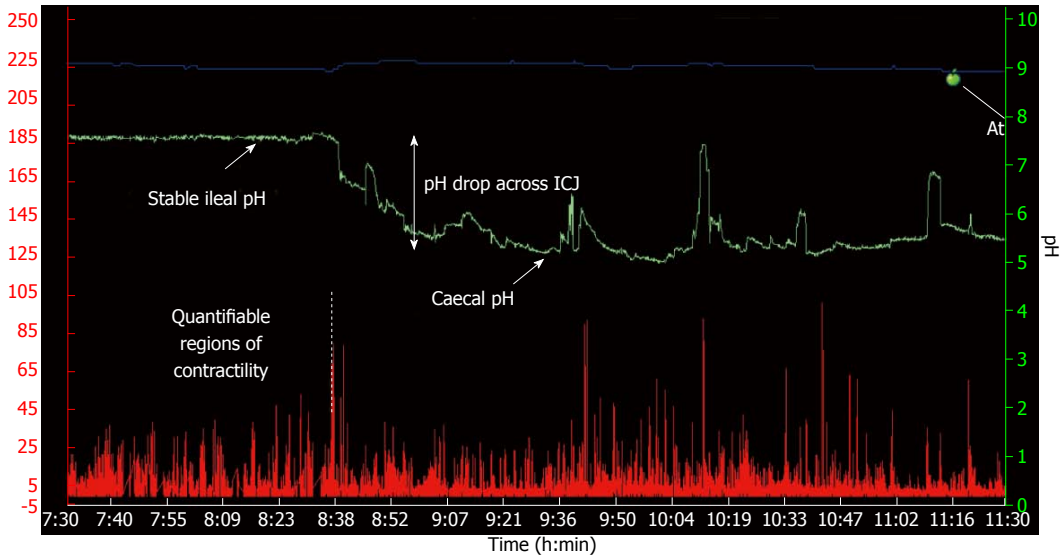

Figure 1 A typical wireless motility capsule trace demonstrating temperature (blue line), pH (green line) and contractility (red line) across the ileo-caecal junction. The pH drop was defined as the difference between the stable ileal pH and the caecal pH nadir. ICJ: Ileo-caecal junction.

Table 1 A comparison of the regional transit times between patients and healthy controls

Transit time (min)	Patients (mean ± SD)	Controls (mean ± SD)	*P* value
GET	290 ± 76	306 ± 55.9	0.86
SBTT	305 ± 14.5	270 ± 25.9	0.24
CTT	1443 ± 192.8	1861 ± 263.7	0.21
WGTT	2039 ± 202	2437 ± 279.4	0.26

GET: Gastric emptying time; SBTT: Small bowel transit time; CTT: Colonic transit time; WGTT: Whole gut transit time.

CTT or WGTT between patients and controls, see Table 1.

Motility comparisons

There were no appreciable differences in antral, duodenal, ileal, caecal and colonic motility between patients and controls, see Table 2.

Regional pH comparisons

There were no appreciable differences in ileal pH between patients and controls, 7.7 ± 0.1 *vs* 7.6 ± 0.1, $P = 0.17$. However, caecal pH was significantly lower in patients in comparison to controls, 5.12 ± 0.05 *vs* 6.16 ± 0.15, $P < 0.0001$.

Δchange ileo-caecal:caecal pH and relationship of caecal pH to caecal contractility

Δ%change ileo-caecal:caecal pH was significantly higher in patients compared to controls (-33.8% ± 0.84 *vs* -18.7 ± 1.5, $P < 0.0001$), see Figure 2. For the whole cohort, there was a moderate correlation between caecal pH and right colonic contractility ($r = 0.54$, $P = 0.002$), see Figure 3.

Table 2 A comparison of the regional motility pattern, given by areas under the curve, between patients and healthy controls

Motility (AUC)	Patients (mean ± SD)	Controls (mean ± SD)	*P* value
Antral	3590 ± 708.3	4710 ± 850.1	0.32
Duodenal	3909 ± 919	6000 ± 1310	0.20
Ileal	13414 ± 2203	12679 ± 2131	0.81
Cecal	4071 ± 531.2	5176 ± 878	0.29
Recto-sigmoid	20504 ± 4583	11894 ± 215	0.09

AUC: Areas under the curve.

DISCUSSION

In this study, we have demonstrated that patients with lower abdominal symptoms typically associated with, but not limited to, conditions such as IBS, have a significantly lower caecal pH compared to controls. This relatively acidic environment is maintained by fermentation and subsequent SCFA production. In addition, we have observed that excessive fermentation in the cecum is correlated with a reduction in caecal contractility.

The measurement of bacterial fermentation products demonstrate marked regional differences in their production across the colon such that SCFA concentrations are greatest in the caecum (127 mmol/L) falling progressively in the transverse (117 mmol/L) and distal colon (90 mmol/L)[17]. These differences in SCFA concentration indicate that fermentation occurs maximally within the right colon, presumably where concentrations of the substrate, arriving from the small bowel, are at their highest[27]. Studies in IBS patients, in which bloating and distension are prevalent symptoms, have demonstrated alterations in colonic fermentation[28,29]. Mortensen *et al*[28] observed that SCFA concentrations are increased in diarrhoea predominant-

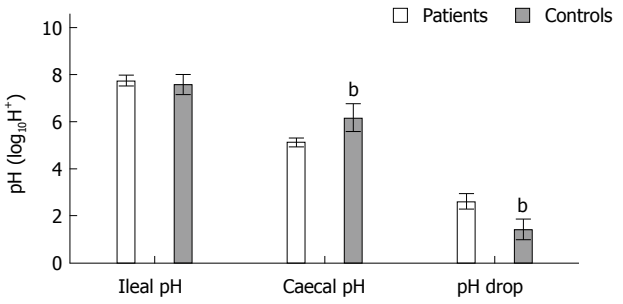

Figure 2 Differences (mean ± SD) in ileal, caecal and ileo-caecal junction pH drop between patients and controls. Caecal pH significantly lower in patients than in controls and pH drop across the ICJ was lower in patients than in controls. $^bP < 0.01$ *vs* patients group. ICJ: Ileo-caecal junction.

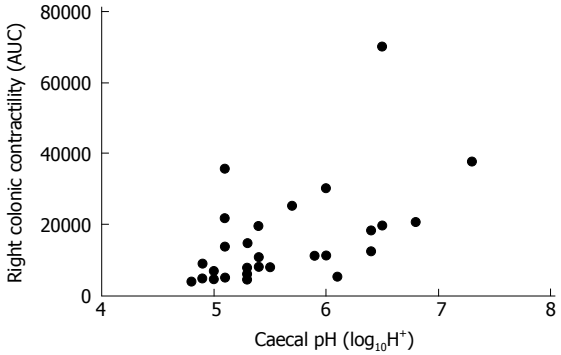

Figure 3 Caecal pH and caecal contractility was positively correlated (r = 0.54, P = 0.002). AUC: Area under the curve.

IBS (IBS-D) and decreased in constipation predominant IBS (IBS-C) although Treem and colleagues reported conflicting results in IBS-C[29]. These observations have engendered the application of a diverse array of sophisticated molecular and culture independent approaches to the evaluation of the GI microbiota in FGID. For instance, Tana *et al*[30] determined SCFA concentrations using high-performance gas chromatography, and found that IBS patients had significantly higher concentrations, which were associated with increased GI symptoms and quality of life burden. A recent important study by Jeffery *et al*[31] performed a detailed pyrosequencing analysis of faecal microbiota composition and demonstrated two species specific subtypes of IBS, independent of symptom based classification derived from the Rome Ⅲ criteria. The first of these showed a microbial composition similar to normal whereas the second was characterized by an increase in *Firmicutes*-associated taxa in association with a relative depletion of *Bacteroides*-related taxa. The implication of this data is that in future GI microbial enterotyping may facilitate stratifications of IBS subpopulations. However, at the present time such methods have limited practicality as a routine clinical biomarker as they are resource and labour intensive[32]. However, given that a raised *Firmicutes*:*Bacteroides* has been positively correlated with increased concentrations of SCFA in a preclinical human model[33], an alluring speculation is that the measurement of caecal pH, using the WMC, may provide an attractive, readily available, surrogate marker obviating many of the limitations of the current microbial enterotyping techniques whilst also assessing for motility disorders.

Another potential application of measuring fermentation by caecal pH may enable the early identification of patients in whom a particular treatment may be more efficacious, particularly given recent laudable progress in therapeutic interventions. Nevertheless, given the heterogeneous nature of FGID populations, it is not surprising that the degree of therapeutic gain conferred by these advances is somewhat variable. In this respect, two areas where considerable furtherance has been made are those of non-absorbable antibiotics and dietary modifications. Firstly, Pimentel *et al*[34] reported the pooled results of two phase 3, double-blind, placebo-controlled trials comparing adequate global relief of symptoms and bloating in IBS patients without constipation who were randomly assigned to received either rifaximin, a minimally absorbed oral broad spectrum antimicrobial agent, or placebo. Patients treated with rifaximin had a significant reduction in global symptoms of IBS and it can be derived that at 3 mo the numbers needed to treat (NNT) weekly bloating symptoms is approximately 10. Secondly, the quantity of poorly absorbed short chain carbohydrates, which exert an intraluminal osmotic effect and are rapidly fermented by bacteria, entering the colon can be modified by dietary restriction[35]. Collectively these short chain carbohydrates are known as fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) and reducing their intake has proven successful in reducing bloating and abdominal pain in IBS patients, with an approximate NNT with respect to the former of 3[36]. However, these novel and emerging interventions are notwithstanding concerns regarding implications for healthcare expenditure, short and long-term safety and patient compliance[35,37,38]. The absolute practical utility of caecal pH measurement as a marker of fermentation remains to be fully determined but it may facilitate the identification of FGID patients who may preferentially benefit from antibiotic or dietary interventions.

In humans, 90%-95% of SCFA are composed of acetate, propionate and butyrate and are plurifunctional through their contribution to the maintenance of mucosal integrity[39], stimulation of salt and water absorption[40], regulation of colonic mucosal blood flow[18] as well as having anti-carcinogenic[41] and immuno-modulatory properties[42]. Moreover, SCFAs may also act as intraluminal chemical stimuli modifying GI motility but whether these effects are stimulatory or inhibitory is unclear and varies according to different experimental paradigms, species and the region of the GI tract being considered[43]. In humans, intra-colonic SCFA infusion has not been shown to influence motility although it is plausible to suggest that the infusion concentrations used were insufficient to activate any putative sensorimotor mechanisms[44]. Our results demonstrate, in an ambulatory relatively physiological setting, a correlation between pH and caecal contractility. Therefore in this

situation it is possible that heightened fermentation in the cecum with associated with elevated concentrations of SCFA, inhibit proximal colonic motor activity potentially leading to a degree of stasis, or "caecoparesis." In an animal model, Dass *et al*[43] investigated the hypothesis that SCFA may modulate colonic motility through the G protein-coupled receptors. Interestingly they demonstrated that SCFA enhanced neuronally mediated contractions of rat distal colon yet increased the frequency of peristaltic contractions in guinea-pig terminal ileum. These data therefore suggest differential regional actions of SCFA such that there is inhibition of motility in the right colon followed by a pro-motility effect in the more distal colon. Combined with increased fermentation in the cecum leading to distension, caecoparesis may be the long sort after alteration in motor function which differentiates IBS patients from healthy subjects and explains why IBS preferentially experience pain in the right colon and upper abdomen in response to balloon distension[45]. Whilst we were unable to accurately assess segmental colonic transit times using the WMC (due to technical limitations discussed earlier), further studies with techniques such as MRI may help to prove this concept further.

The role of SCFA in modulating visceral perception and nociception has been afforded considerable interest. Whilst several investigators have examined the effect of intra-colonic instillation of butyrate, results are conflicting. Bourdu *et al*[46] showed that the administration of butyrate enemas in a rat model caused a sustained, dose-dependent increase in sensitivity to colorectal distension, in the absence of demonstrable microscopic or histological abnormality in the colonic mucosa, closely mimicking what is seen in a proportion of IBS patients. In studies of healthy human volunteers the converse effect has been demonstrated where the administration of butyrate rectal enemas, at physiologically relevant concentrations, caused a dose-dependent decrease in rectal sensitivity[47]. However, a pertinent fundamental limitation of this type of study remains as to whether distal colonic administration of SCFA, even in physiologically relevant concentrations, alters the composition in the proximal colon where SCFA concentration is at its highest as retrograde colonic spread of rectal enemas is irregular and formulation dependent[48]. Whether these data, derived from a small group of healthy volunteers, are applicable to larger cohorts of community-based patients with a FGID currently is uncertain.

This study is not without significant limitations. Firstly, we did not actively screen for diabetes mellitus, thyroid dysfunction or smoking through HbA1c, thyroid stimulating hormone or serum cotinine respectively. Secondly, the control group was marginally older, although this difference did not reach statistical significance. Thirdly, this was an unselected sample, SIBO not actively screened for and being a retrospective analysis. In addition, our findings were unexpected. A further valid criticism is the lack of dietary control but as all subjects had fasted for at least 12 h prior to the study, and subsequently for a further 6 h after the standardized test meal, by the time the WMC capsule reached the cecum, it is improbable, although not impossible, that further intake could influence GI microbiota, fermentation and thus caecal pH[49]. Nevertheless, whilst further validation needed, the overall concept presented herein plausible concept in IBS/functional bloating.

In conclusion the measurement of caecal pH using the WMC provides a quantifiable biomarker of fermentation. In future, this may be used to sub-classify patients with a broad spectrum of FGID and identify those that may benefit most from antibiotic and dietary interventions providing novel insights into the pathophysiology of lower GI symptoms and mechanism of actions of novel treatments.

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COMMENTS

Background

Irritable bowel syndrome is a common disorder of gastrointestinal function whose pathophysiology is incompletely understood.

Research frontiers

There is an increasing body of evidence to suggest that the gastrointestinal microbiota play a significant role in the genesis of symptoms in irritable bowel syndrome. Within the colon, the microbiota ferments a number of substrates whose main by products are short chain fatty acids. To date, the measurement of short chain fatty acids in humans has been invasive and technically difficult.

Innovations and breakthroughs

In this study, authors have utilised wireless motility capsule technology to measure the pH as it traverses the gastrointestinal tract. They have demonstrated that patients with lower abdominal symptoms typically associated with, but not limited to, conditions such as irritable bowel syndrome, have a significantly lower caecal pH compared to controls. This relatively acidic environment is maintained by fermentation and subsequent short chain fatty acid production. In addition, they have observed that excessive fermentation in the cecum is correlated with a reduction in caecal contractility.

Applications

By using such methodology, it may be possible to stratify patients with irritable bowel syndrome in based on their caecal pH. For instance, patients with a low caecal pH, thereby suggesting heightened fermentation and thus bacterial load, may preferentially benefit from antibiotic and "substrate lowering" dietary interventions.

Terminology

The gastrointestinal microbiota is a complex symbiotic ecosystem, whose interactions with the host are complex and largely remain to be fully characterized. This microbiota, in the right colon, ferment substrate arriving from the proximal gastrointestinal tract whose end products are short chain fatty acids.

Peer review

It is an interesting and innovative paper of great clinical impact.

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BRIEF ARTICLE

Prediction of Crohn's disease aggression through *NOD2/CARD15* gene sequencing in an Australian cohort

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Abstract

AIM: To investigate the association between mutations in oligomerisation domain 2/caspase recruitment domains 15 (*NOD2/CARD15*) and the natural history of Crohn's disease (CD) to identify patients who would benefit from early aggressive medical intervention.

METHODS: We recruited thirty consecutive unrelated CD patients with a history of ileo-caecal or small bowel resection during the period 1980-2000; Fifteen patients of these had post-operative relapse that required further surgery and fifteen did not. Full sequencing of the *NOD2/CARD15* gene using dHPLC for exons 3, 5, 7, 10 and 12 and direct sequencing for exons 2, 4, 6, 8, 9 and 11 was conducted. CD patients categorized as carrying variants were anyone with at least 1 variant of the *NOD2/CARD15* gene.

RESULTS: About 13.3% of the cohort (four patients) carried at least one mutant allele of 3020*ins*C of the *NOD2/CARD15* gene. There were 20 males and 10 females with a mean age of 43.3 years (range 25-69 years). The mean follow up was 199.6 mo and a median of 189.5 mo. Sixteen sequence variations within the *NOD2/CARD15* gene were identified, with 9 of them occurring with an allele frequency of greater than 10 %. In this study, there was a trend to suggest that patients with the 3020*ins*C mutation have a higher frequency of operations compared to those without the mutation. Patients with the 3020*ins*C mutation had a significantly shorter time between the diagnosis of CD and initial surgery. This study included Australian patients of ethnically heterogenous background unlike previous studies conducted in different countries.

CONCLUSION: These findings suggest that patients carrying *NOD2/CARD15* mutations follow a rapid and more aggressive form of Crohn's disease showing a trend for multiple surgical interventions and significantly shorter time to early surgery.

Key words: Inflammatory bowel disease; Oligomerisation domain 2/caspase recruitment domains 15; Genotyping; Crohn's disease; Natural history

Core tip: This study conducted a full gene sequencing of nNucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 (*NOD2/CARD15*) within an Australian cohort of patient with Crohn's disease (CD). In this study, there was a trend to suggest that patients with the 3020*ins*C mutation have a higher frequency of operations compared to those without the mutation. Patients with the 3020*ins*C mutation had a significantly shorter time between the diagnosis of CD and initial surgery. The clinical significance of understanding pathogenic *NOD2/CARD15* mutations is to

shift management to a top down approach whereby active medical therapy could be introduced at an early stage to impact on aggressive disease behaviour in mutation positive patients.

Bhullar M, Macrae F, Brown G, Smith M, Sharpe K. Prediction of Crohn's disease aggression through *NOD2/CARD15* gene sequencing in an Australian cohort. *World J Gastroenterol* 2014; 20(17): 5008-5016 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5008.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5008

INTRODUCTION

The pathogenesis of inflammatory bowel disease (IBD) is complex and is thought to result from the interaction of environmental factors with genetic predisposition[1]. Familial aggregation of the disease and studies of twins have strongly suggested that genetic factors contribute to IBD, especially Crohn's disease[2] - a hypothesis that was substantiated with the discovery of a susceptibility locus in the pericentromeric region of chromosome 16 called IBD1[3]. Subsequently, two independent research groups reported that the nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 (*NOD2/CARD15*) gene located on chromosome 16 within the IBD-1 region is associated with increased susceptibility to CD[1,4] . This association was later confirmed by other research groups[5-13].

Approximately 30% of Crohn's patients carry one copy of a mutated *NOD2/CARD15* allele and about 17% of Crohn's patients carry two mutated *NOD2/CARD15* alleles[14], conferring a 2 to 4 fold and 20-40 fold increased risk of developing CD respectively.

Studies have demonstrated three mutations or sequence variants which exhibit the strongest association with the development of CD[7]. These are "3020*ins*C" on exon 11, which is a frameshift mutation[15], and the missense mutations located on exons 4 and 8; these lead to the amino acid substitutions Arg702Trp and Gly908Arg respectively. However, whilst it has been noted for 9 years that these mutations are associated with Crohn's disease, the consequences of these mutations on their protein function is yet unknown.

The only consistent finding to date regarding the clinical impact of *NOD2/CARD15* variants concerns disease localization[6,8,10,16-21]. Some studies have also reported an association between *NOD2/CARD15* mutations and fibrostenotic behaviour[6,11,22], whilst others have reported a higher incidence of fistulas in *NOD2/CARD15* variant patients[11,19,22,23]. The reasons for these observations are unknown but are important for further investigation as it will help in understanding the pathophysiology of *NOD2/CARD15* mutations.

Several lines of evidence are compatible with a significant role of *NOD2/CARD15* variants in determining an association with earlier age of disease onset[6,7,20]. This is consistent with genetic evidence that a younger age of diagnosis identifies families with greater linkage to the IBD1 locus[24].

Currently, little is known about the association between *NOD2/CARD15* mutations and the requirement for initial surgery and for surgical recurrences in CD. Studies conducted to evaluate the direct association of *NOD2/CARD15* mutations with surgical requirements have reported variable findings but generally there is support for prediction of subsequent requirements for surgery. Most of the published data have shown an increased association between *NOD2/CARD15* mutations with ileal surgery, and the mutation with the strongest association was found to be the 3020*ins*C mutation[5,19,25-28]. In three independent studies, one carried out in Germany[20], another in Spain[21] another in Italy[29], patients with mutations of the gene presented an increased risk of repeated surgery, and such surgery was required earlier. These findings were even reinforced within the pediatric population whereby 2 studies have demonstrated that *NOD2/CARD15* mutations were a predictor of earlier age of surgery within a pediatric population[30,31].

Perhaps the greatest shortcoming, though, is that all associations between *NOD2/CARD15* mutations and the requirement of surgery have been based only on the analysis of the three most common mutations within the gene. One of the research studies only evaluated the most common insertion mutation. None of these studies have fully sequenced the *NOD2/CARD15* gene. The current study was designed with the intention of fully sequencing the *NOD2/CARD15* gene in all recruited patients to establish the frequency of variant alleles and unravel other mutations in the aforementioned gene. With that, this research aimed to determine if *NOD2/CARD15* mutations in CD patients are able to predict disease progression including the need for early surgery as well as post-operative relapse requiring re-operation, with a view to predict those who may need early interventions to prevent a relapse from occurring. Full sequencing in itself will provide unique information with regard to the distribution spectrum of *NOD2/CARD15* mutations within the ethnically heterogeneous Australian population.

MATERIALS AND METHODS

The study was approved by the Human Resource Ethics Committee at The Royal Melbourne Hospital and followed consent and privacy procedures.

Thirty unrelated Crohn's disease patients were ascertained consecutively through a search of The Royal Melbourne medical records and private records of RMH consultants, filtering on patients with a history of ileocaecal or small bowel resections during the time period of 1980-2000. The diagnosis of CD was based on standard clinical, radiological and histological criteria.

The patients were divided into two main groups: (1)

Fifteen patients who had post-operative relapse that required surgical interventions; and (2) Fifteen patients who did not have post-operative relapse that required surgical interventions, composing the control group.

We defined surgery as an active procedural intervention including bowel resection, stricturoplasty and balloon dilatation of strictures. We included patients only with a history of ileo-caecal or small bowel resections as their initial type of surgery, as *NOD2/CARD15* mutations have been associated with this group of patients, and we wanted to enrich our genotyped population with mutation carriers.

Although the recruited patients underwent initial small bowel or ileo-caecal resection(s), we did not exclude patients with disease involvement in other parts of the digestive tract. The location of disease was recorded from information obtained in radiological, endoscopic and histological examination, and updated in association with later consultations.

Indication for the requirement of surgical interventions (initial or subsequent surgeries) was based according to clinical judgement of the caring consultant and/or surgeon according to clinical presentation, pre-operative diagnostic findings and intra-operative findings without access to genotyping of *NOD2/CARD15*. Inconsistency in surgical decision making was minimized because the overwhelming majority of surgical procedures were done by the same integrated surgical team.

The indications for surgical interventions composed 5 main groups: (1) failed medical treatment; (2) symptomatic stricturing disease if persistent intestinal obstruction was found on radiological, endoscopic, clinical or intra-operative findings; (3) perforating disease if complicated enterocutaneous fistulae, intra-abdominal fistulae or acute free perforations were present; (4) the presence of intra-abdominal abscesses; and (5) others, including cancer, haemorrhage, toxic dilation.

Group 2 patients were selected from patients who had one or more subsequent surgeries following the initial resection for the recurrence of CD, with the requirement for surgery being consistent with the indications described above. Surgical procedures as a result of complications of the previous surgery, incomplete/abandoned surgery, ileostomy, colostomy and CD-unrelated surgery were not included in interventions analysed in the series. The number of subsequent surgeries undertaken and the time between the surgeries were recorded.

Disease behaviour was based on the Vienna classification: (1) perforating for enterocutaneous/intra-abdominal fistulae, abscesses and inflammatory masses; (2) stricturing for narrowing of the intestinal lumen from fibrostenotic lesions; and (3) inflammatory for the rest of CD patients. The Crohn's disease activity index was noted if present in the medical records.

Family history is of significance if one first or second degree relative has CD. Smoking habit refers to smoking behaviour in July 2005 as reported by the patient, with patients being divided into 3 groups: current smokers, non-smokers (patients who never smoked) and ex-smokers (patients who had given up smoking at least 1 year prior). Extra-intestinal manifestations were defined as follows: Type 1 peripheral arthralgia/arthritis,primary sclerosing cholangitis, affections of the skin (pyoderma gangrenosum) or eye (iritis, uveitis, *etc.*).

The medical history of the patient was collected through retrospective review of medical records and updated during later consultations and/or by phone. The clinical and demographical data were collected in concordance to an established regional database. The demographic data included age, gender, race, smoking habits, family history of IBD and dietary history. The clinical data included disease phenotype, age of onset, age at initial and subsequent surgical interventions, date and type of surgery, location of disease, disease phenotype, extra-intestinal manifestations, and intra and post-operative complications.

A sample of blood was collected from all patients for the full sequencing of the *NOD2/CARD15* gene using dHPLC for exons 3, 5, 7, 10 and 12 and direct sequencing for exons 2, 4, 6, 8, 9 and 11. The investigators who determined the genotypes were blinded to the clinical characteristics of the patients. CD patients categorized as carrying variants were anyone with at least 1 variant of the *NOD2/CARD15* gene.

Statistical analysis

Analysis was carried out using Minitab or R statistics package. Categorical variables were compared using Fishers exact test. Continuous variables were analysed using Student *t* test (and some non-parametric equivalents, namely the Mann-Whitney test). The Mann-Whitney test was often used as the distribution of data was often skewed and this test is a distribution-free test. All *P* values were two sided, and a value of less than 0.05 was considered to be a statistically significant difference.

RESULTS

This cohort of 30 patients were CD patients with a history of ileo-caecal or small bowel resection during the time period of 1980-2000. Group 1 consisted of 15 patients who had post-operative relapses requiring further surgery and Group 2 consisted of 15 patients who did not require further surgery. There were 20 males and 10 females with a mean age of 43.3 years (range 25-69 years). All patients were followed up until December 2005, with a mean follow up of 199.6 mo and a median of 189.5 mo. Nine patients (30%) were current smokers, 3 (10%) were ex-smokers and 18 (70%) were non-smokers. There was no evidence of an association between smoking behaviour and disease progression. None of the patients reported a family history of CD in a first or second degree relative.

In total, 16 sequence variations within the *NOD2/CARD15* gene were identified, with 9 of them occurring with an allele frequency of greater than 10 %. The sequence variations are S178S nt534C>G (Exon 2), IVS2-25G>T (Exon 2), P268S nt 802C>T (Exon 4), R702W

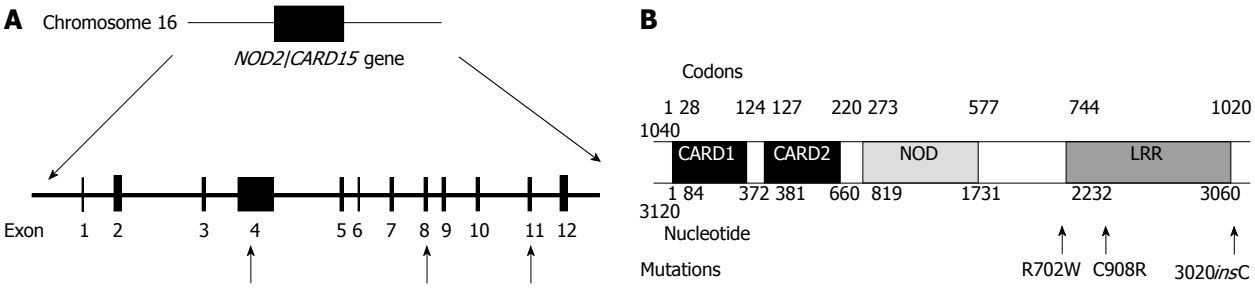

Figure 1 Pathogenic mutation within the nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 gene. A: Physical map of the exon-intron organization of the nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 (*NOD2/CARD15*) gene and the approximate positioning of the mutations within the gene; B: Structural domain of the *NOD2/CARD15* gene. Like the other members of the NOD/leucine rich repeat domain (LRR) protein family, *NOD2/CARD15* consists of three distinct functional domains: CARD, a centrally located NOD and a LRR.

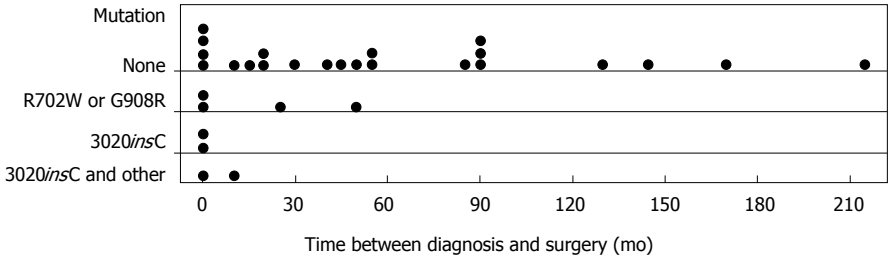

Figure 2 Time between Crohn's disease diagnosis and initial surgery according to nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 status.

Table 1 Number of surgical interventions according to nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 gene status

Surgical interventions	Pathogenic mutation positive (n = 8)	Pathogenic mutation negative (n = 22)
1	2	12
2	1	5
3	1	3
4	3	1
5	1	1

nt 2104C>T (Exon 4), R459R nt 1377C>T (Exon4), R587R nt 1761 T>G (Exon 4), L248R nt 743T>G (Exon 4), R703C nt 2107C>T (Exon 4), T294T nt882 T>A (Exon 4), A611A nt 1833 C>T (Exon 4), IVS4+10 A>C (Exon 4), IVS5+27G>A (Exon 5), IVS4-43C>T (Exon 5), G908R nt 2722G>C (Exon 8), V955l nt 2863G>A (Exon 9), 3020*ins*C (Exon 11). Of these mutations, IVS2-25G G>T on Exon 2 has not been previously reported.

In total, 8 patients (26.6% of the CD patients) carried at least one known pathogenic mutation within the *NOD2/CARD15* gene located on chromosome 16 (Figure 1). The allele frequencies for the pathogenic mutations R702 W (Exon4), G908R (Exon 8) and 3020*ins*C (Exon 11) were 16.7%, 3% and 13.3%. One of the patients was a simple homozygote, 2 patients were compound heterozygotes (R702W and 3020*ins*C) and (G908R and 3020*ins*C) and the remainder 5 patients were simple heterozygotes.

There were 12 patients with the P268S sequence variation. Nine of these patients were heterozygous for the P268S variation and 3 were homozygous. All 8 patients with the pathogenic mutations possessed the P268S variant and both the patients who were compound heterozygotes for the pathogenic mutations were also homozygous for the P268S variation. The P268S change was not found to independently reduce the time between surgery and diagnosis (P = 0.067).

Patients with any pathogenic mutation showed a trend to a higher frequency of operations. According to the Mann-Whitney test performed, we found that patients without any pathogenic mutation had a median of 0.79 surgical interventions per 100 mo whilst those with any pathogenic mutation had 1.09 surgical interventions per 100 mo. However, this was not statistically substantiated (P = 0.096). Among the remainder of the mutation negative patients, 12 patients had 1 surgical intervention, 5 patients had 2 surgical interventions, 3 patients had 3 surgical interventions and only 1 patient had 4 and 5 surgical interventions (Table 1). As all the patients had different lengths of follow-up time, we assessed the number of operations per 100 mo in relation to the *NOD2/CARD15* mutation to investigate whether the mutation predicted patients at a higher risk of operation than those without.

Patients with any pathogenic mutation had a significantly reduced time between their diagnosis of CD and initial surgery. The effect was even more pronounced with the 3020*ins*C mutation, with 3 out of 4 mutation positive patients requiring their first resection almost immediately after CD diagnosis. The mean time between age at diagnosis and 1st surgical resection for patients

Table 2 Analysis of phenotypic characteristics of Crohn's disease patients according to nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 gene status[1]

Characteristics	3020*ins*C mutation positive (n = 4)	3020*ins*C mutation negative (n = 26)
Disease behaviour		
Perforating	1	5
Stricturing	4	14
Inflammatory	3	7
Location of CD lesions		
Small bowel	4	7
Ileocaecal	6	19
Colonic	1	7
Anorectal	1	1
Others	0	0
No of locations involved		
1	4	13
2	4	7
3	0	2
4+	0	0
Extraintestinal manifestations	0	3

[1]Some patients had more than one clinical variable. CD: Crohn's disease.

with any pathogenic mutation was 0.5 mo compared to a median time of 48.5 mo for patients who were negative for a *NOD2/CARD15* pathogenic mutation (P = 0.027). The mean time between time of diagnosis and surgery for patients with a 3020*ins*C mutation was 2.25 mo, with a median of 0.5 mo whereas for patients with R702W or G908R mutation, the median was 12 mo. The distribution of the patients, both positive and negative for the 3020*ins*C mutation was skewed (Figure 2), as expected.

We analysed the phenotypic characteristics presented by all 30 CD patients to determine if a positive 3020*ins*C mutation is associated with clinical variables that could serve as predictors of patients at high risk of disease relapse and re-operation. These clinical phenotypes include disease behaviour, location of disease, number of diseased locations and extra-intestinal manifestations of CD (Table 2). The data on clinical features were recorded from time of diagnosis to December 2005. In our series, there was no significant association between a positive pathogenic mutation and any of the aforementioned clinical features with a $P < 0.05$. Also, biallelic carriers did not have more aggressive behaviour than mono allelic carriers.

Interestingly, though not statistically significant, all eight gene positive patients had stricturing disease behaviour. None of the 3020*ins*C mutation positive patients had extra-intestinal manifestations. Three patients without the mutation displayed extra-intestinal manifestations. These were arthritis, marginal keratitis and Sweets syndrome.

DISCUSSION

In this study, it was found that a *NOD2/CARD15* mutation predicted initial disease aggressiveness in CD with mutation positive patients having a significantly reduced time between the diagnosis of CD and initial surgery.

Table 3 Comparison of allele frequencies of nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 mutations

Study	Population	*NOD2/CARD15* mutation		
		3020*ins*C	Exon 8	Exon 4
Present study	Australian	13.3%	3%	16.7%
Hugot *et al*[4]	European	12%	6%	11%
Hampe *et al*[5]	German/British	16%	-	-
Vermeire *et al*[10]	Canadian	10.3%	5.2%	12.9%
Cavanaugh *et al*[13]	Australian	7%	2%	11%

NOD2/CARD15: Nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 gene.

There was also a trend to suggest that patients with the pathogenic mutations have a higher frequency of operations compared to those without the mutation. These findings were particularly significant in the presence of the 3020*ins*C mutation. If confirmatory studies establish these relationships, active medical therapy could be introduced at an early stage to impact on aggressive disease behaviour.

There was a statistically significant association between the pathogenic *NOD2/CARD15* mutations and the requirement for early surgical intervention. Patients with the mutation had a significantly reduced time between their diagnosis and 1st surgical resection. This association was found to be more significant in patients with the 3020*ins*C mutation. In fact, 3 out of 4 patients with the 3020*ins*C mutation required their first surgical resection immediately after diagnosis. This data would suggest that that the mutation leads to a more aggressive form of disease, which rapidly progresses requiring early surgical intervention. *NOD2/CARD15* mutations seem to act by either impairing the regulatory immune response (allowing patients to be vulnerable to infections that set off CD at an earlier age) or initiating an enhancement of the effector limb of the immune response to bacterial invasion (acting as an initiating factor in early onset CD). In the same way, the mutation could cause a more rapid and aggressive clinical course of disease because either an impaired immune response is unable to adequately contain the bacterial invasion or the mutation itself is fuelling the excessive inflammatory response. The 3020*ins*C mutation is a frameshift mutation resulting in the truncation of the protein in the leucine rich repeat region, which is the main region implicated in the immune regulation pathway, which may explain why the 3020*ins*C mutation leads to an even more aggressive progression of disease compared to the other 2 pathogenic mutations.

We then assessed the association between gene status and the pattern of disease, as previous studies have reported that *NOD2/CARD15* mutations are associated with fibrostenosing disease. Our current results are inconclusive to date in analysing the relationship between *NOD2/CARD15* mutations and the behaviour of the disease. Interestingly, however, all mutation positive patients have a stricturing disease behaviour, either alone or

in combination with a perforating and/or inflammatory pattern, irrespective of the nature of the indication for their initial surgery. This means that our finding is consistent with previously reported data, though it has not been statistically substantiated possible due to the small sample size.

Following the clinical observation that one third of patients need re-operation after initial surgery[21], we questioned whether the *NOD2/CARD15* mutation was responsible for post operative relapses requiring further surgery. A Spanish study published after the commencement of this research found that *NOD2/CARD15* mutations are a predictive risk factor for surgical requirement due to stricturing lesions[21]. The proposed mechanism is that *NOD2/CARD15* mutations predispose to stricturing and fibrotic lesions, and these altered repair mechanisms at the site of surgery would lead to earlier surgical recurrences, irrespective of the cause of initial surgery. It is also controversial whether the relationship between the CARD15 variants and both stenosing phenotype and increased need for surgery in CD patients is a true association or only reflects the high proportion of ileal CD developing bowel stenosis and, therefore, requiring surgery[32]. In our sample population, we found that patients with a *NOD2/CARD15* pathogenic mutation had a higher frequency of operations than those without the mutation. This difference however was not statistically significant (P = 0.096) but showed a trend to more operations in mutation carriers. One limitation of the Spanish study is that it only analysed 23 patients who had subsequent surgery. Also, none of the recruited patients had more than 2 surgical resections. Although some of our patients have had up to 5 operations, our study is also limited by a small sample size. The lack of statistical significance in our study for this parameter of multiplicity of operations could therefore be explained by a Type II error.

These findings add strength to the argument supporting the use of *NOD2/CARD15* genotyping as a prognostic tool in stratifying CD patients with a high risk of initial or subsequent operations. The ability to predict the natural history of high risk patients could prove useful in the application of top-down therapy, which may not just prevent complications but also modify the natural history of the disease[33,34]. Even if preventative therapy is ineffective, these high risk patients should be managed with close collaboration between physicians and surgeons. Most clinicians are not willing to adopt unrestricted top-down approach because a substantial proportion of patients never develop aggressive disease requiring biological therapy. The ability to predict those that will would be a great asset in the management of IBD given the effectiveness described by Oldenburg and Hommes[33]. This is in contrast to the conventional practice of starting with less invasive interventions and working up the therapeutic ladder, which in the long term may subject the patient to unnecessary and invasive investigations, medications and procedures. Genotyping may prove to have a place in contributing to what often is a quite complex decision-making especially since genetic-based classifications are stable compared to exclusive use of phenotypic characteristics which are subject to change over time.

However, it remains to be determined whether full *NOD2/CARD15* gene sequencing during the diagnosis of CD will be clinically useful and cost effective. Most of the *NOD2/CARD15* sequence variations identified in this study are rare and have not been found to influence the natural history of the disease. Our studies do suggest a place for at least the mutational analysis of the most common pathogenic mutations which can be done cheaply. From here, it would be useful to analyse whether *NOD2/CARD15* mutations are able to predict response to therapy, particular biologics. Currently, there are no *NOD2/CARD15* mutations that predict which patients might have sustained remission and which will relapse rapidly after stopping infliximab[35].

Furthermore, this study is of significant importance in highlighting the Australian population of Crohn's patients who are ethically heterogenous and genetically diverse. In the present study, we observed allele frequencies of 13.3%, 3% and 16.7% for the 3020*ins*C, *G908*R mutation and *R702*R mutations respectively. We were the first Australian study to conduct a full genotype sequencing of the *NOD2/CARD15* gene (Table 3). We now know that there is great ethnic and geographical differences in the prevelance of NOD2/CARD15 mutations. The multicenter study published by Lesage *et al*[6], which included several European countries, reported a mutation carrier frequency of 50%. These findings have been replicated by other European studies .In Great Britain, the carrier frequency was found to be 38.5%[7], 46.3% in Belgium[36], 38.2% in Italy[25] , 38% in France[37], and 36.5% in Germany[20]. Conversely, there was a lack of mutant variants in the Asian populations of Japan[38], Korea[39], China[40,41] and India[42]. In one Malaysian study, none of the Malaysian patients with CD carried any of the *NOD2/CARD15* pathogenic mutations[43,44].

We would expect the frequencies of the *NOD2/CARD15* risk alleles found in our patients to be higher than in other studies due to the enrichment of *NOD2/CARD15* variants within our cohort of patients with small bowel and ileo-caecal disease, both of which are disease locations associated with *NOD2/CARD15* mutations. Furthermore, all patients in this cohort have had at least one surgical resection and have more severe disease (with surgery being a marker of severity), another clinical feature suggested to be a result of NOD2 mutations.

In our study, the presence of a *NOD2/CARD15* mutation especially the 3020*ins*C mutation, predicted a more aggressive form of disease which rapidly progressed and showed a trend towards the need for multiple surgical interventions and a significantly shorter time to surgery after diagnosis. Full sequencing may not be relevant for clinical management based on current information, but our results do suggest a place for initial genotyping of

the 3 pathogenic mutations. Further confirmatory studies may suggest that a top-down therapy approach could be considered in mutation positive. There is substantial optimism that *NOD2/CARD15* genotyping could be used in the development of clinical paradigms in the management of Crohn's disease, especially guiding the need for more aggressive medical therapy or surgical intervention after diagnosis of CD.

COMMENTS

Background

Despite advances in the understanding of the pathogenesis of Crohn's disease (CD), little is known about the influence of the nucleotide-binding oligomerisation domain 2/caspase recruitment domains 15 (*NOD2/CARD15*) gene on the natural history of the disease with regard to the requirement for initial or subsequent surgery.

Innovations and breakthroughs

Most research studies conducted tend to carry out gene sequencing for the known pathogenic mutations, mainly due to cost limitations. This study was the first Australian study to conduct a full gene sequence of *NOD2/CARD15* to try to identify novel mutations that may have a pathogenic role. The study was rewarded with new sequence variations of which there were 12 patients with a P268S sequence variation. 9 of these patients were heterozygous for the P268S variation and 3 were homozygous. Whilst not previously identified, the P268S change was not found to independently reduce the time between surgery and diagnosis ($P = 0.067$). It remains to be determined whether full *NOD2/CARD15* gene sequencing during the diagnosis of CD will be clinically useful and cost effective. Most of the *NOD2/CARD15* sequence variations identified in this study are rare and have not been found to influence the natural history of the disease.

Applications

In this study, it was found that a *NOD2/CARD15* mutation predicted initial disease aggressiveness in CD with mutation positive patients having a significantly reduced time between the diagnosis of CD and initial surgery. There was also a trend to suggest that patients with the pathogenic mutations have a higher frequency of operations compared to those without the mutation. These findings were particularly significant in the presence of the 3020*ins*C mutation.

Peer review

This is an interesting manuscript. This study identify a new mutation INV2-25G in *NOD2/CARD15* gene, which is reported previously.

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BRIEF ARTICLE

Routine diagnosis of intestinal tuberculosis and Crohn's disease in Southern India

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Abstract

AIM: To investigate whether routinely measured clinical variables could aid in differentiating intestinal tuberculosis (ITB) from Crohn's disease (CD).

METHODS: ITB and CD patients were prospectively included at four South Indian medical centres from October 2009 to July 2012. Routine investigations included case history, physical examination, blood biochemistry, ileocolonoscopy and histopathological examination of biopsies. Patients were followed-up after 2 and 6 mo of treatment. The diagnosis of ITB or CD was re-evaluated after 2 mo of antituberculous chemotherapy or immune suppressive therapy respectively, based on improvement in signs, symptoms and laboratory variables. This study was considered to be an exploratory analysis. Clinical, endoscopic and histopathological features recorded at the time of inclusion were subject to univariate analyses. Disease variables with sufficient number of recordings and $P < 0.05$ were entered into logistic regression models, adjusted for known confounders. Finally, we calculated the odds ratios with respective confidence intervals for variables associated with either ITB or CD.

RESULTS: This study included 38 ITB and 37 CD patients. Overall, ITB patients had the lowest body mass index (19.6 *vs* 22.7, $P = 0.01$) and more commonly reported weight loss (73% *vs* 38%, $P < 0.01$), watery diarrhoea (64% *vs* 33%, $P = 0.01$) and rural domicile (58% *vs* 35%, $P < 0.05$). Endoscopy typically showed mucosal nodularity (17/31 *vs* 2/37, $P < 0.01$) and histopathology more frequently showed granulomas (10/30 *vs* 2/35, $P < 0.01$). The CD patients more frequently reported malaise (87% *vs* 64%, $P = 0.03$), nausea (84% *vs* 56%, $P = 0.01$), pain in the right lower abdominal quadrant on examination (90% *vs* 54%, $P < 0.01$) and urban domicile (65% *vs* 42%, $P < 0.05$). In CD, endoscopy typically showed involvement of multiple intestinal segments (27/37 *vs* 9/31, $P < 0.01$). Using logistic regression analysis we found weight loss and nodularity of the mucosa were independently associated with ITB, with adjusted odds ratios of 8.6 (95%CI: 2.1-35.6) and

18.9 (95%CI: 3.5-102.8) respectively. Right lower abdominal quadrant pain on examination and involvement of ⩾ 3 intestinal segments were independently associated with CD with adjusted odds ratios of 10.1 (95%CI: 2.0-51.3) and 5.9 (95%CI: 1.7-20.6), respectively.

CONCLUSION: Weight loss and mucosal nodularity were associated with ITB. Abdominal pain and excessive intestinal involvement were associated with CD. ITB and CD were equally common.

Key words: Diagnosis; Differential; Tuberculosis; Gastrointestinal; Crohn's disease; India; Signs and symptoms; Endoscopy; Histopathology

Core tip: Intestinal tuberculosis (ITB) and Crohn's disease (CD) may easily be confused with one another in terms of clinical, laboratory, endoscopic and histopathological features. In this prospective multi-centre study from Southern India, we found weight loss and mucosal nodularity were associated with ITB. Furthermore, right lower abdominal pain and multi-segment intestinal involvement were associated with CD. The random inclusion of as many CD as ITB patients suggests that CD may be increasing in the region. Current diagnostic modalities for differentiating ITB from CD are imperfect and simple inexpensive tools for diagnosis are needed.

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INTRODUCTION

Intestinal tuberculosis (ITB) is commonly encountered by gastroenterologists in India, and is presumably seen more often than Crohn's disease (CD)[1-3]. Nevertheless, CD seems to be increasing in the area, as in other regions of improved hygiene, sanitation and health care[4].

The presentation and pathologic findings in ITB may vary, be non-specific and can easily be confounded with other abdominal or gastrointestinal diseases such as CD or ulcerative colitis[5]. CD is most commonly mistaken for ITB, because the clinical appearance and the radiological, endoscopic and histopathological manifestations may be identical[2,6]. This poses diagnostic problems due to the lack of awareness and the difficulty of confirming TB by bacteriological methods[7]. Skilled clinicians may make the correct diagnosis in approximately half of the patients based on medical history, clinical signs and symptoms alone. By adding results of radiological, endoscopic, histopathological and microbiological investigations, the correct diagnosis may be achieved in up to 80% of the patients[8].

Failing to diagnose ITB represents a considerable risk of morbidity and mortality. However, misdiagnosing and treating ITB as CD could potentially be lethal given the immunosuppressive nature of CD therapy. Conversely, empiric antituberculous chemotherapy (ATT) may complicate diagnosis of ITB at a later stage and facilitate the development of *Mycobacterium tuberculosis* (*M. tuberculosis*) drug resistance. Ultimately, ATT may critically delay proper CD treatment and patients may be at risk of exacerbation or complications.

During the past decade, new diagnostic methods, such as *M. tuberculosis* PCR, immunohistochemistry and interferon-γ release assays have been evaluated for the differentiation of the two diseases. Unfortunately, studies on their sensitivity and specificity have been conflicting and their diagnostic utility is uncertain. New diagnostic algorithms or recommendations have emerged with nearly every paper published[9-13].

Traditional diagnostic modalities, such as acid-fast staining of biopsies or sputum, *M. tuberculosis* culturing, Mantoux tuberculin skin testing, and chest X-rays, are often negative in extra-pulmonary TB[1,5,14]. Currently, empiric prescription of standard ATT for eight weeks followed by re-evaluation of the diagnosis remains the commonly applied diagnostic modality[2,12,14,15].

In this prospective multi-centre study, we evaluated routine clinical, endoscopic and laboratory variables currently applied for diagnosing ITB in Southern India. Using these variables we evaluated the ability to differentiate ITB from CD.

MATERIALS AND METHODS

Thirty-eight patients with ITB and 37 patients with CD were prospectively included from four South Indian medical centres in a consecutive manner from October 2009 to July 2012. Centre investigators identified patients with ITB or CD. The inclusion/exclusion criteria were thoroughly discussed at meetings and clarified in the study protocol (Table 1). Although generally recommended in the literature, *M. tuberculosis* specific microbiologic diagnostics were not routinely applied as our study was descriptive and sought to reflect clinical practice in the region. All ITB patients were prescribed ATT for six months according to the Indian Revised National Tuberculosis Control Program[16].

A detailed medical history was acquired prior to physical examination and further investigations, all of which were recorded in standardised electronic questionnaires.

C-reactive protein (CRP) was analysed in blood serum. Retrograde video ileocolonoscopy with biopsies was performed in each centre after proper colon preparation. Any observed pathology was recorded on video and described according to standardised criteria: anatomic location of lesion(s), aphthous and/or deep ulcers nodularity of mucosa, fissures, and strictures. All four centre investigators were senior consultant gastroenterologists and classified pathology according to their clinical experience. Tissue biopsies obtained during ileocolonoscopy were

Table 1 Inclusion and exclusion criteria for study participants

Criteria	
Inclusion criteria	
Intestinal tuberculosis (as per modified Paustian's criteria[31,32]: (a), and one or more of (b) and (c) had to be fulfilled)	(a) Endoscopic apparent intestinal tuberculosis: transverse ulcers, pseudopolyps, involvement of fewer than four intestinal segments, patulous ileo-coecal valve (b) Histological evidence of tubercles/granulomas with caseation necrosis in intestinal biopsies (c) Clinical response to antituberculous chemotherapeutic drug treatment (ATT) trial
Crohn's disease (as per ECCO guidelines 2010[33] and management consensus of inflammatory bowel disease for the Asia-Pacific region 2006[34])	Exclusion of infectious enterocolitis Endoscopic: ileal disease, rectal sparing, confluent deep linear ulcers, aphthoid ulcers, deep fissures, fistulae, skip lesions (segmental disease), cobble-stoning Histological: focal (discontinuous) chronic (lymphocytes and plasma cells) inflammation and patchy chronic inflammation, focal crypt irregularity (discontinuous crypt distortion) and granulomas (not related to crypt injury) Samples from ileum: irregular villous architecture
Exclusion criteria	
Malignancy	
HIV positive	
Age below 18 yr	

fixated in 10% formalin and preserved in blocks. Sections of 4 μm thickness were cut from blocks and stained by Haematoxylin-Eosin and then evaluated by pathologists.

Patients were scheduled to follow-up clinical evaluation after 8 wk of ATT or immune suppressive therapy. Treatment response was evaluated on clinical grounds, with improvement in signs and symptoms being regarded as confirmatory for diagnosis.

Ethics

The study was approved by The Ethical Committee of Sree Gokulam Medical College and Research Foundation, Trivandrum, India. Written informed consent was obtained after explaining the study to the participants in their preferred language.

Statistics

Due to the skewed distribution of data and limited sample size, the continuous variables were described with median and range, while the categorical variables were listed as counts and percentages. Crude differences between disease type and selected disease associated variables were assessed with a Mann-Whitney Wilcoxon test for the continuous variables. χ^2 tests were applied to compare pairs of categorical variables.

First, all variables possibly associated with disease type (ITB or CD) were subject to univariate logistic regression. Then, variables with sufficient number of recordings and $P < 0.05$ were entered into a multiple logistic regression model (MLR). The results were expressed as odds ratios (OR) with 95% confidence intervals (CI) and adjusted for known confounders. ITB was used as a reference for the ease of interpretation. All tests were two-sided. We considered our study to be an exploratory analysis, therefore $P < 0.05$ was considered statistically significant and we did not perform any correction for multiple testing.

All data were analysed in cooperation with a biomedical statistician using SPSS statistical software Version 18.1.

RESULTS

Baseline data were completed for all participants. In total, 24 of 75 patients were lost to follow-up clinical evaluation: 19 ITB and 5 CD patients. Follow-up endoscopy was only achieved in the minority of the patients, as many either refused repeat endoscopy or were lost to follow-up after clinical evaluation at 2 mo. Two patients initially treated with ATT had their diagnosis revised to CD after endoscopy at 6 mo. None of the CD patients had their diagnosis revised to ITB.

Univariate analyses

Any possible associations between disease type (ITB or CD) and symptoms, signs and other disease associated factors are presented in Table 2. We found an association ($P < 0.05$) between having ITB and experiencing weight loss and watery diarrhoea. A diagnosis of CD was associated with pain in the right lower abdominal quadrant at examination, malaise and nausea. Weight measurements at examination revealed lower body weight in the ITB patients (median: 52 kg, IQR 13) than in the CD patients (median: 59 kg, IQR 12), $P = 0.001$.

ITB patients had a lower body mass index than the CD patients, but cachexia observed by the centre investigators on physical examination was equally distributed between the two groups. The duration of illness prior to receiving care did not differ between the groups. Abdominal pain and altered bowel habits within the last year were commonly reported by both patient groups. The patients had the same average number of bowel evacuations per day. Chest symptoms were noted in four ITB patients and none of the CD patients.

The median CRP was higher in ITB patients (10.7 mg/L, normal reference < 6.0 mg/L) than in CD patients (4.3 mg/L). However, the difference did not reach statistical significance (Table 3).

Ileocolonoscopy showed that CD patients had a more widespread disease with involvement of multiple intestinal segments compared to the ITB patients' more localised

Table 2 Symptoms, signs and disease associated factors of intestinal tuberculosis and Crohn's disease *n* (%)

Symptoms, signs and factors	ITB (*n* = 38)	CD (*n* = 37)	*P* value
Duration of illness prior to care (mo), median	6	6	0.58[1]
Min-Max	0-60	1-78	
Body mass index, median	19.6	22.7	0.01[1]
Min-Max	11.2-26.0	15.6-31.2	
Waist circumference (cm), median (inter quartile range)	77 (11)	80 (15)	0.82[1]
Change in bowel habit	28/37 (76)	34 (92)	0.11[2]
Weight loss in history of current complaint	27/37 (73)	14 (38)	< 0.01[2]
Malaise in history of current complaint	23/37 (62)	32 (87)	0.03[2]
Abdominal pain in history of current complaint	35 (92)	33 (89)	0.71[2]
Nausea in history of current complaint	20/37 (54)	31 (84)	0.01[2]
Recent fever	17/37 (47)	9/36 (25)	0.06[2]
Cachexia at examination	22/36 (61)	21 (57)	0.71[2]
Current watery diarrhoea	21/33 (64)	12/36 (33)	0.01[2]
Daily bowel evacuations, median, max (inter quartile range)	3 (3)	3 (0)	0.24[1]
Pain in right lower abd. quadrant on exam	15/28 (54)	27/30 (90)	< 0.01[2]

[1]Mann Whitney Wilcoxon Test; [2]Pearson χ^2 Test OR Fischer's exact test if n in any cell < 5. ITB: Intestinal tuberculosis; CD: Crohn's disease.

Table 3 Laboratory, endoscopic and histopathological variables in intestinal tuberculosis and Crohn's disease

Clinical variables	ITB (*n* = 38)	CD (*n* = 37)	*P* value
CRP (mg/L), median	10.7	4.3	0.13[1]
Min-max	0.2-70.5	0.3-49.8	
Features on ileocolonoscopy, *n* endoscopy reports	31/38	37	
Localised intestinal inflammation, *n*	12	4	0.01[2]
3 or more inflamed intestinal segments, *n*	9	27	< 0.01[2]
Aphtous ulcers, *n*	12	16	0.71[2]
Deep ulcers, *n*	19	24	0.90[2]
Mucosal nodularity, *n*	17	2	< 0.01[2]
Histopathological evidence of granulomas(s), *n*	10/30	2/35	< 0.01[2]

[1]Mann Whitney Wilcoxon Test; [2]Pearson χ^2 Test OR Fischer's exact test if *n* in any cell < 5. ITB: Intestinal tuberculosis; CD: Crohn's disease.

Table 4 Demographic data of intestinal tuberculosis and Crohn's disease patients *n* (%)

Demographic data	ITB (*n* = 38)	CD (*n* = 37)	*P* value
Age, median	33	33	0.62[1]
(min-max)	(21-68)	(18-76)	
Male gender	22 (58)	24 (65)	0.54[2]
Average monthly household income (€), median	64	114	0.10[1]
(min-max)	(4-508)	(25-953)	
Urban domicile	15 (42)	24 (65)	0.05[2]
High school degree or higher	20 (53)	27 (73)	0.07[2]

[1]Mann Whitney Wilcoxon Test; [2]Pearson χ^2 Test OR Fischer's exact test if *n* in any cell < 5. ITB: Intestinal tuberculosis; CD: Crohn's disease.

disease, $P < 0.01$. Conversely, mucosal nodularity was more frequent in the ITB patients, $P < 0.01$. None of the other disease associated macroscopic features differed between the groups (Table 3). Lesions observed on endoscopy were predominantly located in the ileocoecal area and the ascending colon in both patient groups. However, the number of lesions observed was higher in the CD group of patients (110 lesions *vs* 64 lesions) (Figure 1).

Granulomas were more commonly detected in the biopsies from the ITB patients than the CD patients, $P < 0.01$ (Table 3).

There were no differences between the groups with regard to patient age and sex distribution. In the ITB group, 42% of the patients reported living in urban areas, and 53% held a high school degree or higher. Conversely, 65 % of the CD patients were urban dwellers and 73% held a high school degree or higher. The median household income in the CD group was nearly twice the median household income of those with ITB [9000 rupees (128 €) *vs* 5000 rupees (71 €)] (Table 4).

Logistic regression analyses

Logistic regression analysis was performed to evaluate possible associations between selected disease variables and the outcome (ITB or CD). Variables such as malaise, nausea and watery diarrhoea were excluded from the models due to their confounding potential.

Our first model contained clinical and epidemiological data and included four independent variables: weight loss within last year prior to care, pain in the right lower abdominal quadrant at examination, age and sex. The distributions of the latter two were equal between the groups and were excluded from the model.

Weight loss and abdominal pain remained independent predictors of ITB or CD. Patients with weight loss but no abdominal pain were nearly 9 times more likely to have ITB than patients with the opposite features, OR = 8.6, 95%CI: 2.1-35.6. Conversely, patients with pain in the right abdominal quadrant on examination but no weight loss were 10 times more likely to have CD, adjusted OR 10.1, 95%CI: 2.0-51.3 (Table 5).

Our second model contained endoscopic and epidemiological data and included four independent variables: 3 or more involved intestinal segments, nodularity

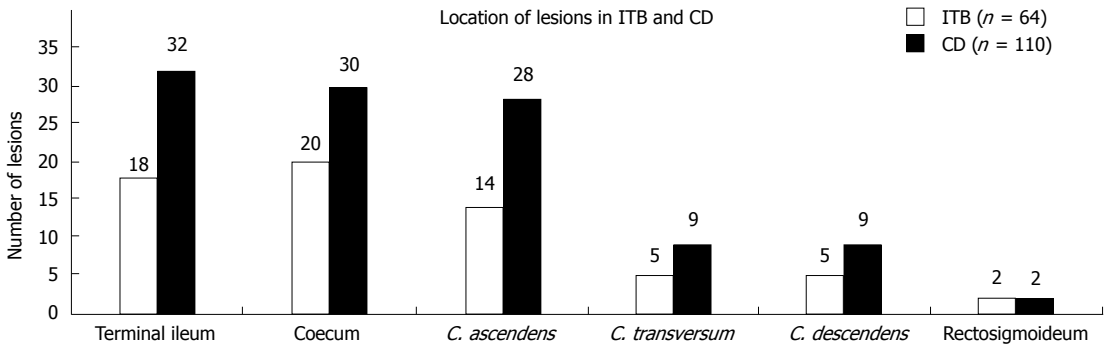

Figure 1 Location of lesions in intestinal tuberculosis and Crohn's disease. ITB: Intestinal tuberculosis; CD: Crohn's disease.

Table 5 Multiple logistic regression analysis of symptoms, signs and endoscopic features of intestinal tuberculosis and Crohn's disease

	P value	OR	95%CI	Associated with
Multiple logistic regression model 1				
Weight loss	0.003	8.6	2.1-35.6	ITB
Pain in right lower abdominal quadrant on examination	0.005	10.1	2.0-51.3	CD
Multiple logistic regression model 2				
Mucosal nodularity	0.001	18.9	3.5-102.8	ITB
Multi-segment involvement (3 or more)	0.005	5.9	1.7-20.6	CD

ITB: Intestinal tuberculosis; CD: Crohn's disease.

of mucosa, age and sex. The age and sex distributions were equal between the groups and these variables were excluded from the model. Involvement of 3 or more intestinal segments and nodularity of mucosa remained associated with the type of diagnosis (ITB or CD). The strongest independent predictor was mucosal nodularity. ITB patients were 19 times more likely to present with this feature on endoscopy when controlling for the other factors in the model, OR = 18.9, 95%CI: 3.5-102.8. Conversely, a patient with involvement of 3 or more intestinal segments on endoscopy was 6 times more likely to suffer from CD, adjusted OR = 5.9, 95%CI: 1.7-20.6 (Table 5).

DISCUSSION

Weight loss is typically found in TB disease in general and is traditionally referred to as "wasting". The reasons for loss of weight in TB may be multi-factorial. As cachectic patients lose body fat, their leptin production subsequently drops, resulting in loss of appetite and further weight reduction. Energy expenditure has not been found to be increased in TB patients. Thus, wasting has been explained by decreased energy uptake rather than increased energy consumption[17,18]. Weight loss as a presenting symptom of TB has previously been found in 45% of patients with pulmonary TB[19]. Interestingly, 73% of our ITB patients experienced weight loss prior to care. Intestinal involvement may result in malabsorption of nutrients and decreased intestinal transit time[20,21]. Therefore, weight loss in ITB patients may not only reflect the general wasting observed in TB, but also the intestinal involvement itself.

Weight loss may occur in patients with CD as well and has been investigated in several previous studies. In patients without small intestinal involvement and malabsorption other mechanisms may lead to weight reduction. As in TB, a relative decrement in energy intake rather than energy loss has been found to be a major contributor to loss of body mass in colonic CD[22]. Intestinal inflammation may result in inadequate protein sparing mechanisms. Weight loss may therefore be more pronounced in patients with low protein intake and intestinal disease. CD patients with active disease also require excessive energy consumption compared to healthy people.

With respect to the drop in leptin production and decreased energy intake, weight loss in ITB seems stronger than in CD. Weight loss was reported more frequently in ITB patients, and their body weight at examination was lower than in the CD patients. Data of our patients' weight prior to receiving care was unavailable. Thus, whether the ITB patients' baseline body weight prior to illness was lower than in the CD patients is unknown.

Pain in the right lower abdominal quadrant was more frequently reported on physical examination in our CD patients than in the ITB patients ($P < 0.01$). We made a distinction between abdominal pain as a symptom in the patient's history and localised abdominal pain as a sign on physical examination. Our findings of abdominal pain on examination, predominantly in CD patients and only to a lesser degree in ITB patients, may reflect a higher level of inflammation and a lower threshold for pain in CD compared with ITB. This is further supported by the finding of relatively more lesions in the ileocecal and ascending colon areas in our CD patients. Abdominal pain as a

symptom of ITB is well known from the literature, but distinguishing it from pain on physical examination has not been described previously.

Previous reports have described skip lesions and extensive disease to be a more frequent finding of CD[2,23]. Our study confirms this with more CD patients suffering from involvement of three or more intestinal segments compared to those with ITB ($P < 0.01$). Thus, ITB is localised to the predilection site of *M. tuberculosis* infection, usually the terminal ileum or coecum. Distal segments are more frequently involved in CD. Although the phenotype of CD in India seems similar to CD elsewhere, environmental triggers of disease may vary between different geographical regions and possibly affect the distribution of lesions[1,4,8].

Nodularity of the intestinal mucosa is a well known feature of ITB[2,3,24]. We identified nodularity in 17 ITB patients and two CD patients. Although nodular mucosa may be a typical finding in ITB, a range of other aetiologies should be kept in mind[25].

With regards to patient demographics, a greater proportion of our CD patients were living in urban communities. We found a trend of CD patients having a higher household income and level of education. This is somewhat similar to the so-called "blue collar findings" reported in studies on IBD, where CD as opposed to ulcerative colitis seems more prevalent in persons with higher socioeconomic status[4,26]. The median age of 33 years at diagnosis in both ITB and CD patients is equivalent to observations in previous reports[2,8,27].

Males were over-represented in our cohort with a male to female ratio of 3 to 2. Other reports on CD in Asia have described a similar 3:2 male predominance[8,27]. In India, this may reflect inequity in access to health care[28].

Our ITB patients were mainly living in rural areas, with a rural to urban ratio of 3 to 2. Epidemiological studies on TB in India have shown different case detection between rural and urban dwellings[29,30]. It is known that TB transmits more easily in communities with lower socioeconomic status because people live more densely with poorer ventilation and hygienic facilities. The relatively lower household income and educational level in our ITB patients may simply reflect that our ITB patients were predominantly rural dwellers with limited access to higher education and income.

By chance, we recruited equal numbers of ITB and CD patients during the 33-mo inclusion period. This result may be a random finding, but it could also suggest that the incidence of CD in South India is higher than previously assumed. According to epidemiological studies throughout Asia the incidence of IBD including CD could be increasing[4].

The study has some limitations. Of the four variables found to be predictive of disease, "pain" and "nodularity" could not be further sub-classified. Conversely, "weight loss" and "number of involved intestinal segments" allowed for objective estimation: a significant difference in body weight was found between the groups and the endoscopists scored the number of involved segments according to pre-defined anatomic locations.

Our cohort of 75 patients only allows for crude statistical analyses. This is reflected by the wide confidence intervals of the ORs established by logistic regression analysis. Therefore, although statistically significant differences were found between the groups with regard to selected variables, the precision of the estimates is limited. Furthermore, to draw any conclusions on differences between the study groups or the background population with regard to demographics or epidemiology is not feasible. Despite repeated attempts to contact patients for scheduling appointments, one third of the patients were lost to follow-up. Hence, we are unaware of their clinical course and could not confirm their diagnosis.

As we sought to describe the current clinical practice in Southern India, only diagnostic modalities routinely available were evaluated. Although positive acid-fast staining and/or culturing of *M. tuberculosis* from intestinal biopsies have high positive predictive values for TB diagnosis, these methods were not applied, possibly due to their low yield shown in previous studies[1,5,14]. Additionally, it may be unfavourable to leave a patient untreated pending the result of a slow growing *M. tuberculosis* culture. As negative chest radiographs may not rule out ITB, routine chest X-ray was not performed in our cohort. Chest symptoms were recorded in four of our 38 ITB patients. CT or MR enterography was not applied in the participating centres during the study period.

Our multi-centre design including four study sites strengthens the study as selection bias was avoided. The standardisation of histopathological evaluation of biopsies between the different pathologists was limited and microscopic features could only partially be pooled for analysis. Similarly, although inter-observer variability was reduced by use of standardised endoscopy report forms, interpretations of pathology may have varied between the endoscopists, Apart from endoscopy and histopathology, all laboratory analyses were conducted by one person and all case record forms were standardised to multiple choice answers.

Of all clinical features, weight loss favoured a diagnosis of ITB while right lower abdominal pain on physical examination favoured a diagnosis of CD.

Endoscopically, nodularity of the mucosa was found to be a hallmark of ITB. Involvement of three or more intestinal segments favoured a diagnosis of CD, whereas the majority of our ITB patients had localised disease. The features of our patients are consistent with previous descriptions of ITB and CD from the Indian subcontinent. Interestingly, nearly as many CD patients as ITB patients were recruited from the secondary and tertiary centres of this study. This result suggests that CD is increasing in South India.

Currently, differentiating between ITB and CD is time consuming, labour intensive and costly. Clinical features distinguishing between the diseases may overlap, and a

range of other diseases may present identically to ITB and CD. Simple, inexpensive and rapid diagnostic modalities applicable in populations of both high and low TB endemicity are needed for the differentiation and diagnosis of ITB and CD.

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COMMENTS

Background

Crohn's disease (CD) is on the increase worldwide while tuberculosis (TB) is re-emerging in areas of low TB endemicity. Intestinal TB (ITB) may easily be confounded with CD, a mimicry that could pose diagnostic and therapeutic challenges. Authors prospectively evaluated routine clinical, endoscopic and laboratory variables currently available in Southern India. By employing these variables, they evaluated the ability to differentiate ITB from CD.

Research frontiers

Differentiating between ITB and CD may be time consuming, labour intensive and costly. Modern tools using PCR, immunohistochemistry and interferon-γ release assays have been studied. However, their diagnostic yield remains uncertain and new diagnostic algorithms or recommendations emerge with nearly every paper published. Traditional diagnostic methods such as acid-fast staining of biopsies or sputum, *Mycobacterium tuberculosis* (*M. tuberculosis*) culturing, Mantoux tuberculin skin testing, and chest X-rays, are often negative in extrapulmonary TB. Today, empiric prescription of antituberculous chemotherapy for eight weeks followed by re-evaluation of the diagnosis is commonly applied as a diagnostic modality. Simple, inexpensive and rapid diagnostic methods applicable in populations of both high and low TB endemicity are needed for the differentiation and diagnosis of ITB and CD.

Innovations and breakthroughs

Of all clinical features, weight loss favoured a diagnosis of ITB while right lower abdominal pain on physical examination favoured a diagnosis of CD. Endoscopically, mucosal nodularity was found to be a hallmark of ITB, while involvement of three or more intestinal segments favoured a diagnosis of CD. The features of their patients are consistent with previous descriptions of ITB and CD from the Indian subcontinent. However, they also demonstrated that clinical features overlap. The same number of CD patients as ITB patients were recruited from the secondary and tertiary centres of this study and authors stress clinicians should be highly cautious when trying to distinguish between the diseases.

Applications

By understanding the differences and the similarities between these two diseases co-existing on the Indian sub-continent, authors may apply new diagnostic modalities to the diagnostic work-up and further evaluate their performance in the field. Their study adds valuable clinical information that may help target the development of simple and inexpensive diagnostics in the future.

Terminology

Polymerase chain reaction, or *PCR*, is a laboratory technique used to make multiple copies of a segment of DNA, *i.e.*, mycobacterial DNA; Immunohistochemistry is a molecular biological method which detects antigens in tissue sections by means of immunological and chemical reactions; Interferon-γ release assays detect latent tuberculosis infection by measuring in vitro interferon-γ release in response to antigens present in *M. tuberculosis*; Leptin is an adipocyte derived hormone which regulates energy intake and expenditure, metabolism and behaviour.

Peer review

This is a prospective, multi-center exploratory study analysing whether routinely used clinical variables could help to differentiate patients with ITB from CD. In a small series of patients the authors find a marginally significant difference in four variables - they found weight loss and nodularity of the mucosa to be independently associated with ITB. Right lower abdominal quadrant pain on examination and involvement of $\geqslant$ 3 intestinal segments were independently associated with CD.

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BRIEF ARTICLE

Dietary habits of colorectal neoplasia patients in comparison to their first-degree relatives

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Abstract

AIM: To compare the dietary habits between colorectal neoplasia patients, their first-degree relatives, and unrelated controls.

METHODS: From July 2008 to April 2011, we collected epidemiological data relevant to colorectal cancer from patients with colorectal neoplasias, their first-degree relatives, and also from a control group consisting of people referred for colonoscopy with a negative family history of colorectal cancer and without evidence of neoplasia after colonoscopic examination. The first-degree relatives were divided into two groups following the colonoscopic examination: (1) patients with neoplasia or (2) patients without neoplasia. Dietary habits of all groups were compared. A χ^2 test was used to assess the association between two dichotomous categorical variables.

RESULTS: The study groups consisted of 242 patients with colorectal neoplasias (143 men, 99 women; mean age: 64 ± 12 years) and 160 first-degree relatives (66 men, 94 women; mean age: 48 ± 11 years). Fifty-five of the first-degree relatives were found to have a neoplastic lesion upon colonoscopy, while the remaining 105 were without neoplasia. The control group contained 123 individuals with a negative family history for neoplastic lesions (66 men, 57 women; mean age: 54 ± 12 years). Two hypotheses were tested. In the first, the dietary habits of first-degree relatives with neoplasia were more similar to those of patients with neoplasia, while the dietary habits of first-degree relatives without neoplasia were similar to those of the control group. In the second, no sex-related differences in dietary habits were expected between the particular groups. Indeed, no significant differences were observed in the dietary habits between the groups of patients, controls and first-degree relatives with/without neoplastic lesions. Nevertheless, statistically significant sex-related differences were observed in all groups, wherein women had healthier dietary habits than men.

CONCLUSION: In all groups examined, women had healthier dietary habits than men. Modification of screening guidelines according to sex may improve the efficiency of screening programs.

Key words: Colorectal neoplasms; Family; Food habits; Risk factors; Mass screening

Core tip: We compared the dietary habits of patients with neoplasia (patients and their first-degree relatives with neoplasia) and without neoplasia (first-degree relatives without neoplasia and an unrelated control group). We did not identify significant differences in dietary habits between the groups; however, we did identify statistically significant differences between the

dietary habits of men and women in all groups. In all groups, women had healthier dietary habits. Modification of screening guidelines according to sex may improve the efficiency of screening programs, although further studies are needed to support this hypothesis.

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INTRODUCTION

Colorectal cancer is the second leading cause of cancer-related death in developed countries. The Czech Republic has the highest prevalence of colorectal cancer in the world. In 2008, the incidence of colorectal cancer in the Czech Republic was 94.2/100000 men and 61.8/100000 women[1]. It is well established that colonoscopic screening reduces both the occurrence and mortality of colorectal cancer[2]. In 2000, the Czech Republic introduced a nationwide cancer-screening program that included fecal occult blood testing of people over 50 years of age. The program was then updated in 2009 to include the possibility of a primary colonoscopy screening for those over 55 years of age[3,4].

Colorectal neoplasias (CRN) are associated with non-hereditary as well as hereditary risks. Colorectal cancer is the most common familial form of cancer. More than 30% of cases can be attributed to hereditary causes, of which only 5% are due to hereditary cancer syndromes such as familial adenomatous polyposis syndrome and hereditary non-polyposis colorectal cancer[5]. First-degree relatives (FDR) of patients with CRN (either colorectal cancer or advanced adenomas) show up to a 4-fold increased risk for CRN when compared with the general population and are at increased risk for advanced or multiple adenomas[6-9].

Non-hereditary risk factors for colon cancer include advanced age, male sex, alcohol consumption and smoking[10-12]. Dietary factors, such as elevated red meat consumption and low intake of fruit, vegetables, dairy products and dietary fiber, have been associated with an increased risk for CRN[13]. Obesity, sedentary lifestyle, inflammatory bowel diseases and several other conditions such as acromegaly, diabetes mellitus and ischemic heart disease have also been shown to increase risk for colon cancer[14-17].

The goal of this study was to compare the dietary habits of patients with CRN and a control group with the dietary habits of FDR with regard to the findings obtained after a colonoscopy screening. The first tested hypothesis was that dietary habits of FDR with neoplasia are similar to those of patients with CRN and that the dietary habits of FDR without neoplasia are similar to those of the control group. The second tested hypothesis was that there are no sex-related differences of dietary habits between the particular groups.

MATERIALS AND METHODS

Study subjects and clinical data

From July 2008 to April 2011, we collected epidemiological data relevant to colorectal cancer, both from patients with CRN and their FDR as well as from a control group. Epidemiological data, including smoking status (current/former *vs* never), fat intake (low *vs* high), body mass index (BMI; < 30 *vs* ≥ 30 kg/m^2), beer consumption (daily/occasionally *vs* never), consumption of dairy products, fruits, vegetables and red meat (daily *vs* less frequent) and education attainment (primary *vs* secondary/tertiary), were collected from the patients with CRN, FDR and controls by a medical doctor. A single specialist in gastroenterology and nutrition performed the interview about the respondent's dietary habits (the amounts of red meat, fat, dairy products, *etc.*) and made a categorization according to the answers (high intake/low intake in each category). Collection of epidemiological data was part of The Family Project, a unique direct medical counseling project targeting FDR that took place at a single center (non-university), Hospital Frydek-Mistek. The goals of the project were to promote proper colonoscopic surveillance of FDR and to identify FDR at highest risk for CRN. The project was approved by the local ethics committee. All participants signed an informed consent. Simultaneously, an informative campaign was launched in the local media to promote and support public awareness of the project.

FDR were referred to colonoscopic examinations and, dependent on the findings, were divided into FDR with or FDR without neoplasia. The control group contained people with a negative family history that had been referred for colonoscopy and were confirmed to be without neoplasia according to the findings from the colonoscopic examination.

Statistical analysis

Ages are presented as mean ± SD. The dietary habits of all groups (patients with CRN, FDR with neoplasia, FDR without neoplasia, and control group) were compared. A χ^2 or Fisher's exact test was used to assess the association between two dichotomous categorical variables. Because of a heterogeneous representation of men and women in the FDR without neoplasia group, the men and women in all groups were compared separately.

RESULTS

The study groups consisted of 242 patients with CRN (143 men, 99 women; 64 ± 12 years) and 160 FDR (66 men, 94 women; 48 ± 11 years). Fifty-five patients in the FDR group were found to have neoplastic lesions upon colonoscopy, while 105 patients had no evidence of neo-

Table 1 Characteristics of the study groups *n* (%)

Characteristics	Patients	FDR with neoplasia	FDR without neoplasia	Controls	*P* value (χ^2)
Male sex	143/242 (59)	30/55 (56)	36/105 (34)	66/123 (54)	0.001
Obesity	68/242 (28)	15/55 (27)	23/105 (22)	27/123 (22)	0.478
Smoking, current/former	123/242 (51)	28/55 (51)	32/105 (30)	48/123 (39)	0.006
High fat intake	102/242 (42)	28/55 (51)	35/105 (33)	52/123 (42)	0.175
High red meat consumption	171/242 (71)	37/55 (67)	65/105 (62)	62/123 (50)	0.002
Beer consumption	155/242 (64)	35/55 (64)	54/105 (51)	83/123 (67)	0.070
Low intake of dairy products	81/242 (33)	22/55 (40)	27/105 (26)	45/123 (37)	0.219
Low fruit and vegetable consumption	72/242 (30)	14/55 (25)	25/105 (24)	46/123 (37)	0.128
Primary education attainment	134/242 (55)	15/55 (27)	27/105 (26)	47/123 (38)	0.001

FDR: First-degree relatives.

Table 2 Comparison of dietary habits between colorectal neoplasias patients and first-degree relatives with/without neoplasia (χ^2/Fisher's exact test)

Comparison	Male patients *vs* FDR with neoplasia	Male patients *vs* FDR without neoplasia	Female patients *vs* FDR with neoplasia	Female patients *vs* FDR without neoplasia
Obesity	0.274	0.101	0.207	0.642
Smoking	0.975	0.001	0.727	0.645
High fat intake	0.247	0.912	0.451	0.460
High red meat consumption	0.621	0.738	0.956	0.474
Beer consumption	0.674	0.263	0.558	0.316
Low intake of dairy products	0.932	0.976	0.143	0.328
Low fruit and vegetable consumption	0.553	0.794	1.000	0.707
Education attainment	0.002[1]	0.004[1]	0.260	0.001[1]

[1]Higher education attainment in first-degree relatives (FDR).

Table 3 Comparison of dietary habits between controls and first-degree relatives with/without neoplasia (χ^2/Fisher's exact test)

Comparison	Male controls *vs* FDR with neoplasia	Male controls *vs* FDR without neoplasia	Female controls *vs* FDR with neoplasia	Female controls *vs* FDR without neoplasia
Obesity	0.816	0.833	0.379	0.959
Smoking	0.281	0.078	0.289	0.578
High fat intake	0.281	0.090	0.375	0.685
High red meat consumption	0.284	0.187	0.052	0.041[2]
Beer consumption	0.045[1]	0.749	0.456	0.535
Low intake of dairy products	0.618	0.522	0.215	0.315
Low fruit and vegetable consumption	0.049[1]	0.780	1.000	0.794
Education attainment	0.095	0.188	0.444	0.199

[1]Male controls have higher beer consumption and lower consumption of fruits and vegetables; [2]Female controls have higher red meat consumption. FDR: First-degree relatives.

plasia. The control group consisted of 123 individuals with a negative family history of colon cancer and without neoplastic lesion following colonoscopic examination (66 men, 57 women; 54 ± 12 years). Characteristics of all groups are presented in Table 1.

We first tested the hypothesis that dietary habits of FDR with neoplasia are similar to those of patients with CRN and that dietary habits of FDR without neoplasia are similar to those of the control group. We next tested the hypothesis that there are no sex-related differences in the dietary habits between the particular groups. Comparisons of the groups are presented in Tables 2 and 3. The comparison between men and women in all groups is shown in Table 4.

In summary, both of our hypotheses were disproven. There were no significant differences in the dietary habits between the groups of patients, controls and FDR with/without neoplastic lesions. In all groups, however, there were statistically significant differences in the dietary habits between men and women, despite no differences in education attainment among them.

DISCUSSION

Our study was based on epidemiological data relevant to colorectal cancer that was obtained from patients with CRN, their FDR with neoplasia, FDR without neoplasia, and from a control group.

Table 4 Comparison of dietary habits in all groups: males *vs* females (χ^2/Fisher's exact test)

Comparison	Males *vs* females			
	Patients	FDR with neoplasia	FDR without neoplasia	Controls
Obesity	0.023	0.472	0.659	0.831
Smoking	0.001	0.044	0.646	0.002
High fat intake	0.001	0.044	0.002	0.004
High red meat consumption	0.004	0.294	0.045	0.005
Beer consumption	0.001	0.102	0.001	0.001
Low intake of dairy products	0.024	1.000	0.026	0.028
Low fruit and vegetable consumption	0.016	0.537	0.098	0.001
Education attainment	0.756	0.104	0.727	0.508

FDR: First-degree relatives.

It is well established that risks for colorectal cancer can be either hereditary or non-hereditary. Non-hereditary risks are well described, as mentioned in the Introduction. There is also an association of colorectal cancer with the gut microbiome. Intestinal microbiota can transform food compounds into genotoxic agents, activate proto-oncogenes, or inactivate tumor suppressor genes[18-20].

Genetic factors associated with an increased risk for CRN include low-penetrant susceptibility loci and specific polymorphisms. Certain genetic variants and polymorphisms in a number of genes have been associated with increased colon cancer risk; APC-I1307K, HRAS1-VNTR and MTHFR variants represent the strongest candidates for low penetrance susceptibility alleles[21,22]. In genome-wide association studies, as many as 170 common but separate genetic variations have been implicated in CRN susceptibility[23]. Based on current data, there are three main pathways of colorectal carcinogenesis: chromosomal instability, microsatellite instability, and hypermethylation[24,25]. One important question, however, is how hereditary risks may be confounded by familial similarities in diet, physical activity level, or other environmental exposures.

Our first tested hypothesis was that the dietary habits of FDR with neoplasia are similar to those of CRN patients, while the dietary habits of FDR without neoplasia are different and more similar to those of the control group. We hypothesized that both the controls and FDR without neoplasia have a healthier lifestyle, while patients with CRN and FDR with neoplasia have worse, shared dietary habits. Because of the heterogeneous representation of men and women FDR without neoplasia, men and women in all groups were compared separately.

To our surprise, all groups had very similar dietary habits. We only observed a difference in the male CRN patients, where there were significantly more smokers than in the group of FDR males without neoplasia. It has been shown that smoking can increase risk of colorectal cancer by up to 18%[12]. Paradoxically, male controls consumed more beer and lower amounts of fruits and vegetables than FDR males with neoplasia. Female controls consumed more red meat than FDR females without neoplasia. It is surprising that we did not observe any association between poor dietary habits and occurrence of neoplasia in patients with CRN and their FDR with neoplasia, despite all the proven non-hereditary risk factors.

The second tested hypothesis was that there would be no sex-related differences between the particular groups. Regardless of the colonoscopic findings in all groups, however, males had worse dietary habits than females, despite no difference in education attainment between the men and women. It is well known that women gain more health resources in their screening programs. This fact, together with a known higher incidence of CRN in men, places men at a disadvantage. Thus, we can assume that the one-third higher incidence of colorectal cancer in men could be, in part, attributed to their less healthy lifestyle. Media campaigns should, therefore, be targeted to the male population, since there is a great need for improvement of their lifestyle and dietary habits.

This study has several limitations. The sample size of each group was relatively small and made up of individuals stemming from a population with the highest prevalence of colorectal cancer in the world. The results, therefore, are specific and may only apply to the Czech population surveyed. Diabetes mellitus was not observed throughout all groups (only in the CRN group of patients), so we cannot evaluate obesity and dietary habits with respect to diabetes mellitus. The mean ages across the groups examined were different and represent another weakness of the study.

In conclusion, we did not find significant differences between patients and their FDR with/without neoplastic lesions, although we did identify statistically significant differences between the habits of men and women in all groups. Women in all groups had healthier dietary habits. We propose that media campaigns should be targeted to the male population, due to a need to improve their lifestyle. Modification of screening guidelines according to sex may improve the efficiency of screening programs but further studies are needed to support this hypothesis.

COMMENTS

Background

Colorectal neoplasias are associated with hereditary and non-hereditary risks. Colorectal cancer is the most common familial form of cancer. First-degree relatives of patients with colorectal neoplasia, both colorectal cancer and advanced

adenomas, show up to a 4-fold increased risk for colorectal neoplasias when compared to the general population.

Research frontiers

It is important to understand how hereditary risks may be confounded by familial similarities in diet, physical activity level or other environmental exposures and whether it is possible to modify screening programs according to different risk groups to achieve higher efficiency in reduction of colorectal neoplasia.

Innovations and breakthroughs

The authors did not find significant differences between healthy controls, patients and their first-degree relatives with/without neoplastic lesions. The authors identified statistically significant differences between the dietary habits of men and women in all groups. In all groups examined, women had healthier dietary habits.

Applications

The authors propose a media campaign to target the male population and promote ways to improve the health-related aspects of their lifestyle. Modification of screening guidelines according to sex may improve the efficiency of screening programs, but further studies are needed to support this hypothesis.

Terminology

First-degree relatives: a family member who shares approximately 50% of their genes with a particular individual in a family; first-degree relatives include parents, offspring and siblings.

Peer review

This is an important epidemiological study comparing the dietary habits of persons with and without colorectal neoplasia. This is a well-designed study and has clinical applications for understanding the risks of colorectal cancer.

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BRIEF ARTICLE

Utility of serum TNF-α, infliximab trough level, and antibody titers in inflammatory bowel disease

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Abstract

AIM: To assess tumor necrosis factor-α (TNF-α), infliximab (IFX) concentrations, and antibodies against IFX molecules in patients with inflammatory bowel disease (IBD) who develop loss of response, side effects, or allergic reaction during anti TNF-α therapy.

METHODS: Blood samples of 36 patients with response loss, side effects, or hypersensitivity to IFX therapy (Group Ⅰ) and 31 patients in complete clinical remission (Group Ⅱ) selected as a control group were collected to measure trough serum TNF-α level, IFX, and anti-IFX antibody (ATI) concentration. We examined the correlation between loss of response, the development of side effects or hypersensitivity, and serum TNF-α, IFX trough levels, and ATI concentrations.

RESULTS: The serum TNF-α level was shown to be correlated with the presence of ATI; ATI positivity was significantly correlated with low trough levels of IFX. ATIs were detected in 25% of IBD patients with loss of response, side effects, or hypersensitivity, however no association was revealed between these patients and antibody positivity or lower serum IFX levels. Previous use of IFX correlated with the development of ATI, although concomitant immunosuppression did not have any impact on them.

CONCLUSION: On the basis of the present study, we suggest that the simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may further help to optimize the therapy in critical situations.

Key words: Tumor necrosis factor-α; Infliximab; Antibody; Inflammatory bowel disease

Core tip: The clinical utility of measuring serum tumor necrosis factor-α (TNF-α), infliximab, and anti-infliximab levels in the therapeutic decisions of patients with inflammatory bowel disease is still an outstanding question. In this study we assessed TNF-α, infliximab concentrations, and antibodies against infliximab molecules in patients with inflammatory bowel disease who developed loss of response, side effects, or allergic reaction during anti TNF-α therapy. Our results showed that the simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may further help to optimize therapy in critical situations.

Pallagi-Kunstár É, Farkas K, Szepes Z, Nagy F, Szűcs M, Kui R, Gyulai R, Bálint A, Wittmann T, Molnár T. Utility of serum TNF-α, infliximab trough level, and antibody titers in inflammatory bowel disease. *World J Gastroenterol* 2014; 20(17): 5031-5035 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5031.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5031

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the two types of inflammatory bowel disease (IBD) characterized by alternating periods of relapse and remission. The introduction of anti-tumor necrosis factor (TNF)-α therapy caused a dramatic change in the management of IBD. Approximately 40% of patients that initially responded to anti-TNF-α therapy will subsequently lose that response, thus requiring dose intensification or drug change[1]. The presence of antibodies against anti-TNF-α agents and low drug serum concentrations have been implicated as predisposing factors for therapeutic failure. Dose intensification as a possible method for managing therapeutic failure is only viable in cases of low anti-TNF-α drug trough levels, while switching to another drug may be useful if antibodies develop against the biological agents[2]. Immunogenicity (the formation of antibodies to the biological agents) is the major cause of loss of response and adverse reactions. Scheduled maintenance therapy, concomitant immunomodulators therapy, and pretreatment with high-dose corticosteroids may help to reduce immunogenicity[3].

Although anti-drug antibodies were initially considered to play a role in shorter response duration, recent studies revealed that detectable infliximab (IFX) trough levels, irrespective of anti-drug antibody status, may predict to clinical response and endoscopic improvement[4,5]. The role of TNF-α measurement, together with antibody and drug serum concentration in therapeutic decisions, has not previously been investigated in everyday practice; furthermore, pharmacokinetic monitoring of IFX to control disease activity and optimize the treatment of IBD is not standardized in the daily routine. The aim of this study was to assess TNF-α, IFX concentrations, and antibodies against IFX molecules in patients with IBD who developed loss of response, side effects, or allergic reaction during anti TNF-α therapy.

MATERIALS AND METHODS

Study population

Sixty-seven patients with CD and UC treated in our center with IFX between 2011 and 2012 were enrolled in this prospective observational study and categorized into two groups. Blood samples of 36 patients with response loss, side effects, or hypersensitivity to IFX therapy (Group I) and 31 patients in complete clinical remission (Group Ⅱ) selected as a control group were collected to measure trough serum TNF-α level, IFX, and anti-IFX antibody (ATI) concentration. The study was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged. The 3 infusion induction phase was followed by maintenance therapy in every patient. Data on patient demographics, clinical characteristics, concomitant corticosteroid and azathioprine therapies, need of surgery, C-reactive protein level, erythrocyte sedimentation rate (ESR), hematocrit, leukocyte and serum iron levels, and details on biological therapy were prospectively registered. Disease activity was measured by using the Crohn's disease activity index (CDAI) and partial Mayo score. The patients' demographic and clinical data are summarized in Table 1.

Table 1 Demographic and clinical data of patients participating in the study

Therapy	IBD patients with loss of response, side effects, hypersensitivity (n = 6)	Control IBD patients (n = 1)
Mean age at diagnosis, yr	34.9 (17-67)	36.4 (17-66)
Mean disease duration at biological therapy, yr	7.1 (1-20)	7.7 (1-21)
CD/UC	19/17	17/14
Male/female	14/22	14/17
Previous biological therapy	22	15
Concomitant steroid therapy	5	3
Concomitant thiopurine therapy	18	16
Previous surgery	16	7
Active disease	25	0

CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease.

We examined the correlation between loss of response, side effects, or hypersensitivity and serum TNF-α, IFX trough levels, and ATI concentrations.

Measurement of serum TNF-α, IFX trough levels, and ATI

Enzyme-linked immunosorbent assay (ELISA) was applied to determine the serum levels of TNF-α, infliximab trough levels, and ATI. Blood samples were obtained prior to application of IFX infusion. Q-INFLIXI ELISA, Q-ATI ELISA, and Q-TNF-α ELISA kits were obtained from Matriks Biotek, Ankara, Turkey.

Statistical analysis

Continuous data were analyzed using medians with an interquartile range (IQR). All categorical data were compared between groups of patients using the Pearson χ^2 statistic. Mann-Whitney U and Fisher's exact tests were used for comparison of infliximab trough levels and ATIs in a subgroup of patients. Relation between laboratory parameters, IFX trough levels, and ATI was analyzed by Mann-Whitney U test. A P value less than 0.05 was considered to be significant.

Table 2 Serum infliximab and antibody levels in cases of antibody positivity

Patients	Serum IFX level (μg/mL)	ATI level (μg/mL)
1	2.75	3194.90
2	2.68	258.55
3	2.67	1056.25
4	2.66	3055.04
5	2.93	3712.82
6	2.26	3343.07
7	2.66	129.54
8	2.49	4540.33
9	12.40	58.92
10	2.66	3679.21
11	2.65	536.57
12	1.90	555.53
13	1.71	810.87
14	4.67	46.34

IFX: Infliximab; ATI: Antibody.

RESULTS

The median CDAI in groups I and Ⅱ were 138 (IQR 68-186) and 50 (IQR 34-70), respectively; the partial Mayo score in the two groups were 5 (IQR 3-6) and 1 (IQR 0-1), respectively. The median serum TNF-α levels were 10.5 (IQR 3.2-18-9) and 6.3 (IQR 1.5-15.7) pg/mL in groups I and Ⅱ, respectively. The median IFX trough level was 3.1 (IQR 2.6-5.04) and 3.5 (IQR 2.6-4.7) μg/mL in the two groups, respectively. Fourteen patients were found to have ATI positivity with a median of 933 μg/mL (IQR 328-3306). ROC analysis revealed that the cut off value of serum IFX for detecting ATI was 3.01 μg/mL. The serum TNF-α level was significantly higher in the presence of ATI (24.23 pg/mL *vs* 6.28 pg/mL, $P = 0.005$). ATI positivity correlated significantly with low trough levels of IFX (2.66 μg/mL *vs* 3.86 μg/mL, $P = 0.015$). However, no difference was detected in serum IFX and antibody levels between the two groups (2.67 μg/mL *vs* 2.66 μg/mL, $P = 0.821$). Serum IFX and ATI levels in patients with ATI positivity are summarized in Table 2. Two of the IBD patients with antibodies against anti TNF-α developed side effects, 5 patients lost response, and an allergic reaction occurred in 3 patients. 37 patients were previously treated with biologicals, with development of ATI being more frequent those patients ($P = 0.048$). Dose intensification was required in 9 patients. No association was found between dose intensification and the development of ATI. Concomitant immunosuppression had no impact on IFX trough levels or on the development of ATI formation. Increased ESR and C-reactive protein correlated significantly with lower serum IFX level ($P = 0.04$ and $P = 0.002$). The serum TNF-α level was higher in patients not treated concomitantly with steroids ($P = 0.038$).

DISCUSSION

In this prospective observational study, serum TNF-α level was shown to be correlated with the presence of ATI, and ATI positivity correlated significantly with low trough levels of IFX. ATIs were detected in 25% of IBD patients with loss of response, side effects, or hypersensitivity, however no association was revealed between these patients and antibody positivity or lower serum IFX levels. Previous use of IFX correlated with the development of ATI, although concomitant immunosuppression did not have any impact on them.

The prevention and management of therapeutic failure with IFX is a significant challenge for clinicians in the field of IBD. One of the major reasons for loss of response is the development of ATI, which is frequently caused by immunogenicity[6]. Immunogenicity induced by IFX can be determined by measuring antibodies, concentrations of TNF-α, and IFX levels[7]. Use of concomitant immunomodulators and maintenance *vs* episodic IFX therapy has previously been shown to decrease the incidence of ATI[8,9]. Baert *et al*[4] revealed that ATIs reduce serum IFX level, as well as increase the risk of infusion reactions and loss of response. The role of ATI in loss of response to IFX and the lower efficacy of IFX re-treatment have also been confirmed by a study by Farrell *et al*[5]. In this study, both increased TNF-α and decreased IFX levels correlated with the presence of ATI, although neither ATI nor serum IFX influenced the outcome of the therapy. A recent meta-analysis also concluded that the presence of ATIs is associated with a significantly higher risk of loss of clinical response to IFX and lower serum IFX levels in patients with IBD[10]. Although these statements and consequences are logical, the results of clinical practice are conflicting.

In a recently published systematic review, Chaparro *et al*[2] assessed the relationship between the efficacy of TNF-α blockers and their serum levels and the clinical utility of testing for antibodies against TNF-α. A close relationship was revealed between trough levels of anti-TNF-α and maintenance of response. Maser *et al*[11] did not find any difference in the duration of clinical response in patients with detectable IFX serum levels with or without ATI. A higher serum IFX level was proved to predict a longer duration of response and clinical remission by some studies both in CD and UC[9-12]. In contrast, a Japanese study shown that the median trough levels of IFX did not differ significantly in patients who maintained and lost response, suggesting a faster clearance in cases of loss of response[13]. Because of these controversial data, the usefulness of monitoring the trough levels and ATI concentrations in therapeutic decisions may be questionable. Our results do not confirm the clinical utility of trough level and antibody measurement in the differentiation of "problematic" patients with loss of response or adverse reactions *vs* those who respond appropriately to the biological therapy.

In a recently published study by Bortlik *et al*[14], the median trough levels of IFX were significantly higher and antibody titers significantly lower in patients with concomitant thiopurines. In our study, previous biological therapy had a more significant effect on the outcome of IFX therapy than the concomitant use of

thiopurines. According to a study by Afif *et al*[15], dose escalation was associated with a high clinical response in patients with subtherapeutic IFX levels and negative ATI, and better clinical outcome was achieved in ATI positive patients switching to another anti TNF-α. On the basis of previous studies, concomitant corticosteroid therapy is suggested to decrease the effect of TNF-α blocker[16,17], which was confirmed by our results regarding the higher TNF-α level in patients receiving steroids. In conclusion, we found significant association between serum TNF-α level and the presence of ATI, as well as between ATI positivity and low trough levels of IFX. However, antibody positivity and lower serum IFX levels did not correlate with loss of response, side-effects, and hypersensitivity. Previous use of IFX correlated with the development of ATI. When previous studies determined only ATI positivity or negativity, detectable IFX serum concentration suggested many were false-negative results. This factor was decreased by the quantification of ATI titers in our study. On the basis of the present work, we suggest that further prospective studies are needed to determine whether the simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may help to optimize therapy in critical situations.

COMMENTS

Background

The prevention and the management of therapeutic failure with infliximab (IFX) is a significant challenge for clinicians in the field of inflammatory bowel disease (IBD). One of the major reasons for loss of response is the development of anti-IFX antibody (ATI). Due to controversial data, the usefulness of monitoring IFX trough levels and ATI concentrations in therapeutic decisions may be questionable.

Research frontiers

Previous studies have suggested that ATIs reduce the serum IFX level, and therefore increase the risk of infusion reactions and loss of response. The role of ATI in the lower efficacy of IFX retreatment has also been previously confirmed.

Innovations and breakthroughs

Simultaneous measurement of serum tumor necrosis factor-α (TNF-α) level, serum anti TNF-α concentration, and antibodies against anti TNF-α may help to optimize therapy in critical situations of IBD. However, no previous studies have been performed in this topic.

Applications

Significant association was revealed between serum TNF-α level and the presence of ATI, as well as between ATI positivity and low trough levels of IFX. However, antibody positivity and lower serum IFX levels did not correlate with loss of response, side-effects, and hypersensitivity.

Peer review

This is an interesting study with the important message that simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may help to optimize therapy in critical situations.

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BRIEF ARTICLE

Ecological study of gastric cancer in Brazil: Geographic and time trend analysis

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Abstract

AIM: To investigate the geographic distributions and time trends of gastric cancer (GC) incidence and mortality in Brazil.

METHODS: An ecological study of the DATASUS registry was conducted by identifying hospitalizations for GC between January 2005 and December 2010. The data included information on the gender, age, and town of residence at the time of hospital admission and death.

RESULTS: The GC rates, adjusted according to available hospital beds, decreased from 13.8 per 100000 in 2005 to 12.7 per 100000 in 2010. The GC rates decreased more among the younger age groups, in which the male-to-female difference also decreased in comparison to the older age groups. Although the lethality rates tended to increase with age, young patients were proportionally more affected. The spatial GC distribution showed that the rates were higher in the south and southeast. However, while the rates decreased in the central-west and south, they increased in the northern regions. A geographic analysis showed higher rates of GC in more urbanized areas, with a coast-to-inland gradient. Geographically, GC lethality overlapped greatly with the hospital admission rates.

CONCLUSION: The results of this study support the hypothesis of a critical role for environmental factors in GC pathogenesis. The declining rates in young patients, particularly males, suggest a relatively recent decrease in the exposure to risk factors associated with GC. The spatial distribution of GC indicates an ongoing dynamic change within the Brazilian environment.

Key words: Gastric cancer; Ecological study; Hospitalization; Lethality rate

Core tip: Declining rates in young patients and changes in the geographic distribution of gastric cancer suggest a recent decrease in the exposure to risk factors within the Brazilian environment.

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INTRODUCTION

Gastric cancer (GC) is among the most common cancers worldwide and is estimated to be the second-leading cause of cancer-related death[1]. At the time of diagnosis, most patients already present with late-stage disease, resulting in an overall low survival rate[1,2]. Although the mechanisms that underlie gastric carcinogenesis development are not completely understood, GC likely results from complex interactions between host and environmental factors[3].

Multiple risk factors for GC have been recognized, including smoking and diet[4,5], but *Helicobacter pylori* (*H. pylori*) infection and the resulting development of intestinal metaplasia appear to be of crucial importance[6,7]. Chronic inflammation secondary to *H. pylori* infection is thought to be responsible for the development of a stepwise progression from chronic gastritis to pre-malignant changes that eventually result in GC[8,9].

Although epidemiological data appear to indicate a progressive reduction in the incidence of GC, particularly in developed countries within North America and Western Europe, significant geographical[10] and ethnic[11] variations exist. However, data from developing countries remain limited, which renders global analyses difficult. The aim of this study was to analyze the geographic distributions and time trends of GC incidence and mortality in Brazil to identify the areas with differential risks and outcomes for GC.

MATERIALS AND METHODS

Data source

Data from the Health Informatics Department of the Brazilian Ministry of Health (DATASUS) were utilized for this study (http://www2.datasus.gov.br/DATASUS). DATASUS is a fundamental tool for the coordination of the National Health Information System and maintains reference tables and vocabularies used in information systems throughout the entire country. This population-based health and disease registry includes important information such as medical procedures, hospital admission and discharge, and mortality, and it covers approximately the entire Brazilian population. In addition, demographic data, such as age, gender, and municipality, collected from the Instituto Brasileiro de Geografia e Estatística (IBGE; Brazilian Institute of Geography and Statistics), are also available at the DATASUS website.

In this study, we used hospital discharge records for GC patients as an estimate of the GC incidence. We assumed that hospital-based procedures for either GC diagnosis or treatment would in fact reflect the actual disease numbers. Variables for which DATASUS does not collect information, such as co-morbidities and treatment details, were not evaluated.

Study design, population, and variables

We conducted an ecological study with the DATASUS registry by identifying hospitalizations for GC patients diagnosed between January 2005 and December 2010. The analysis period was based on the most recent and consistent information and contained complete data entries. We included patients for whom a diagnosis of malignant neoplasm of the stomach was assigned; these patients were classified as C16 (from C16.0 to C16.9), according to the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10). The data included information on the town of residence at the time of hospital admission and death, and the patients were categorized by gender and age; for the latter, patients were stratified as < 20 years, 20-49 years, 50-69 years, and > 70 years of age (for whom coexisting co-morbidities might render meaningful comparisons more difficult).

For the geographic distribution analysis, the GC hospitalization rates were obtained per 100000 inhabitants in the individual municipalities. The results from this exploratory model were included in a platform to plot maps depicting the estimates and distribution of GC.

Statistical analysis

The statistical analysis was performed with the statistical software package SPSS for Windows (Version 10.0.1, SPSS Inc., Chicago, IL, United States). Exploratory procedures were applied to the data, and summary descriptive statistics and graphical displays were generated either for all cases or separately for groups of cases.

RESULTS

In this study, we first present an overall picture of GC in Brazil over time (from 2005-2010) by showing the total cases registered per year, the rates per 100000 inhabitants, and the lethality rates, including the distribution by gender.

Hospitalizations for gastric cancer

The total numbers of hospitalizations for GC were 19085 in 2005 and 17602 in 2010, of which 3232 and 3305 resulted in death, respectively. Nevertheless, in terms of cases per 100000 inhabitants, the rates adjusted according to available hospital beds decreased from 13.8 per 100000 in 2005 to 12.7 per 100000 in 2010 (Figures 1 and 2 and Table 1). Regarding gender, the Brazilian numbers confirm the world trend for male predominance, as male patients accounted for 65% of all GC cases (Figure 1).

Next, we analyzed the population distribution of GC according to the age at diagnosis, using nationwide age-standardized rates of hospitalizations (Figure 3). Relatively low rates of GC cases were detected within the population < 50 years of age, whereas the rates were higher among those > 50 years, as expected. However, given the differences between 2005 and 2010, GC-related

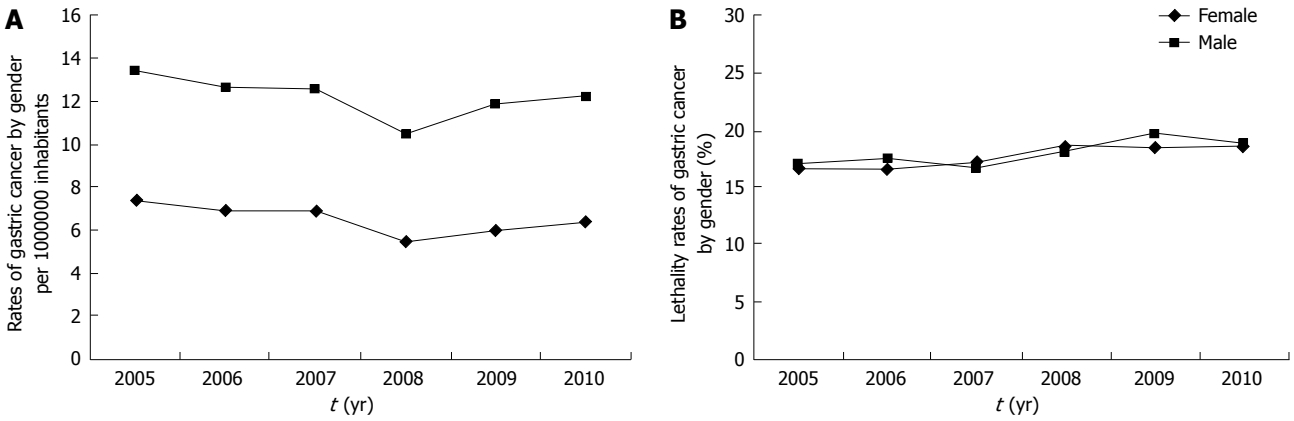

Figure 1 Gastric cancer incidence by gender (A), estimated from hospitalizations and lethality trends (B), in Brazil from 2005-2010.

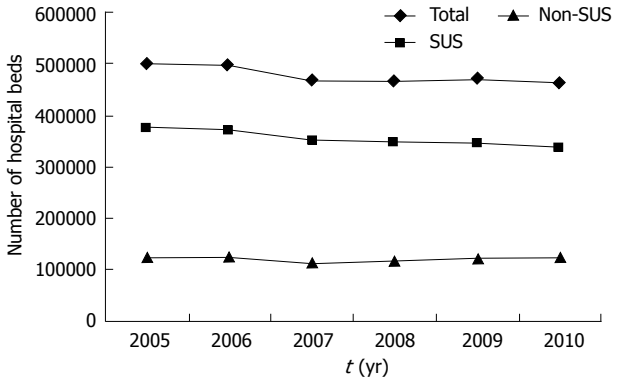

Figure 2 Number and distribution of hospital beds in Brazil from 2005-2010.

hospitalizations decreased proportionally more among the < 50 years groups, in which the male-to-female difference also markedly decreased in comparison with the older age groups (Table 2).

Lethality rates from gastric cancer

Regarding GC-associated mortality, although the differences were not significant, the lethality rates appeared to indicate an increasing trend during the period from 2005-2010 (Figure 1 and Table 1). Although GC lethality was not related to gender, the use of standardized age groups seemed to reveal differences. As expected, the mortality rates increased linearly with age, ranging from approximately 10% among patients < 50 years to 25% among patients > 70 years (Figure 3). Nevertheless, lethality was proportionally greater among patients < 50 years, compared to the older groups (Table 2).

Geographic distribution of gastric cancer

Regarding the geographic distribution, this study analyzed the GC hospitalization rates per 100000 inhabitants within the individual municipalities. The obtained results were plotted in maps that depicted the estimates and distributions of GC per region, including the south, southeast, north, northeast, and central-west regions, in the years 2005 and 2010. The standardized rates defined three ranges of 0-10, 10-65, and > 65. The spatial distribution of GC showed that the rates were higher in the south and southeast, in contrast to those in the north and northeast regions. However, while the GC hospitalization rates decreased in the central-west and southern regions from 2005 to 2010, they increased in the northern regions. A geographic analysis of the distribution of GC cases indicated the presence of higher rates in municipalities with urbanized residency, those located in more industrialized areas, and those with greater urban population concentrations. Moreover, the GC rates also demonstrated a coast-to-inland gradient, which greatly overlaps with the urban-to-rural areas of the country (Figure 4). With respect to GC lethality, the standardized rates defined four ranges of < 25, 25-50, 50-75, and > 75. The geographic distribution of lethality overlapped considerably with the GC hospitalization rates (Figure 5).

In 2005, the towns with the highest rates of GC per 100000 inhabitants were Cuiabá (38.0) in Mato Grosso (central-west) and Curitiba (36.0) in Paraná (south), whereas in 2010, the highest rates were observed in Vitória (42.1) in Espírito Santo (southeast) and Boa Vista (24.9) in Roraima (north). Notably, of the municipal areas with the highest GC rates, 2 of the top 4 areas in 2005 were located in the south, in contrast to 2 of the top 6 that were located in the north in 2010. Table 1 includes the hospitalization and lethality rates and populations of each country region, federal unity, and capital.

DISCUSSION

This study was the first to analyze the spatial distributions and demographics of GC incidence rates and lethality estimates over time in Brazil to identify the areas with differential risks and outcomes. Overall, the results of this study indicate that the nationwide GC incidence declined overall, whereas the lethality rate increased between 2005 and 2010. In particular, we observed a proportional decrease in GC hospitalization rates and the male-to-female ratio among young individuals. Nevertheless, the GC lethality rates proportionally increased among the younger population between 2005 and 2010. The geographic

Table 1 Distributions of gastric cancer hospitalization rates and lethality in 2005 and 2010

Region, Federal Unity and Capital	2005				2010			
	Hospitalization rate (per 10^5)	Population	Adjusted hospitalization rates (per 10^5)	Lethality	Hospitalization rate (per 10^5)	Population	Adjusted hospitalization rates (per 10^5)	Lethality
Brazil	10.4	184184074	13.8	17.0%	9.2	190755799	12.7	18.8%
North	6.0	14698834	7.4	16.0%	6.7	15864454	8.4	16%
Rondônia	2.9	1534584	4.2	11.4%	4.7	1562409	6.5	10.8%
Porto Velho	2.4	373917	4.0	33.3%	4.2	428527	6.1	11.1%
Acre	9.7	669737	10.5	13.8%	7.1	733559	7.8	9.6%
Rio Branco	11.4	305730	13.1	17.1%	11.0	336038	12.9	13.5%
Amazonas	8.9	3232319	10.8	12.1%	8.9	3483985	10.3	9.1%
Manaus	16.2	1644688	22.0	11.6%	16.4	1802014	21.0	9.5%
Roraima	1.5	391318	1.6	0.0%	18.0	450479	18.5	12.3%
Boa Vista	1.7	242179	1.7	0.0%	23.9	284313	24.9	11.8%
Pará	5.4	6970591	6.7	22.0%	4.6	7581051	6.1	28.3%
Belém	11.9	1405873	17.4	23.4%	9.6	1393399	17.2	31.3%
Amapá	2.9	594577	3.3	29.4%	6.9	669526	8.1	28.3%
Macapá	2.8	355405	3.4	30.0%	8.0	398204	10.0	34.4%
Tocantins	6.8	1305708	7.9	10.1%	11.3	1383445	13.0	6.4%
Palmas	9.1	208168	11.6	5.3%	4.8	228332	6.2	0.0%
Northeast	6.4	51018983	7.6	16.0%	6.5	53081950	7.8	17%
Maranhão	5.5	6103338	6.4	12.5%	4.2	6574789	4.9	17.9%
São Luís	9.4	978822	12.3	21.7%	12.3	1014837	15.8	18.4%
Piauí	4.5	3006886	5.0	12.7%	6.8	3118360	7.8	9.0%
Teresina	4.7	788770	6.0	18.9%	9.8	814230	13.0	18.8%
Ceará	9.4	8097290	11.4	13.1%	8.9	8452381	11.2	13.7%
Fortaleza	15.2	2374944	20.3	15.8%	9.5	2452185	13.3	14.2%
Rio Grande do Norte	15.7	3003040	18.7	20.0%	10.2	3168027	11.9	22.5%
Natal	20.1	778038	29.5	32.7%	12.6	803739	16.9	25.7%
Paraíba	8.8	3595849	10.5	13.2%	7.1	3766528	8.3	11.3%
João Pessoa	8.9	660797	11.9	15.3%	6.8	723515	9.1	6.1%
Pernambuco	2.9	8413601	3.5	10.5%	7.4	8796448	9.0	15.2%
Recife	3.7	1501010	4.7	16.4%	8.7	1537704	11.4	20.9%
Alagoas	7.6	3015901	8.7	13.9%	3.0	3120494	3.5	13.7%
Maceió	10.0	903464	12.2	10.0%	3.1	932748	3.9	13.8%
Sergipe	3.3	1967818	3.8	27.7%	2.9	2068017	3.6	25.4%
Aracajú	6.6	498618	8.1	30.3%	2.5	571149	3.6	21.4%
Bahia	5.2	13815260	6.3	19.2%	5.8	14016906	6.9	22.1%
Salvador	6.2	2673557	8.7	25.7%	8.5	2,675,656	11.8	27.3%
Southeast	11.9	78472036	17.0	18.0%	11.0	80364410	16.6	20.0%
Minas Gerais	14.3	19237434	18.7	17.0%	12.0	19597330	16.5	18.9%
Belo Horizonte	14.5	2375330	22.7	20.0%	12.3	2375151	20.5	21.2%
Espírito Santo	9.3	3408360	12.4	13.6%	10.6	3514952	14.9	21.7%
Vitória	13.1	396324	18.0	12.2%	29.3	414586	42.1	16.7%
Rio de Janeiro	8.8	15383422	12.5	26.4%	7.6	15989929	11.5	23.9%
Rio de Janeiro	9.0	6094182	14.5	33.9%	7.1	6320446	12.6	24.8%
São Paulo	12.1	40442820	18.4	17.1%	11.9	41262199	18.8	19.5%
São Paulo	11.1	10927985	19.6	19.6%	11.2	11253503	21.1	21.8%
South	15.9	26973432	21.4	16.0%	11.5	27386891	16.1	18.0%
Paraná	17.2	10261840	22.7	15.7%	14.3	10444526	19.9	16.9%
Curitiba	20.0	1757903	36.0	13.6%	11.5	1751907	20.1	17.8%
Santa Catarina	17.1	5866590	21.9	16.8%	5.6	6248436	7.6	17.0%
Florianópolis	22.2	487047	28.0	23.9%	1.7	515288	2.2	42.9%
Rio Grande do Sul	14.1	10845002	19.8	15.1%	12.1	10693929	17.4	18.3%
Porto Alegre	11.9	1428694	16.7	21.8%	9.9	1409351	14.9	24.5%
Central-West	10.2	13020789	13.8	15.0%	7.9	14058094	11.1	21.0%
Mato Grosso do Sul	10.6	2264489	13.8	17.0%	11.0	2449024	17.1	22.6%
Campo Grande	8.0	749770	11.9	23.3%	13.5	786797	22.7	24.5%
Mato Grosso	13.2	2803272	17.4	14.0%	7.4	3035122	10.1	22.1%
Cuiabá	23.6	533801	38.0	17.5%	10.3	551098	15.9	26.3%
Goiás	8.0	5619919	10.9	12.7%	6.0	6003788	8.3	20.6%
Goiânia	8.8	1201007	13.4	17.9%	7.5	1302001	12.0	22.4%
Distrito Federal	11.2	2333109	16.2	19.8%	9.8	2570160	13.9	17.9%
Brasília	11.2	2333109	16.2	19.8%	5.0	2570160	7.1	10.9%

distribution of GC indicates a higher concentration throughout the south, southeast and central-west regions, in contrast to the lower rates in the northern regions. Interestingly, this study detected a decrease in the GC inci-

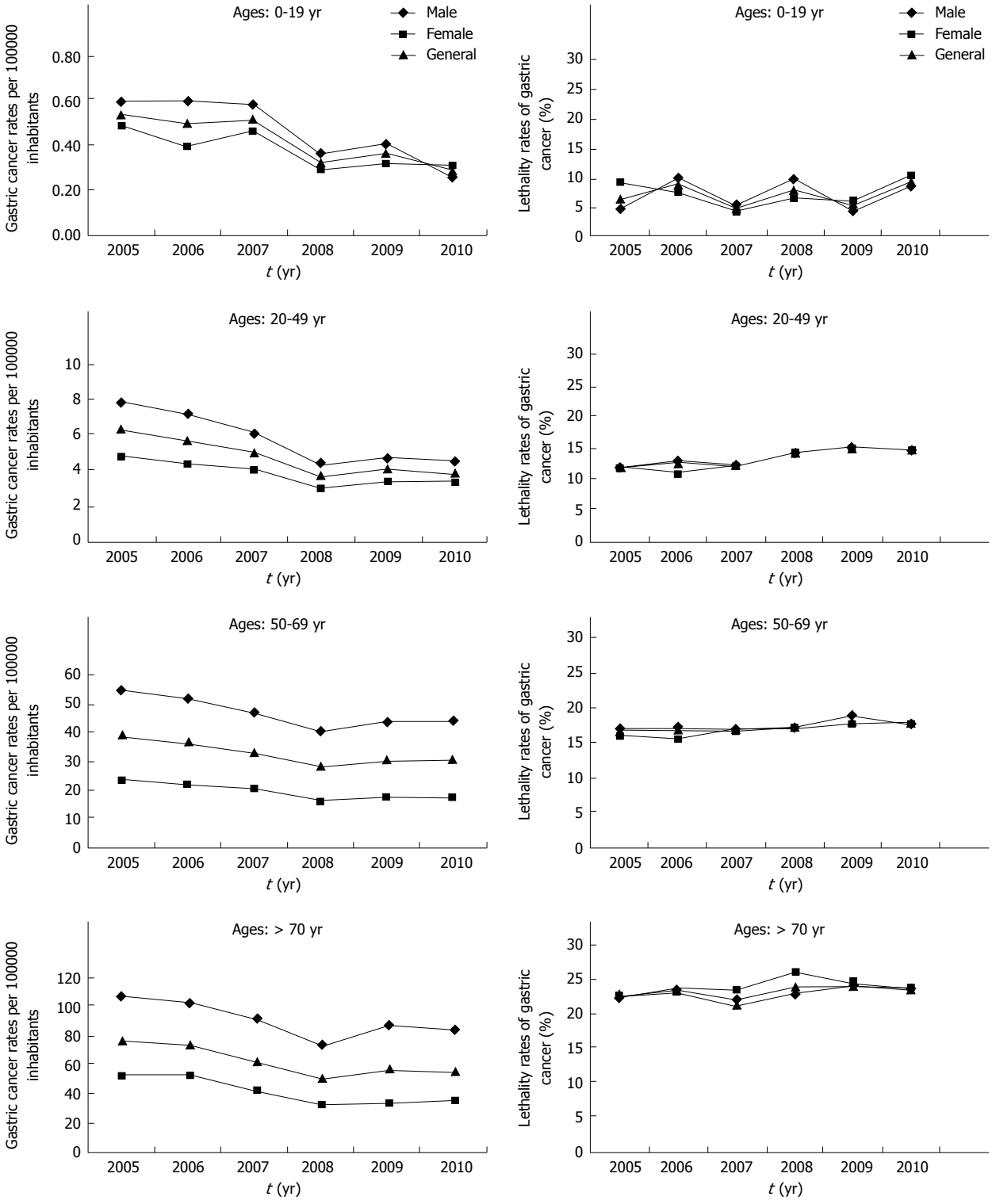

Figure 3 Age-standardized gastric cancer incidences by gender, estimated from hospitalizations and lethality trends, in Brazil from 2005-2010.

dence in regions of higher concentration over time, but it also unveiled a contrasting relative increase in cases in the northern regions during the same period.

Several studies support the notion that the GC incidence is declining worldwide[12,13]. In accordance with those findings, in the current study, we have presented data from estimates of the GC incidence in Brazil to show that although the population increased from 2005 to 2010, hospitalizations for GC consistently declined. The time trends of the age-stratified groups show an increase in the GC incidence in patients > 50 years with an overall male predominance, a well-recognized phenomenon in GC that is usually attributed to possible differential environmental exposure[14,15]. This suggests that the risk factors for GC have been present in our environment, but the exposure statuses to the relevant

Table 2 Changes in gastric cancer-associated hospitalizations and lethality rates among the standardized age ranges from 2005-2010

	Age ranges	2005	2010	Difference
Hospitalization	< 20 yr	0.54	0.28	-48.1%
	20-49 yr	6.23	3.91	-37.2%
	50-69 yr	38.06	29.67	-22.0%
	> 70 yr	75.97	55.40	-27.1%
Lethality	< 20 yr	6.5	9.5	+46.2%
	20-49 yr	12.1	14.8	+22.3%
	50-69 yr	16.9	17.8	+5.3%
	> 70 yr	22.5	23.5	+4.4%

factors most likely differed between male and female patients. However, our finding of decreasing GC rates, especially in the younger age groups and proportionally more among males, support the idea of relatively recent environmental modifications in Brazil and appear to be in accordance with a previous Japanese study, in which the decreasing trend in younger groups was additionally associated with intestinal-type GC[16].

Overall, more than 90% of the GCs are adenocarcinomas and comprise two well-differentiated types, namely intestinal and diffuse[17]. The intestinal type, to which the decrease in GC has been attributed[18,19], is usually more prevalent among men and the elderly and is predominant in the lowest socioeconomic groups; diet and *H. pylori* infection are major risk factors. The diffuse type, in contrast, is more frequent among the young, with an equal male-female distribution, and has been associated with constitutive-related factors[15]. Considering the well-known differences in the pathogenic mechanisms of the two GC subtypes, our results appear to corroborate the trends of a 25-year study of a reference center in Southern Brazil that showed a decrease in the intestinal subtype paralleled by a steady increase in the diffuse subtype of GC, predominantly in women < 45 years[20]. This fact might explain the tendency to select more severe or advanced cases with increasing lethality, which are also compatible with the diffuse subtype of GC[21]. In agreement with our results, young patients in a large population-based study of age-related GC outcomes in the United States also presented with more advanced disease. However, the prognosis of young patients with GC remained better than that of the older patients[22]. Although this information appears paradoxical and might also contradict our results, it is likely that the adjusted stage-stratified relative survival would be more favorable among young patients, compared to older patients, due to their general health condition and possibly to differences in the availability and quality of health care.

With respect to the incidence and associated mortality, marked geographic differences are characteristic of worldwide GC epidemiology. The highest incidence rates have been reported in Japan and Korea, whereas the lowest are in the United States and Western and Northern European countries; Brazil has been considered to have an intermediate-high pattern[10,23,24]. Although the precise etiology of GC remains unknown, studies have identified potential genetic, environmental, and lifestyle risk factors that might also account also for the geographical differences. Several risk factors have been associated with GC, including *H. pylori* infection, smoking, and diet[4,15,25]. Regarding dietary factors, ecologic studies have suggested associations of GC incidence and mortality with salt consumption[26-28]. Studies of immigrants have shown that changing patterns of incidence according to the location where people live appear to reinforce the critical role of dietary habits in the development of GC[28,29].

The differential occurrence in urban versus rural municipalities constitutes another intriguing factor that could contribute to additional geographical variations in GC. In contrast to our results, a study conducted in southern Spain showed a reduction in the mortality rates in the most rural municipalities[30,31]. However, geographic GC clustering remains evident in China, and high-risk areas are located in rural areas, especially in the north[32,33]. Similar results from studies conducted in Lithuania[34], South Korea[35], and Brazil[36], which reported higher risks of GC incidence and mortality in rural areas, also appear to agree with our findings and with those of another previous Brazilian study that estimated a specific reduction in GC would occur in the state capitals ten years earlier than in the inland municipalities[24]. These differences might be explained at least in part by gaps in education and preventive measures, together with lower access to health-care services for populations residing outside of larger urban centers.

H. pylori infection has been established as the most important risk factor for GF, but its presence alone is not sufficient to explain GC development[9,37]. *H. pylori* is commonly observed and widely distributed throughout the populations of developing countries[38,39], including Brazil[40], and the low socioeconomic level, which includes variables indicative of a crowded environment and deficient sanitation or habitation conditions, have been confirmed in population-based studies to be critical for infection acquisition[41,42]. Therefore, it is reasonable to suppose that *H. pylori* infection would also account for the high prevalence of GC in Brazil. In fact, GC has been associated with socioeconomic status and, at an individual level, this variable might be linked not only with *H. pylori* infection but also with dietary patterns, smoking, and possibly environmental and occupational exposures[15,31,43,44]. However, the socioeconomic condition and its influence on *H. pylori* infection alone cannot explain, for example, the high frequency of GC in Japan or Korea, both of which have a high socioeconomic level. Because of these discrepancies, potential synergistic associations of *H. pylori* infection with different variables have also been investigated with respect to GC development. Previously, in a cross-sectional study in Japan, investigators found an association between *H. pylori* infection and the consumption of salted food[29]. Nevertheless, the potential association between salt consumption and *H. pylori* infection with respect to GC development was also

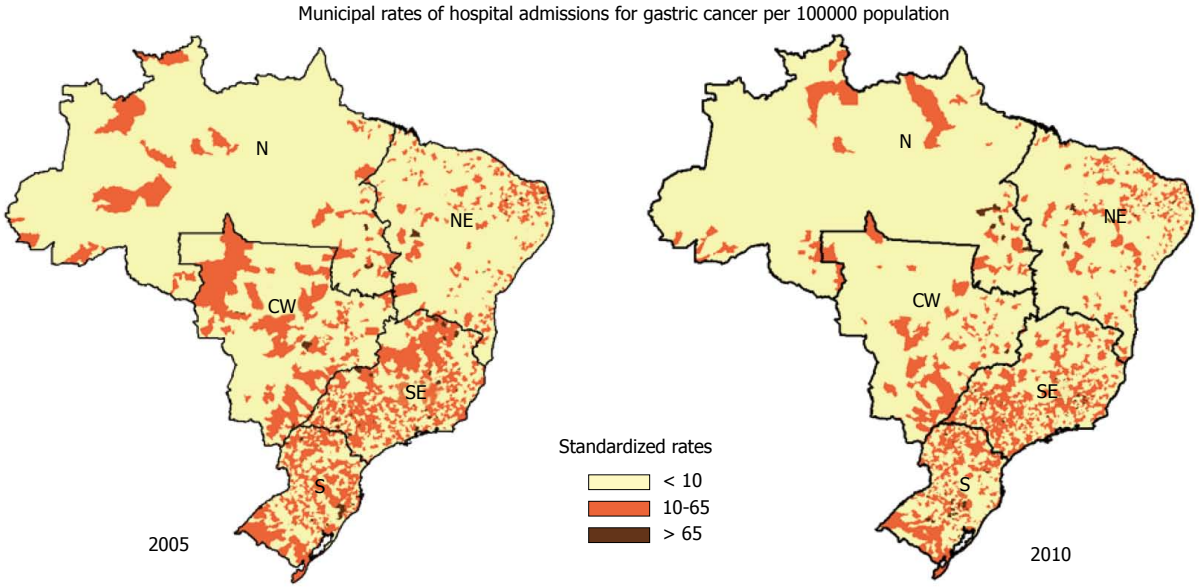

Figure 4 Geographic distribution of gastric cancer in Brazil according to the municipal hospitalization rates (per 100000 inhabitants) in the years of 2005 and 2010. Standardized rates defined three ranges of 0-10, 10-65 and > 65. S: South; SE: Southeast; N: North; NE: Northeast; CW: Central-west.

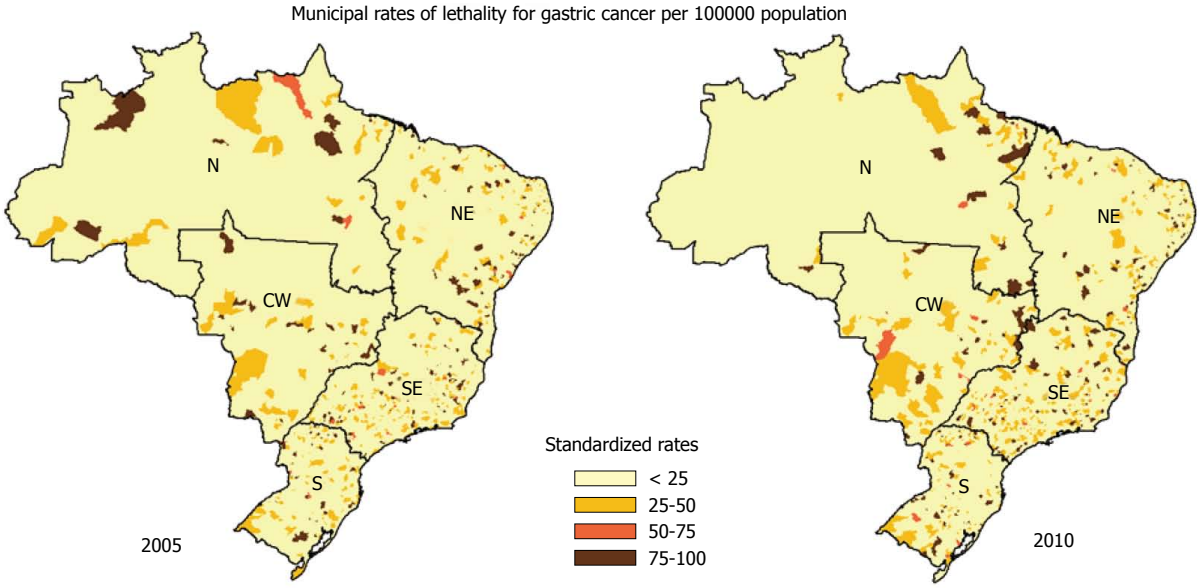

Figure 5 Geographic distribution of gastric cancer lethality in Brazil according to the municipal rates (per 100000 inhabitants) in the years of 2005 and 2010. Standardized rates defined four ranges of < 25, 25-50, 50-75 and > 75. S: South; SE: Southeast; N: North; NE: Northeast; CW: Central-west.

investigated in epidemiological studies and yielded inconsistent results[14,45,46].

Despite the potential limitations of this study, which are basically related to the lack of disease details and information regarding co-morbidities and therapy, and the fact that the data were based on hospitalization rates and mortality, all procedures were thoroughly applied to generate a large database that contained nationwide information that was collected in a single, common, electronic data-based system. Therefore, although an ecological fallacy would still be possible in this study, this possibility was mitigated by the use of municipalities and the simplicity and straightforwardness of the data entered in the electronic system. However, it is important to cautiously interpret the results of this study because of some additional methodological limitations. For example, the concept of a municipal unit might be very different in Brazil, compared to most countries. In addition to clear geographic heterogeneities, Brazilian cities might present great differences in terms of population. In addition to the well-known global tendency towards urban concentration, considerable differences are observed among the 5435 Brazilian municipalities. For example, populations might vary from one thousand to twenty million inhabitants per city and yet, these would be considered municipal units of the same level in our database. An-

other potential caveat of this study is the precision of the registries, which is likely related to the geographic socioeconomic differences, with repercussions on health-care quality. Nevertheless, it is clear that GC will remain a significant societal burden and will unfortunately continue to constitute a critical health issue in Brazil for many years. Therefore, despite all the potential limitations and criticism regarding this type of study, the information generated by this unified database might allow the first insights regarding the dynamics of GC in Brazil.

In conclusion, the results of this study appear to support the hypothesis that environmental elements are fundamental determinants of GC pathogenesis. Furthermore, the decline in GC incidence among younger patients, along with less discrepancy in the gender distribution during the analysis period, likely suggests a relatively recent decrease in the population-level exposure to the environmental risk factors associated with GC. However, the differential spatial distribution of GC suggests a possible ongoing dynamic change within the Brazilian environment, with shifts in incidence from the south towards the north and from more urbanized coastal areas to inland areas.

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COMMENTS

Background

Gastric cancer (GC) is among the most common cancers worldwide and is the second leading cause of cancer-related deaths. The underlying pathogenic mechanisms of GC are not completely understood, but they likely result from host-environmental factor interactions.

Research frontiers

Although epidemiological data indicate a progressive reduction in the GC incidence in developed countries, remarkable geographical and ethnic variations exist. However, data from developing countries remain limited, which renders global analysis difficult.

Innovations and breakthroughs

This study is the first to analyze the spatial distributions and demographics of the GC incidence and lethality estimates over time in Brazil to identify the areas with differential risks and outcomes. An ecological study of data from the Health Informatics Department of the Brazilian Ministry of Health registry identified the GC hospitalization rates between January 2005 and December 2010. The data included information about the town of residence at hospital admission and death, and the patients were categorized by gender and age. For the geographic distribution analysis, the GC hospitalization rates were obtained per 100000 inhabitants in the individual municipalities. The results from this exploratory model were included in a platform to plot maps that depicted the GC estimates and distributions.

Applications

The ecological analysis provided by this study identified changes in the GC geographic distribution and declining GC rates in young patients, suggesting a recent decrease in the exposure to risk factors within the Brazilian environment.

Peer review

This database-based study explores variations in the GC rates and consequent deaths in Brazil from 2005-2010. In addition, this study provides information about the nationwide geographic localization. Hospital discharge records associated with a GC diagnosis, regardless of the duration or reason for hospitalization and including outpatient procedures, were utilized to estimate the GC rate, distribution, and lethality. Another novel finding of this study is that of a coast-to-inland gradient that greatly overlaps with the urban-to-rural areas of the country. The authors believe that this observation reflects the geographic and social-economic features of Brazilian society within the analysis period. In addition, this information might reinforce the notion that the interaction of people with their geographic environments could determine or at least modulate economic and social development, with consequent influences on demographics and health issues.

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BRIEF ARTICLE

Evaluation of preferable insertion routes for esophagogastroduodenoscopy using ultrathin endoscopes

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Abstract

AIM: To evaluate the discomfort associated with esophagogastroduodenoscopy (EGD) using an ultrathin endoscope through different insertion routes.

METHODS: This study (January 2012-March 2013) included 1971 consecutive patients [male/female (M/F), 1158/813, 57.5 ± 11.9 years] who visited a single institute for annual health checkups. Transnasal EGD was performed in 1394 patients and transoral EGD in 577. EGD-associated discomfort was assessed using a visual analog scale score (VAS score: 0-10).

RESULTS: Multivariate analysis revealed gender (M *vs* F: 4.02 ± 2.15 *vs* 5.06 ± 2.43) as the only independent predictor of the VAS score in 180 patients who underwent EGD for the first time; whereas it revealed gender (M *vs* F 3.60 ± 2.20 *vs* 4.84 ± 2.37), operator, age group (A: < 39 years; B: 40-49 years; C: 50-59 years; D: 60-69 years; E: > 70 years; A/B/C/D/E: 4.99 ± 2.32/4.34 ± 2.49/4.19 ± 2.31/3.99 ± 2.27/3.63 ± 2.31), and type of insertion as independent predictors in the remaining patients. Subanalysis for gender, age group, and insertion route revealed that the VAS score decreased with age regardless of gender and insertion route, was high in female patients regardless of age and insertion route, and was low in males aged over 60 years who underwent transoral insertion.

CONCLUSION: Although comprehensive analysis revealed that the insertion route may not be an independent predictor of the VAS score, transoral insertion may reduce EGD-associated discomfort in elderly patients.

Key words: Esophagogastroduodenoscopy; Ultrathin endoscope; Visual analog scale

Core tip: To evaluate the effects of insertion route for unsedated surveillance esophagogastroduodenoscopy (EGD), this retrospective study included 1971 consecutive patients who visited a single institute for annual health checkups. EGD-associated discomfort was as-

sessed using a visual analog scale (VAS). Statistical analysis using VAS revealed that the VAS score decreased with age regardless of gender and insertion route, was high in females regardless of age and insertion route, and was low in males aged over 60 years who underwent transoral insertion.

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INTRODUCTION

Recently, because of the development of endoscopic treatment, the importance of early detection of gastrointestinal neoplasms has become extremely important[1-5]. In addition, gastrointestinal endoscopic technology has advanced considerably with improved resolution and image enhancement[6-10]. Inevitably, the importance of surveillance esophagogastroduodenoscopy (EGD) in detecting upper gastrointestinal neoplasms has become noticeable, particularly for superficial squamous cell carcinomas and early gastric cancers.

On the other hand, remarkable breakthrough in technology has led to the development of endoscopes of smaller diameter with high-resolution pictures. Ultrathin endoscopes have enabled us to perform surveillance EGD through transnasal insertion, and their role in minimally invasive EGD has been reported from various institutes[11-19]. Therefore, transnasal EGD has been accepted as a preferable choice for surveillance EGD, particularly among younger patients.

However, transnasal insertion sometimes cannot be performed because of various reasons such as pain or nasal hemorrhage, resulting in a switch to transoral insertion using the same ultrathin endoscope in daily clinical practice. Our previous study revealed that elderly patients prefer transoral EGD to transnasal EGD[20]. Although patients are reluctant to undergo EGD because of uncomfortable insertion, even if an annual check-up is recommended, a preferable choice of ultrathin endoscope insertion route in patients with different profiles has not been evaluated.

Therefore, this study aimed to evaluate preferable insertion routes during unsedated EGD using an ultrathin endoscope in patients with different profiles.

MATERIALS AND METHODS

This study was conducted at the Center for Epidemiology and Preventive Medicine in the University of Tokyo Hospital from January 2012 to March 2013. After excluding patients with invalid data, the study included patients who had an endoscopic procedure including biopsy, patients with a past history of upper gastrointestinal tract surgery, or patients with a change of insertion route because of nasal hemorrhage or intolerable pain; 1971 consecutive patients who underwent EGD with the use of ultrathin endoscopes during a medical checkup were enrolled. The profiles of these patients are shown in Table 1.

Table 1 Profiles of all patients

	Patients who underwent 1st EGD	Patients who underwent 2nd or subsequent EGD	*P* value
Gender			NS
Male	98	1060	
Female	82	731	
Operator			NS
a	128	1144	
b	49	599	
c	0	28	
d	2	16	
e	1	4	
Age group (yr)	49.3 ± 13.2 54	58.3 ± 11.5	< 0.05
< 39	39	115	
40-49	45	320	
50-59	29	464	
60-69	13	588	
> 70		304	
Insertion route			< 0.05
Transnasal	139	1255	
Transoral	41	536	
VAS score	4.50 ± 2.33	4.11 ± 2.35	< 0.05
Examination time (s)			NS
	306.0 ± 60.0	302.1 ± 61.8	
Type of scope			NS
a	78	690	
b	66	739	
c	19	162	
d	8	69	
e	4	36	
f	3	67	
g	2	28	

EGD: Esophagogastroduodenoscopy; VAS: Visual analog scale score; NS: Not significant.

Each patient was allowed to choose their insertion route. Pre-EGD preparation for both insertion routes included an oral administration of dimethicone (Gascon drop; Kissei Pharmaceutical Co., LTD.; Nagano, Japan) and pronase (PronaseMS; Kaken Pharmaceutical Co., LTD.; Tokyo, Japan). For local anesthesia, oral administration of a viscous gel of 2% lidocaine hydrochloride and modified spray method was provided for both transoral and transnasal insertion routes. The modified spray method involved spraying 0.05% naphazoline nitrate into each nostril, followed by an injection with a viscous gel of 2% lidocaine hydrochloride. Conscious sedation was not performed in any patient. For transoral insertion, a thin-type mouthpiece and tongue depressor (Endo-leader; Top Corp.; Tokyo, Japan) was used[21].

All EGDs were performed by well-trained endos-

Table 2 Univariate and multivariate analyses against the visual analog scale scores in patients who underwent 1st esophagogastroduodenoscopy

Patients who underwent 1st EGD	VAS score	Univariate	Multivariate
Gender		$P < 0.05$	$P < 0.05$
Male	4.02 ± 2.15		
Female	5.06 ± 2.42		
Operator		NS	NS
a	4.47 ± 2.30		
b	4.51 ± 2.49		
c	-		
d	6		
e	4		
Age group		NS	NS
< 39	4.93 ± 2.12		
40-49	4.44 ± 2.54		
50-59	4.11 ± 2.33		
60-69	4.34 ± 2.18		
> 70	4.54 ± 2.93		
Insertion route		NS	NS
Transnasal	4.50 ± 2.26		
Transoral	4.46 ± 2.59		
Examination time (s)		$P < 0.05$	NS
	$r^2 = 0.0336$		
Type of scope		NS	NS
a	4.60 ± 2.36		
b	4.76 ± 2.33		
c	3.95 ± 2.41		
d	3.13 ± 1.64		
e	5.25 ± 2.75		
f	2.33 ± 1.53		
g	4		

EGD: Esophagogastroduodenoscopy; VAS: Visual analog scale score; NS: Not significant.

Table 3 Univariate and multivariate analyses against the visual analog scale scores in patients who underwent 2nd or subsequent esophagogastroduodenoscopy

Patients who underwent 2nd or subsequent EGD	VAS score	Univariate	Multivariate
Gender		$P < 0.05$	$P < 0.05$
Male	3.60 ± 2.20		
Female	4.84 ± 2.37		
Operator		$P < 0.05$	$P < 0.05$
a	3.95 ± 2.36		
b	4.43 ± 2.34		
c	3.57 ± 2.04		
d	4.69 ± 2.06		
e	2.50 ± 1.00		
Age-group		$P < 0.05$	$P < 0.05$
< 39	4.99 ± 2.32		
40-49	4.34 ± 2.49		
50-59	4.19 ± 2.31		
60-69	3.99 ± 2.27		
> 70	3.63 ± 2.31		
Insertion route		$P < 0.05$	NS
Transnasal	4.19 ± 2.27		
Transoral	3.93 ± 2.53		
Examination time (s)		NS	NS
	$r^2 = 0.000843$		
Type of scope		NS	NS
a	4.22 ± 2.35		
b	4.11 ± 2.36		
c	3.93 ± 2.29		
d	3.98 ± 2.48		
e	4.64 ± 2.11		
f	3.25 ± 2.24		
g	3.82 ± 2.57		

EGD: Esophagogastroduodenoscopy; VAS: Visual analog scale score; NS: Not significant.

Table 4 Jonckheere-Terpstra test of the visual analog scale score and age group in addition to gender and insertion route

Jonckheere-Terpstra test	*P* value		
Male-transnasal insertion	NS	NS	$P < 0.05$
Female-transnasal insertion	NS		
Male-transoral insertion	NS ($P = 0.0833$)	$P < 0.05$	
Female-transorai insertion	$P < 0.05$		

NS: Not significant.

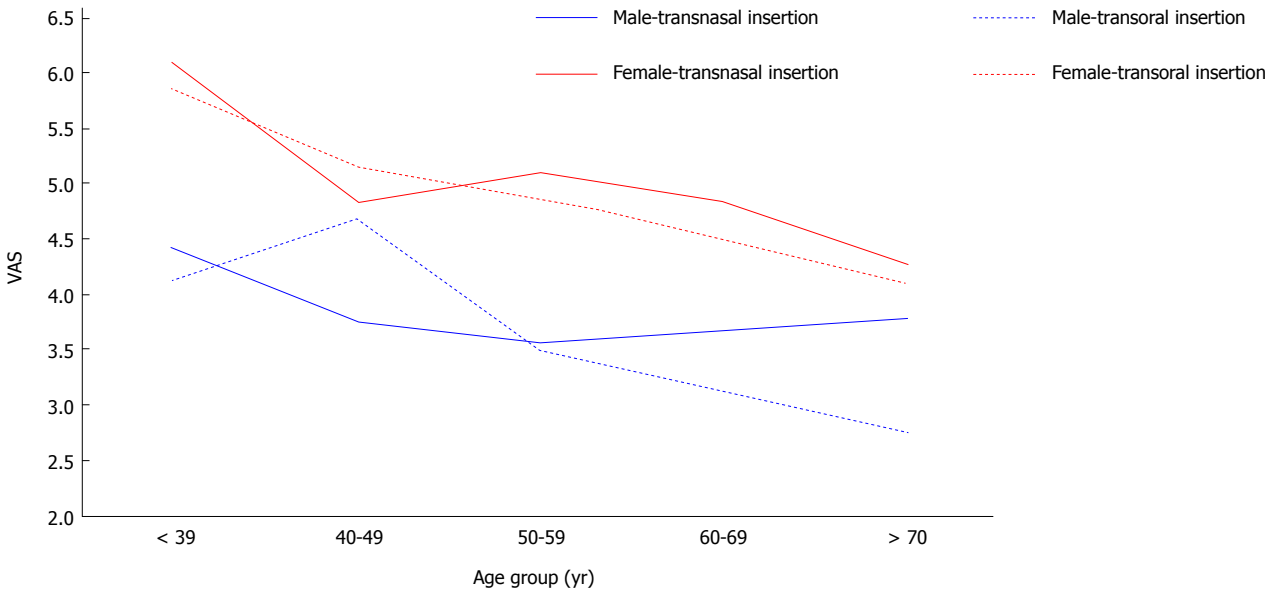
copists who has performed more than 1000 EGDs respectively and were certified by the Japanese Gastroenterological Endoscopy Society. Seven types of ultrathin endoscopes were used in this study: GIF-XP260N and GIF-XP260NS (Olympus Corp, Tokyo, Japan), EG-580NW, EG-530NW, and EG-530N (Fujifilm Holdings Corp, Tokyo, Japan), and EG16-K10 and prototype EG17-K10 (Hoya Corp, Tokyo, Japan). The Prototype EG17-K10 was used as a part of collaborative effort by the University of Tokyo Hospital and Hoya Corporation. These endoscopes are indicated as a, b, c, d, e, f, and g, respectively, in the tables.

Each patient rated EGD-associated discomfort on a visual analog scale (VAS) score of 0-10, with ten being rated as maximum discomfort[22-24]. These questions were part of examination routines and the feedback was used to improve our clinical practice. This study was conducted as a retrospective chart review of consecutive patients and was approved by the Ethics committee.

The parameters, such as gender, age group, previous experience with EGD, insertion route, operator, examination time, and the VAS score, were evaluated. Age groups were defined as A, B, C, D, and E in patients aged below 40 years, 40-49 years, 50-59 years, 60-69 years, and over 70 years, respectively. Statistical analyses were performed using the student's t-test for numerical variables, the Chi-square test for categorical variables, and the Jonckheere-Terpstra test for trend analysis. Multivariate analyses were performed using a stepwise regression analysis. All analyses except for the Jonckheel-Terpstra test were performed using a JMP software (SAS Institute Inc., Cary, NC, United States). A P value < 0.05 was considered significant.

RESULTS

Of 1971 patients (male/female: 1158/813, mean age, 57.5 ± 11.9 years, range, 25-89 years), 180 and 1791 patients underwent a 1st EGD and a 2nd or subsequent EGD, respectively. Patients who underwent 1st EGD were significantly younger than other patients. Furthermore, the number of patients receiving transnasal EGD and the



Age group	< 39		40-49		50-59		60-69		> 70	
Male-transnasal insertion (n = 769)	4.41 ± 2.00 (n = 58)	4.37 ± 2.24 (n = 71)	3.75 ± 2.19 (n = 154)	3.91 ± 2.30 (n = 185)	3.56 ± 2.20 (n = 207)	3.54 ± 2.17 (n = 261)	3.65 ± 2.04 (n = 255)	3.51 ± 2.15 (n = 357)	3.77 ± 2.09 (n = 95)	3.27 ± 2.18 (n = 186)
Male-transoral insertion (n = 291)	4.15 ± 3.21 (n = 58)		4.68 ± 2.68 (n = 31)		3.38 ± 2.04 (n = 54)		3.15 ± 2.36 (n = 102)		2.76 ± 2.16 (n = 91)	
			[a]		[a]		[a]		[a]	[a]
Female-transnasal insertion (n = 486)	6.06 ± 2.09 (n = 31)	6.00 ± 2.09 (n = 44)	4.84 ± 2.58 (n = 94)	4.93 ± 2.64 (n = 135)	5.10 ± 2.12 (n = 139)	5.03 ± 2.23 (n = 203)	4.84 ± 2.21 (n = 161)	4.74 ± 2.27 (n = 231)	4.28 ± 2.35 (n = 61)	4.20 ± 2.41 (n = 118)
Female-transoral insertion (n = 245)	5.85 ± 1.91 (n = 13)		5.15 ± 2.79 (n = 41)		4.86 ± 2.45 (n = 64)		4.50 ± 2.41 (n = 70)		4.12 ± 2.49 (n = 57)	

([a]P < 0.05)

Figure 1 Visual analog scale score score and age groups in addition to gender and insertion route. [a]P < 0.05 between male and female groups.

VAS score were significantly higher in those who underwent their 1st EGD than in other patients.

Although univariate analysis revealed a significantly higher VAS score in females than in males and a positive correlation with examination time, multivariate analysis revealed gender as the only independent predictor of the VAS score (Table 2).

For patients who underwent their 2nd or subsequent EGD, multivariate analysis revealed gender, operator, and age group as independent predictors of the VAS score. Although the VAS score for transnasal insertion was significantly higher than that for transoral insertion, multivariate analysis indicated that the insertion route may not be an independent predictor of the VAS score (Table 3).

For further evaluation, subanalysis performed by combining gender, age group, and insertion route (Figure 1) revealed that the VAS scores were significantly higher in females than in males, regardless of age group and insertion route. With regard to the insertion route, among the male patients aged over 60 years old, the VAS scores were significantly lower in patients receiving transoral insertion than in those receiving transnasal insertion. The Jonckheere-Terpstra test revealed that the VAS scores decreased with age (Table 4). In particular, these scores markedly decreased with age in patients who underwent transoral insertion.

DISCUSSION

This study revealed the relationship between the profiles of patients and EGD-associated discomfort using an ultrathin endoscope. To minimize the discomfort during a surveillance EGD, it may be better to recommend transnasal insertion for younger patients and transoral insertion for elderly patients, particularly in males aged over 60 years.

Although the reason why gender difference affected the VAS score in this study is not clear, higher VAS scores in females have previously been reported with regard to postoperative pain[25]. Aubrun *et al*[25] evaluated postoperative pain using VAS scores and morphine dos-

age and reported a significantly higher VAS score and dosage in females. The authors speculated that women had a lower pain threshold and less tolerance to experimental pain compared with men. Our study also supports their speculation.

With regard to the decreasing trend in the VAS score with age, we speculated that it may be due to weakening of the gag reflex. On the other hand, any discomfort associated with transnasal insertion to the hypopharynx primarily includes nasal pain rather than weakening of the gag reflex. We speculated that age is more strongly associated with weakening of the gag reflex than with nasal pain. In addition, male gender has been reported to be a risk factor for aspiration pneumonia in a systematic review[26]. This report indicates that age-related weakening of the gag reflex is greater in males than in females. Nasal pain does not seem to be related to age, which results in lower VAS scores for transoral insertion in elderly individuals, particularly males.

The main limitation of this study is a lack of objectivity when assessing discomfort using the VAS score. EGD-associated discomfort also includes anxiety, abdominal fullness due to insufflation, and various other factors in addition to nasal pain and weakening of the gag reflex. However, it may be difficult to objectively evaluate each factor. In addition, the difference in discomfort associated with transnasal and transoral insertions may be limited to discomfort associated with insertion to the hypopharynx. With regard to this short route, the difference in discomfort associated with both insertion routes is primarily attributed to nasal pain and weakening of the gag reflex. Therefore, we used the VAS score as a relatively reliable and simple objective assessment method to compare these two insertion routes.

In conclusion, this study demonstrated age-related and gender-related discomfort associated with transoral and transnasal EGD using ultrathin endoscopes. Although further data collection is necessary, the appropriate choice of insertion route may easily convince patients who are reluctant to undergo surveillance EGD.

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COMMENTS

Background

The importance of surveillance esophagogastroduodenoscopy (EGD) in detecting upper gastrointestinal neoplasms has become very evident in the context of clinical daily practice where the importance of detection of early stage gastrointestinal neoplasms has received more emphasis.

Research frontiers

Although the ultrathin endoscopes for transoral or transnasal insertion during medical checkups has been accepted as a less invasive technique, because of the discomfort due to an uncomfortable insertion route, patients may become reluctant to undergo EGDs during annual health checkups.

Innovations and breakthroughs

The authors' study investigating discomfort that accompanies unsedated EGD using ultrathin endoscopes demonstrated a correlation between discomfort and insertion route with regard to gender and age group.

Applications

To decrease unsedated EGD-associated discomfort while using ultrathin endoscopes, transnasal insertion should be chosen except for elderly males. For elderly males aged over 60 years, transoral insertion may be preferred rather than transnasal insertion.

Terminology

An ultrathin endoscope is an endoscope with a tip diameter of approximately 6 mm. It enables transnasal insertion and is widely used for a medical checkup using EGD in Japan.

Peer review

This study demonstrated age-related and gender-related discomfort associated with transoral and transnasal EGD using ultrathin endoscopes. It's a good study with important clinical applications.

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BRIEF ARTICLE

Simplified fistula dilation technique and modified stent deployment maneuver for EUS-guided hepaticogastrostomy

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Abstract

AIM: To evaluate the success rates, procedural time and adverse event rates of the modified methods in endoscopic ultrasonography-guided hepaticogastrostomy (EUS-HGS).

METHODS: Twenty-eight patients in a prospective case series who underwent EUS-HGS (phase Ⅰ). Forty-six patients in a matched case-control study (phase Ⅱ). The simplified technique for fistula dilation was the primary use of a 4 mm balloon catheter with a stainless steel stylet. The stent deployment was modified by deploying the metal stent inside a bile duct (half of the stent) under EUS and fluoroscopic guidance and gently pulling the echoendoscope after full deployment of the stent inside the echoendoscope channel (remaining portion of the stent) under fluoroscopic guidance. This cohort was compared with a matched historical cohort.

RESULTS: In phase Ⅰ, the technical and clinical success with the modified method was 96% (27/28) and 89% (24/27 as per-protocol analysis). The overall adverse event rate was 7%. In phase Ⅱ, there was no difference in technical and clinical success, stent patency and overall adverse events in each group. However, the procedural time (15.3 ± 5.2 min *vs* 22.3 ± 6.0 min, $P < 0.001$) and early adverse events (0% *vs* 26%, $P = 0.02$) were statistically improved in case cohort compared with control cohort.

CONCLUSION: Compared with the conventional EUS-HGS technique, the procedural time was shorter and early adverse events were less frequent with our simplified and modified technique.

Key words: Endoscopic ultrasonography; Biliary drainage; Hepaticogastrostomy; Treatment outcome; Adverse event

Core tip: Endoscopic ultrasonography-guided hepaticogastrostomy (EUS-HGS) with direct transluminal stenting is a complex procedure in terms of guidewire manipulation, fistula dilation and stent deployment. We prospectively evaluated our simplified and modified EUS-HGS technique; fistula dilation with a 4 mm balloon dilation catheter with a stainless steel stylet and stent deployment maneuver with an 8 mm fully covered metal stent with dual flaps. The technical and clinical success was 96% (27/28) and 89% (24/27). The overall adverse event rate was 7%. Compared with

the conventional EUS-HGS technique, the procedural time was shorter and early adverse events were less frequent with our modified technique.

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INTRODUCTION

Endoscopic ultrasonography-guided biliary drainage (EUS-BD) is an emerging alternative to percutaneous transhepatic biliary drainage (PTBD) or surgery after failed endoscopic retrograde cholangiopancreatography (ERCP)[1-3]. EUS-BD can be completed in 3 ways: EUS-guided hepaticogastrostomy, choledochoduodenostomy, and rendezvous therapy[2,4-10]. EUS-guided rendezvous therapy seems to be the safest of all 3 approaches[11]. However, this technique is not always successful. Although an enhanced guidewire manipulation protocol has been introduced, 44% of patients may still require EUS-guided hepaticogastrostomy or choledochoduodenostomy after failed ERCP[12]. EUS-guided hepaticogastrostomy with direct transluminal stenting (EUS-HGS) may be a viable option for patients with surgically altered anatomy, proximal bile duct obstruction, and duodenal invasion after failed ERCP[2]. EUS-guided hepaticogastrostomy is one of the most complex procedures in terms of guidewire manipulation, fistula dilation, and stent deployment[11,13]. With regard to guidewire manipulation, the intrahepatic approach appears to present a challenge because the overall technical success rate of EUS-guided rendezvous and antegrade biliary stenting/balloon dilation has been reported to be lower than that of the extrahepatic rendezvous method[14]. Thus, a substantial number of patients in which the intrahepatic approach of guidewire manipulation was used may eventually require EUS-HGS[12].

For fistula dilation in EUS-HGS, graded dilation with a 4 F cannula and a 6 F and 7 F bougie dilator may be preferred because this step-by-step procedure seems to be safe[12]. However, this procedure is not always successful. The procedural time may also be increased because of the need for accessory changes and difficulties with the advancement of each bougie dilator. Furthermore, accidental loss of the guidewire may occur during this step-by-step maneuver. A needle knife may eventually have to be used in some cases due to the difficulty of graded dilation of the fistula tract. The use of a needle knife for fistula dilation in EUS-BD may be associated with postprocedure adverse events[4]. Another difficulty in EUS-HGS is transgastric stent deployment[4,15]. Scope position will be backward for identification of the distal end of the deploying stent, and the stent will be placed in a more inner side of the intrahepatic duct during stent deployment[16]. This placement may result in proximal stent migration after stent deployment[2]. Furthermore, distal stent migration in EUS-HGS may occur during follow-up periods[9].

Thus, a simple step for fistula dilation, troubleshooting of stent deployment, and a stent with a modified design are needed to prevent proximal or distal migration in EUS-HGS. During EUS-guided drainage of pancreatic pseudocysts, we found that a direct 4 mm balloon catheter (Hurricane RX, Boston-Scientific, Natick, MA) was usually successful for fistula dilation. This device has a low-profile 5.8 F balloon shaft designed to reduce resistance and increase pushability. It also features a 4 F tip and stainless steel stiffening stylet inserted in the proximal portion of the catheter shaft, thereby ensuring less trauma and pushability (Figure 1A). We introduced this device for fistula dilation in EUS-HGS. In our previous study[17], a fully covered self-expandable metal stent (FCSEMS) with an anchoring flap showed excellent antimigration compared with FCSEMS with flared ends in patients with benign distal biliary stricture. In the present study, we prospectively evaluated the role of a 4 mm balloon dilation catheter with a stainless steel stylet and modified stent deployment maneuver with an 8 mm fully covered metal stent with dual flaps for EUS-HGS (Figure 1B). To determine whether this simplified and modified technique affects the procedural time or adverse events, we also performed a case-control study.

MATERIALS AND METHODS

Patients

Between August 2012 and August 2013, 2042 ERCPs were carried out by a single experienced endoscopist (Park DH). In this study period, 1109 cases needed biliary decompression for benign or malignant biliary obstruction. Of these 62 (5.6%) patients were candidates for alternative or complementary techniques other than ERCP for biliary decompression. Among the 62 patients, 59 underwent EUS-BD as follows: 28 hepaticogastrostomy, 11 antegrade biliary stenting, 9 rendezvous technique, 8 choledochoduodenostomy, and 3 hepaticoduodenostomy. Trainees were not involved in the ERCP. The EUS-BD was performed at the time of a failed ERCP in the same session.

The inclusion criteria for EUS-HGS were failure of initial biliary cannulation or bile duct decompression through ERCP or EUS-guided rendezvous because of accompanying duodenal obstruction, surgically altered anatomy, high-grade hilar biliary stricture, or failed guidewire manipulation in EUS-guided rendezvous or antegrade therapy. Patients who refused PTBD were also included. The exclusion criteria were (1) refusal to participate in the study protocol; (2) pregnancy; and (3) patient age younger than 18 years. All enrolled patients were given antibiotics pre- and postprocedure. CO_2 insufflation was routinely applied during ERCP and EUS before

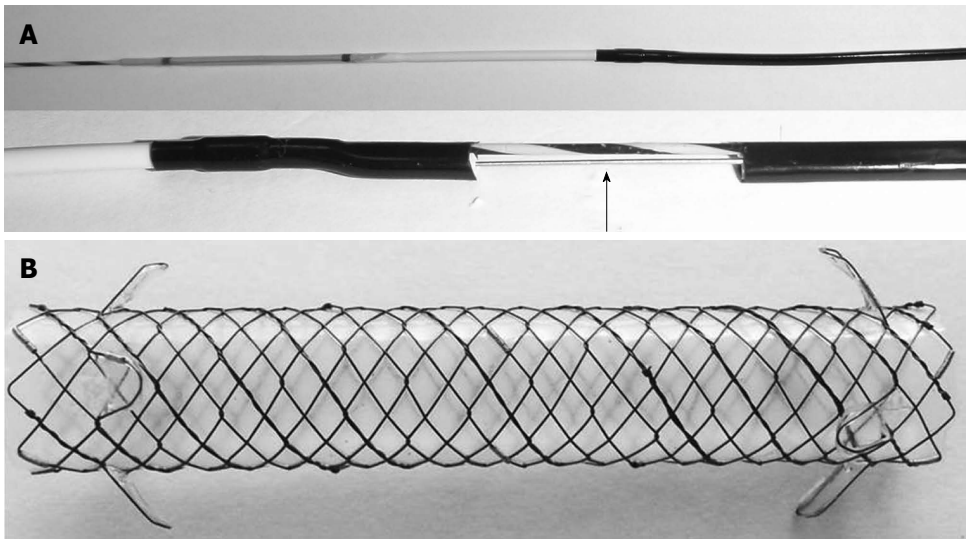

Figure 1 Balloon dilation catheter and metal stent. A: A 4 mm balloon dilation catheter with stainless steel stiffening stylet to augment pushability (arrow); B: An 8 mm in diameter, dual-flap fully covered metal stent.

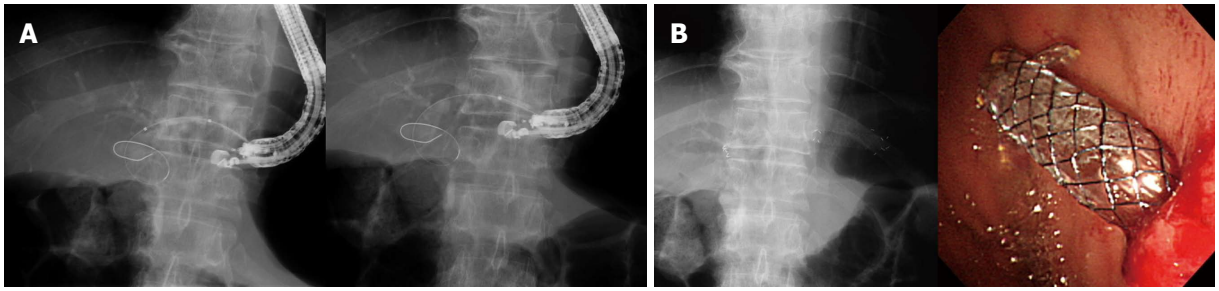

Figure 2 Endoscopic ultrasonography-guided hepaticogastrostomy using a 4 mm balloon dilation catheter with stainless steel stylet and an 8 mm fully covered metal stent with dual flaps. A: A 0.035-inch guidewire was introduced into the bile duct and fistula dilation was performed in two sites (liver parenchyma and fistula tract) using a 4 mm biliary balloon dilatation catheter; B: The dual-flap fully covered metal stent was deployed within the bile duct (half of metal stent) under echoendoscopic and fluoroscopic guidance, and echoendoscope channel (remained portion of metal stent) under fluoroscopic guidance, and then placed in hepaticogastrostomy site.

the commencement of this study (January 2010).

Prospective EUS-HGS protocol

EUS was performed by using a GF-UCT 240 linear-array echoendoscope (Olympus Medical System, Tokyo, Japan). The echoendoscope was placed in the cardia or in the lesser curvature of the stomach and was oriented to view the intrahepatic duct. Color Doppler imaging was used to identify the regional vasculature. Dilated intrahepatic bile duct puncture was carried out with a 19-gauge needle (EUSN-19-T, Cook Endoscopy, Winston-Salem, NC), and bile juice was aspirated. Then, a contrast medium was injected into the punctured bile duct to confirm successful biliary access. Tract dilation was then carried out. To simplify the tract dilation, a 4 mm balloon dilation catheter was used initially. For the facilitation of the advance of a balloon catheter to intrahepatic bile duct, the balloon catheter was in a plane with the axis of the wire as it entered the bile duct on EUS. If the guidewire was placed in the right intrahepatic duct, hilum, or proximal left intrahepatic duct, the 4 mm balloon dilation catheter was directly used for fistula dilation. If the guidewire was placed toward the left peripheral intrahepatic duct instead of the hilum, a 4 F cannula (Glo-tip; Cook Medical, Winston-Salem, NC) was used as a stiff instrument to advance through the fistula tract and into the left intrahepatic duct[12]. When the guidewire was placed into the desired intrahepatic duct (proximal left, right intrahepatic duct, or hilum), the 4 mm balloon catheter was applied for fistula dilation. After full expansion was confirmed under fluoroscopy, the balloon was kept for 5-10 s. This 4 mm balloon catheter was dilated in two points (between the left hepatic duct and the hepatic parenchyma and between the hepatic parenchyma and the stomach) to facilitate the deployment of the metal stent (Figure 2). In the case of resistance to the advance of the 4 mm balloon catheter, graded dilation with 4 F cannula and tapered biliary dilators was applied. If 4 F cannula was failed to advance repeatedly, needle knife was used finally to prevent the loss of mounted guidewire.

Modified technique for the deployment of the transluminal stent

To stabilize the position/attachment of the endoscope in

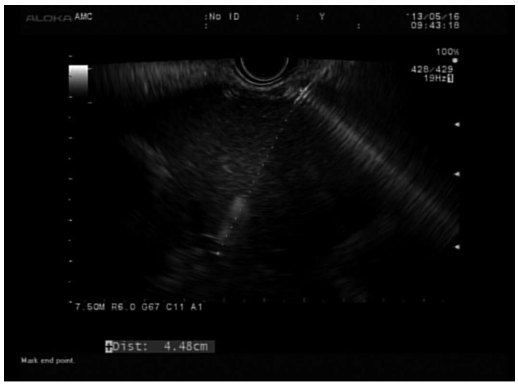

Figure 3 Determination of stent length. The length of an endoscopic ultrasonography needle between gastric wall and punctured left hepatic duct was 4.48 cm (4.48 cm × 2 + 1 cm = 9.96 cm). Based on our formula, a 10 cm fully covered metal stent was placed for this patient.

the high body of the stomach or the cardia during insertion of the stent in EUS-HGS, we deployed the front one-half of the dual-flap metal stent (8 mm in diameter, 5-10 cm in length, dual-flap fully covered metal stent, M.I. Tech, Gyeonggi-do, South Korea) with a 8.5 F introducer under echoendoscopic and fluoroscopic guidance. After deploying the remainder of the stent within the channel of echoendoscope under fluoroscopic guidance, the echoendoscope was pulled out gently, and the stent was left in the hepaticogastrostomy site. The length of a stent was determined by our formula [the length (cm) of an EUS needle between gastric wall and punctured left hepatic duct on the EUS (representative as approximately half of a stent in hepatic parenchyma) multiply two (representative as remaining half of a stent in deploying inside the echoendoscope, including possible stent shortening) plus 1 cm] (Figure 3). This 1 cm was considered as the intrahepatic bile duct portion of a metal stent. To reduce the resistance of stent deployment inside the channel of echoendoscope, an 8 mm in diameter FCSEMS was applied. Clinical data, including the technical success, adverse events, and other variables, were prospectively recorded and evaluated.

Follow-up

We followed the patients in phase I after the procedure until October 2013. To check migration of the stent, biochemical parameters and a simple abdominal film were assessed 1, 3 and 6 mo after the EUS-HGS. Follow-up data were collected prospectively.

Definitions

The procedural time was defined as the time between the puncture of the intrahepatic bile duct with the EUS needle and the placement of FCSEMS. The procedural time is important because it represents the technical difficulty. Shortening the procedural time would help to increase success rates and reduce procedure-related adverse events. Overall adverse events included early adverse events (up to 14 d) and late adverse events (later than 14 d). Adverse events included complications of abdominal pain, pneumoperitoneum, bleeding and stent migration. Abdominal pain was defined as pain not caused by pancreatitis or perforation[18]. Proximal stent migration was defined as any migration of the FCSEMS into the bile duct or abdominal cavity, preventing its easy removal[19]. Distal stent migration was classified as spontaneous or gastric migration. Spontaneous migration was defined as distal migration without the stent becoming lodged in the bowel[17]. Gastric migration was defined as a partially migrated stent impacting in the distal hepaticogastrostomy site.

Technical success was defined as follows: (1) success of fistula dilation with the 4 mm balloon catheter and deployment of the metal stent with the modified method; and (2) the passage of the metal stent across the stomach or esophagus, along with the flow of contrast medium and/or bile through the stent. Functional success was defined as a decrease of bilirubin to < 75% of the pretreatment value within the first month[7]. The functional success rate was calculated for the patients for whom the procedure was technically successful (as per-protocol analysis). Stent occlusion was defined as the recurrence of jaundice and cholestasis and/or evidence of a dilated biliary system on images, which in all cases would require biliary intervention.

Conventional method was performed as follows. Graded dilation with 4 F cannula and tapered biliary dilators (6 F, and 7 F, catheter tip, 4 F; Cook) was applied initially for fistula dilation. In the case of resistance to the advance of the bougie dilator, graded dilation with a 4 F cannula was repeated again. At that time, a needle knife was used only when repeated graded dilation failed and the risk of failing guidewire maintenance was increased because our previous study found that the use of a needle knife was a risk factor for postprocedure adverse event after EUS-BD[4]. The metal stent was deployed under echoendoscopic and fluoroscopic guidance as per previous studies[4,7].

All the patients provided written informed consent to participate in this study. For the two phases of the study, our institutional review board approved the study protocol (phase I , IRB No. 2012-0608) and the retrieval and analysis of previous collected data for comparison with the prospective cohort (phase Ⅱ, matched case-control study, IRB No. 2013-0664).

Statistical analysis

In phase I , our previous study showed 74% of successful graded dilation for fistula dilation in EUS-HGS[4]. To detect a 21% increase in successful fistula dilation with the 4 mm balloon dilation catheter in EUS-HGS, we needed 25 patients to have an 80% chance of rejection the hypothesis of no difference at the 0.05 level (2-tailed). As a 10% dropout rate was considered, 28 patients were invited to participate in this study. This sample size was then used as the stopping rule for patient recruitment in the phase I study. For the phase Ⅱ study, clinical data were retrieved on 36 patients who underwent EUS-HGS

Table 1 Baseline characteristics of the study population *n* (%)

Characteristics	EUS-BD (*n* = 28)
Median age (range, yr)	63 (29-87)
Male	20 (71)
Indication	
Malignant (*n* = 25)	
Cholangiocarcinoma	10
Pancreatic cancer	5
Gallbladder cancer	2
Stomach cancer	2
Ampulla of Vater cancer	1
Colon cancer	1
Duodenal cancer	1
Hepatocellular carcinoma	1
Intraductal papillary neoplasm of bile duct	1
Lymphoma	1
Benign (*n* = 3)	
Benign IHD stricture	1
Postoperative anastomosis site stricture	1
IgG4-related sclerosing cholangitis	1
Surgically altered anatomy	6 (21)

EUS-BD: Endoscopic ultrasonography-guided biliary drainage.

with conventional technique from January 2010 to July 2012 (historical cohort). The patients in historical cohort were 1:1 blindly matched with our prospective cohort by age and etiology of biliary obstruction by a statistician (Lee JB) who was unaware of the purpose of this study. Finally, 23 patients in each cohort were enrolled in phase Ⅱ study. As the focus of this research was to highlight potential differences, the resultant significant *P* values were not corrected for the multiple testing of data. Categorical and binary variables were tested using the χ^2 test or Fisher's exact test according to the EUS-HGS methods. The Mann-Whitney *U* test or Wilcoxon signed-rank test was used for continuous variables. Stent patency and overall survival were compared using the Kaplan-Meier method with a log-rank test. All statistical analyses were performed with SPSS v.18.0 (IBM, Armonk, NY), with results considered significant at a *P* value < 0.05.

RESULTS

Outcomes of the modified technique for EUS-HGS (phase Ⅰ)

In phase Ⅰ, 28 patients underwent EUS-HGS with the 4 mm balloon dilation catheter with a stainless steel stiffening stylet and an 8 mm fully covered metal stent with dual flaps. The median age of the study population was 63 years (range, 29 to 87 years), and the male to female ratio was 2.5 (Table 1). The indications for EUS-HGS are described in Table 1. The technical and clinical success of EUS-HGS with the modified method was 96% (27/28) and 89% (24/27) as per-protocol analysis (Table 2). The fistula dilation with the 4 mm balloon catheter was successful in 27 patients. A needle knife was used in one patient since the 4 mm balloon catheter and 4 F cannula were not able to be advanced into the puncture site. The 4 F cannula was initially used in 7 patients (25%) to manipulate the guidewire in place in the desired intrahepatic duct. In the remaining patients, direct use of the 4 mm balloon catheter for fistula dilation was performed after the guidewire was placed in the desired left hepatic duct. Serum total bilirubin decreased significantly within 1 mo after EUS-HGS (10.3 ± 9.4 to 3.7 ± 5.1 mg/dL, $P < 0.001$). During mean follow-up period of 5.2 mo, no patients received the second treatments such as pancreaticoduodenectomy or gastrojejunostomy after EUS-HGS.

Table 2 Outcome of endoscopic ultrasonography-guided hepaticogastrostomy *n* (%)

Outcome	EUS-HGS (*n* = 28)
Technical success rate	27 (96)
Clinical success rate	24 (89)
Median length of stent (range), cm	8.5 (5-10)
Procedure time, mean ± SD, min	15.6 ± 5.8
Median stent patency (95%CI), d	150 (5-295)
Early adverse event	0
Late adverse event	2 (7)
Overall survival (95%CI), mo	7.5 (5.0-12.0)

Late adverse events: Pseudoaneurysm (1), partial distal migration (1). Data are expressed as *n* (%) or mean ± SD. EUS-HGS: Endoscopic ultrasonography-guided hepaticogastrostomy.

The overall adverse event rate was 7% (2/28). There were no early adverse events after EUS-HGS with the modified technique. However, two late adverse events were reported: gastric migration of the stent and bleeding from a pseudoaneurysm. The partially migrated stent was removed successfully with a rat tooth forceps, and a new FCSMES was inserted through the fistula tract. The patient with pseudoaneurysm was presented as hematemesis 8 mo after EUS-HGS. There was huge fresh blood clot attached to stent in endoscopic finding (Figure 4A). Since pseudoaneurysm from left hepatic artery was noted around the proximal end of hepaticogastrostomy stent in CT (Figure 4B), hemostasis was achieved by urgent embolization of the feeding vessel from the left hepatic artery (Figure 4C). After embolization, the patient fully recovered and remained alive without any biliary re-intervention 6 mo later.

Matched case-control study (phase Ⅱ)

In the age and etiology matched case-control study, there was no difference in baseline characteristics, technical success, clinical success, median stent patency, and overall adverse events in each group (Table 3). However, the procedural time (15.3 ± 5.2 *vs* 22.3 ± 6.0 min, $P < 0.001$) and early adverse events (0% *vs* 26%, $P = 0.02$) were statistically improved in the case cohort compared with the control cohort (Table 3).

DISCUSSION

Although many studies of EUS-BD have shown promising results in terms of the technique's efficacy and safety[4,6,7,20], studies on standard techniques or dedicated

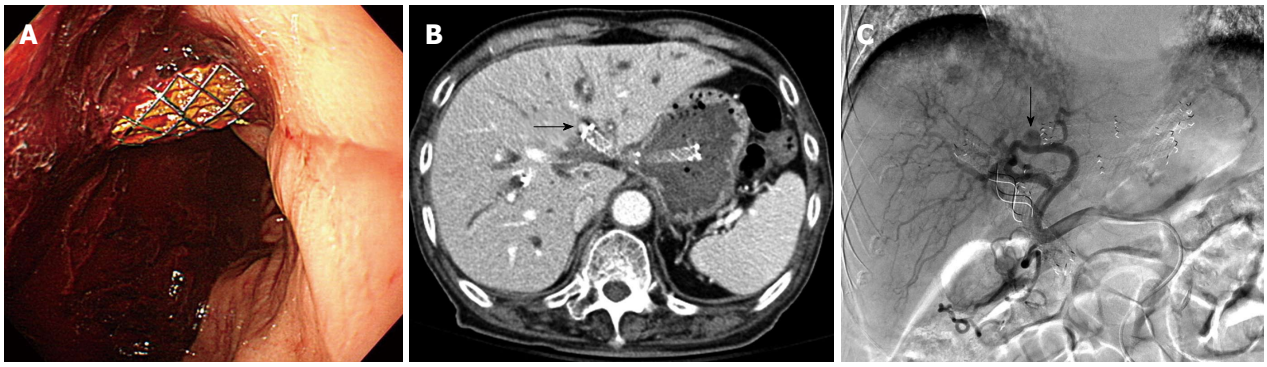

Figure 4 Development of pseudoaneurysm as a late adverse event after endoscopic ultrasonography-guided hepaticogastrostomy. A: There was huge fresh blood clot attached to stent in endoscopic finding; B and C: Since pseudoaneurysm from left hepatic artery was noted in CT and angiography, hemostasis was achieved by embolization of the feeding vessel from the left hepatic artery (arrow).

Table 3 Age and etiology matched case-control results *n* (%)

Characteristics	Modified method (*n* = 23)	Conventional method (*n* = 23)	*P* value
Age mean ± SD, yr	62.9 ± 14.6	64.1 ± 12.8	0.88
Male	17 (74)	12 (52)	0.13
Etiology of bile duct obstruction			> 0.99
Benign	1	1	
Perihilar lesion	15	15	
Periampullary lesion	5	5	
Peribiliary or metastatic lymph node	2	2	
Surgically altered anatomy	6	6	> 0.99
Failed guidewire manipulation during ERCP or EUS-guided rendezvous	9	7	0.54
Technical success rate[1]	22 (96)	21 (91)	> 0.99
Functional success rate[2]	20 (91)	16 (76)	0.24
% of needle knife use in two groups	1 (4)	1 (4)	> 0.99
Procedure time, mean ± SD, min	15.3 ± 5.2	22.3 ± 6.0	< 0.001
Median stent patency (95%CI), d	216 (73-359)	129 (64-194)	0.73
Total adverse event (%)	2 (9)	8 (35)	0.07
Early	0	6 (26)[3]	0.02
Late	2 (9)	2 (9)[4]	> 0.99
Overall survival (95%CI), mo	7.5 (5.6-9.4)	4.3 (1.8-6.8)	0.27

[1]Intention-to-treat analysis; [2]Per-protocol analysis; [3]Pneumoperitoneum (2), proximal migration (1), partial proximal migration (1), partial distal migration (1), abdominal pain (1); [4]Distal migration (2).

devices for EUS-BD are lacking. The absence of such studies may limit the general popularity of EUS-BD and cause it to be performed only by experts in a few tertiary centers. Graded dilation may be preferred to cautery dilation for the creation and dilation of the fistula in EUS-BD in terms of safety. However, graded dilation is not always successful, and it can result in a longer procedural time. Although needle knife cautery may reduce the procedural time, postprocedure adverse events may occur[4]. In the present study, one-stage fistula dilation was possible in 96% of cases by using a 4 mm-balloon dilation catheter with a stainless steel stylet. This simple step may shorten the procedural time in EUS-HGS without increasing postprocedure adverse events. We used a modified deployment technique with a metal stent with dual flaps for the prevention of proximal and distal migration during the procedure and follow-up periods. As in our previous study[17], this stent showed a reliable antimigration effect.

In spite of repeated graded tract dilations with bougie catheter were done to prevent the use of a needle knife in patients with conventional method, the adverse events were not decreased compared to previous study[4]. The dilation force of a bougie dilator or a needle knife is axial. The axial force by bougie dilator may widen the gap between the liver and the stomach during fistula dilation[21], which can lead to increase the stent migration or pneumoperitoneum. Actually, almost postprocedural adverse events occurred in conventional group were stent migration (3/6) or self-limited pneumoperitoneum (2/6). In contrast, because the dilation force of a balloon catheter is radial, the separation of tissue planes is minimized and the tract dilation can be done stably within a short period of time. Since the radial force tends to increase the risk of perforation[21], we performed the tract dilation with a balloon catheter in limited points shortly and used CO_2 insufflation during procedure. Furthermore, this minimal dilation in the fistula tract may result in easy deployment of the metal stent because 4 mm balloon in the fistula

tract and fistula site may obviate the resistance of the deployed metal stent and reduce the distance between the gastric wall and liver during a stent placement. This may minimize the chance of a bile leak or pneumoperitoneum.

Our previous two studies showed a comparable procedural time and no reduction in the procedural time, despite possible technical proficiency with time trends[6,7]. The simple dilation method in the present study with the modified stent deployment, which shortened the procedural time, may be used by endoscopists with various experiences of EUS-BD. Although there are various sizes of balloon catheter, we chose the smallest, a 4 mm (12 F) balloon catheter, because the minimal fistula dilation obtained with this size of catheter is sufficient to insert any commercially used catheters or stent delivery devices without resistance.

During stent deployment, echoendoscopic and fluoroscopic findings may be more important than endoscopic findings because the latter in the high body of the stomach or cardia during stent deployment may lead to instability of the proximal and distal position of the metal stent. In this study, we used an 8 mm diameter metal stent with dual flaps for stent deployment inside the bile duct (half of metal stent) and echoendoscope channel (remaining portion of the metal stent). This modified deployment maneuver may secure the stable position of the deployed metal stent and reduce the chance of proximal and distal migration. Usually, longer stents (up to 12 cm in length) are used to prevent proximal or distal migration[2]. However, such stents may result in the need for stent revision or encourage sludge formation[22]. Using the modified technique of stent deployment described herein, a stent with a more appropriate length may be placed.

In one patient with malignant hilar stricture, hematemesis from a left hepatic artery pseudoaneurysm occurred 8 mo after successful EUS-HGS (8 mm in diameter, 7 cm in length). Urgent feeding artery embolization was performed. As a hepatic artery pseudoaneurysm may occur after PTBD and after PTBD-assisted metal stent placement[23-25], EUS-HGS with long-dwelling metal stents may be associated with left hepatic artery pseudoaneurysm. After intrahepatic biliary decompression, the relatively large diameter of FCSEMS may erode the intrahepatic bile duct, resulting in a left hepatic artery pseudoaneurysm. The presence of pseudoaneurysm at the tip of the stent may suggest the possibility of its development due to compression of the arterial wall by the metal stent. Further larger studies of metal stents with a modified proximal tip (*e.g.,* an uncovered portion without flared ends or a flap or with a smaller diameter) may be needed to address this issue.

This study has some limitations. In phase Ⅱ, our prospective case series was retrospectively compared with a matched control. Although a matched case-control study may eliminate various confounding factors, it may provide less evidence for causal inference than randomized controlled trials. As our previous study showed a relatively higher postprocedural adverse event rate with the use of a needle knife when graded dilation was not feasible for fistula dilation and one intraperitoneal stent migration with a conventional stent deployment[12], a randomized trial comparing our modified with conventional method in EUS-BD may be impractical. Therefore, we performed a matched case-control study rather than a randomized trial.

The difference of stent design might have affected the postprocedural stent migration. With regard to the antimigration effect of FCSEMS for benign biliary stricture, the anchoring flap design was superior to the flared end design[17]. However, the FCSEMS with a flared end may be enough preventing migration for very tight biliary stricture[6,7]. There is a waist in the middle of the stent at the site of hepaticogastrostomy site when the metal stent is initially inserted. Therefore, it seems that the difference of stent design would not be a significant factor affecting early stent migration in EUS-HGS. However, if the hepaticogastrostomy site is dilated by the expansion of metal stent, there could be a difference of stent migration between anchoring flap and flared end like benign biliary strictures. Since the follow-up period was not enough in this study, there was no significant difference of late stent migration between two groups. In order to verify about this issue, further long-term studies will be required.

The procedural time and adverse event rate may be associated with proficiency in time trend. After about 60 cases of EUS-BD were performed, cohort control with prospectively maintained database were collected before the phase I period. Therefore, the effect of the difference in the technical proficiency of the operator on procedural time trends is likely minimal. Furthermore, CO_2 insufflation was applied in both the case and the control cohort. These efforts may minimize the selection bias from the two groups in terms of safety.

PTBD is still standard care in patients with unsuccessful biliary decompression of ERCP[26]. For randomized trials of EUS-BD and PTBD for biliary decompression after failed ERCP, a standard protocol with a dedicated device for EUS-BD may be a prerequisite because previous multicenter studies with nonstandardized protocols and dedicated devices for EUS-BD showed relatively higher adverse event rates[27-29]. EUS-BD is still an evolving technique. Thus, standard and internationally accepted protocols for EUS-BD may be required before randomized trials of EUS-BD and PTBD.

In summary, our simplified and modified technique for EUS-HGS with a 4 mm balloon catheter with a stainless steel stylet and stent deployment maneuver inside the bile duct and echoendoscope channel appears to shorten the procedural time and reduce postprocedure adverse events compared with the conventional method. A multicenter study may be warranted to confirm our results. Based on our experience, a 4 mm balloon catheter with a stainless steel stylet may be considered as a platform for a dedicated EUS-HGS device for fistula dilation.

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COMMENTS

Background

Endoscopic ultrasonography-guided hepaticogastrostomy with direct transluminal stenting (EUS-HGS) is a complex procedure in terms of guidewire manipulation, fistula dilation, and stent deployment.

Research frontiers

EUS-guided biliary drainage is an emerging alternative to percutaneous transhepatic biliary drainage or surgery after failed endoscopic retrograde cholangiopancreatography. A substantial number of patients in which the intrahepatic approach of guidewire manipulation was used may eventually require EUS-HGS. However, the standard technique for EUS-HGS has not been established yet.

Innovations and breakthroughs

To increase the success rate and to decrease the adverse events rate in EUS-HGS, maintaining the scope position during the procedure and shortening the procedural time are important. The simplified and modified technique for EUS-HGS with a 4 mm balloon catheter with a stainless steel stylet and stent deployment maneuver inside the bile duct and echoendoscope channel appears to shorten the procedural time and reduce postprocedure adverse events compared with the conventional method.

Applications

The study results suggest that a 4 mm balloon catheter with a stainless steel stylet may be considered as a platform for a dedicated EUS-HGS device for fistula dilation.

Terminology

EUS-HGS: The dilated intrahepatic bile duct is punctured under EUS-guidance. Then bile duct is opacified by injection of contrast medium under fluoroscopic guidance. After identifying the biliary obstruction, the fistula is dilated and a stent is placed across the fistula to drain the bile juice.

Peer review

The authors suggested the simplified and modified EUS-HGS technique, and compared with conventional method. The simplified technique for fistula dilation with 4 mm balloon catheter with stainless steel stylet and modified stent deployment maneuver in EUS-HGS was associated with shortened procedural time and reduced postprocedure adverse event.

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BRIEF ARTICLE

Hepatitis B and C viruses are not risks for pancreatic adenocarcinoma

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Abstract

AIM: To investigate whether hepatitis B virus (HBV) and hepatitis C virus (HCV) increase risk of pancreatic ductal adenocarcinoma (PDAC).

METHODS: We recruited 585 patients with cytological and/or pathologically confirmed PDAC in National Taiwan University Hospital from September 2000 to September 2013, and 1716 age-, sex-, and race-matched controls who received a screening program in a community located in Northern Taiwan. Blood samples were tested for the presence of HCV antibodies (anti-HCV), HBV surface antigen (HBsAg), antibodies against HBsAg (anti-HBs), and hepatitis B core antigen (anti-HBc) in all cases and controls. The odds ratio (OR) of PDAC was estimated by logistic regression analysis with adjustment diabetes mellitus (DM) and smoking.

RESULTS: HBsAg was positive in 73 cases (12.5%) and 213 controls (12.4%). Anti-HCV was positive in 22 cases (3.8%) and 45 controls (2.6%). Anti-HBs was positive in 338 cases (57.8%) and 1047 controls (61.0%). The estimated ORs of PDAC in multivariate analysis were as follows: DM, 2.08 (95%CI: 1.56-2.76, $P < 0.001$), smoking, 1.36 (95%CI: 1.02-1.80, $P = 0.035$), $HBsAg^{+}/anti\text{-}HBc^{+}/anti\text{-}HBs^{-}$, 0.89 (95%CI: 0.89-1.68, $P = 0.219$), $HBsAg^{-}/anti\text{-}HBc^{+}/anti\text{-}HBs^{+}$, 1.03 (95%CI: 0.84-1.25, $P = 0.802$).

CONCLUSION: HBV and HCV infection are not associated with risk of PDCA after adjustment for age, sex, DM and smoking, which were independent risk factors of PDAC.

Key words: Hepatitis B virus; Hepatitis C virus; Pancreatic ductal adenocarcinoma; Risk; Endemic disease; Diabetes mellitus

Core tip: Previous studies on hepatitis B virus (HBV) status and pancreatic cancer risk have produced conflicting results. This study is the first study to use controls from the general population compared to previous hospital-based case-controls studies with age- and sex-matched controls. HBV infection was determined by measuring antibodies against hepatitis B core antigen and HBV surface antigen. The risk of HBV/hepatitis C virus infection was evaluated after adjustment for im-

portant risk factors such as age, sex, diabetes mellitus and smoking in a high-endemic HBV area.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignant tumors with an average 5 year survival rate of < 5%. Early diagnosis followed by surgery with curative intent is the only way to improve the outcome of PDAC. Diabetes mellitus (DM), smoking, chronic pancreatitis, and family history of PDAC are well known risk factors of PDAC[1]. Screening high-risk individuals for PDAC might shed some light on detection of the early stage of the disease.

Several studies on hepatitis B virus (HBV) status and pancreatic cancer risk have produced conflicting conclusions[2-6]. The previous studies were conducted with a different design and population in regions with different HBV or hepatitis C virus (HCV) prevalence rate. The prevalence of HBV and HCV infection varies widely among different regions of the world, ranging from < 0.5% in western countries to 8%-25% in endemic countries in East Asia[7,8]. HBV or HCV infection is a global challenge[9] and it is nowadays regarded as a major risk for hepatocellular carcinoma (HCC)[8]. In Taiwan, a HBV-endemic area with about 15% carrier rate, chronic HBV infection has the strongest association with the development of HCC and accounts for 75%-80% of all HCC cases[8,10,11]. Besides, an association between HBV or HCV infection and extrapancreatic cancers has been reported in non-Hodgkin's lymphoma[12]. One study from the United States has demonstrated that past exposure to HBV may be associated with pancreatic cancer development[2] with low prevalence of chronic HBV or HCV infections (< 2%)[13]. Studies from an HBV-endemic area have shown contradictory results[4,5]. A cohort study by Iloeje *et al*[14] conducted in Taiwan suggested that chronic HBV carriers had an increased risk of pancreatic cancer, with an adjusted hazard ratio of 1.95 (95%CI: 1.01-3.78). However, only about 69% of cases of pancreatic cancer were proven histologically to be adenocarcinoma. Furthermore, the incidence rate of pancreatic cancer in that cohort was 12.7 cases/100000 persons, even among those without hepatitis B infection (20.4 cases/100000 person among those with hepatitis B). In fact, the officially reported incidence rate of pancreatic cancer in Taiwan was 2.15-6.18 cases/100000 persons in the same period during the REVEAL-HBV cohort study, provided by Health Promotion Administration, Ministry of Health and Welfare, http://www.hpa.gov.tw/. More importantly, the REVEAL-HBV cohort study did not adjust for DM. DM is a well-known risk factor for PDAC and accounted for about two times the risk, which might have confounded their result. The REVEAL-HBV cohort study gave an odds ratio (OR) of 1.95, which meant that those with hepatitis B were about twice as likely to develop pancreatic cancer as those who were not infected. However, only a small increase in absolute risk, about 0.08%, was observed in the REVEAL-HBV cohort study[15]. It is mandatory to validate the association of HBV infection in histologically proven PDAC with adjustment for important risk factors including DM and smoking.

Taiwan is an endemic area of HBV infection with a prevalence of about 15%[8,10,11]. The prevalence and incidence of PDAC in Taiwan (http://www.hpa.gov.tw/) are lower than in the United States[1]. It is important to know whether there is an association between HBV infection and PDAC in HBV-endemic areas because it could influence the attitude and strategy to screening for PDAC. Therefore, we conducted a large case-control study to evaluate whether HBV and HCV infections are possibly associated with histologically proven PDAC in Taiwan, with adjustment for known risk factors.

MATERIALS AND METHODS

Study population

Cases were patients with histologically or cytologically proved PDAC in National Taiwan University Hospital between September 2000 and September 2013. Controls were individuals recruited from a free screening program in a community located in Northern Taiwan supported and sponsored by the Liver Disease Prevention and Treatment Research Foundation. A total of 1716 age-, sex- and race-matched controls were randomly selected from 6000 individuals who participated in the screening activity. The patients and controls were frequency matched by age and sex in a narrow-confined area in Taiwan, which was considered to have the same ethnicity (Taiwanese). The study was approved by our Institutional Review Board. All the cases and controls were born before the launch of the vaccination program for HBV in Taiwan[16].

Serology and definition of HBV and HCV infection

Blood samples were collected from patients and controls. Serum samples were tested for aspartate aminotransferase, alanine aminotransferase, HCV antibodies (anti-HCV), HBV surface antigen (HBsAg), antibodies against HBsAg (anti-HBs), and antibodies against HBV core antigen (anti-HBc) (General Biologicals Corporation, Hsinchu, Taiwan)[17]. Anti-HBs was considered positive if the titer was ≥ 10 mIU/mL. The laboratory researcher running these assays was blinded to the disease status (cases or controls) of the participants' blood samples. Blood samples were drawn from patients with pancreatic cancer before treatment (operation or chemotherapy). HBV

infection was defined by the presence of HBsAg. Positivity of anti-HBs confers protective immunity and was detectable in patients who recovered from HBV infection or were immunized with HBV vaccine[16,18]. Patients who were positive for anti-HBc were defined as previously exposed to HBV. Individuals with chronic HCV infection were defined as those with positive HCV antibodies[19].

Statistical analysis

Statistical analysis was performed by SPSS version 17 (SPSS, Chicago, IL, United States). We compared the demographic characteristics among patients and controls. The *t* test was used to compare the mean age between patients and controls. The χ^2 test was used to compare proportions. We performed multivariate unconditional logistic regression analyses using all variables significant at $P < 0.05$ in the single-factor analyses. For each factor, we calculated the OR and 95%CI using maximum likelihood estimation.

RESULTS

There were no significant difference in age and sex between cases and controls (Table 1). The mean age (± SD) for PDAC patients was 63.81 ± 13.73 years and 63.11 ± 8.89 years for controls ($P > 0.05$). Male sex accounted for 51.2% and 53.3% in cases and controls, which did not have a significant difference. About 9% of the controls had DM and 23.4% of the PDAC patients had DM ($P < 0.0001$). Smokers accounted for 14.0% in the controls and 20.0% in the PDAC group ($P < 0.001$) (Table 1).

The prevalence of anti-HCV was not significantly higher among patients with PDAC (3.8%) than in the controls (2.6%) (Table 2). With respect to HBV, HBsAg was detected in 73 (21.5%) and 213 (12.4%) in the PDAC and control groups, respectively, which showed a similar frequency of positivity for HBsAg between the two groups (Table 2). The overall prevalence of anti-HBs with evidence of protective immunity in PDAC and controls was 338 (57.8%) and 1047 (61.0%), respectively. In univariate analysis, HBsAg, anti-HBs and anti-HBc positivity was not associated with an increased risk of PDAC (Table 2).

HBV infection status in a high-endemic area was determined by HBsAg, anti-HBs and anti-HBc, therefore, we further divided the cases and controls into subgroups for further analysis according to the serological markers: $HBsAg^-/anti\text{-}HBc^-/anti\text{-}HBs^-$: HBV infection naïve; $HBsAg^+/anti\text{-}HBc^+/anti\text{-}HBs^-$: chronic HBV infection; $HBsAg^-/anti\text{-}HBc^+/anti\text{-}HBs^+$: past infection with acquired immunity to HBV. In both univariate and multivariate analyses, chronic HBV infection ($HBsAg^{+/}anti\text{-}HBc^+/anti\text{-}HBs^-$) and past infection with acquired immunity to HBV ($HBsAg^-/anti\text{-}HBc^+/anti\text{-}HBs^+$) were not associated with risk of PDAC (Table 2).

DM was associated with PDAC in both univariate analysis (OR: 3.08, 95%CI: 2.39-3.97, $P < 0.001$) and multivariate analysis (OR: 2.08, 95%CI: 1.56-2.76, $P < 0.001$). Smoking was also associated with risk of PDAC in univariate analysis (OR: 1.54, 95%CI: 1.20-1.96, $P = 0.01$) and multivariate analysis (OR: 1.36, 95%CI: 1.02-1.80, $P = 0.035$).

We also stratified the age into two groups, > 50 years and < 50 years. The risk of PDAC was not significantly different in those with or without HBV or HCV infection, regardless of age > 50 or < 50 years.

Table 1 Characteristics of study population *n* (%)

Characteristics	Cases (*n* = 585)	Controls (*n* = 1716)
Age, yr		
Median	62.00	62.00
mean ± SD	63.81 ± 13.73	63.11 ± 8.89
Sex		
Female	273 (46.7)	837 (48.8)
Male	312 (53.3)	879 (51.2)
DM[b]	137 (23.4)	155 (9.0)
Smoking[a]	117 (20.0)	240 (14.0)

[a]$P < 0.05$, [b]$P < 0.01$ *vs* the control group.

DISCUSSION

We did not find any positive association between HBV or HCV infection and PDAC after adjustment for age, sex, DM and smoking. This result differed from the previous REVEAL-HBV cohort study[14]. First, in the REVEAL-HBV cohort study, the researchers did not adjust DM as a risk factor, which might have confounded the result. In our study, DM was an independent risk factor for PDAC in multivariate analysis, which increased the risk by twofold. This was similar to previous studies from China and South Korea[4,5,20]. Second, the incidence rate of pancreatic cancer in patients without HBV infection in the REVEAL-HBV cohort study was 12.7/100000 person-years, which was nearly twice the incidence rate officially reported by the Health Promotion Administration, Ministry of Health and Welfare, Taiwan. Not all the PDAC cases in both databases were verified by cytology or histopathology. This observation raises an important issue; namely, that studies from registered databases where the results are not confirmed by histopathology or cytology should be used with care and require verification. With the increased use of abdominal imaging in health checkups, an increase in the number of incidentally found pancreatic neoplasms, such as neuroendocrine tumors and solid pseudopapillary neoplasms, could confound the use of the database if the disease register is not based on histological confirmation. Concern about histologically proven types of pancreatic cancer was also emphasized[3,15].

One recent meta-analysis of HBV or HCV infection and risk of pancreatic cancer described some problems with previous observational studies[21]. They concluded that the findings underscore the need for more studies to confirm this potential relationship[14]. One of the problems came from the selection of controls. The five

Table 2 Pancreatic cancer risk factors and association of pancreatic cancer with hepatitis C virus and hepatitis B virus infection: univariate and multivariate logistic regression analysis

Variables	Cases (n = 585)	Controls (n = 1716)	Univariate analysis		Multivariate analysis	
			OR	95%CI	OR	95%CI
Hepatitis C						
Anti-HCV$^+$	3.8%	2.6%	1.45	0.86-2.43	1.36	0.80-2.31
Hepatitis B						
HBsAg$^+$	12.5%	12.4%	1.01	0.86-1.34		
Anti-HBs$^+$	57.8%	61.0%	1.14	0.95-1.39		
Anti-HBc$^+$	66.0%	66.4%	1.02	0.84-1.24		
HBsAg$^-$/anti-HBc$^-$/anti-HBs$^-$	15.2%	13.6%	1.14	0.88-1.49	1.00	Ref
HBsAg$^+$/anti-HBc$^+$/anti-HBs$^-$	10.3%	12.4%	1.24	0.92-1.68	1.22	0.89-1.68
HBsAg$^-$/anti-HBc$^+$/anti-HBs$^+$	42.7%	41.0%	1.07	0.89-1.29	1.03	0.84-1.25
Age					1.01	0.99-1.02
Male sex	53.3%	51.2%	1.08	0.89-1.30	1.13	0.91-1.40
DM	23.4%	9.0%	3.08[b]	2.39-3.97	2.08[b]	1.56-2.76
Smoking	20.0%	14.0%	1.54[a]	1.20-1.96	1.36[a]	1.02-1.80

[a]P < 0.05, [b]P < 0.01 *vs* the control group in regression analysis. Blood samples were tested for the presence of anti-HCV, HBsAg, anti-HBs, and anti-HBc in all cases and controls. HBsAg$^-$/anti-HBc$^-$/anti-HBs$^-$: HBV infection naïve; HBsAg$^+$/anti-HBc$^+$/anti-HBs$^-$: chronic HBV infection; HBsAg$^-$/anti-HBc$^+$/anti-HBs$^+$: acquired immunity to HBV infection.

previous case-control studies were all based on hospitalized cases and controls[2,4,20-22]. Therefore, the controls selected from hospitalized patients could not represent the general population. In our study, the controls came from a screening program in Taiwan but not from hospitalized patients or outpatients. The frequencies of HBsAg, anti-HBs and anti-HBc in our controls were similar to those reported in the general population in Taiwan[8,11]. Our study is the first case-control study to address the association of HBV and HCV infection with histologically proven PDAC and with age- and sex-matched controls from the general population.

Cohort studies theoretically might be more informative than case-control studies in terms of design. There were three cohort studies regarding this issue. The first two cohort studies, which did not adjust for DM as a risk factor[14,23], drew a positive conclusion. However, a recent study did not show a significant positive association between HBV infection and pancreatic cancer after adjustment for DM as a risk factor[6]. To the best of our knowledge, DM is a risk factor of pancreatic cancer with an average OR of about 2[1]. Whether the association of DM, HBV infection and PDAC was the same as in the previous two cohorts awaits our attention.

We defined HBsAg$^-$/anti-HBc$^-$ as "never exposed to HBV", and HBsAg$^-$/anti-HBc$^+$ as "past exposure to HBV". In a relatively low-prevalence region in the United States, only 1/879 (0.1%) controls were HBsAg$^+$, 20/879 (2.3%) were anti-HBs$^+$, and 54/879 (6.14%) were anti-HBc$^+$. In our study, the positive frequency of HBsAg, anti-HBs and anti-HBc was 12.4% (213/1716), 61.1% (1048/1716) and 66.4% (1140/1716) in our controls. Besides, all negative serology markers (HBsAg$^-$/anti-HBc$^-$/anti-HBs$^-$) was considered seronegative for HBV infection in Taiwan rather than only HBsAg$^-$/anti-HBc$^-$[18,24]. It was more appropriate to do subgroup analysis including all these three serology markers (HBsAg, anti-HBc and anti-HBs) simultaneously to calculate the risk of HBV in PDAC in a high-endemic area. In the analysis by El-Serag *et al*[23] from the United States, only the cohort studies included all three of these markers. They did not stratify DM as a risk factor, which could have confounded the results because the study was in a region with a low prevalence of HBV and high incidence of pancreatic cancer. Three case-control studies have taken anti-HBc into consideration in their analysis. All three came from hospital-based population in an HBV-endemic area but with conflicting results[4,5,22]. Only one of these studies concluded that HBV was a risk factor for pathologically confirmed PDCA after adjustment for DM[4]. The other two studies showed contradictory results and neither of them adjusted for factors such as DM and smoking.

Our study is believed to be the first to address the association between HBV/HCV infection and PDAC in a high-endemic HBV area by using population-based controls and histopathologically or/and cytologically confirmed PDAC, with adjustment for age, sex, DM and smoking. We showed that HBV or HCV infection was not associated with the development of PDAC. HBV and HCV infections are considered major risk factors for HCC, and HBV accounts for 75%-80% of all HCC cases in Taiwan[8,10,11]. It is reasonable that the incidence of PDAC in Taiwan should be higher if HBV infection is really associated with increased risk of PDAC. According to the National Cancer Registry data, the incidence of HCC in Taiwan is about 30 per 100000 persons in contrast to 7-8 per 100000 persons for PDAC[25]. In our PDAC patients, we did not find any differences in the age at diagnosis with cancer staging, clinical outcome, and survival between patients with or without HBV infection. We also analyzed the hepatitis B e antigen (HBeAg) and anti-HBe status and/or HBV DNA in our PDAC cases who were infected by HBV. There was still no difference between patients with or without HBeAg. It is difficult to understand the biological explanation for PDAC risk in patients with HBV infection. In the meta-analysis, posi-

tive HBeAg was not a risk factor for pancreatic cancer, in contrast to $HBsAg^+$ or $HBsAg^-$/anti-HBc^+/anti-HBs^-[21]. The progression from active hepatitis virus infection to chronic inflammatory response in PDAC carcinogenesis is still unknown.

There were some merits to our study. First, we had a good case-control study matched by age and sex. All the PDAC cases were diagnosed with histological and/or cytological confirmation. The risk of HBV infection was evaluated by using HBsAg, anti-HBc and anti-HBs simultaneously with adjustment for DM and smoking. Second, the controls were from the general population and not a hospital-based population. Our controls were from a community taking part in a free hepatitis screening program in Northern Taiwan, which could have represented the normal population better, without selection bias caused by socioeconomic differences in recruitment, such as control data from those taking part in paid health checkups.

Our study had some limitations. It was a retrospective case-control study. The reported risks of pancreatic cancer such as family history and body mass index[1,26] could not be obtained in our controls because the aim of the screening campaign was for detection of HBV and HCV infection. In determining the HBV status, we could now examine the HBV DNA to clarify the HBV status more clearly. Analysis of viremia is more accurate to determine HBV status compared to serological markers. Only some of our PDAC patients were checked for HBV DNA. Our study could not establish whether the viral load increased the risk of PDAC.

In conclusion, our study is believed to be the first to address the association of HBV and HCV infection with histologically proven PDAC in an endemic area of Taiwan, with controls coming from the general population. HBV and HCV infection was not associated with the risk of PDAC. The exact role and association of risk of HBV infection and PDAC awaits further studies conducted in other endemic and non-endemic areas, with adjustment for well-documented risk factors of PDAC.

COMMENTS

Background

Previous studies on hepatitis B virus (HBV) status and pancreatic cancer risk have produced conflicting results.

Research frontiers

Previous studies have addressed the association of HBV/hepatitis C virus (HCV) with pancreatic ductal adenocarcinoma (PDAC) with some limitations. Some of the analyses did not include important risk factors such as diabetes mellitus (DM) and smoking, which might have confounded the results. Some studies included pancreatic malignancy from databases in which PDAC was not verified by cytology or histopathology. Some of the studies determined HBV status only by the presence of HBV surface antigen (HBsAg).

Innovations and breakthroughs

The present study is believed to be the first to use controls from the general population, compared to previous hospital-based case-controls studies with age- and sex-matched controls. All the PDAC in the study was confirmed by histology and/or cytology. Authors included antibodies against HBV core antigen in addition to HBsAg and antibodies against HBsAg in defining the HBV status. The risk of HBV/HCV infection was evaluated after adjustment for age, sex, DM and smoking in a high-endemic HBV area.

Applications

Negative results in a good quality controlled study are important. It is useful to know whether there is an association between HBV infection and PDAC in endemic area for HBV because it could influence the attitude and strategy to screen for PDAC.

Peer review

The manuscript is well presented and of interest, and the design of this study was appropriate. The study was done well and the results could contribute to our knowledge of this topic.

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BRIEF ARTICLE

Solitary fibrous tumors in abdomen and pelvis: Imaging characteristics and radiologic-pathologic correlation

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Abstract

AIM: To describe the imaging features of solitary fibrous tumors (SFTs) in the abdomen and pelvis, and the clinical and pathologic correlations.

METHODS: Fifteen patients with pathologically confirmed SFTs in the abdomen and pelvis were retrospectively studied with imaging techniques by two radiologists in consensus. Patients underwent unenhanced and contrast-enhanced imaging, as follows: 3 with computed tomography (CT) and magnetic resonance imaging (MRI) examination, 8 with CT examination only, and 4 with MRI examination only. Image characteristics such as size, shape, margin, attenuation or intensity, and pattern of enhancement were analyzed and correlated with the microscopic findings identified from surgical specimens. In addition, patient demographics, presentation, and outcomes were recorded.

RESULTS: Of the 15 patients evaluated, local symptoms related to the mass were found in 11 cases at admission. The size of the mass ranged from 3.4 to 25.1 cm (mean, 11.5 cm). Nine cases were round or oval, 6 were lobulated, and 10 displaced adjacent organs. Unenhanced CT revealed a heterogeneous isodense mass in 7 cases, homogeneous isodense mass in 3 cases, and punctuated calcification in one case. On MRI, most of the lesions (6/7) were heterogeneous isointense and heterogeneous hyperintense on T1-weighted images and T2-weighted images, respectively. All tumors showed moderate to marked enhancement. Heterogeneous enhancement was revealed in 11 lesions, and 7 of these had cysts, necrosis, or hemorrhage. Early non-uniform enhancement with a radial area that proved to be a fibrous component was observed in 4 lesions, which showed progressive enhancement in the venous and delayed phase. No statistical difference in the imaging findings was observed between the histologically benign and malignant lesions. Three patients had local recurrence or metastasis at follow-up.

CONCLUSION: Abdominal and pelvic SFTs commonly appeared as large, solid, well-defined, hypervascular masses with variable degrees of necrosis or cystic change that often displaced adjacent structures.

Key words: Abdominal imaging; Solitary fibrous tumors; Computed tomography; Magnetic resonance imaging

Core tip: Few studies have investigated the imaging features of solitary fibrous tumors (SFTs) in the abdomen and pelvis. We present the computed tomography and/or magnetic resonance imaging features of fifteen cases, and correlated them with histopathological results. We found that the imaging features of abdominal and pelvic SFTs predominantly appeared as large, well-defined, hypervascular masses with variable degrees of necrosis, cystic change, or hemorrhage that tended to displace adjacent structures. SFTs usually manifested as heterogeneous hyperintensity on T2-weighted images with low signal intensity areas representing flow voids, fibrosis, or collagen.

SFTs should be considered when the aforementioned imaging features are encountered.

Li XM, Reng J, Zhou P, Cao Y, Cheng ZZ, Xiao Y, Xu GH. Solitary fibrous tumors in abdomen and pelvis: Imaging characteristics and radiologic-pathologic correlation. *World J Gastroenterol* 2014; 20(17): 5066-5073 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5066.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5066

INTRODUCTION

Solitary fibrous tumors (SFTs) were first described by Klemperer and Rabin in 1931 as a localized fibrous mesothelioma[1]. The origin of SFTs has been controversial, and it is now considered to be a pathologically diverse, ubiquitous mesenchymal neoplasm of fibroblastic or myofibroblastic origin that can be either benign or malignant[2,3]. Clinically, they are often misdiagnosed as other hypervascular tumors by radiological and pathologic examination. Although SFTs may occur in any site of the body, they have been predominantly localized in the pleura, followed by the head and neck, and their presence in the abdomen and pelvis is rare.

Although the histopathological features of SFTs are well characterized, the appearance of these tumors on imaging is not well documented. To date, the most extensive reports regarding the imaging characteristics of SFTs in the abdomen and pelvis is by Shanbhogue *et al*[4], Zhang *et al*[5], and Ginat *et al*[6], and others focused on case reports[7-10]. To better characterize the radiological features of this rare disease, we present the computed tomography (CT) and/or magnetic resonance imaging (MRI) features of 15 cases of SFTs within the abdomen and pelvis, and correlated them with the histopathological findings. To our knowledge, this is the largest and most detailed radiologic case series reported to date for SFTs.

MATERIALS AND METHODS

Study population and imaging protocol

CT and MRI findings of 15 patients with pathologically confirmed SFTs in the abdomen and pelvis were retrospectively reviewed. Of these 15 patients, three underwent both CT and MRI examination, 8 had CT examination only, and 4 had MRI examination only. This study was conducted according to the ethical standards of our institution and was approved by our review board.

Due to the retrospective nature of the study, the CT and MR images were acquired with varied parameters. Contrast-enhanced CT was acquired 45-60 s after intravenous injection of Ominipaque-based CT contrast agent; and contrast enhanced MRI scans were acquired 25-30 s (arterial phase), 45-60 s (venous phase), and 3 min (delayed phase) after intravenous administration of gadolinium-based MRI contrast agent. For CT examinations, patients were scanned using a 64 multi-detector CT (Lightspeed VCT, GE Healthcare, Chalfont St Giles, United Kingdom) with an X-ray tube voltage of 120 kV, current of 200 mA, width of collimator 64 mm × 0.625 mm, and collimation and intervals of 5-10 mm. MRI scans were performed with a 1.5 T scanner (Avanto, Siemens Medical Systems, Munich, Germany). The scanning parameters were as follows: a T1-weighted gradient-echo sequence (TR: 90-180 ms, TE: 2-5 ms, flip angle: 70°), T2-HASTE sequence (TR: 4000-6000 ms, TE: 80-100 ms, flip angle: 20°), and T1-weighted VIBE sequence (TR/TE: 4.8/2.2 ms, flip angle: 70°).

Imaging analysis

The images were reviewed by two radiologists working in consensus-one with 20 years of experience in abdominal imaging and one a fellow. Each was only aware of the histological diagnosis, but did not review the official preoperative radiology reports. The CT and MR images were evaluated for location, size, shape, margin, internal architecture, CT density, and MRI signal intensity compared with adjacent muscle, pattern of enhancement, and changes in adjacent structures. The degree of mass enhancement was assessed subjectively and categorized as follows: mild, when the enhancement was similar to that of adjacent muscle; moderate, when the enhancement was higher than that of muscle but lower than that of blood vessels; marked, when the enhancement was approaching that of blood vessels. These imaging findings were correlated with the microscopic findings of the surgically obtained specimens, and compared between the histologically benign and malignant groups. In addition, review of the patients' charts was performed to determine demographic data, clinical presentation, and postsurgical outcome.

RESULTS

Clinical results

The study group consisted of 12 men and 3 women with a median age of 65.2 years (range, 1-76 years). Of these 15 patients, 11 (73%) had local symptoms at admission, including a palpable mass (n = 4), abdominal pain or discomfort to various degrees (n = 5), difficulty in defecation (n = 3), urinary frequency or retention (n = 4), gross hematuria (n = 1), and a sexual disorder (n = 1). A firm and non-tender mass was revealed in 10 patients upon digital rectal examination. However, none had systemic symptoms; and all the routine laboratory studies were unremarkable. A Foley catheter was placed in the urethra with complete resolution of urinary symptoms in patients with urinary retention. Surgical excision was successfully performed in all patients; and embolization prior to surgery was performed in one patient to prevent excessive surgical hemorrhage. At follow-up 0-62 mo (median, 20.3 mo) after surgery, 2 patients had local recurrence and one had hepatic metastasis, and all were classified as malignant at initial pathological examination.

Table 1 Radiological findings of the 15 cases with solitary fibrous tumors

No/age/sex	Benign/ malignant	Location	Size (cm^2)	Shape, margin	CT density/MRI intensity	Degree, pattern of enhancement
1/1/M	Benign	Adrenal region-L	3.4 × 3.4	Rounded, well-defined	Isodensity with dot calcification	Moderate, homogeneous
2/76/M	Benign	Pararectal space-L	4.0 × 4.1	Oval, well-defined	Homogeneous isodensity	Moderate, homogeneous
3/39/M	Benign	Rectovesical space	11.3 × 11.5	Lobulated, well-defined	Isodensity with patchy necrosis	Marked, heterogeneous
4/41/M	Benign	Paravesical space-R	10.8 × 14.0	Oval, well-defined	Isodensity with patchy necrosis	Moderate, heterogeneous
5/29/F	Benign	Paravesical space-L	8.1 × 6.3	Oval, well-defined	Homogeneous isodensity Homogeneous isointensity (T1WI) Homogeneous hyperintensity (T2WI)	Marked, homogeneous
6/43/F	Benign	Presacral space	15.3 × 18.0	Oval, well-defined	Isodensity with patchy necrosis Heterogeneous isointensity with patchy hyperintensity (T1WI) Heterogeneous hyperintensity with patchy hypointensity (T2WI)	Marked, heterogeneous
7/60/F	Benign	Intraperitoneal	18.0 × 20.2	Lobulated, well-defined	Heterogeneous isointensity with patchy hypointensity (T1WI) Heterogeneous hyperintensity (T2WI)	Marked, heterogeneous Progressive enhancement
8/33/M	Benign	Presacral space	11.3 × 12.0	lobulated, well-defined	Isointensity with radial hypointensity (T1WI) Heterogeneous hyperintensity with radial hypointensity (T2WI)	Marked, heterogeneous Progressive enhancement
9/69/M	Malignant	Retroperitoneal	15.3 × 20.8	Lobulated, ill-defined	Isodensity with patchy necrosis	Moderate, heterogeneous
10/59/M	Malignant	Prevesical space	9.3 × 10.2	Oval, well-defined	Homogeneous isodensity	Moderate, homogeneous
11/52/M	Malignant	Intraperitoneal	7.4 × 8.1	Lobulated, well-defined	Isodensity with patchy necrosis	Marked, heterogeneous
12/61/M	Malignant	Paravesical space-R	11.6 × 12.0	Oval, well-defined	Isodensity with patchy necrosis	Marked, heterogeneous
13/47/M	Malignant	Rectovesical space	10.4 × 11.3	Oval, well-defined	Isodensity with patchy necrosis Heterogeneous isointensity with patchy hypointensity (T1WI) Heterogeneous hyperintensity (T2WI)	Moderate, heterogeneous
14/51/M	Malignant	Retroperitoneal	15.0 × 25.1	Lobulated, ill-defined	Heterogeneous isointensity with patchy hypointensity (T1WI) Heterogeneous hyperintensity (T2WI)	Marked, heterogeneous Progressive enhancement
15/57/M	Malignant	Rectovesical space	4.7 × 4.8	Oval, well-defined	Isointensity with radial hypointensity (T1WI) Heterogeneous hyperintensity with radial hypointensity (T2WI)	Marked, heterogeneous Progressive enhancement

M: Male; F: Female; L: Left; R: Right; CT: Computed tomography; MRI: Magnetic resonance imaging; T1WI: T1-weighted imaging; T2WI: T2-weighted imaging.

Imaging findings

The CT and MR imaging features of the 15 patients with SFTs are shown in Table 1. Three masses were found in the upper abdominal retroperitoneum, 2 in the peritoneal cavity, and 10 in the pelvis including 2 in the presacral space, one in the pararectal space, 3 in the rectovesical space, 3 in the paravesical space, and one in the prevesical space. All patients demonstrated a solitary mass with sizes ranging from 3.4 cm × 3.4 cm to 25.1 cm × 15.0 cm, with 10 masses larger than 10 cm in maximum diameter. Thirteen cases were well-defined, and 2 were ill-defined; 9 cases were round or oval, and the other 6 were lobulated. Thirteen cases displaced and compressed the adjacent organs, such as the pancreas, prostate, uterus, bladder, intestine, or vessels, and could not be delineated on CT and MR imaging. In addition, one of the patients with ill-defined margins showed invasion of the posterior stomach wall and envelopment of the celiac trunk and its branches (Figure 1). However, no lymphadenopathy or distant metastases were detected on the CT and MR images at initial evaluation.

On unenhanced CT images, all 11 cases with CT scans manifested as an isodense mass, of which 7 had relatively hypodense necrotic or cystic areas, 3 were homogeneous, and one had punctuated calcification. In total, 7 patients underwent MRI scans. On T1-weighted images, almost all lesions were isointense, in which 5 cases exhibited mostly isointense signals with patchy or radial areas of low intensity (Figure 2A), one case had mostly an isointense signal with a patchy area of mild hyperintensity (Figure 3A), and one case had homogeneous isointensity. On T2-weighted images (T2WI), one lesion exhibited homogeneous hyperintensity, and the others had heterogeneous hyperintensity, in which 3 cases had a patchy or radial area of hypointensity (Figures 2B, 3B). In addition, intra- or extra-tumoral flow voids were found in 4 cases on T2WI (Figure 2B).

After intravenous injection of contrast material, feeding vessels (vascular pedicle) were seen in 4 of the 15 cases (26.7%) (Figure 2C). The degree of mass enhancement on CT and MRI varied from moderate to marked; and in patients with MRI examination, it was marked in the arterial phase and persisted in the portal venous and delayed phase. Of these 15 cases, homogenous enhancement was

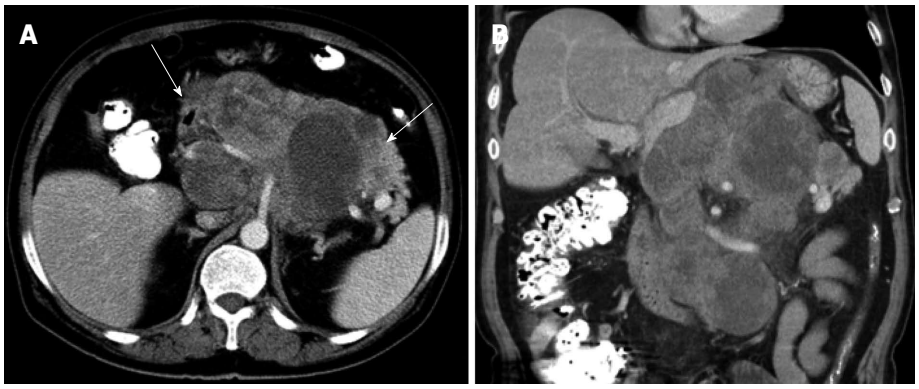

Figure 1 Computed tomography findings of a solitary fibrous tumor in the retroperitoneum. A: Contrast-enhanced axial image showing a lobulated, ill-defined, and heterogeneously moderate enhancing mass in the retroperitoneum. The mass invades the pancreas and stomach (arrows), and the celiac trunk and its branches were enveloped; B: The contrast-enhanced coronal reconstruction image shows envelopment of the branches of the celiac trunk.

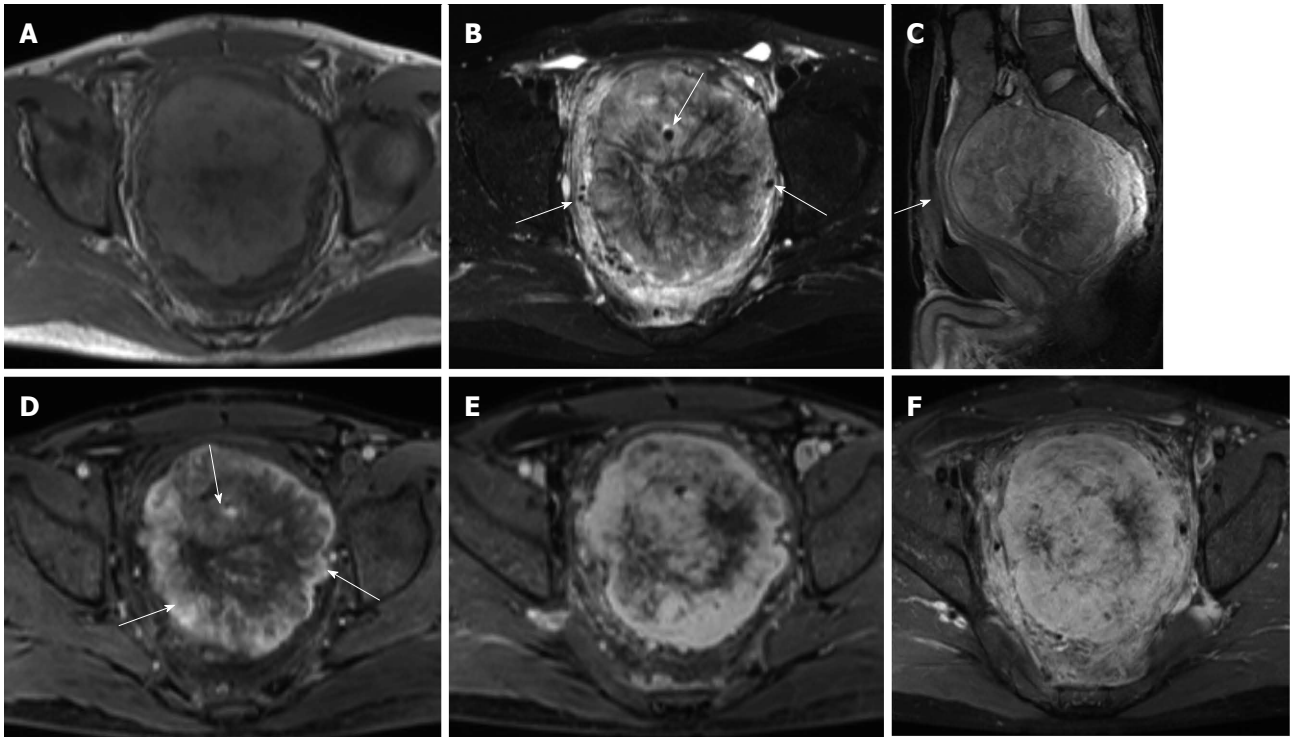

Figure 2 Magnetic resonance images of a solitary fibrous tumor in the presacral space. A: T1-weighted Magnetic resonance (MR) image showed that the mass is lobulated and almost isointense to the muscle. Radial hypointensity is seen in the center; B: Axial fat-suppressed T2-weighted MR image shows that the mass is predominantly hyperintense with radial areas of low signal intensity. In addition, intra- and extra-tumoral flow voids can be detected (arrows); C: Sagittal fat-suppressed T2-weighted MR image showing that the rectum is compressed anteriorly by the mass (arrow); D: The mass demonstrated intense heterogeneous enhancement in the arterial phase. The feeding vessels (vascular pedicle) can be demonstrated clearly (arrows); E, F: The mass showed persistent and progressive enhancement in the portal venous (E) and delayed phase (F).

observed in 4 cases and heterogeneous enhancement in 11 cases. In the latter cases, 7 lesions showed necrotic or cystic areas on enhanced CT and/or MR images, and the other 4 lesions demonstrated early non-uniform enhancement with the center showing a radial or fissured region which showed progressive enhancement spreading from the periphery to the center in the portal venous and delayed phases on enhanced MR images (Figures 2D-F, 3C). However, in our case series, there was no statistical difference in the imaging findings between the histologically benign and malignant lesions.

Pathological findings

Of the surgical specimens, 9 tumors were rounded or ovoid, 6 were lobulated, and 10 were encapsulated. The cut surfaces were grayish-white or yellowish-white in color, and fish meat-like in texture. Radial or fissured regions of the abundant fibrotic component were revealed in the center of 4 cases, cystic or necrotic foci in 7 cases, and hemorrhage in one case.

Histological examination demonstrated that the tumors had a haphazard patternless architecture of spindle or ovoid cells with a varying degree of collagenous tis-

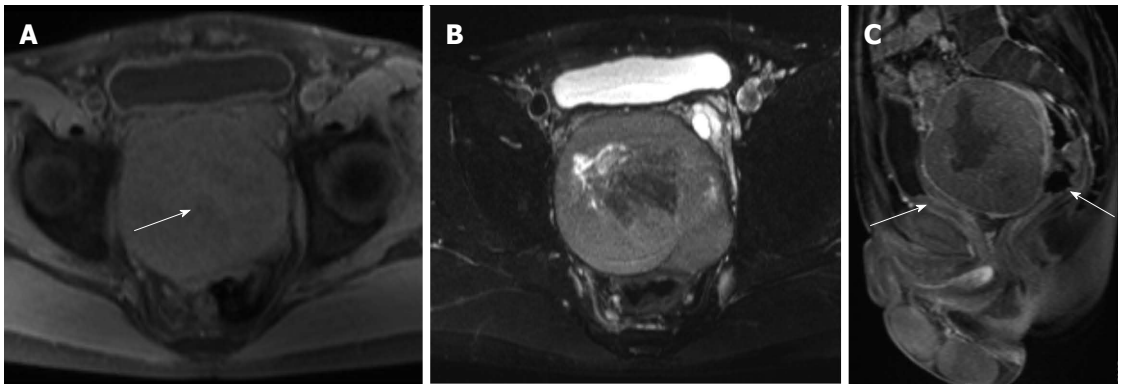

Figure 3 Magnetic resonance images of a solitary fibrous tumor in the rectovesical space. A: T1-weighted Magnetic resonance (MR) image showing an oval isointense mass with patchy mild hyperintensity (arrow). The patchy mild hyperintensity was proven to be a hemorrhage; B: Fat-suppressed T2-weighted MR images reveal a mass with heterogeneous hyperintensity and patchy hypointensity; C: Contrast-enhanced MR images demonstrate moderate heterogeneous enhancement of the mass, which displaces the bladder anteriorly and rectum posteriorly (arrows).

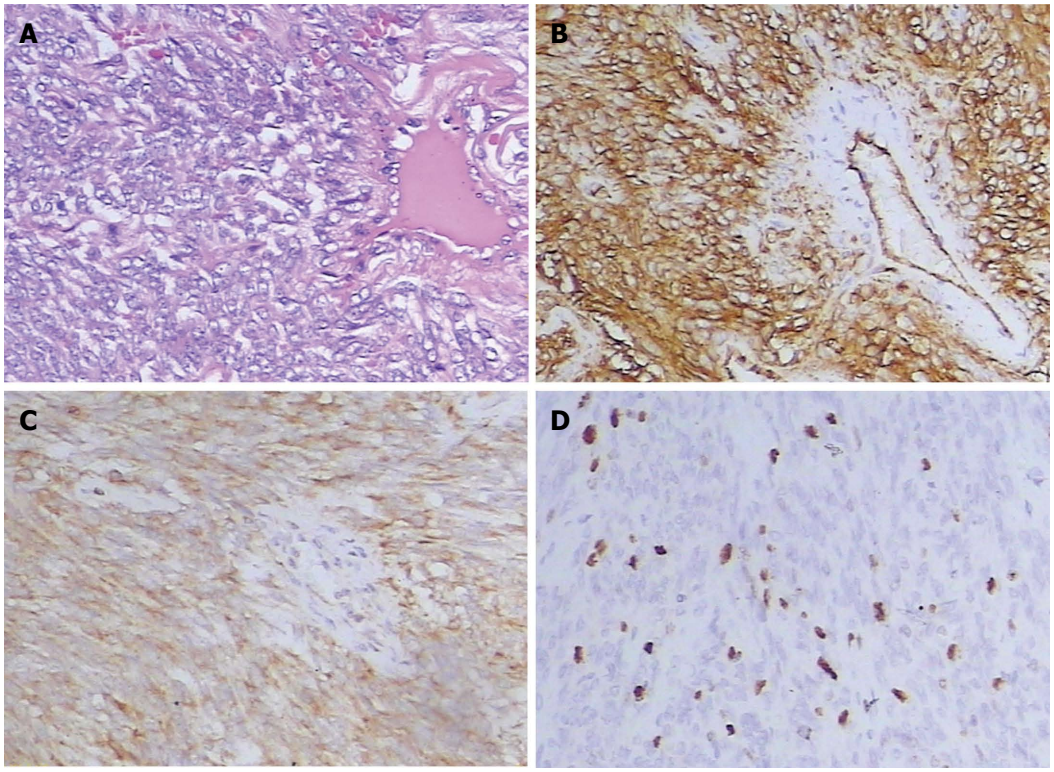

Figure 4 Pathologic features of the solitary fibrous tumor. A: Hematoxylin and eosin staining, × 40, indicates monotonous spindle cell proliferation with a hemangiopericytoma-like vascular growth pattern; B-D: Immunohistochemical staining, x 40, indicates that the tumor is diffusely positive for CD34 (B) and Bcl-2 (C), and the proportion of Ki67 positive cells is 15% (D).

sues and a hemangiopericytoma-like appearance with prominent thin-walled vascular vessels (Figure 4A). The degree of cellularity varied for each tumor and was inversely related to the collagenous tissues. Immunohistochemical staining revealed that CD34 (Figure 4B) was positive in all cases, CD99 in 12 cases, B-cell lymphoma 2 (bcl-2) (Figure 4C) in 14 cases, S-100 in 4 cases, while all lesions were negative for desmin. The proportion of Ki-67 positive cells was greater than 10% in 4 cases (Figure 4D), and less than 5% in the other cases. In total, 8 patients were classified as benign and 7 presented malignant criteria at pathological analysis of the surgical specimens. All 7 malignant tumors were hypercellular lesions, in which 3 each had mild to moderate atypia, and 4 displayed marked atypia.

DISCUSSION

SFT is a rare spindle cell mesenchymal neoplasm with age of onset around 50-60 years, and equal distribution in men and women. Patients in our series ranged from 1 to 76 years, but the incidence was higher in men than in women with a sex ratio of 4:1. To the best of our knowledge, we report the first case of SFT in an infant.

Although SFTs were thought to occur most frequently in the pleura, they may develop virtually anywhere throughout the body, while involvement of the abdomen and pelvis is particularly rare[2]. Despite its characteristic histological and immunohistochemical features, SFTs in the abdomen and pelvis remain a diagnostic challenge to both clinicians and radiologists; it is often poorly recognized and frequently confused with other neoplasms that more commonly occur at this site. Although rare, radiologists and surgeons must be aware of SFTs in the abdomen and pelvis.

Extrapleural SFTs were typically demonstrated as large, slow-growing soft tissue tumors[4]. Symptoms related to the site are frequent in these locations, such as a palpable mass, pain, gross hematuria, bowel obstruction, and urinary retention or obstruction[11,12], as seen in some of our patients. Systemic symptoms such as hypoglycemia (< 5% patients), arthralgia, hypertrophic osteoarthropathy, and clubbing have also been documented in the literature[4,13]; however, they were not present in any of our patients. These symptoms usually resolve upon removal of the tumor.

In the last 20 years, the classification of SFTs and hemangiopericytoma has changed, and most hemangiopericytomas are now thought to be cellular variants of SFTs[2,3]. Overall, SFTs are usually well-demarcated and partially encapsulated in neoplasms[2]. These tumors are highly vascular and have a propensity to undergo hemorrhage, necrosis, and myxoid degeneration. Microscopically, they show a wide range of morphological features, from predominantly fibrous lesions containing alternating fibrous areas and hyalinized thick-walled vessels (fibrous variants) to more cellular and less fibrous neoplasms with a "patternless pattern" (a monotonous appearance) and thin-walled branching vessels (cellular variant)[2,3,14]. Immunohistochemically, both of them variably express CD34, CD99, and bcl-2 antigens; and the fibrous form demonstrates strong reactivity with CD34, whereas the cellular form demonstrates weak reactivity[2].

General histological features that may help to identify a malignant lesion include large size, infiltrative margins, hypercellularity, nuclear atypia, mitotic activity (≥ 4/10 high-power fields), and the presence of necrosis and hemorrhage[2,15,16]. In addition, malignant SFTs tend to lose CD34 immunoreactivity and overexpress Ki-67, P53, and S-100[17]. In our cases, 8 tumors were classified as benign, and 7 as malignant. Although most extrapleural SFTs have been reported to be benign histologically, approximately 10%-15% of SFTs demonstrate malignant behavior in the form of recurrence or metastasis[2,18]. Local recurrences and distant metastases may be expected in malignant SFTs, as seen in three of our patients. Although none were found in our patients, some cases of "histologically benign" SFTs that do recur or metastasize have been reported in the literature[19,20]. The most important risk factor for recurrence is invaded surgical margins. Therefore, complete resection with negative surgical margins is the treatment of choice, and long-term follow-up of all patients is highly recommended, regardless of anatomic location[20,21]. The vascular nature and the presence of large feeding vessels made surgical removal technically difficult and preoperative embolization that can reduce intraoperative hemorrhage may be required[22], as seen in one of our patients.

Radiological information provides useful information, such as detection, characterization, and localization of tumors. In addition, it can depict the local extent, possible invasion into adjacent structures, and locoregional and distant metastases. Importantly, these images provide a road map in the future for the operating surgeons. The most common imaging finding, recently reported in the literature, was a large, well-defined, round, oval, or lobulated hypervascular mass that tended to displace or invade adjacent structures such as the bowel, urinary bladder, seminal vesicle, ureter, and vessels[4,5,12]. The largest mass measured in our cases was 25.1 cm × 15.0 cm. The site of origin of the mass could not be defined on some images because margins blended in with adjacent structures. We inferred that the lobulated shape was due to different growth rates of the tumor. In addition, the difference in the resistance against the growth of the tumor may also be an important factor.

Most of our patients with plain CT scans demonstrated an isodense mass with patchy hypodensity. The attenuation likely depended upon the collagen content, as hyperdense lesions have abundant collagen[6]. Calcification is rare and can be seen in large benign or malignant tumors, but its presence or absence is not necessarily a helpful distinguishing feature[4,6]. On MRI, SFTs were usually isointense or slightly hyperintense on T1WI and heterogeneously hyperintense on T2WI, relative to muscle. Heterogeneous signal intensity was observed in some cases and was likely related to the components of hemorrhage, necrosis, cystic, or myxoid degeneration, and hyalinized stromal content[4,5,7]. However, the presence of low-signal-intensity foci on both T1WI and T2WI were mainly attributed to the components of dense collagen and fibrosis, low cellularity, and associated reduced proton mobility[6]. In addition, lesions with high fibrous content may demonstrate progressive enhancement during the arterial and portal phases that become marked on delayed images[5,8], as seen in some of our patients.

SFTs were highly vascular and vigorously enhancing on both enhanced CT and MR images. These tumors are usually heterogeneous, with hypervascular areas showing early intense enhancement, hypercellular areas showing moderate enhancement, and areas of necrosis or of cystic or myxoid degeneration showing no enhancement[5,23]. In addition, hypercellular areas demonstrated persistent enhancement in the venous and delayed phases. However, there is considerable overlap in the type of enhancement, during which 100% of malignant and 60% of benign SFTs show heterogeneous enhancement[23], as seen in our patients. Enhanced CT and MR images can depict the supplying arteries, and intra- or extra-tumoral flow voids can be readily revealed on unenhanced T2WI.

Wignall *et al*[24] and Garcia-Bennett *et al*[25] thought that the presence of a vascular pedicle, although not specific, is a useful distinguishing feature of SFTs; it was seen in 4 of our patients.

The differential diagnosis for these imaging characteristics includes other hypervascular tumors or tumors with predominant fibrous content, such as leiomyosarcoma, neurogenic tumor, pheochromocytoma, lymphoma, desmoid tumor, malignant fibrous histocytoma, mesothelioma, and fibroma. Using imaging findings alone, differentiating among these is not possible. Therefore, complete excision and histopathologic examination are necessary to establish the diagnosis.

In conclusion, the imaging features of SFTs in the abdomen and pelvis predominantly appeared as well-defined, hypervascular masses with variable degrees of necrosis, cystic change, or hemorrhage. They usually manifested as heterogeneous hyperintensities on T2WI with low signal intensity areas representing flow voids, fibrosis, or collagen. Although we believe that the radiologist may diagnose SFT when a mass presents with the aforementioned imaging features, histopathologic examination remains necessary to confirm the diagnosis.

COMMENTS

Background

Solitary fibrous tumors (SFTs) may occur in any site of the body, but their presence in the abdomen and pelvis is rare. Clinically, they are often misdiagnosed as other hypervascular tumors by radiological and pathologic examination. Few studies to date have investigated the imaging features of SFTs in the abdomen and pelvis.

Research frontiers

The imaging appearance of SFTs on imaging is much less well reported than the histopathologic features. To better characterize the radiological features of this rare disease, the authors present the computed tomography and/or magnetic resonance imaging features of 15 cases of SFTs within the abdomen and pelvis, and correlated them with histopathological results.

Innovations and breakthroughs

This is the largest and most detailed radiologic case series of SFTs in the abdomen and pelvis reported to date.

Applications

This study indicated that the imaging features of SFTs in the abdomen and pelvis predominantly appear as a well-defined, hypervascular mass with a variable degree of necrosis, cystic change, or hemorrhage. They usually manifest as heterogeneous hyperintensity on T2-weighted images with low signal intensity areas representing flow voids, fibrosis, or collagen. The radiologist may suggest the diagnosis of SFTs when a mass with the above imaging features is encountered.

Terminology

SFTs can be benign or malignant and are considered a pathologically diverse, ubiquitous mesenchymal neoplasm of fibroblastic or myofibroblastic origin.

Peer review

This is a retrospective clinical analysis of 15 cases of SFTs in the abdomen and pelvis. SFTs in the abdomen and pelvis are rarely encountered in clinics. Only anecdotal case reports are given in the medical literature. This case series is important and sheds light on these uncommon neoplasms.

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BRIEF ARTICLE

Evaluation of routine biopsies in endoscopic screening for esophagogastric junction cancer

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Abstract

AIM: To explore whether routine biopsies at the high incidence spot of esophagogastric junction (EGJ) cancer are justified in endoscopic screening.

METHODS: This was a multicenter population-based study conducted in eight high-risk areas in China. A total of 37396 participants underwent endoscopic examination. Biopsies were obtained from visible mucosal abnormalities or from normal-appearing mucosa at the high incidence spot of esophagogastric junction cancer when no abnormality was detected. Specimens showing high-grade intraepithelial neoplasia (HIN) or higher grade lesions were deemed as pathologically "positive". The ratios of positive pathologic diagnosis between participants with abnormal and normal-appearing mucosa were compared using the Pearson χ^2 test. Odds ratios and 95% confidence intervals, adjusted for potential confounders, were calculated using logistic regression.

RESULTS: A total of 37520 individuals participated in this study and 37396 (99.7%) participants had full information and were suitable for analysis. During endoscopic examinations, 9.11% (3405/37396) participants were found to have visible mucosal lesions. Of the participants who had normal-appearing mucosa at the EGJ, only 0.28% (94/33991) were diagnosed with HIN or higher grade lesions, whereas 6.05% (206/3405) of participants with abnormalities at the EGJ had a positive pathologic result. After controlling for other variables, visible abnormal mucosa detected under endoscopy strongly predicted a positive pathologic result (OR = 32.51, 95%CI: 23.96-44.09). The proportion of participants with "positive" pathologic diagnoses increased as the total number of endoscopic examinations performed by the doctors increased (< 5000 cases *vs* 5000-10000 cases *vs* > 10000 cases, Z = -2.7207, P = 0.0065, Cochran Armiger trend test). The same trend was found between the proportion of participants with positive pathologic diagnoses and the total number of years the doctors performed endoscopy (< 5 years *vs* 5-10 years *vs* > 10 years, Z = -10.3222, P < 0.001,

Cochran Armiger trend test).

CONCLUSION: Additional routine biopsies from the high incidence spot of EGJ cancer are of limited value and are unjustified.

Key words: Esophagogastric junction cancer; High incidence spot; Screening; Endoscopy; Biopsy

Core tip: Our findings offer population-level evidence for the high incidence spot of esophagogastric junction (EGJ) cancer. It is also the first study to evaluate whether the findings at the high incidence spot could be used in endoscopic screening in high-risk populations to increase the detection rate. We found that visible mucosal abnormalities of the EGJ at endoscopy were strongly associated with pathologic diagnoses of high-grade intraepithelial neoplasia or higher grade lesions. When no abnormalities were detected, routine biopsies from normal-appearing mucosa at the high incidence spot in endoscopic screening were unjustified in high-risk populations.

Niu X, Wei WQ, Hao CQ, Song GH, Li J, Hua ZL, Li YW, Chang J, Wang XZ, Zhao DL, Wang GQ, Hsieh E, Qiao YL. Evaluation of routine biopsies in endoscopic screening for esophagogastric junction cancer. *World J Gastroenterol* 2014; 20(17): 5074-5081 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5074.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5074

INTRODUCTION

In the past two decades, studies from Western countries[1,2], China[3], Iran[4] and Japan[5] have all reported an increasing incidence of cancers arising from the esophagogastric junction (EGJ). In China, cancer registry data from 1998 to 2002 showed that the world age-standardized incidence rates of EGJ cancers for males were $46.7/10^5$, $82.8/10^5$, $40.8/10^5$ and $89.1/10^5$ in Cixian, Yangcheng, Linzhou and Shexian, respectively[6], making it the third most common malignancy in these regions. Recently, an asymmetrical circumferential distribution of EGJ cancers has been described in Chinese patients. Wang *et al*[7] found that over 75% of EGJ cancers were located at the right anterior side of the EGJ at endoscopy, specifically at the most proximal gastric rugae of the lesser curvature. Subsequent observations indicated that 91%-95% of early stage EGJ cancers and precancerous lesions originated from this specific site[8-10]. Thus, this circumferential asymmetry in the formation of pathological lesions at the EGJ led to the identification of the high incidence spot of junctional lesions in China. Similar findings of such an asymmetrical circumferential distribution of lesions at the EGJ have also been found in Iran[4] and Japan[11,12] where a high incidence of upper gastrointestinal cancers (UGIC) have been reported. These findings were adopted by the Chinese Ministry of Health's cancer screening guidelines (2005), named The Practice of Screening, Early Diagnosis and Early Treatment for Esophageal and Cardiac Cancer. The preliminary version of the guidelines recommended endoscopists take multiple biopsies from visible abnormal mucosa at the EGJ at endoscopy or a routine biopsy at the high incidence spot of EGJ when no mucosal abnormality was detected[13].

However, the additional routine biopsy at the high incidence spot of the EGJ remains controversial among doctors conducting these screening programs in high-risk populations. Several recent investigations have shown that 90% of EGJ cancers could be detected *via* mucosal abnormalities at endoscopy, including congestion, roughness, erosion, and nodularity[14,15], and have suggested that contrary to the current guidelines, biopsies should only be taken from visible gross lesions at the EGJ, thereby avoiding excessive biopsies. However, other studies have pointed out that a significant proportion of early stage EGJ adenocarcinomas were diagnosed from biopsies of asymptomatic mucosa *via* endoscopy[16]. In addition, it was observed that 5% of normal-appearing and pathologically-confirmed healthy mucosa progressed to EGJ cancers in a high-risk Chinese population taking part in a 15-year prospective study[17]. Moreover, the detection of endoscopically recognizable lesions usually requires examination by highly skilled and experienced endoscopists, whereas village doctors conducting these screening projects are typically from community-based hospitals in rural China and have only received basic medical training. In order to reduce the missed diagnoses among these endoscopists, they continued to use the first version of the guidelines given that additional routine biopsy played a crucial role in the early detection of EGJ cancers. These inconsistent findings were mainly from hospital-based studies that were limited in sample size or restricted to only one high-risk area in China. To provide more population-based evidence to determine whether additional routine biopsies at the high incidence spot of EGJ cancer are necessary, we performed a population-based study in eight high-risk areas for upper gastrointestinal tract cancers in China.

MATERIALS AND METHODS

Study design and participants

This was a multicenter population-based, cross-sectional study conducted in eight counties with a high incidence of upper gastrointestinal tract cancers, namely Linzhou, Cixian, Shexian, Feicheng, Yangzhong, Taixing, Yangcheng and Yanting. Based on previous field studies and population registry data, each county has 10-20 villages. The target population (aged 38-72 years) accounted for approximately 25% of total residents in each county.

The number of villages (two to five, respectively)

selected was based on the health resources and research capacity of each county. One study investigator visited the local health facility in each village to notify them of the impending study and arranged a date to drive the participants to the hospitals. Eligible participants were healthy locals, who were aged 38 to 72 years without contraindications for endoscopic examinations (*e.g.*, severe cardiovascular or respiratory disease). Participants with a history of upper gastrointestinal surgeries, liver cirrhosis, esophageal varices, hematemesis, severe bleeding diathesis, unstable angina, psychiatric diseases or allergy to lidocaine were excluded. All eligible subjects in each village were invited to participate. In total, 27 villages were selected and over 70% of eligible residents from these villages participated in our study and signed a voluntary written informed consent document at the time of entry into the study.

All phases of this study were approved by the Institutional Review Board of the Cancer Foundation of China.

Endoscopic examination

All endoscopic examinations were conducted in eight local cancer hospitals, respectively, using the following procedure: after signing the informed consent, participants were anesthetized with 5 mL 1% lidocaine per os for 5 min. Participants were then placed in the left lateral position. The entire esophagus and stomach were examined, but only the EGJ was analyzed. The EGJ was recognized as the most proximal extent of the gastric fold at endoscopy. Tumors with an epicenter lying at the EGJ, or within 1 cm proximally and distally were defined as EGJ cancers. During examination, suspicious lesions showing congestion, bleeding, roughness, erosion, plaque and nodularity were targeted and 3-4 endoscopic biopsies were taken. If no mucosal abnormality was detected, 1-2 additional routine biopsies were obtained from the high incidence spot, which is at the right side of the EGJ from the axial view, specifically at the most proximal gastric rugae of the lesser curvature. Visual endoscopic manifestations of the EGJ mucosa were recorded as "normal-appearing" and "abnormal". All endoscopic examinations were performed by board-certified endoscopists trained by the Cancer Institute/Cancer Hospital, Chinese Academy of Medical Sciences. The skill and experience of the endoscopists were measured by the total number of endoscopic examinations performed (< 5000 cases; 5000-10000 cases; > 10000 cases) and the total number of years performing endoscopy (< 5 years; 5-10 years; > 10 years). Twenty four endoscopists were involved in this study. All endoscopic examinations were performed using the same type of endoscope (Olympus GIF-H260).

Pathologic diagnosis

Biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin and eosin. Each biopsy slide was read by two unified-trained local pathologists (16 in total) without knowledge of the visual endoscopic findings, and pathologic diagnoses were made according to the definitions in the World Health Organization (WHO) Classification of Tumours Pathology and Genetics Tumours of the Digestive System (2000). Findings with clinical implications, specifically high-grade intraepithelial neoplasia (HIN) or higher grade lesions were deemed as pathologically "positive" in our study. These patients were offered endoscopic mucosal resection or surgery, depending on the pathological grade. Other participants, including those with low-grade intraepithelial neoplasia (LIN), were regarded as pathologically "negative". Different follow-up schemes were provided to participants according to their pathologic diagnoses.

Statistical analysis

All analyses were performed using SAS 9.2 (SAS Institute Inc, Cary NC, United States). $P \leqslant 0.05$ was considered statistically significant. Proportions of diagnoses between participants with abnormal and normal-appearing mucosa were compared using the Pearson χ^2 test. Evidence for associations between pathologic diagnoses and the total number of endoscopic examinations performed by endoscopists and years of performing endoscopy were further assessed using the Cochran Armiger trend test. Univariate binary logistic regression was performed to choose variables that may be associated with the presence of "positive" pathology diagnosis. These variables were: age (continuous), gender (dichotomous), county (eight categories), endoscopic manifestations (dichotomous), total number of endoscopic examinations performed by a given endoscopist (three categories) and total number of years performing endoscopy (three categories). Variables showing a significant association ($P \leqslant 0.05$) were further analyzed using multivariate logistic regression.

RESULTS

From 2006 to 2008, a total of 37520 individuals participated in this study and 37396 (99.7%) participants had full information and were suitable for analysis. The mean age of the 37396 participants was 51.52 ± 7.92 years. During endoscopic examinations, 9.11% (3405/37396) of participants were found to have visible mucosal lesions. Endoscopic records also showed the percentages of participants with abnormal mucosa by individual county, which ranged from 1.00% (Yangting, 59/5911) to 33.96% (Feicheng, 1016/2992). Pathologic results indicated that there were 300 positive cases, 144 of which came from Linzhou. As shown in Table 1, Linzhou also had the largest proportion of total participants (18.08%, 6763/37396), while only 8.02% (2999/37396) were from Yangcheng.

Table 2 shows the frequency distribution in terms of different pathologic and endoscopic results. Among participants who had normal-appearing mucosa at the EGJ, only 0.28% (94/33991) were diagnosed with HIN or higher grade lesions, whereas 6.05% (206/3405) of participants with abnormalities at the EGJ had a positive

Table 1 Distribution of demographic characteristics, pathology and endoscopy results by county *n* (%)

County	Median age (range, yr)	Gender		Endoscopy		Pathology		Total
		Male	Female	Normal	Abnormal	Positive	Negative	
Linzhou	51 (32)	2876 (42.53)	3887 (57.47)	6159 (91.07)	604 (8.93)	144 (2.13)	6619 (97.87)	6763 (18.08)
Cixian	50 (33)	3004 (46.47)	3461 (53.53)	5512 (85.26)	953 (14.74)	45 (0.70)	6420 (99.30)	6465 (17.29)
Shexian	50 (32)	1933 (45.82)	2286 (54.18)	3588 (85.04)	631 (14.96)	7 (0.17)	4212 (99.83)	4219 (11.28)
Feicheng	52 (32)	1552 (51.87)	1440 (48.13)	1976 (66.04)	1016 (33.96)	30 (1.00)	2962 (99.00)	2992 (8.00)
Yangzhong	49 (34)	2367 (49.59)	2406 (50.41)	4721 (98.91)	52 (1.09)	41 (0.86)	4732 (99.14)	4773 (12.76)
Taixing	52 (34)	1345 (41.08)	1929 (58.92)	3219 (98.32)	55 (1.68)	20 (0.61)	3254 (99.39)	3274 (8.75)
Yangcheng	51 (34)	1521 (50.72)	1478 (49.28)	2964 (98.83)	35 (1.17)	3 (0.10)	2996 (99.90)	2999 (8.02)
Yanting	53 (29)	3118 (52.75)	2793 (47.25)	5852 (99.00)	59 (1.00)	10 (0.17)	5901 (99.83)	5911 (15.81)
Total	51 (34)	17716 (47.37)	19680 (52.63)	33991 (90.89)	3405 (9.11)	300 (0.80)	37096 (99.20)	37396 (100.00)

Table 2 Distribution of pathologic diagnoses by mucosa manifestation, total number of endoscopies performed and number of years performing endoscopy *n* (%)

Distribution	Pathology		Total	*P* value
	Positive	Negative		
Mucosa manifestation (at endoscopy)				< 0.001[1]
Abnormal	206 (6.05)	3199 (93.95)	3405	
Normal	94 (0.28)	33897 (99.72)	33991	
Total number of endoscopies performed				0.0065[1]
< 5000	46 (0.55)	8275 (99.45)	8321	
5000-10000	19 (0.92)	2053 (99.08)	2072	
> 10000	235 (0.87)	26768 (99.13)	27003	
Years of performing endoscopy				< 0.001[1]
< 5	11 (0.18)	6009 (99.82)	6020	
5-10	86 (0.50)	16952 (99.50)	17038	
> 10	203 (1.42)	14135 (98.58)	14338	

[1]Statistically significant ($P < 0.05$).

pathologic result. This difference was significant by Pearson's χ^2 test ($\chi^2 = 1296$, $P < 0.001$).

Overall, 72% (27003) of endoscopies in this study were conducted by senior endoscopists who had performed more than 10000 endoscopic examinations and 83.9% of the doctors had practiced endoscopy for more than 5 years. A statistically significant trend between pathologic diagnoses and total number of endoscopies performed by a given endoscopist was found. The proportion of participants with "positive" pathologic diagnoses increased as the total number of endoscopic examinations performed increased (< 5000 cases *vs* 5000-10000 cases *vs* > 10000 cases, $Z = -2.7207$, $P = 0.0065$, Cochran Armiger trend test). The same trend was found between the proportion of participants with positive pathologic diagnoses and the total number of years the doctor had performed endoscopy (< 5 years *vs* 5-10 years *vs* > 10 years, $Z = -10.3222$, $P < 0.001$, Cochran Armiger trend test). Biopsies taken by more experienced or skilled endoscopists had a higher proportion of positive pathologic diagnoses.

Univariate logistic regression analysis demonstrated significant associations ($P \leqslant 0.05$) between positive pathologic diagnoses and six variables, respectively: age, gender, county, endoscopic manifestations, the total number of endoscopic examinations performed by a given endoscopist and his/her total number of years performing endoscopy. Thus, these six variables were all included in the subsequent multivariate logistic regression model. Using a stepwise approach (SLE = 0.05, SLS = 0.05), age, gender, county, endoscopy manifestations and the total number of endoscopic examinations performed by a given endoscopist were significantly associated with a positive pathologic diagnosis in the final multivariable model (Table 3). After controlling for other variables, visible abnormal mucosa detected at endoscopy strongly predicted a positive pathologic result (OR = 32.51, 95%CI: 23.96-44.09).

DISCUSSION

In China, government-sponsored screening projects for seven cancers have been initiated in high-risk areas since 2005, namely cancers of the cervix, esophagus, colorectum, liver, nasopharynx, stomach and breast. The technical support unit of these projects, the Cancer Foundation of China, established the committee of experts and developed the preliminary version of screening guidelines for each cancer. These preliminary versions were largely based on limited clinical studies and experience from cancer screening programs in other countries. However, most of the recommendations lacked solid evidence from large Chinese populations. Therefore, revisions based on evidence from research and clinical practice in China is of great importance. The aim of our study was to clarify the existing controversy among doctors regarding the use of routine biopsy of the high incidence spot of the EGJ using population-level data.

The findings from our study suggest that routine biopsies from the high incidence spot of EGJ cancers are of limited value and are unjustified. Taking biopsies from the 3405 participants with visible abnormal mucosa at endoscopy enabled the detection of 6.05% (206/3405) of patients with HIN or higher grade lesions at the EGJ. When adjusted for potential confounders, a strong association between visible mucosal abnormality and positive pathologic result was also observed in the multivariate analysis. Compared with participants who showed normal-appearing mucosa at endoscopy, the risk of HIN or higher grade lesions was 32 times higher in patients with visible lesions at the EGJ. In contrast, routine biopsies of

Table 3 Multivariate logistic regression analysis: Independent variables and corresponding odds ratios for positive pathologic diagnosis

Selected variables	*b*	OR	OR, 95%CI	*P* value
Age (yr)	0.08	1.09	1.07, 1.10	< 0.0001[1]
Gender				
Male	0.42	2.32	1.75, 3.06	< 0.0001[1]
Female	0.00	1.00		
County				
Linzhou	0.00	1.00		
Cixian	-0.39	0.20		0.0508
Shexian	-1.76	0.05	0.02, 0.11	< 0.0001[1]
Feicheng	-0.72	0.14	0.09, 0.22	0.0004[1]
Yangzhong	2.03	2.19	1.27, 3.76	< 0.0001[1]
Taixing	0.66	0.56	0.33, 0.93	0.0069[1]
Yangcheng	-0.55	0.17		0.3177
Yanting	-0.51	0.17		0.0975
Endoscopy				
Abnormal	1.74	32.51	23.96, 44.09	< 0.0001[1]
Normal	0.00	1.00		
Total number of endoscopies performed				
< 5000	-0.47	0.45	0.22, 0.91	0.0110[1]
5000-10000	0.00	1.00		
> 10000	0.13	0.81		0.3014

[1]Statistically significant ($P < 0.05$).

33991 patients with normal-appearing mucosa only identified 94 additional pathologically positive cases (0.28%). Thus, following the original guidelines unnecessarily exposed 33897 (90.6% of the total population) participants to the risks of biopsy, compared with taking biopsies only from those with visibly abnormal mucosa. Furthermore, a diagnostic biopsy for high risk areas of upper gastrointestinal carcinomas (UGIC) typically costs 100 Yuan (approximately $16.0) before taking into account medical resources; therefore the cost of 33897 excess biopsies is considerable.

Although 31.33% (94/300) of the patients with pathologically positive biopsies in this study were identified *via* routine biopsy of the high incidence spot of the EGJ, this finding should be interpreted carefully. Results from several prospective studies have suggested that precancerous lesions, such as HIN were reversible[18,19]. Therefore, the actual number of patients who may have developed clinically significant junctional cancers is likely to be less than 94, this regression of premalignant lesions may be attributed to treatment such as *Helicobacter pylori* (*H. pylori*) eradication given the positive association between EGJ cancers and *H. pylori* infection in the Chinese population[20,21]. Further research is needed to elucidate the mechanisms of this regression using a globally consistent diagnostic criterion. In lieu of performing biopsies in participants with normal-appearing mucosa as previously recommended, a more plausible approach for identifying the additional cases missed by traditional endoscopy would be the development of alternative screening strategies to detect patients at risk of EGJ cancers. Ideally, endoscopy should be performed in individuals found to be positive in a filter selection test.

Given the strength of evidence from our study, the Chinese Expert Committee of Early Detection and Early Treatment of Cancer accepted our unpublished findings and promptly revised the recommendation for routine biopsy at the high incidence spot in 2011. At this time, patients undergoing endoscopic screening for EGJ cancers have biopsies taken only in the setting of visible mucosal lesions[22]. In order to reduce the percentages of missed diagnoses, an additional retroflex view of EGJ mucosa is now recommended when screening for junctional cancers at endoscopy. Following the straight view examination, the additional retroflexion may increase the chances of doctors detecting visible mucosal lesions, especially when the endoscopists are less skilled or experienced. The procedure is easily performed without causing additional complications and is well tolerated by patients. Based on limited feedback from doctors performing endoscopy according to the revised guidelines, the EGJ is easier to identify and mucosal abnormalities of the EGJ are clearer and more apparent when utilizing the retroflex maneuver. In fact, since adopting the revised guidelines, no obvious decrease in the detection rate has been reported by endoscopists from these high-risk regions. Further studies are needed to provide more population-level evidence on the revised guidelines for screening EGJ cancers.

The eight study sites chosen were formerly known as high-risk areas of esophageal squamous cell carcinoma (OSCC), but recent studies have found that EGJ cancers and distal gastric carcinomas are increasingly prevalent and the clustering of UGIC was observed in all of these regions[6]. For instance, Cancer registry data from 2009 showed that the age-standardized incidence of gastric cancer and esophageal cancer was 198.55 and 111.99 per 100000 for men and 69.50 and 53.61 per 100000 for women, respectively, in Shexian[23]. Cancers of the EGJ are still classified as gastric cardia cancer (GCC) in the cancer registry and accounted for over 50% of new cases of gastric cancer in four high-risk areas of UGIC, namely Cixian, Shexian, Linzhou and Feicheng, from 2006 to 2008[24]. Our results showed that 0.802% of the target population had HIN or higher-grade lesions, which is consistent with previous reports regarding high EGJ cancer prevalence among these high-risk areas.

Despite the consistent findings of a remarkably high and rapidly rising incidence of junctional cancers, the etiology of cancer at this site varies geographically. In most Western countries, junctional cancers are strongly associated with esophagogastric reflux and intestinal metaplasia[25-27]. Nevertheless, other studies, mainly from Eastern countries, demonstrated a significant association between EGJ cancers and *H. pylori*-induced atrophic gastritis, which is more similar to distal gastric cancer[20,21,28-30]. Thus, some gastroenterologists postulated that cancers of the esophagus-gastric junction may have two distinct etiologies, with some resembling esophageal adenocarcinomas and others resembling non-cardia gastric cancers[31-33]. Different classification systems for junctional tumors may contribute to these different findings, however, whether

adenocarcinomas of the EGJ have heterogeneous etiologies, although of great academic and surgical importance, is not the main concern of this article. In this study, we are interested in the rationality of guidelines for cancer screening programs carried out in these high-risk regions for UGIC. Currently, there are two screening schemes for EGJ cancers: one is combined with esophageal cancer screening *via* endoscopic biopsy examinations with Lugol' s iodine staining; the other is combined with gastric cancer screening using the serum pepsinogen test and endoscopy. Regardless of the clustering of UGIC and similar endoscopy methods adopted in screening, no program or study has focused on integrating screening for esophageal, junctional and gastric cancers together in one examination. In our study population, for example, the number of missed diagnoses may have been reduced if a serum pepsinogen test and/or questions regarding family history of UGIC and gastrointestinal symptoms had been used as a primary screening method before endoscopy.

Observations of an asymmetrical circumferential distribution of lesions in the gastrointestinal tract are not uncommon. For instance, the uneven distribution of benign gastric ulcers, primarily found on the lesser curvature or the anterior and posterior walls close to the lesser curvature, has offered crucial information to distinguish benign and malignant gastric ulcerative lesions. When it comes to the circumferential asymmetry in the formation of pathological lesions at the EGJ, the mechanism is largely unknown. One possible explanation is that the pressure on the left posterior side of the lower esophageal sphincter (LES), an important antireflux barrier, is higher compared to the right anterior side, which may also be the reason why reflux esophagitis, Barrett' s esophagus and Barrett's esophagus-related cancers are more frequently found on the right anterior wall[12]. However, the prevalence of esophagogastric reflux disease, Barrett's esophagus and esophageal adenocarcinoma are relatively low in East Asia compared to Western countries[34,35]. EGJ cancers are significantly associated with *H. pylori*-induced atrophic gastritis in Chinese patients[20,21]. Whether the asymmetrical circumferential pressure of the LES could also play a significant role in the development of EGJ lesions in Asian high-risk populations requires further investigation. The main concern in this study is whether identification of the asymmetrical circumferential distribution of lesions at the EGJ or the so-called high incidence spot could be used in the design of screening guidelines for this cancer in high-risk populations. Our study assessed the effect of additional routine biopsies from this specific site at the EGJ and found that routine biopsies can improve the detection rate. However, biopsies from 125 (37396/300) subjects are needed before identifying one positive case based on our study, while biopsies from 17 (3405/206) subjects are needed to identify one positive case if biopsies are only taken from areas with mucosal abnormalities at the EGJ. These findings provide crucial information for other countries with a high incidence of EGJ cancers, especially when national health authorities attempt to adopt endoscopy as a primary detection procedure in mass screening programs, such as in Iran, Japan or South Africa. Instead of trying to identify all potential patients, training enough qualified endoscopists as well as pathologists for understaffed local hospitals may be a more cost-effective and wise option.

Certain weaknesses in our study merit further discussion. First, this was a cross-sectional study conducted in eight high-risk regions, however, the number of villages selected varied among the counties. In Linzhou, one of the most well-established cancer prevention centers, five villages were selected and enrolled the largest proportion of total participants (18.08%) due to their very experienced screening team with sufficient personnel and working capacity. Whereas, in Feicheng two villages were selected and only made up 8.00% of total subjects due to limited study resources. This uneven sampling could have resulted in heterogeneity within our study population. However, the purpose of this study was not to compare frequency distributions by counties, and the descriptive analysis of demographic characteristics showed similar mean ages and sex ratios (Table 1). Second, biopsies obtained from subjects with normal-appearing mucosa were all targeted at the high incidence spot of EGJ cancers, whereas biopsies from participants with mucosal abnormalities were only taken from visible lesions regardless of the anatomic sites at the EGJ. If all subjects had been biopsied from the high incidence spot of junctional cancers, we would have had more information regarding the prevalence of lesions at this site. Nevertheless, the aim of our study was not to investigate the prevalence of lesions originating from the high incidence spot. Furthermore, it would not have been ethical or efficient to obtain unnecessary biopsies from participants given the primary goal of our study was to reduce the overall risk involved with endoscopic screening for EGJ cancers. Third, the diagnostic criterion for EGJ cancers in this study was tumors with an epicenter lying at the EGJ, or within 1 cm proximally and distally. This diagnostic criterion is largely based on the assumption that endoscopists can identify the EGJ at endoscopy, which is defined as the most proximal extent of the gastric folds. However, this landmark is affected by patients' constantly changing physical conditions such as respiration, gut motor activity, and the degree of distention of the esophagus and stomach[36]. Our diagnostic criteria for EGJ cancers were mainly derived from the previous widely used concept, gastric cardia cancer, and revised according to the Siewert type Ⅱ junctional cancer. However, it is conceptually unclear why the definition of EGJ cancer should be limited to within 1 cm distal and proximal to the EGJ. Despite its shortcomings and given the absence of compelling evidence for a universally accepted definition or diagnostic criteria, we continued to use the current definitions described above.

In summary, this is the first multicenter, population-based study to investigate the role of routine biopsies from normal-appearing mucosa at the high incidence spot of EGJ cancers when screening for junctional adenocarcinomas. Our findings did not support the use of routine biopsies from this specific site to detect HIN or

higher-grade lesions in high-risk populations without visible mucosal lesions. In contrast, visible mucosal abnormalities of the EGJ are strongly associated with positive pathologic diagnoses and it is advisable to consider these pathological manifestations as an indication for biopsies during endoscopic examinations. As a direct result of our findings, the guidelines for EGJ cancer screening in China have already been adapted to reduce unnecessary risks and expenditure. However, further studies are needed to identify less invasive methods for the primary prevention of junctional cancers in order to reduce the overall rates of missed diagnoses. In addition, follow-up studies are necessary to examine the impact of screening guidelines on cancer mortality, the ideal age of screening initiation, the optimal intervals for repeat examinations, and appropriate follow-up for patients with different precancerous lesions.

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COMMENTS

Background

An asymmetrical circumferential distribution of esophagogastric junction (EGJ) cancers has been described in Chinese, Iranian and Japanese patients. This circumferential asymmetry of pathological lesions at the EGJ led to the identification of a high incidence spot of junctional lesions which is at the right anterior side of the EGJ at endoscopy, specifically at the most proximal gastric rugae of the lesser curvature. However, whether identification of the high incidence spot could be used in mass endoscopic screening in high-risk populations to increase the detection rate of high-grade intraepithelial neoplasia or higher grade lesions is largely unknown.

Research frontiers

Observations of an asymmetrical circumferential distribution of lesions in the gastrointestinal tract are not uncommon. However, the mechanism of the circumferential asymmetry in the formation of pathological lesions at the EGJ is largely unknown. One possible explanation is that the pressure on the left posterior side of the lower esophageal sphincter (LES), an important antireflux barrier, is higher compared to the right anterior side, which may also be the reason why reflux esophagitis, Barrett's esophagus and Barrett's esophagus-related cancers are more frequently found on the right anterior wall. However, the prevalence of esophagogastric reflux disease, Barrett's esophagus and esophageal adenocarcinoma are relatively low in East Asia compared to Western countries. EGJ cancers are significantly associated with *Helicobacter pylori*-induced atrophic gastritis in Chinese patients. Whether the asymmetrical circumferential pressure of the LES could also play a significant role in the development of EGJ lesions in Asian high-risk populations requires further investigation.

Innovations and breakthroughs

The aim of the study was to clarify the existing controversy among doctors regarding the use of routine biopsy of the high incidence spot of the EGJ using population-level data. This study determined whether identification of the asymmetrical circumferential distribution of lesions at the EGJ or the so-called high incidence spot could be used in the design of screening guidelines for this cancer in high-risk populations. The study assessed the effect of additional routine biopsies from this specific site at the EGJ and found that routine biopsies can improve the detection rate, but unnecessarily exposed the majority of participants to the risks of biopsy, compared with taking biopsies only from those with visibly abnormal mucosa. When adjusted for potential confounders, a strong association between visible mucosal abnormality and a positive pathologic result was also observed in the multivariate analysis. Compared with participants who showed normal-appearing mucosa at endoscopy, the risk for high-grade intraepithelial neoplasia or higher grade lesions was 32 times higher in patients with visible lesions at the EGJ.

Applications

This study suggests that routine biopsies from the high incidence spot of EGJ cancers are of limited value and are unjustified. Visible mucosal abnormalities of the EGJ are strongly associated with positive pathologic diagnoses, and it is advisable to consider these pathological manifestations as an indication for biopsies during endoscopic examinations. The findings provide crucial information for other countries with a high incidence of EGJ cancers, especially when national health authorities attempt to adopt endoscopy as a primary detection procedure in mass screening programs, such as in Iran, Japan or South Africa. Instead of trying to identify all potential patients, training enough qualified endoscopists as well as pathologists for understaffed local hospitals may be a more cost-effective and wise option.

Terminology

Esophagogastric junction cancers: esophagogastric junction cancers are tumors with an epicenter lying at the esophagogastric junction, or within 1 cm proximally and distally.

Peer review

This is a multicenter study that was aimed to explore whether routine biopsies at the high incidence spot of oesophagogastric junction cancer were justified in endoscopic screening.

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RETROSPECTIVE STUDY

Prognostic analysis and comparison of colon cancer in Han and Hui patients

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Abstract

AIM: To investigate the relevant prognostic factors and their differences between colorectal cancer (CRC) patients of Chinese Han and Hui ethnicities in the Beijing region.

METHODS: A retrospective analysis of 880 patients diagnosed with CRC at Xuanwu Hospital, Capital Medical University between September 2001 and September 2011 was performed. Among the 880 patients, 398 and 482 were Hui and Han, respectively. Characteristics including sex, age, diet, tumor size, primary tumor site, Dukes' stage and degree of differentiation were analyzed for their influence on prognosis. Data on dietary structures were recorded through a questionnaire survey conducted during the patient's first visit, return visit or follow-up checkups.

RESULTS: Among patients with colon cancer, the 5-year survival rate for patients of Hui ethnicity was lower than that for Han patients (P = 0.025). Six risk factors (age of onset, dietary structure, tumor size, Dukes' stage, location of cancer and degree of differentiation) in both Han and Hui patients were identified as prognostic factors (P < 0.05). Multivariate analysis showed that age of onset (P = 0.002), diet (P = 0.000), Dukes' stage (P = 0.000) and degree of differentiation (P = 0.000) are prognostic factors affecting both ethnic groups. Comparison of prognostic factors between Han and Hui patients with CRC showed that dietary structure was a statistically significant factor, and diet varied significantly between the two ethnic groups.

CONCLUSION: Dietary structure has a significant influence on colon cancer prognosis among Han and Hui patients with colon cancer in Beijing, which may cause a difference in their survival rates.

Key words: Colon cancer; Colorectal cancer; Han patients; Hui patients; Prognosis; Multivariate analysis

Core tip: Beijing is one of the cities with a high incidence rate of colorectal cancer (CRC) in China. No prior investigation has been made, based on Han and Hui ethnicities in the Beijing region. Our study compares the clinical features and prognosis of CRC patients of these two ethnicities. The results showed a difference in the 5-year survival rate between the two ethnicities, which may be contributed by various risk factors like age, sex and dietary habits. This is the first retrospective study conducted in Beijing region to investigate the clinical features and prognosis of CRC patients of two major ethnicities, Han and Hui.

Zhang M, Zhao QC, Liu YP, Yang L, Zhu HM, Chhetri JK. Prognostic analysis and comparison of colon cancer in Han and Hui patients. *World J Gastroenterol* 2014; 20(17): 5082-5086 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5082.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5082

INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors of the gastrointestinal tract, representing the third and second most common cancer worldwide and in Western developed countries, respectively[1,2]. In China, the incidence rate of CRC ranks fourth in men and third in women[3]. Beijing is one of the cities with a high incidence of CRC in China, with an upward trend in both incidence rate and mortality rate[4]. Beijing has a population of diverse ethnicities including Han and Hui, which are among the most populous ethnicities. As the only Tier 3 hospital in the district with the biggest Hui community in Beijing, our hospital receives the highest number of Hui patients in Beijing, which facilitates this study on the clinical characteristics of Hui patients. There has been limited research on clinical features of Han and Hui people who exhibit significant differences in aspects such as religious practices, environment, lifestyle and dietary habits. Therefore, we collected clinical information from 398 and 482 CRC patients of Hui and Han ethnicity, respectively, in the Beijing region and performed a 10-year retrospective study of prognostic factors in Hui and Han patients.

MATERIALS AND METHODS

Study group

From September 2001 to September 2011, 398 Hui and 482 Han inpatients who had been histopathologically diagnosed as having CRC at Xuanwu Hospital, Capital Medical University were studied; there were 259 men and 139 women in the Hui group *vs* 302 men and 180 women in the Han group. The median age in the Hui group was 63 years with a range of 30-93 years. The median age in the Han group was 65 years with a range of 25-95 years.

Methods

Medical records were collected, and CRC clinical characteristic forms were formulated so that patients were categorized based on their clinical characteristics, including sex, age of onset, dietary structure, tumor size, Dukes' stage, tumor location and degree of differentiation. Data on dietary structures were recorded through a questionnaire survey conducted during the patient's first visit, return visit or follow-up checkups. The prognostic factors under investigation were categorized as follows: age (three groups: $\leqslant$ 40, 41-60 or > 60 years); dietary structure (four groups: fat > 35% and dietary fiber < 35%, fat > 35% and dietary fiber > 35%, fat < 35% and dietary fiber > 35%, and fat < 35% and dietary fiber < 35%); tumor size (two groups: $\leqslant$ 5 and > 5 cm); tumor location (three groups: rectum, left-sided colon and right-sided colon); and degree of differentiation (three groups: high, medium and low). Follow-up phone calls started from the date of discharge till the date of death due to recurrence or metastasis or June 2012.

Statistical analysis

The Kaplan-Meier statistical method was employed to determine the probability of survival and log-rank test to compare those. Cox regression was used to determine prognosis factors. All calculations were carried out with SPSS ver. 13.0 statistical software. Data were collected into Excel before statistical analysis. Skewed distribution of measurement data was described by M (Q_R). The Kaplan-Meier statistical method was employed to calculate the survival rates which were compared by log-rank test in univariate analysis. The significant variables from univariate analysis were then assessed by stepwise methods in Cox regression which isolated the effects of other variables to determine the independent prognostic factors. $P < 0.05$ was considered to indicate a statistically significant result for all analyses.

RESULTS

Follow-up result and survival rates

One hundred and sixty of the 398 Hui patients (40.2%) and 186 of the 482 Han patients (38.6%) died of CRC recurrence or metastasis. The follow-up rate was 91.7%, with 73 cases lost. The 3-, 5- and 10-year survival rates for Hui patients were 62.1%, 49.7% and 25.2%, respectively, and those for Han patients were 63.2%, 53.3% and 25.9%, respectively. Hui patients showed a significantly lower 5-year survival rate than Han patients ($P = 0.025$).

Univariate analysis of prognostic factors for CRC

For univariate analysis, we identified six risk factors (age of onset, dietary structure, tumor size, Dukes' stage, location of cancer and degree of differentiation) in both Han and Hui patients as prognostic factors ($P < 0.05$) (Tables 1 and 2).

Multivariate analysis of prognostic factors for CRC

Through running the multivariate Cox regression on the six prognostic factors from univariate analysis, we identified age of onset ($P = 0.002$), dietary structure ($P = 0.000$), Dukes' stage ($P = 0.000$) and degree of differentiation ($P = 0.000$) to be independent prognostic factors ($P < 0.05$) for CRC, whereas tumor size ($P = 0.632$) and location of tumor were not independent factors ($P > 0.05$).

Comparison of prognostic factors between Han and Hui patients with CRC

Student's *t* test showed that dietary structure was a statistically significant factor between Han and Hui patients, while other prognostic factors like age of onset, Dukes' stage and degree of differentiation were not.

DISCUSSION

Relationship of age of onset, Dukes' stage and degree of differentiation with CRC prognosis

Despite controversy over whether age could be an inde-

Table 1 Results of univariate analysis of clinical data from Han colorectal cancer patients

Characteristic		*n*	Average survival time	3-yr survival rate	5-yr survival rate	10-yr survival rate	*P* value
Sex	Male	302	72.81 ± 1.96 × 2.45	63.5%	52.2%	23.0%	0.954
	Female	180	74.03 ± 1.96 × 3.08	62.6%	55.0%	30.9%	
Age	≤ 40 yr	18	69.10 ± 1.96 × 8.07	56.8%	46.5%	19.3%	0.000
	41-60 yr	147	86.65 ± 1.96 × 3.30	72.8%	68.1%	33.4%	
	> 60 yr	317	65.77 ± 1.96 × 2.30	59.1%	47.0%	15.4%	
Dietary structure	Fat > 35% and dietary fiber < 35%	106	59.71 ± 1.96 × 6.05	53.7%	39.6%	23.1%	0.000
	Fat > 35% and dietary fiber > 35%	136	63.75 ± 1.96 × 2.24	60.8%	48.2%	24.0%	
	Fat < 35% and dietary fiber > 35%	80	86.79 ± 1.96 × 5.15	80.2%	66.3%	32.2%	
	Fat < 35% and dietary fiber < 35%	160	76.79 ± 1.96 × 5.15	63.7%	54.3%	25.6%	
Tumor size	≤ 5 cm	322	77.24 ± 1.96 × 2.24	66.9%	59.1%	26.9%	0.000
	> 5 cm	160	64.22 ± 1.96 × 3.65	54.8%	38.5%	23.8%	
Dukes' stage	A	82	100.60 ± 1.96 × 3.45	97.0%	90.0%	53.0%	0.000
	B	148	96.63 ± 1.96 × 3.29	87.9%	71.4%	48.9%	
	C	169	70.25 ± 1.96 × 3.37	58.9%	51.8%	25.2%	
	D	83	23.90 ± 1.96 × 0.66	4.2%			
Location of tumor	Rectal cancer	247	77.82 ± 1.96 × 2.69	68.7%	58.4%	23.8%	0.005
	Left-sided colon cancer	98	73.06 ± 1.96 × 4.14	62.5%	57.6%	27.9%	
	Right-sided colon cancer	137	65.77 ± 1.96 × 3.55	54.1%	41.5%	23.4%	
Degree of differentiation	High	83	88.96 ± 1.96 × 4.08	83.5%	69.4%	34.3%	0.000
	Medium	329	78.30 ± 1.96 × 2.42	68.4%	59.5%	28.6%	
	Low	70	35.92 ± 1.96 × 3.11	19.6%	12.8%	6.3%	

pendent risk factor for CRC in previous studies[5,6], this study shows that age of onset is a risk factor for CRC in both ethnic groups through univariate and multivariate analyses. The data show that the survival rate of the young group is on the low side. Possible causes include ignorance of symptoms, late presentation to medical care and misdiagnosis of malignant tumors as benign in the young group of patients. Besides, young patients are often diagnosed with undifferentiated or low differentiated CRC at a relatively late Dukes' stage with a lower chance of eradication by surgery[7,8], which leads to a lower survival rate than the middle age group. This suggests that clinicians should pay greater attention to young patients and change their traditional means of diagnosis in order to enhance the rate of early diagnosis and treatment, improving the prognosis for young CRC patients. On the other hand, the survival rate for the elderly group was also lower than that for the middle age group due to older age, lower tolerance of surgery and chemotherapy, and higher rates of complications and postoperative complications. This study also identifies Dukes' stage as an important prognostic factor for CRC in the two ethnic groups. The survival rate drops as Dukes' stage goes up, consistent with most other previous studies on Dukes' stage in CRC patients[9,10]. For each degree that Dukes' stage increases, the mortality rate rises by 2.92-fold. Therefore, Dukes' stage should be determined as early as possible in order to decide if early surgical treatment is given, because the prognosis improves as the opportunity of radical excision increases. However, the chance of undergoing radical excision decreases as Dukes' stage increases. When patients can only receive palliative surgeries or even lose the chance to receive any surgery, their prognosis is poorer. Degree of differentiation is another important prognostic factor for CRC[11,12]. Some studies show a high correlation between degree of differentiation in CRC and postoperative survival. The survival rate is lower for cancer of low differentiation compared to that for cancer of medium and high differentiation[13]. The lower the degree of differentiation, the higher the malignancy and probability of metastasis. The low degree of differentiation also lowers the opportunity for radical excision and leads to poorer prognosis.

Influence of dietary structure on prognosis of CRC in the two ethnic groups

Hui people practice the Islamic religion, one of the three major world religions along with Buddhism and Christianity. As of the end of 2009, the population of Muslims, the adherents of Islam, is 1.57 billion, accounting for 23% of the world's population, which are distributed across 204 countries[14]. Thus, research on the clinical features for this group is not without its significance. CRC results from polygenic alterations of colonic epithelium caused by multiple factors such as genetic and environmental causes. Much epidemiological research on CRC shows that it can be caused by economic development, changes in lifestyle, especially dietary structure, and other factors including environmental and hereditary factors[15,16]. The present study indicated that the 5-year survival rate of CRC patients from the Hui group was significantly lower than that of patients from the Han group, suggesting the important role that dietary structure played in determining the difference in survival between the two ethnic groups[17]. Based on survey results, the Hui people were found to have higher consumption of beef and lamb while abstaining from consumption of pork. Their daily fat intake is higher and dietary intake in each meal is lower than the Han people. Despite the fact that Hans consume a diverse range of meat types, including pork, chicken, beef and lamb, their daily intake of meat is lower while their intake of vegetable, fruits and grains is

Table 2 Results of univariate analysis of clinical data from Hui colorectal cancer patients

Characteristic		*n*	Average survival time	3-yr survival rate	5-yr survival rate	10-yr survival rate	*P* value
Sex	Male	259	69.70 ± 1.83 × 2.14	62.9%	48.5%	24.2%	0.836
	Female	139	71.22 ± 1.83 × 3.25	61.8%	51.2%	30.1%	
Age	≤ 40 yr	16	67.28 ± 1.83 × 7.17	55.6%	43.4%	18.9%	0.000
	41-60 yr	127	82.04 ± 1.83 × 4.45	70.2%	64.6%	34.0%	
	> 60 yr	255	66.36 ± 1.83 × 3.50	58.4%	46.8%	16.2%	
Dietary structure	Fat > 35% and dietary fiber < 35%	159	58.23 ± 1.83 × 7.12	50.6%	38.9%	22.4%	0.000
	Fat > 35% and dietary fiber > 35%	104	62.05 ± 1.83 × 3.04	59.7%	45.2%	24.9%	
	Fat < 35% and dietary fiber > 35%	51	87.66 ± 1.83 × 4.97	79.5%	65.6%	33.0%	
	Fat < 35% and dietary fiber < 35%	84	73.98 ± 1.83 × 5.77	64.2%	53.5%	25.1%	
Tumor size	≤ 5 cm	266	78.13 ± 1.83 × 2.88	67.1%	58.0%	25.8%	0.000
	> 5 cm	132	62.74 ± 1.83 × 2.96	55.3%	37.2%	22.7%	
Dukes' stage	A	69	99.96 ± 1.83 × 4.36	95.6%	86.7%	50.3%	0.000
	B	126	96.63 ± 1.83 × 3.29	86.1%	64.4%	44.1%	
	C	130	70.25 ± 1.83 × 3.37	56.3%	51.8%	23.6%	
	D	73	23.90 ± 1.83 × 0.66	4.0%			
Tumor location	Rectal cancer	199	76.29 ± 1.83 × 2.33	68.2%	56.1%	22.3%	0.015
	Left-sided colon cancer	80	72.13 ± 1.83 × 4.65	61.2%	56.4%	26.9%	
	Right-sided colon cancer	119	63.55 ± 1.83 × 3.26	53.6%	40.3%	23.6%	
Degree of differentiation	High	66	89.04 ± 1.83 × 5.07	82.4%	68.4%	32.8%	0.000
	Medium	273	76.25 ± 1.83 × 2.02	67.8%	57.9%	27.0%	
	Low	59	33.28 ± 1.83 × 2.96	20.3%	13.1%	5.1%	

higher. Americans, who have a high CRC incidence rate, consume fat, which is made up of primarily saturated fat, as 41.8% of their daily caloric intake[18-20]. Japanese who have low incidence of CRC, consume fat, which is mainly unsaturated fat, as 12.2% of daily caloric intake. Some animal studies show that high consumption of fat can increase CRC incidence, induce earlier tumor formation, exacerbate tumor malignancy, increase the rate of metastasis and shorten survival time in experimental animals. A high-fat diet can increase the incidence rate of CRC[21-23], possibly through the following mechanisms: (1) changing the concentration of cholic acid in feces; (2) increasing intestinal bacterial enzyme activities and promoting the formation of carcinogens; and (3) incorporating a higher intake of meat which produces carcinogenic heterocyclic amine in the frying or grilling process. A high-fiber diet is negatively correlated to, and has significant dose-response relationship with, the risk of morbidity in CRC[24-26]. It possibly works through the following mechanisms: (1) increasing the bulk of the feces, diluting carcinogens, shortening the passage time through the intestinal tract, reducing the contact between colonic mucosa and the carcinogen in the feces, and thereby reducing the risk of CRC; (2) regulation of lipid metabolism by lowering the concentration of deoxy bile acid in the colon through control of reabsorption, dilution and effects of absorption and chelation; (3) changing colonic flora which influences the structure and function of colonic mucosa and colonic epithelial growth and regulation of pH of the intestinal tract; and (4) alleviation of the damage to colonic epithelium by toxic agents through strengthening protective barrier function of the colonic mucosa. Hence, we recommend a low-fat and high-fiber diet for prevention of CRC and improvement of prognosis.

In summary, this study concludes that age of onset, dietary structure, Dukes' stage and degree of differentiation are the common prognostic factors for CRC in both Han and Hui patients. There are significant differences in dietary structure between the two ethnic groups, which thereby cause a difference in their survival rates. The above prognostic factors for CRC strongly emphasize the importance of early diagnosis and treatment and suggest reasonable and healthy dietary habits so as to improve the prevention and prognosis of CRC.

COMMENTS

Background

Colorectal cancer (CRC) is one of the most common malignant tumors of the gastrointestinal tract worldwide. It is one of the major causes of morbidity and mortality in China, and Beijing is one of the cities with a high incidence rate. Beijing has a population of diverse ethnicities including Han and Hui which are among the most populous ethnicities. Studies have shown the CRC incidence rate vary from different races and ethnic population. There has been limited research on clinical features of Han and Hui people who exhibit significant differences in aspects such as religious practices, environment, lifestyle and dietary habits.

Research frontiers

Various studies have shown the CRC incidence and mortality vary from different races, ethnicities, age and gender. Familial and hereditary factors could be the major risk factors, while environmental factors like nutritional practices, physical activity, obesity, use of alcohol and tobacco could be the other risk factors. No prior research has been conducted regarding the differences in clinical features and prognosis of Han and Hui CRC patients in the Beijing region.

Innovations and breakthroughs

This study investigated the different clinical features and prognosis of Han and Hui CRC patients in the Beijing region. The sample size was relatively large and reliability was strong. The study concluded that age of onset, dietary structure, Dukes' stage and degree of differentiation are the common prognostic factors for both Han and Hui CRC patients. The 5-year survival rate for patients of Hui ethnicity was lower than that for Han patients. Significant dietary differences between the two ethnic groups exist, which may cause a difference in their survival rates.

Applications

This study strongly emphasizes the importance of early diagnosis and treatment in CRC patients and suggests reasonable and healthy dietary habits so as

to improve the prevention and prognosis of CRC. This study can act as a guide for clinical prevention and further research of CRC.

Peer review

In the present manuscript, the authors analyzed the differential factors between colorectal cancer patients of Han and Hui ethnicities in the Beijing region. This is the first retrospective study conducted in Beijing region to investigate the clinical features and prognosis of CRC patients of two major ethnicities, Han and Hui. Overall, the manuscript is very well written.

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CLINICAL TRIALS STUDY

Value of a new stick-type rapid urine test for the diagnosis of *Helicobacter pylori* infection in the Vietnamese population

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Abstract

AIM: To assess the value of a new test for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection, Rapirun® *H. pylori* Antibody Stick (Rapirun® Stick), in a Vietnamese population.

METHODS: Eligible patients without previous history of *H. pylori* eradication were recruited. Rapid urease test (RUT) and histologic examination were used to diagnose the *H. pylori* infection. Patients were considered *H. pylori* positive when the RUT results were positive and/or the bacteria were detected histologically. Rapirun® Stick tests were performed using urine samples, and the results were compared with the other 2 methods.

RESULTS: We enrolled 200 patients with a mean age of 36 (range, 18-76) years. There were 116 females and 84 males. Of the 200 patients, 111 (55.5%) were diagnosed as being *H. pylori* positive. The sensitivity, specificity, and accuracy of the Stick test were 84.7%, 89.9%, and 87.0%, respectively. There were 17 (8.5%) false-negative patients and 9 (4.5%) false-positive patients.

CONCLUSION: The Rapirun® Stick test has high sensitivity, specificity, and accuracy for the diagnosis of *H. pylori* infection in the Vietnamese population. The test can be clinically applied in Vietnamese populations.

Key words: *Helicobacter pylori*; Urine test; Rapirun® Stick; Vietnamese; Rapid urease test

Core tip: The Rapirun® *Helicobacter pylori* (*H. pylori*) Antibody Stick (Rapirun® Stick) has recently been developed to detect anti-*H. pylori* antibody in urine. This test requires fewer processing steps and provides quicker results. This study attempted to assess the value of this new test for the diagnosis of *H. pylori* infection in a Vietnamese population. The sensitivity, specificity, and accuracy of the Stick test were 84.7%, 89.9%, and 87.0%, respectively. The Rapirun® Stick test has high sensitivity, specificity, and accuracy for the diagnosis of *H. pylori* infection in the Vietnamese population. The test can be clinically applied in Vietnamese populations.

Quach DT, Hiyama T, Shimamoto F, Le QD, Ho LX, Vu NH, Yoshihara M, Uemura N. Value of a new stick-type rapid urine test for the diagnosis of *Helicobacter pylori* infection in the Vietnamese population. *World J Gastroenterol* 2014; 20(17): 5087-5091 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5087.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5087

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection plays an important role in the pathogenesis of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma[1]. Recent studies have demonstrated that a strategy to test and treat *H. pylori* in uninvestigated, dyspeptic patients in primary care is safe and reduces the need for endoscopy[2,3]. In addition, the indications to test and eradicate *H. pylori* have expanded even to subjects who do not have upper gastrointestinal symptoms, including first-class relatives of patients with gastric cancer and patients requiring long-term therapy with aspirin or non-steroidal anti-inflammatory drugs[4]. Therefore, there is an increasing need for non-invasive methods to diagnose *H. pylori* infection.

Several methods to diagnose *H. pylori* infection have been developed, among which the urea breath test (UBT) is currently regarded as the most accurate assay. However, the UBT is still expensive and not widely available in many countries, including Vietnam. An ideal non-invasive diagnostic test should be simple, inexpensive, rapid, and processed without special equipment and expertise but which delivers acceptably accuracy. A rapid urine test based on enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of anti-*H. pylori* antibody in urine. One of these urine-based ELISA kits, the Rapirun® *H. pylori* Antibody Detection Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), has been reported to have high sensitivity and specificity in several trials in different geographic areas, including Vietnam[5-11]. Recently, a new stick-type rapid urine test, Rapirun® *H. pylori* Antibody Stick (Rapirun® Stick) (Otsuka Pharmaceutical Co., Ltd.), has been developed that requires less labour and which provides results more rapidly than the conventional Rapirun® kit. It takes 15 min to evaluate the result with the Rapirun® Stick, whereas 20 min is required for the conventional Rapirun® kit. This method was reported to have an agreement rate of 98.4% compared with the conventional method in a Japanese population[12]. However, it has not been evaluated in other populations. This study therefore aimed to assess the value of the Rapirun® Stick test for the diagnosis of *H. pylori* infection in a Vietnamese population.

MATERIALS AND METHODS

Patient population

From October 2012 to December 2012, patients undergoing upper gastrointestinal endoscopy at the Department of Endoscopy, University Medical Center in Ho Chi Minh, Vietnam, were recruited. Exclusion criteria for the patients included those with a past history of *H. pylori* eradication therapy or previous gastric surgery and patients taking any type of antibiotics, H_2-receptor blockers, bismuth or proton pump inhibitors in the last 4 weeks before endoscopy. Informed written consent was obtained from all patients participating in the trial. This study was approved by the local ethics committee.

Gastric biopsies

During upper gastrointestinal endoscopy, endoscopic lesions were recorded. Three biopsies were taken from each patient: 2 for histologic examination and 1 for rapid urease test (RUT). The 2 biopsies for histological examination were taken from the greater curvature, one in the antrum and the other in the corpus, and were sent for Haematoxylin and Eosin and Giemsa staining. Tissue specimens were examined by an experienced pathologist (FS) who was blind to all clinical information.

The biopsy for RUT was taken from the greater curvature of the corpus, about 2 cm above the atrophic border. This biopsy location has been reported to optimise the sensitivity of the RUT to detect *H. pylori* in a Vietnamese population[13]. PyloriTek® (Serim Research Co., Elkhart, IN, United States) was used and the colour change was read within 1 h after incubation. This RUT has been validated in several previous studies and has shown very high sensitivity and specificity (90%-98.5% and 97%-100%, respectively)[14-17].

Rapirun® *H. pylori* Antibody Stick test

After endoscopy, urine samples were collected and were processed within 1 h of collection for the detection of antibodies against *H. pylori* using the Rapirun® Stick. The test measures human immunoglobulin G (IgG) antibodies against *H. pylori* in urine using the principle of immunochromatography.

The antigen used is a crude extract from a clinically isolated *H. pylori* strain, the OHPC-040 strain, taken from a Japanese patient with chronic gastritis. A previous report demonstrated that OHPC-040 was the most suitable isolate to detect urinary antibodies to *H. pylori* among 20 clinical isolates extracted from patients with disorders of the upper digestive tract and that OHPC-040 was positive for the *vacA*, *ureB*, and *cagA* genes based on the results of DNA analysis[18]. The test stick contains colloidal gold-conjugated anti-human IgG (Fc) polyclonal antibody (goat). The test line and control line in the evaluation section of the stick are immobilised with *H. pylori* antigen and anti-human IgG polyclonal antibody, respectively.

The Rapirun® Stick test procedure consisted of 2 steps: (1) taking approximately 0.3 mL of the urine sample, as indicated by the measurement guide on the pipette included in the kit, then adding it to a container holding the sample diluent (0.3 mL) and mixing them (an approximately 2-fold dilution); and (2) standing a test stick in the container that holds the mixture of urine and diluent (as described above) with the sample absorption section of the test stick submerged in the diluted sample. After leaving the kit undisturbed for 15 min at room temperature (25 ℃-30 ℃), we then confirmed visually whether any red lines appeared in the evaluation section. The appearance of 2 distinct red bands (one control and one test line) indicates a positive test (Figure 1). The appearance of the control line only indicates a negative result. The absence of a control line indicates an invalid result.

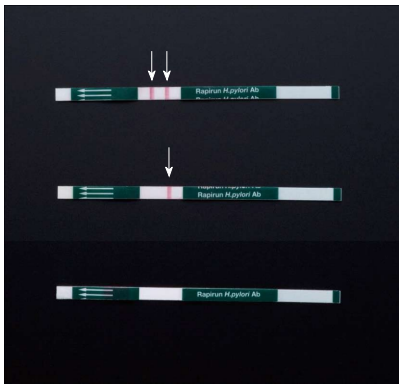

Figure 1 Rapirun® *Helicobacter pylori* Antibody Stick. The sample is considered positive when 2 red bands at the test line and control line (arrows) are observed 15 min later and is considered negative when only the control line is observed. The absence of a control line indicates an invalid result.

Table 1 Demographic characteristics and clinical diagnostics of the patients evaluated

Characteristics	*H. pylori* positive (n = 111)	*H. pylori* negative (n = 89)	Total (n = 200)
Mean age (range) (yr)	35.5 (18-62)	36.6 (18-76)	36 (18-76)
Sex (female/male)	59/52	57/32	116/84
Diagnosis			
Normal gastroduodenal tract	1	6	7
Gastritis and/or duodenitis	83	66	149
Gastric ulcer	4	1	5
Duodenal ulcer	16	1	17
Reflux esophagitis	5	15	20
Reflux esophagitis and peptic ulcer disease	2	0	2

H. pylori: *Helicobacter pylori*.

Definition of H. pylori infection

The results of the RUT and Rapirun® Stick test were read by different researchers who were not aware of the results of the other methods used to diagnose *H. pylori* infection. The definition of *H. pylori* infection in this study required at least one positive test of 2 tests, the RUT and histologic examination. Absence of *H. pylori* infection required both of these tests to be negative. Equivocal tests were excluded from the analysis.

Statistical analysis

Analysis to determine the sensitivity, specificity, positive and negative predictive values, and accuracy of the Rapirun® Stick test was performed with SPSS software for Windows, version 20 (SPSS Inc., Chicago, IL, United States).

RESULTS

We recruited 200 patients in this study. The quality of gastric biopsies for histologic examination to detect *H. pylori* was excellent in all patients. There were no invalid results with the Rapirun® Stick; therefore, we included data from all 200 patients in the analysis.

Table 2 Diagnostic accuracy of Rapirun® Stick test

Rapirun® Stick test	*H. pylori* infection status	
	Positive	Negative
Positive (103)	94	9
Negative (97)	17	80
Total	111	89

Sensitivity, 84.7% (94/111); specificity, 89.9% (80/89); positive predictive value, 91.2% (94/103); negative predictive value, 82.5% (80/97), and accuracy, 87.0% [(94 + 80)/(111 + 89)]. *H. pylori*: *Helicobacter pylori*.

All patients were ethnic Vietnamese. The demographic characteristics of the patients are indicated in Table 1. The mean age of the patients was 36 (range, 18-76) years. There were 116 (58.0%) females and 84 (42.0%) males.

Of the 200 patients, 111 (55.5%) were diagnosed as being *H. pylori* positive: among them, 16 (14.4%) had duodenal ulcer, 5 (4.5%) had reflux esophagitis, 4 (3.6%) had gastric ulcer, and 2 (1.8%) had both gastro-duodenal ulcer and reflux esophagitis. Eighty-nine (44.5%) patients were *H. pylori* negative: among them, only one (1.1%) had gastric ulcer and one (1.1%) had duodenal ulcer, whereas 15 (16.9%) had reflux esophagitis. Of the 24 patients with gastro-duodenal ulcer, 22 (91.7%) had *H. pylori* infection. However, 7 of 22 (31.8%) patients with reflux esophagitis also had the infection.

The sensitivity, specificity, positive and negative predictive values, and accuracy of the Rapirun® Stick test were 84.7%, 89.9%, 91.2%, 82.5%, and 87.0%, respectively (Table 2). There were 17 (8.5%) false-negative patients including 3 with duodenal ulcer, 1 with reflux esophagitis, and 13 with gastritis/duodenitis. Among them, 14 had both a positive RUT and positive histologic examination, 2 had only a positive histologic result, and 1 had only a positive RUT result. There were 9 (4.5%) false-positive patients including 1 patient with reflux esophagitis and 8 with gastritis/duodenitis.

DISCUSSION

To our knowledge, this study is the first to determine the validity of the Rapirun® Stick in a Vietnamese population. The assay is noninvasive, easy to handle, and the cost of using urine as a sample is low. The test can be clinically applied in populations of developing countries such as Vietnam.

Vietnam is one of the countries with a high prevalence of gastric cancer. The mortality rate for gastric cancer is 18.6/100000 for males and 8.4/100000 for females[19]. Among cancer deaths in Vietnam, gastric cancer is the second leading cause followed by lung cancer for males, and fourth, followed by breast, cervix, uterine, and colorectal cancer, for females during 2006 and 2007[20]. The reason of the high mortality from gastric cancer may mainly be the high prevalence of infection from *H. pylori*, a definite carcinogen of gastric cancer. *H. pylori* infection was detected in 65.6% of the hospital-based population (mean age, 42.5 years)[21].

To reduce the incidence of gastric cancer in Vietnam, a nationwide *H. pylori* eradication treatment may be recommendable because *H. pylori* has been regarded as a definite carcinogen, and several studies have shown that its eradication reduces the incidence of gastric cancer development[22,23].

To carry out *H. pylori* eradication treatment, a simple, low-cost, and accurate method is needed to diagnose the infection. There are various methods to detect the infection so far: RUT, bacteriologic culture, histologic examination, UBT, serum antibody assay, and detection of anti-*H. pylori* antibody in urine and *H. pylori* antigen in stool. RUT, bacteriologic culture, and histologic examination require endoscopic biopsy. UBT is regarded as the most accurate assay; however, it requires special apparatus and is expensive to perform. If sensitive screening for *H. pylori* infection were possible using urine samples, it would not only be more convenient in clinical practice but would also be very useful for mass screening. The Rapirun® Stick, a newly developed detection kit for anti-*H. pylori* antibody in urine, is very simple and requires only 15 min to complete. Furthermore, the test does not require technical expertise, special sample handling, or any additional equipment and thus allows considerable savings of diagnosis-related costs. The kit is a candidate test method that would be applicable for use with the Vietnamese population.

The sensitivity, specificity, and accuracy of the conventional Rapirun® kit in a Vietnamese population were reported to be 79.5%, 90.7%, and 84.5%, respectively[11]. In the present study, the sensitivity, specificity, and accuracy of the new Rapirun® Stick test, were 84.7%, 89.9%, and 87.0%, respectively. The values are relatively better in the present study compared with the study using the conventional Rapirun® kit. This may be due to the difference in the populations tested and the methods used to investigate *H. pylori* infection: bacterial culture, histologic examination, and serum ELISA in the study of the conventional Rapirun® kit, and RUT and histologic examination in the present study. Although the antigen used in the Rapirun® Stick is a crude extract from a clinically isolated *H. pylori* strain taken from a Japanese patient, our study clearly demonstrates the usefulness of the Rapirun® Stick in the Vietnamese population. This is truly the first report on the usefulness of the kit external to the Japanese population.

In the present study, 8.5% were false-negative patients. This may be due to the *H. pylori* polymorphism, the host factors in different geographic areas, and the extremely low level of anti-*H. pylori*-specific IgG in the urine of the patients. In contrast, 4.5% were false-positive patients. Graham *et al*[9] reported that 2 patients who had been treated for *H. pylori* infection more than 32 and 42 mo previously, respectively, had positive Rapirun® test results, suggesting that the urine test results may remain positive for an extended time after successful cure of the infection. *H. pylori* in our false-positive patients might have been eradicated intentionally or unintentionally. The reasons for the incidence of the false-positive and false-negative results should be investigated to improve the sensitivity, specificity, and accuracy of the kit.

Evaluation of the diagnostic performance of the conventional Rapirun® kit in various countries, including Japan, Taiwan, South Korea, Vietnam, United States, and European countries (Austria, France, Germany, and Italy), showed a sensitivity of 77.4%-96.7%, specificity of 83.3%-97.4%, and accuracy of 80.4%-96.1%[11,24]. The present study showed high sensitivity, specificity, and accuracy for the new Rapirun® Stick. In addition, the Rapirun® Stick has been reported to have an agreement rate of 98.4% compared with the conventional Rapirun® kit in a Japanese population[12]. Therefore, the Rapirun® Stick can be applicable in many countries, at least in the above-mentioned countries.

There are several limitations in the present study. First, the patients were enrolled in only one hospital in Ho Chi Minh, in southern Vietnam. There are reports showing differences in the prevalence of gastrointestinal diseases such as peptic ulcer and gastric cancer and of *vacA*-positive *H. pylori* between Hanoi, in northern Vietnam, and Ho Chi Minh[19]. Therefore, the study population may not be representative of the entire Vietnamese population. Second, RUT and histologic examination were used to diagnose the infection in the present study. In several patients, these methods produced false-negative or false-positive results, leading to the possible misdiagnosis of *H. pylori* infection.

In conclusion, we demonstrated the usefulness of the Rapirun® Stick test for the diagnosis of *H. pylori* infection in a Vietnamese population: the sensitivity, specificity, and accuracy of the Rapirun® Stick test were high. The test can be clinically applied in Vietnamese populations.

COMMENTS

Background

The Rapirun® *Helicobacter pylori* (*H. pylori*) Antibody Stick (Rapirun® Stick) has recently been developed to detect anti-*H. pylori* antibody in urine. This test requires fewer processing steps and provides quicker results. This study attempted to assess the value of this new test for the diagnosis of *H. pylori* infection in a Vietnamese population.

Research frontiers

The Rapirun® Stick was reported to have an agreement rate of 98.4% compared with the conventional method in a Japanese population. However, it has not been evaluated in other populations.

Innovations and breakthroughs

This study is the first to determine the validity of the Rapirun® Stick in a Vietnamese population. The assay is noninvasive, easy to handle, and the cost of using urine as a sample is low.

Applications

The Rapirun® Stick can be clinically applied in populations of developing countries such as Vietnam.

Terminology

Rapid urease test is a rapid test for diagnosis of *H. pylori*. The basis of the test is the ability of *H. pylori* to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and carbon dioxide.

Peer review

The authors examined the value of new test for the diagnosis of *H. pylori* infection, Rapirun® Stick, in a Vietnamese population. The Stick test has high sensitivity, specificity, and accuracy for the diagnosis. The results are interesting, and suggest that the test can be clinically applied in Vietnamese populations.

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CLINICAL TRIALS STUDY

Chromoendoscopy of gastric adenoma using an acetic acid indigocarmine mixture

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Abstract

AIM: To investigate the usefulness of chromoendoscopy, using an acetic acid indigocarmine mixture (AIM), for gastric adenoma diagnosed by forceps biopsy.

METHODS: A total of 54 lesions in 45 patients diagnosed as gastric adenoma by forceps biopsy were prospectively enrolled in this study and treated by endoscopic submucosal dissection (ESD) between January 2011 and January 2012. AIM-chromoendoscopy (AIM-CE) was performed followed by ESD. AIM solution was sprinkled and images were recorded every 30 s for 3 min. Clinical characteristics such as tumor size (< 2 cm, ⩾ 2 cm), surface color in white light endoscopy (WLE) (whitish, normochromic or reddish), macroscopic appearance (flat or elevated, depressed), and reddish change in AIM-CE were selected as valuables.

RESULTS: *En bloc* resection was achieved in all 54 cases, with curative resection of fifty two lesions (96.3%). Twenty three lesions (42.6%) were diagnosed as well-differentiated adenocarcinoma and the remaining 31 lesions (57.4%) were gastric adenoma. All adenocarcinoma lesions were well-differentiated tubular adenocarcinomas and were restricted within the mucosal layer. The sensitivity of reddish color change in AIM-CE is significantly higher than that in WLE (*vs* tumor size ⩾ 2 cm, $P = 0.016$, *vs* normochromic or reddish surface color, $P = 0.046$, *vs* depressed macroscopic type, $P = 0.0030$). On the other hand, no significant differences were found in the specificity and accuracy. In univariate analysis, normochromic or reddish surface color in WLE (OR = 3.7, 95%CI: 1.2-12, $P = 0.022$) and reddish change in AIM-CE (OR = 14, 95%CI: 3.8-70, $P < 0.001$) were significantly related to diagnosis of early gastric cancer (EGC). In multivariate analysis, only reddish change in AIM-CE (OR = 11, 95%CI: 2.3-66, $P = 0.0022$) was a significant factor associated with diagnosis of EGC.

CONCLUSION: AIM-CE may have potential for screening EGC in patients initially diagnosed as gastric adenoma by forceps biopsy.

Key words: Acetic acid indigocarmine mixture; Early

gastric cancer; Gastric adenoma; Reddish change; Endoscopic submucosal dissection

Core tip: A novel chromoendoscopy procedure using an acetic acid indigocarmine mixture (AIM) is effective for recognizing the margins of early gastric cancer (EGC). In some cases the color of the EGC area gradually becomes reddish after instillation of the AIM solution. The study shows that the sensitivity of AIM-chromoendoscopy (AIM-CE) in the diagnosis of EGC was significantly higher than that of white light endoscopy, but the specificity and accuracy were not. AIM-CE may have potential for screening EGC in patients initially diagnosed as gastric adenoma by forceps biopsy.

Kono Y, Takenaka R, Kawahara Y, Okada H, Hori K, Kawano S, Yamasaki Y, Takemoto K, Miyake T, Fujiki S, Yamamoto K. Chromoendoscopy of gastric adenoma using an acetic acid indigocarmine mixture. *World J Gastroenterol* 2014; 20(17): 5092-5097 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5092.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5092

INTRODUCTION

Histological diagnosis by forceps biopsy before endoscopic resection (ER), such as endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD), is important to determine whether ER should be performed[1]. However, forceps biopsy specimens represent limited local pathological findings of the whole lesion. In fact, disagreement between the histological results of the forceps biopsy and the pathological results of endoscopically resected specimens has been reported[2-7].

The use of narrow band imaging (NBI) and acetic acid with magnifying endoscopy for the diagnosis of gastric cancer has been reported[8-12],but it is difficult to predict a coexisting gastric cancer component in borderline lesions. We previously reported that a novel chromoendoscopy procedure using an acetic acid indigocarmine mixture (AIM) was effective for recognizing the margins of early gastric cancer (EGC)[13], and that the color of the EGC sometimes turned reddish by degrees after instillation of the AIM solution[14]. It has been reported that the characteristics of gastric adenomas, such as size, red color and depressed type, are significant variables that may be seen by white light endoscopy (WLE)[15]. However, there have been no reports of AIM-chromoendoscopy (AIM-CE) for diagnosing a coexisting gastric cancer component in gastric adenomas.

The aim of this study was to evaluate the usefulness of AIM-CE for lesions initially diagnosed gastric adenoma by forceps biopsy.

MATERIALS AND METHODS

Subjects

This was a prospective study carried out at a single endoscopy unit at the Tsuyama Chuo Hospital**,** following a preliminary study. From January 2011 to January 2012, a total of 54 lesions in 45 patients with an endoscopic diagnosis of gastric adenoma by forceps biopsy were treated by ESD following AIM-CE. All patients were recruited prospectively and each provided written informed consent. The study design was approved by the Tsuyama Chuo Hospital Clinical Ethics Committee on Human Experiments, in accordance with the Helsinki Declaration.

Endoscopic procedures

The lesions were initially observed by WLE, after which 20 mL of 0.4% indigo carmine (IC) solution was sprinkled onto the lesions through the accessory channel of the endoscope. Images from WLE and IC observation were recorded using a digital filing system. After washing away the IC solution, 40 mL AIM solution (0.6% acetic acid with 0.4% IC) was sprinkled onto the lesions and images were recorded again every 30 s for 3 min[14]. Before the lesions were treated, clinical variables such as tumor size (< 2 cm, ⩾ 2 cm), surface color in WLE (whitish, normochromic or reddish), macroscopic appearance (flat or elevated, depressed), and reddish color change in AIM-CE, were evaluated. Images of WLE and AIM-CE were evaluated by two endoscopists (Kono Y and Takenaka R) who were blinded to the final histology.

Endoscopic dissection

ESD was performed using an IT knife 2, needle knife and/or dual knife. The histological diagnosis of biopsy specimens and the curability of ESD were evaluated according to the standards in Japanese Classification of Gastric Cancer[16]. The specimens obtained by forceps biopsy and ESD were reviewed by a single pathologist (Miyake T).

Preliminary evaluation

Before this prospective study, we designed a preliminary retrospective study to estimate the beyond-chance agreement. Twenty lesions in 16 patients, previously diagnosed as gastric adenoma by forceps biopsy, were evaluated between two endoscopists' diagnosis of AIM-CE and the κ-index was calculated. A κ value < 0.4 cases was regarded as representative of poor agreement; 0.41 to 0.60, fair agreement; 0.61 to 0.80, good agreement and > 0.80, excellent agreement.

Statistical analysis

Fisher's exact test or χ^2 test was used for all categorical variables, and the Mann-Whitney *U* test was used for continuous valuables. McNemar's test was used for comparison of diagnostic ability between WLE and AIM-CE. Univariate and multivariate logistic regression analyses were used to determine the significant factors contributing to diagnosis of EGC. Variables found in the univariate analysis to be significantly associated with diagnosis of EGC were included in a multivariable logistic regression analysis. All statistical calculations were carried out using JMP software (for Windows, version 10). *P* values

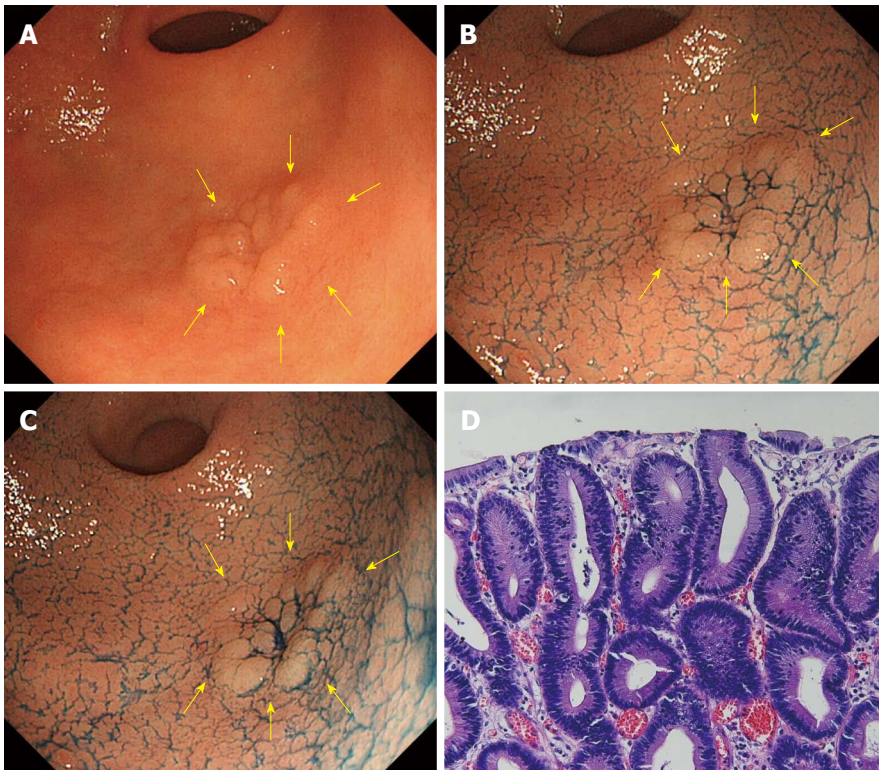

Figure 1 A case of tubular adenoma (no change in acetic acid indigocarmine mixture-chromoendoscopy). A: Whitish superficial elevated lesion is shown at the greater curvature of the antrum in white light endoscopy (indicated by yellow arrows); B: After sprinkling indigo carmine solution; C: 3 min after sprinkling acetic acid indigocarmine mixture (AIM) solution. Compared to B, there was no surface color change (no change in AIM-chromoendoscopy); D: Histology after endoscopic submucosal dissection.

< 0.05 were considered to be statistically significant in all tests.

RESULTS

In AIM-CE, the reddish color change was recorded as positive when the surface color was judged to have turned reddish compared to the surrounding mucosa. Figures 1 and 2 show the typical cases of no change and reddish color change in AIM-CE, respectively.

Clinical parameters in all cases

Table 1 shows the clinical characteristics of the 54 cases. The difference of mean ages between the adenocarcinoma group and the adenoma group was not significant ($P = 0.85$). The gender ratio differed significantly different between the adenocarcinoma group and the adenoma group ($P = 0.047$)

All adenocarcinoma lesions were well-differentiated tubular adenocarcinomas and were restricted within the mucosal layer. The adenocarcinoma lesions were significantly larger in diameter than the adenoma lesions ($Z = 2.3$, $P = 0.019$, Man-Whitney U test). There were no significant differences between the adenocarcinoma group and the adenoma group in tumor location and macroscopic type ($P = 0.33$ and $P = 0.84$, respectively).

Diagnostic ability of WLE and AIM-CE

Before this prospective study, we designed a preliminary pilot study. There was excellent inter-observer agreement on the findings made by AIM-CE (κ index = 0.90).

Table 2 shows the diagnostic ability of WLE and AIM-CE. The sensitivity of reddish color change in AIM-CE is significantly higher than that of WLE (*vs* tumor size $\geqslant$ 2 cm; $P = 0.016$, *vs* normochromic or reddish surface color; $P = 0.046$, depressed macroscopic type; $P = 0.0030$). On the other hand, no significant differences were found in the specificity and accuracy.

In the univariate analysis, normochromic or reddish surface color in WLE (OR = 3.7, 95%CI: 1.2-12, $P = 0.022$) and reddish color change in AIM-CE (OR = 14, 95%CI: 3.8-70, $P < 0.001$) were significantly related to diagnosis of EGC. In the multivariate analysis, only reddish change in AIM-CE (OR = 11, 95%CI: 2.3-66, $P = 0.0022$) is a significant factor associated with diagnosis of EGC (Table 3).

DISCUSSION

Gastric adenomas are reportedly associated with synchronous gastric cancers with varying frequencies, ranging from 8% to 59%[17]. Even using forceps biopsy, a definite diagnosis of cancer may be difficult before endoscopic resection. Discrepancies may exist between forceps biopsy samples and resected specimens[17]. In this study, AIM-CE was found to be useful to predict gastric malignancy in the lesions initially diagnosed as gastric adenoma by forceps biopsy. This provides a treatment strategy for

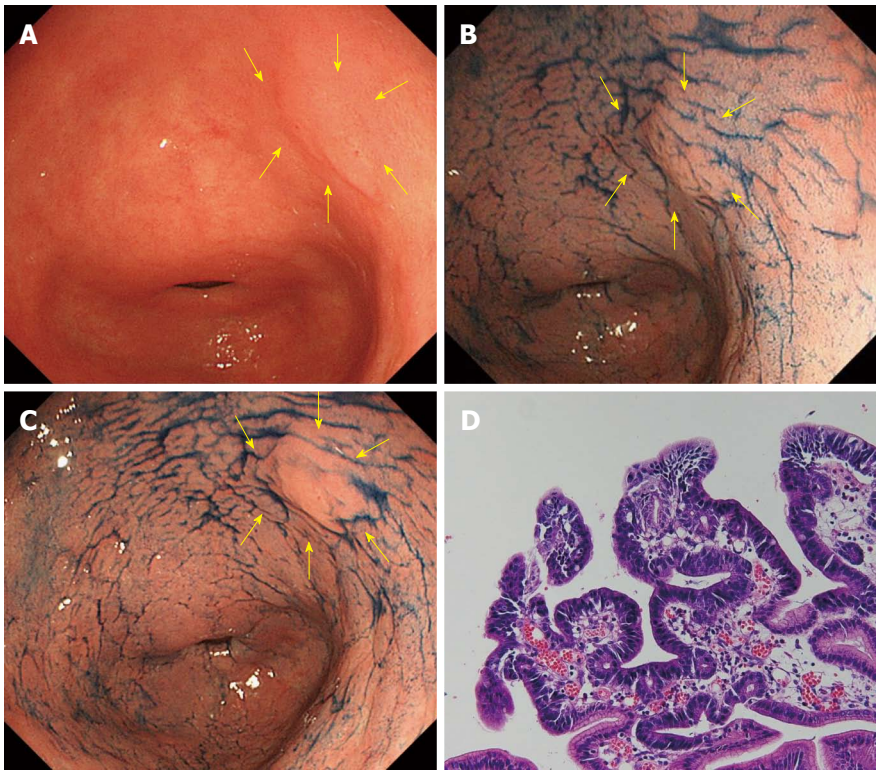

Figure 2 A case of well-differentiated adenocarcinoma (reddish color change in acetic acid indigocarmine mixture-chromoendoscop). A: A normochromic superficial elevated lesion is shown at the lesser curvature of the antrum in white light endoscopy (indicated by yellow arrows); B: After sprinkling indigo carmine solution. The margin of the lesion was not clear; C: 3 min after sprinkling acetic acid indigocarmine mixture (AIM) solution. The margin of the lesion became clear and the surface color turned reddish (reddish change in AIM-chromoendoscopy); D: Histology after endoscopic submucosal dissection.

Table 1 Data from the clinical characteristics of 54 cases *n* (%)

Characteristics	Adenoma (*n* = 31)	Adenocarcinoma (*n* = 23)	*P* value
Age (mean ± SD), yr	71 ± 6.8	71 ± 7.4	0.850
Gender (male/female)	15/16	18/5	0.047
Tumor size (mm, mean ± SD)	12 ± 5.5	19 ± 11	0.019
Location			
Upper third of the stomach	1 (3.2)	3 (13)	0.330
Middle third of the stomach	13 (42)	7 (30)	
Lower third of the stomach	17 (54.8)	13 (57)	
Macroscopic type			
0-Ⅰ	1 (3.2)	1 (4.3)	0.840
0-Ⅱa	25 (80.7)	17 (74)	
0-Ⅱc	5 (16.1)	5 (21.7)	

Table 2 Data from the diagnostic ability of white light endoscopy and acetic acid indigocarmine mixture-chromoendoscopy

Parameters	Sensitivity	Specificity	Accuracy
Tumor size (≥ 2 cm)	47.8%[a]	77.4%	64.8%
Surface color in WLE (normochronic or reddish)	56.5%[a]	74.2%	66.7%
Macroscopic appearance (depressed)	21.7%[b]	83.9%	57.4%
Reddish change in AIM-CE	87.0%	67.7%	75.9%

[a]$P < 0.05$, [b]$P < 0.01$ *vs* Reddish change in AIM-CE using McNemar's test. WLE: White light endoscopy; AIM: Acetic acid indigocarmine mixture; CE: Chromoendoscopy.

such lesions when the surface color of the lesions changes to a reddish color after applying AIM solution.

EMR or ESD allows *en bloc* resection of the entire mucosal lesion[2-7]. However, application of EMR/ESD for all gastric adenoma lesions may excessively increase time and costs. In addition, we should take care of complications such as bleeding and perforation. If we could predict malignant transformation, EMR/ESD could be selectively performed for high-risk gastric adenoma patients. According to the results of several studies, the concordance rates between endoscopic forceps biopsied samples and post-treatment specimens are 65%-90%[18-23]. In this study, lesions were resected in *en bloc* fashion by ESD and, because the sample number was lower and the *en bloc* resection rate was higher, the concordance rate could be lower.

The rate of malignant transformation of gastric adenomas increases with the size of the tumor[17,24,25]. The surface appearance of gastric adenomas also may be an important factor contributing to the diagnosis of carcinoma[17]. In 2005, Kitoh *et al*[26] reported that two endoscopic findings, focal redness and lack of glossiness, were significant factors associated with gastric cancer. In our study, the adenocarcinoma lesions were significantly greater in diameter than the adenomatous lesions and more frequently had a red color in WLE, as described previously. However, these factors did not contribute to diagnosis of EGC in multivariate analysis. Only AIM-CE

Table 3 Data from the logistic regression analysis of factors contributing to diagnosis of early gastric cancer

Parameters	Subgroup	Univariate OR (95%CI)	Multivariate OR (95%CI)
Tumor size	< 2 cm	1.0	1.0
	⩾ 2 cm	3.1 (0.99-11)	1.1 (0.25-5.0)
Surface color in WLE	Whitish	1.0	1.0
	Normochromic or reddish	3.74 (1.2-12)[a]	1.8 (0.44-7.2)
Macroscopic appearance	Flat or elevated	1.0	-
	Depressed	1.4 (0.35-5.9)	-
Reddish change in AIM-CE	No	1.0	1.0
	Yes	14 (3.8-70)[b]	11 (2.3-66)[b]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* Reddish change. OR: Odds ratio; WLE: White light endoscopy; AIM: Acetic acid indigocarmine mixture; CE: Chromoendoscopy.

contributed to predictive diagnosis of borderline lesions, although it was difficult to predict malignant potential from tumor size, surface color, and macroscopic appearance in WLE.

Although the use of NBI and acetic acid with magnifying endoscopy for the diagnosis of gastric cancer is effective[8-12], there are some clinical problems. First, magnifying endoscopy is available only in a limited number of medical institutions. Second, because magnifying endoscopy is used near the lesion, an accidental touch of the mucosa often causes bleeding, making examinations difficult thereafter. We consider that AIM-CE is a safe, easy and cost-effective method for diagnosis of EGC.

It is not fully understood why the cancerous lesions diagnosed by forceps biopsy as borderline malignancy may turn a reddish color after spraying the AIM solution is sprayed. After spraying the acetic acid solution, the duration of whitening differed between the gastric cancer and noncancerous mucosa: aceto-whitening disappeared earlier from the gastric cancer than the noncancerous mucosa. Furthermore, while non-cancerous mucosa whitens when it comes into contact with acetic acid, cancer cells do not, producing a good contrast between a pinkish cancer lesion and the surrounding non-cancerous tissue[27]. We speculate that adding indigocarmine makes this contrast even clearer. However, in this study, a reddish change in AIM-CE was determined by the subjective judgment of two endoscopists. Therefore, we are planning a quantitative analysis of color change in AIM-CE between the adenomas and cancers diagnosed by ESD.

In conclusion, AIM-CE may have potential for screening EGC in patients initially diagnosed as gastric adenoma by forceps biopsy. If the surface color of the lesions changes to reddish after applying the AIM solution, we should consider an endoscopic treatment such as EMR or ESD.

COMMENTS

Background

It is reported that gastric adenomas have a malignant potential, and disagreement between the histological results of the forceps biopsy and the pathological results of endoscopically resected specimens could occur. However, it is difficult to predict a coexisting gastric cancer component before performing endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD).

Research frontiers

In white light endoscopy (WLE), the clinical characteristics of gastric adenomas which are related to malignant potential have been investigated, but there have been no reports of acetic acid indigocarmine mixture-chromoendoscopy (AIM-CE) for diagnosing coexisting gastric cancer components in gastric adenomas. The color of the early gastric cancer (EGC) sometimes changes to reddish by degrees after instillation of the AIM solution. The authors aimed to evaluate the usefulness of AIM-CE for lesions initially diagnosed gastric adenoma by forceps biopsy.

Innovations and breakthroughs

The use of NBI and acetic acid with magnifying endoscopy for the diagnosis of EGC is effective, but magnifying endoscopy is often difficult because it needs an endoscopists' technique. Authors consider that AIM-CE is a safe, easy and cost-effective method for diagnosis of EGC.

Applications

They consider AIM-CE may have potential for screening EGC in patients initially diagnosed as gastric adenoma by forceps biopsy. They recommend EMR or ESD if the color of gastric lesions changes reddish in AIM-CE.

Peer review

For successful treatment (endoscopic mucosal/submucosal resection or laparoscopic gastrectomy) in patients with EGC it is crucial to determinate the lateral extent of the tumor. In this paper the authors evaluated the usefulness of novel chromoendoscopy procedure using AIM-CE for detection of EGC in lesions initially diagnosed as gastric adenoma by forceps biopsy. The procedure was followed by ESD. It was found that the sensitivity of AIM-CE for recognizing the margins of EGC is significantly higher than that of WLE, but there is no difference in specificity and accuracy. The authors conclude that AIM-CE is a safe, easy and cost-effective method for the diagnosis of EGC. The results are interesting and promising for clinical practice as a screening test for EGC.

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CLINICAL TRIALS STUDY

Clinical trial of thalidomide combined with radiotherapy in patients with esophageal cancer

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Abstract

AIM: To investigate the short-term efficacy and tolerability of radiotherapy plus thalidomide in patients with esophageal cancer (EC).

METHODS: Serum samples from 86 EC patients were collected before, during, and after radiotherapy, and the vascular endothelial growth factor (VEGF) level was examined by ELISA. According to the change in serum VEGF level during radiotherapy, the patients were divided into two groups: in the drug group, VEGF level was increased or remained unchanged, and thalidomide was administered up to the end of radiotherapy; in the non-drug group, VEGF level was decreased and radiotherapy was given alone. Thirty healthy volunteers served as controls. The efficacy and safety of radiotherapy plus thalidomide therapy were investigated.

RESULTS: The 86 EC patients had a significantly higher level of VEGF compared with the 30 healthy controls before radiotherapy ($P < 0.01$), and the VEGF level was significantly correlated with primary tumor size, lymph node metastasis, histopathologic type, and clinical stage ($P < 0.01$). Of 83 evaluable cases, VEGF level was significantly decreased after radiotherapy in 32 patients in the drug group ($P < 0.05$), with an effective rate of 71.88%. The incidence of dizziness and/or burnout in the drug group and non-drug group was 62.50% and 15.69%, respectively ($P = 0.000$), and the incidence of somnolence was 12.50% and 0%, respectively ($P = 0.019$). No significant differences were observed.

CONCLUSION: Thalidomide can down-regulate serum VEGF level in EC patients, and combined with radiotherapy may improve treatment outcome. Thalidomide was well tolerated by EC patients.

Key words: Thalidomide; Radiotherapy; Esophageal cancer; Vascular endothelial growth factor

Core tip: Vascular endothelial growth factor (VEGF)-based individualized radiotherapy for esophageal cancer (EC) was achieved in this clinical study. EC patients undergoing radiation treatment may receive different protocols: thalidomide combined with radiation or radiation alone, according to their VEGF level. This study was designed to set appropriate radiotherapy regimens for different patients, improve sensitivity, and decrease resistance in radiation oncology.

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INTRODUCTION

In 1971, Folkman proposed that tumor growth is angiogenesis-dependent, which resulted in a new concept for the control of tumor growth. Previous studies also demonstrated that, of all the angiogenesis-related factors, vascular endothelial growth factor (VEGF) is the most important. Many researchers have reported that VEGF is an independent prognostic factor of esophageal cancer (EC) that plays a decisive role in the recurrence and metastasis of EC[1-4]. Moreover, research has indicated a negative relationship between VEGF expression in tumor tissue and radiosensitivity; tumors with high VEGF expression have poor sensitivity to radiotherapy, and thus predict a poor prognosis[5-8]. Given these findings, radiotherapy combined with an anti-VEGF-mediated anti-angiogenesis protocol is expected to cast new light on EC treatment.

Thalidomide[9-13] was marketed as a non-prescription sedative in Europe in 1956, and was withdrawn from the market in the early 1960s due to strong teratogenic effects. Surprisingly, in the 1990s, thalidomide was reported to be effective in the treatment of AIDS complications, multiple myeloma, and other tumors, which was largely attributable to its anti-neoangiogenetic effect. Therefore, in 1998 thalidomide was again approved by the FDA for the treatment of tumors. Subsequent studies confirmed that thalidomide could inhibit VEGF and basic fibroblast growth factor (bFGF) secretion, and had immunoregulatory, anti-tumor proliferation, and metastasis effects[14-17].

In the present study we used thalidomide to down-regulate VEGF expression in EC patients receiving radiotherapy, with the intention of improving the radiotherapy outcome of EC patients. From July 2009, we divided EC patients receiving radiotherapy into two groups according to the change in serum VEGF levels. Patients with increased or unchanged VEGF levels during radiotherapy were also given thalidomide. The efficacy, side effects (SE), and toxicity of the combination therapy were determined.

MATERIALS AND METHODS

Eligibility and baseline parameters

Between July 2009 and March 2011, 86 patients with pathologically-confirmed EC with no prior treatment were enrolled in this study, including those classified as having early esophageal carcinoma that had rejected surgical therapy for underlying diseases or other personal reasons. The clinical baseline evaluation on admission included general and baseline investigations. General investigations included a complete medical history, physical examination, routine blood examination, blood biochemistry, routine urine examination, routine stool examination, abdominal Doppler ultrasound, and an electrocardiogram (ECG). Baseline investigations included chest computed tomography (CT), upper gastroenterography, and serum VEGF level. The 86 patients included 62 males and 24 females, with a median age of 66.4 years (range, 40-86 years). According to the CT staging strategy for EC proposed by Kienle *et al*[18], 15 cases were at stage Ⅰ, 45 at stage Ⅱ, 9 at stage Ⅲ, and 17 at stage Ⅳ. Histopathologic typing identified squamous cell carcinoma in 81 cases, adenocarcinoma in 3 cases, and small cell carcinoma in 2 cases. X-ray pathologic typing showed marrow type in 76 cases, massive type in 5, ulcer type in 3, and narrow type in 2. The blood specimens from 30 healthy volunteers (including 18 males and 12 females, with a median age of 33.3 years and a range of 26-45 years) were used as controls. An increase or decrease in VEGF of 10% compared with the VEGF level before radiotherapy was deemed clinically significant. The study was approved by the Institutional Review Board (IRB) of the Second People's Hospital of Changzhou. All patients were well-informed of the possible treatment side effects, toxicities, and complications, and informed consent was obtained from each patient.

Radiotherapy

The patients were placed in the supine position, with both hands on their head and fixed using a mold immobilizing technique. Twenty patients received conventional X-ray simulation and three-field radiotherapy; one anterior (width 6 cm) and two posterior (width 4.5-5.5 cm), with both upper and lower margins being 3-5 cm. Forty-seven patients received CT simulation and three-dimensional conformal radiation therapy (3DCRT). The gross target volume (GTV) consisted of thickened esophagus wall according to CT (the results of esophagography and esophagoscopy were also considered) and enlarged lymph nodes with a diameter $\geqslant$ 1 cm. Clinical target volume (CTV) covered the GTV + 2.5-3.0 cm of the craniocaudal margin + 0.5-0.8 cm of the transverse and anteroposterior margin + the corresponding lymphatic drainage region. A 0.5 cm isotropic margin was added to the CTV to make up the planning target volume (PTV). This ensured that the prescribed dose covered 95% of the PTV, with the maximum dose for spinal cord < 45 Gy and for both lung V_{20} < 30%. 1.8-2.0 Gy/fraction, 5 fractions a week, with a total dose of 60-72 Gy, were delivered to all patients by a 6-MV-X-ray linear accelerator.

VEGF determination

Peripheral venous blood samples were obtained within one week before, during (3-4 wk), and within one week after radiotherapy. The samples (2 mL for each) were well mixed and centrifuged (4 ℃, 3000 r/min, radius was 10 cm) for 10 min, and the obtained sera were stored at -70 ℃ until use. Serum VEGF level was determined by enzyme-linked immunosorbent assay (ELISA) (Purchased from 4A Biotech Co., Ltd, Beijing).

Administration of thalidomide

Serum VEGF level was determined in EC patients 3-4 wk after the initiation of radiotherapy, and patients with increased or unchanged VEGF levels compared with levels before radiotherapy were also given thalidomide. In the first week, thalidomide was given at 100 mg/d before

Table 1 Relationship between pre-radiotherapy serum vascular endothelial growth factor level and clinical characteristics of esophageal cancer patients

Variable	Cases	VEGF (ng/L)	t (*F*)	*P* value
Sex			1.11	> 0.05
Male	62	120.78 ± 44.72		
Female	24	135.92 ± 61.00		
Age, yr			0.89	> 0.05
< 55	71	126.96 ± 51.37		
⩾ 55	15	115.73 ± 42.47		
Lesion site			0.40	> 0.05
Cervical and upper thoracic segment	22	131.00 ± 59.42		
Middle thoracic segment	36	119.50 ± 36.04		
Lower thoracic segment	28	127.36 ± 57.71		
Histopathologic type			3.40	< 0.01
Squamous cell carcinoma	81	122.26 ± 45.91		
Adenocarcinoma	3	196.67 ± 115.14		
Small cell carcinoma	2	128.50 ± 19.09		
X-ray pathologic types			0.47	> 0.05
Marrow	76	126.89 ± 52.3		
Massive	5	120.40 ± 14.00		
Ulcer	3	94.67 ± 23.07		
Narrow	2	110.00 ± 31.11		
Primary foci			4.55	< 0.01
T1 + T2	29	99.66 ± 22.64		
T3 + T4	57	137.89 ± 54.95		
Lymph node metastasis			7.50	< 0.01
N0	30	89.80 ± 12.80		
N1-2	56	144.50 ± 51.69		
Distant metastasis			1.02	> 0.05
M0	65	128.15 ± 49.71		
M1	21	115.23 ± 50.36		
Clinical stage			2.52	< 0.01
Ⅰ + Ⅱ	60	115.08 ± 39.76		
Ⅲ + Ⅳ	26	149.40 ± 63.20		

Vascular endothelial growth factor (VEGF) level in 86 esophageal cancer (EC) cases was significantly correlated with primary tumor size, lymph node metastasis, histopathologic type, and clinical stage of EC ($P < 0.01$), but was not correlated with lesion site, distant metastasis, X-ray pathologic type, gender, or age ($P > 0.05$).

sleep; if no side effects were observed, 200 mg/d was started from the next week up to the end of radiotherapy.

Evaluation of short-term effect

Esophageal barium examination was performed in the fourth week and at the end of radiotherapy in all patients. The short-term effect was assessed according to the criteria of the International Union Against Cancer (UICC). Complete response (CR): mass shadow disappeared, mucosa returned to normal or became coarse, barium agent passed smoothly, no or slight rigidity of the esophagus, and no or slight stenosis. Partial response (PR): no obvious distortion or ulceration, tumor volume reduced by more than 50%, barium agent passed fairly smoothly, border not so smooth (with a little filling-defect or crater), or a smooth border, but with obvious stenosis. Minor response (MR): broadened lumen, improved distortion, and ulceration and tumor volume reduced by less than 50%. No change (NC): obvious filling-defect or crater, aggravated stenosis, no noticeable tumor volume reduction or volume increase (by less than 25%). CR and PR were considered to demonstrate effective treatment, while MR and NC denoted ineffective treatment.

Statistical analysis

All data were analyzed by SAS 9.0. Mean values for measurement data were presented as mean ± SD, differences in measurement data and enumeration data were compared using analysis of variance and χ^2 test, respectively. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Relationship between pre-radiotherapy VEGF level and clinical characteristics of EC patients

The mean pre-radiotherapy serum VEGF level in the 86 EC patients was 125.00 ± 49.89 ng/L, which was significantly higher than that in the 30 healthy controls (79.63 ± 39.17 ng/L, $P < 0.01$). The pre-radiotherapy serum VEGF level in the 86 EC patients was significantly correlated with primary tumor size, lymph node metastasis, histopathologic type, and clinical stage, but was not correlated with lesion site, distant metastasis, X-ray pathologic types, gender, or age (Table 1).

Dynamic changes in VEGF level

Serum VEGF levels were determined in all 86 EC patients before radiotherapy. Radiotherapy was discontinued in 3 cases due to intolerable complications. Of the remaining 83 evaluable EC cases, VEGF levels were significantly increased in 32 cases during radiotherapy ($P < 0.01$); these patients were given thalidomide with radiotherapy (drug group), and their VEGF levels were significantly decreased at the end of treatment compared with those during radiotherapy ($P < 0.05$). The other 51 patients, whose VEGF levels were significantly decreased during radiotherapy compared with before radiotherapy ($P < 0.01$), received radiotherapy as initially planned (non-drug group); the VEGF levels in these patients were not significantly different during and after radiotherapy ($P > 0.05$) (Table 2).

Short-term response

The response rate (CR + PR) was 71.88% for 32 patients in the drug group and 78.43% for 51 patients in the non-drug group (Table 3).

Side effects

The incidence of dizziness and/or burnout in the drug and non-drug groups was 62.50% and 15.69%, respectively ($P = 0.000$), and the incidence of somnolence was 12.50% and 0%, respectively ($P = 0.019$). These differences were significant. In the drug and non-drug groups, the incidence of grade Ⅲ-Ⅳ esophagitis was 12.50% and 11.76% ($P = 0.812$), grade Ⅲ-Ⅳ leukocyte decrease was 6.25% and 9.80% ($P = 0.864$), grade Ⅲ-Ⅳ platelet decrease was 3.13% and 5.88% ($P = 0.961$), and grade Ⅲ-Ⅳ

Table 2 Dynamic changes in vascular endothelial growth factor levels in 83 evaluable esophageal cancer patients

Group	*n*	Before radiotherapy	During radiotherapy	After radiotherapy
Drug group	32	98.56 ± 28.74	122.69 ± 43.03[d]	109.53 ± 32.48[b]
Non-drug group	51	141.76 ± 53.78	100.94 ± 22.61[f]	100.31 ± 23.45

[b]$P < 0.01$ *vs* during radiotherapy (drug group); [d]$P < 0.01$ *vs* before radiotherapy (drug group); [f]$P < 0.01$ *vs* before radiotherapy (non-drug group).

nausea and vomiting was 9.38% and 27.45%, respectively ($P = 0.089$). Anaphylaxis was not observed in the two groups ($P = 1.000$) (Table 4). All patients tolerated the side effects, and there were no withdrawals due to them.

DISCUSSION

EC is one of the most common malignant tumors in China, and radiotherapy is currently the main treatment for EC. However, the efficacy of radiotherapy has not improved over the past 30 years, with the 5-year survival rate being 15%-39%[19]. Improvements in treatment efficacy and patient compliance, and a reduction in recurrence, metastasis, and side effects are still the focus of EC studies.

Tumor growth is neo-angiogenesis-dependent, and VEGF has been demonstrated to be an independent prognostic factor for EC, and plays a key role in the recurrence and metastasis of EC. It has been reported that a high expression of VEGF may indicate poor radiosensitivity and prognosis of tumors. Recent research has confirmed that thalidomide can inhibit VEGF secretion, tumor proliferation, and metastasis. In 1999, thalidomide was first reported by Singhal *et al*[14] in the treatment of refractory multiple myeloma, which yielded a clinical remission rate of 32%. Thalidomide was subsequently found to have definite effects on several malignant hematological tumors. Thalidomide, as an angiogenesis inhibitor, has become an important choice in the comprehensive treatment of solid tumors[20-22].

Reports on the relationship between thalidomide and radiosensitivity of esophageal tumors are very rare. Between 2009 and 2010, we explored the relationship between thalidomide and the radiosensitivity of esophageal carcinoma cells, and found that thalidomide enhanced the radiosensitivity of esophageal carcinoma cells both *in vitro* and *in vivo*, probably by down-regulating VEGF expression in esophageal carcinoma cells[23,24]. In addition, our previous clinical research also demonstrated that the response rate to radiotherapy was 61.90% in EC patients, with increased VEGF level during radiotherapy and 90.25% in those with decreased VEGF level, suggesting that patients with increased VEGF were resistant to radiotherapy. Therefore, the dynamic variation in serum VEGF plays a key role in predicting the radiosensitivity of EC patients. In the present study, we found that the

Table 3 Response rate of 83 evaluable esophageal cancer patients *n* (%)

Group	*n*	CR	PR	MR	NC
Drug group	32	10 (31.25)	13 (40.63)	7 (21.87)	2 (6.25)
Non-drug group	51	26 (50.98)	14 (27.45)	7(13.73)	4 (7.84)

CR: Complete response; MR: Minor response; PR: Partial response; NC: No change.

Table 4 Side effects in 83 evaluable esophageal cancer cases

Side effects	Group		χ^2	*P* value
	Drug group	Non-drug group		
Dizziness or burnout	20	8	19.28	0.000
Somnolence	4	0	-	0.019
Grade Ⅲ-Ⅳ esophagitis	4	6	0.06	0.812
Grade Ⅲ-Ⅳ leukocyte decrease	2	5	0.03	0.864
Grade Ⅲ-Ⅳ platelet decrease	1	3	0.02	0.961
Grade Ⅲ-Ⅳ nausea and vomiting	3	14	2.913	0.089
Anaphylaxis	0	0	-	1.000

pre-radiotherapy VEGF level in 86 EC cases was significantly higher than that in the healthy controls, and was correlated with primary tumor size, lymph node metastasis, histopathologic type, and clinical stage of EC, and was not correlated with lesion site, distant metastasis, X-ray pathologic type, gender, or age. These results indicated that high VEGF levels help to maintain hypertonic status, and can increase tumor vessel permeability, thus exerting a significant influence on the invasion and metastasis of EC cells. These findings suggest that VEGF is a key indicator for evaluating biological behavior and predicting the prognosis of EC. In this study, VEGF level was significantly increased in 32 EC patients during radiotherapy, and when thalidomide was given concurrently with radiotherapy, this resulted in a significantly decreased VEGF level at the end of treatment; 32 patients had a response rate (CR + PR) of 71.88%. These results indicate that thalidomide may improve the radiosensitivity of EC patients with high VEGF expression by down-regulating the VEGF level and improving the outcome of radiotherapy.

Teratogenesis is the main side effect of thalidomide, and other frequent side effects include central nervous system symptoms such as dizziness, burnout, and somnolence, followed by peripheral neuropathy; venous thromboembolism (VTE) is the most severe side effect[25]. Neither VTE nor other severe side effects were observed in the 32 EC patients who received thalidomide in this study; only dizziness, burnout and/or somnolence were observed, which were tolerable after expectant treatment. It has been reported that the side effects of thalidomide are dose-related, and 90% of patients could tolerate a dose of 400 mg/d with few severe side effects;

the adverse reactions of thalidomide were alleviated or disappeared in most patients following dose reduction or drug discontinuation[26].

Generally, EC patients have different degrees of psychological disorders, such as depression, anxiety, and dysphoria, resulting in insomnia, decreased quality of life, and in some cases radiotherapy has to be withdrawn. Thalidomide has been shown to have a satisfactory sedative effect, and in this study we noted that EC patients who received thalidomide had a better sleep and diet pattern, and their quality of life and treatment compliance were greatly improved. All patients in the drug group received radiotherapy up to the end of the treatment period without interruption. We also found that thalidomide could achieve a satisfactory sedative effect in advanced EC patients with persistent insomnia. Wijermans *et al*[27] and Tassinari *et al*[28] also demonstrated that thalidomide, as a TNF-α inhibitor, improved the quality of life of EC patients.

Currently available anti-angiogenesis drugs include Avastin and endostatin; however, they are very expensive and most patients cannot afford them, which greatly limits the clinical application of anti-angiogenesis therapy. In comparison, thalidomide has the advantages of low price, oral administration, and fewer side effects, making it an affordable agent for anti-angiogenesis-based target therapy.

These findings indicate that thalidomide has the potential to down-regulate serum VEGF in EC patients during radiotherapy, and the combination of thalidomide and radiotherapy can not only increase the response rate of EC patients to radiotherapy, but also improve their quality of life and treatment compliance. Considering the relatively small sample size of the present study, we plan to conduct a clinical randomized controlled trial with a larger sample size to further investigate the efficacy, side effects, and influence on long-term survival of this combined therapy, with the hope of providing a new treatment strategy for EC patients.

COMMENTS

Background

Angiogenesis is essential for tumor growth, invasion, metastasis, and relapse, and the pro-angiogenic vascular endothelial growth factor (VEGF) is a key factor. Esophageal cancer (EC) tumors which overexpress VEGF may indicate lymph node metastasis and poor prognosis, and VEGF has been shown to be an independent prognostic factor. A VEGF-targeted antiangiogenic agent combined with radiation may be a novel strategy for EC patients.

Research frontiers

VEGF has been demonstrated to be a target for anti-angiogenesis therapy, but few reports on thalidomide as a radiation-sensitizing agent are available, and its influence on VEGF regulation remains undetermined.

Innovations and breakthroughs

In this study, the authors found that the determination of VEGF level in EC patients can indicate whether a patient would benefit from radiation treatment.

Applications

VEGF variation during radiotherapy has significance in predicting prognosis.

Terminology

Thalidomide is a potential radiation-sensitizing agent which may cooperate with radiation to down-regulate VEGF and enhance radiosensitivity in EC patients.

Peer review

This study mainly focused on VEGF change in EC patients during radiation treatment under thalidomide intervention, as well as its influence on the patients' response and tolerance to radiotherapy.

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OBSERVATIONAL STUDY

TNM staging of colorectal cancer should be reconsidered by T stage weighting

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Author contributions: Li J and Ding KF raised doubts about the relationship between the 7th edition of the AJCC TNM staging system and the survival of patients with colorectal cancer, and designed the methods, proposed the revised TNM staging system, and wrote the draft of the manuscript; Guo BC and Sun LR undertook the statistical analysis; Wang JW, Fu XH, Zhang SZ and Poston G discussed the hypothesis that the T stage has greater weight in affecting the survival of colorectal cancer patients and took part in the decision-making process for rearranging the TNM staging system and also participated in the study design and revision of the manuscript; all authors have read, revised, and approved the manuscript.

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Abstract

AIM: To verify that the T stage has greater weight than the N stage in the staging of colorectal cancer.

METHODS: Open data from the Surveillance, Epidemiology, and End Results program were reviewed and analyzed according to the T stage, N stage, and patients' observed survival (OS). The relative weights of the T and N stages were calculated by multiple linear regressions based on their impact on survival. Risk scores for 25 TN categories were then calculated from the T and N stage relative weights, and a rearranged tumor node metastasis (TNM) staging system was proposed *via* a cluster analysis of the TN scores.

RESULTS: Both T and N stages significantly affect the OS of patients with colorectal cancer. Moreover, the T stage has greater weight than the N stage in the TNM staging system of colorectal cancer. For colon cancer, the relative T and N stage weights were 0.58 and 0.42, respectively, and for rectal cancer, the relative T and N stage weights were 0.61 and 0.39, respectively. On the basis of cluster analysis of the TN scores, T1N1a was classified to stage Ⅰ, and T2N1a-1b and T1N1b-2a were classified to stage Ⅱ in our revised TNM staging system for both colon and rectal cancer. For colon cancer, T4bN0 was classified to stage Ⅲa, but for rectal cancer, it was classified to stage Ⅲb.

CONCLUSION: As the T stage affects colorectal cancer survival more significantly than the N stage, the TNM staging should be revised by relative T stage weight.

Key words: Colorectal cancer; Neoplasm staging; Cluster analysis; Survival analysis; Observational study

Core tip: The 7th edition of the American Joint Committee on Cancer (AJCC) tumor node metastasis (TNM) staging system for colorectal cancer can not predict survival linearly by stage. We propose that the T stage has greater weight than the N stage, more especially in rectal cancer than in colon cancer. Moreover, in this article, we propose a revised scheme for the 7th edition of the AJCC TNM staging system. In our revised scheme, T4bN0 is classified to stage Ⅲa in colon cancer, but to

stage ⅢB in rectal cancer. This is the first attempt to revise the established TNM staging system for colorectal cancer by shaking the keystone of present classification based on the lymph nodes status.

Li J, Guo BC, Sun LR, Wang JW, Fu XH, Zhang SZ, Poston G, Ding KF. TNM staging of colorectal cancer should be reconsidered by T stage weighting. *World J Gastroenterol* 2014; 20(17): 5104-5112 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5104.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5104

INTRODUCTION

The American Joint Committee on Cancer (AJCC) tumor node metastasis (TNM) staging system is widely used to predict the prognosis for patients with colorectal cancer and to guide adjuvant therapy after potentially curative surgery. The 7th edition of the AJCC TNM staging system was published in 2010[1]. Patients with colorectal cancer, which directly invades or is adherent to other organs or structures, have poorer prognoses. As a result, stage T4 was stratified to T4a and T4b, and patients with T4bN0 lesions were reclassified from stage ⅡB to ⅡC. Similarly, T1-2N2 was moved from stage ⅢC to ⅢA/ⅢB. These changes reflect the fact that the T stage affects survival in colorectal cancer patients more significantly than previously believed.

The most obvious drawback of the AJCC TNM staging system is that the relative weighting of the N stage is over-estimated. Except for patients in stage Ⅳ, all patients with lymph node involvement are defined as stage Ⅲ. However, data from the Surveillance, Epidemiology, and End Results (SEER) program has shown that the 5-year observed survival (OS) of stage ⅢA patients (T1-2N1 and T1N2a) matches that of stage Ⅰ patients[2,3]. On the other hand, stage ⅡC patients have a poorer prognosis, equivalent to that of stage ⅢB patients (Figure 1). In other words, the 7th edition of the AJCC TNM staging system fails to predict survival linearly by stage. In a study in which survival data for 1165 Japanese colorectal cancer patients were calculated according to 7th edition of the AJCC TNM staging system, it was found that after stage Ⅰ, patients in stage ⅢA unexpectedly showed the best prognosis[4].

As we were concerned that the T stage may have a greater impact on the survival of colorectal cancer patients following potentially curative surgery than that proposed in the updated 7th edition of the AJCC TNM staging system, we analyzed open SEER data further to clarify the impact of different T and N stage weights on survival. In doing so, we propose a revised scheme for the 7th edition of the AJCC TNM staging system.

MATERIALS AND METHODS

Open SEER population-based data from 1992 to 2004 were reviewed[2,3], and data on colon cancer and rectal cancer were analyzed separately. Patients with stage 0 and Ⅳ disease were excluded from the study. 5-year OS rate data were extracted according to 25 combinations of the T stage (1 = T1, 2 = T2, 3 = T3, 4 = T4a, and 5 = T4b) and N stage (0 = N0, 1 = N1a, 2 = N1b, 3 = N2a, and 4 = N2b). Stage N1c (tumor deposit) was also excluded from the study because no data were available.

As T and N stage scores are independent variables, and 5-year OS rates are dependent variables, three-dimensional (3D) scatter plots were constructed to demonstrate the relationships of the T stage, N stage and OS. In addition, multiple linear regressions were calculated to elucidate the quantitative relationships of these parameters. For example:

$OS = (c\text{-}b_1 \times T - b_2 \times N) \times 100\%$

where c is the survival constant, and b_1 and b_2 are the mean coefficients of regression of T and N. According to the coefficients of regression, we get:

$\alpha OS/\alpha T = b_1$, $\alpha OS/\alpha N = b_1$

This means that when the T stage changes by one unit, the relative influence on OS is b_1, and when the N stage changes by one unit, the relative influence on OS is b_2. Therefore, the relative influence on OS of the T stage and N stage is b_1:b_2. Since OS is only influenced by 2 indicators, T and N, according to the relative influence on OS of T and N, we can calculate the normalization weights of the indicators T and N. For example, the weight of T is:

$WT = b_1/(b_1 + b_2) \times 100\%$

and the weight of N is:

$WN = 1 - WT$.

The scores of TN combinations are the scores of each stage multiplied by its weight. For example, TN scores for T4aN0 = WT × 4 + WN × 0, and TN scores for T2N2b = WT × 2 + WN × 4 (WT is the weight of T, and WN is the weight of N). This concept was derived from a comprehensive evaluation of all the available data, which balances the various indicators. Using this method, the scores of 25 TN combinations of T stage and N stage were calculated.

Subsequently, we used cluster analysis (also called group analysis; a statistical analysis method for studying the classification of samples or indicators[5]) of the TN scores to rearrange the TNM staging system.

Statistical analysis

All statistical analyses were performed using SPSS® version 16.0. A P value < 0.05 was considered statistically significant.

RESULTS

Multiple linear regressions of T stage, N stage and OS

Both T and N stages significantly affect the OS of patients with colorectal cancer. The 3D scatter plots of T and N stages and 5-year OS for colon cancer are shown in Figure 2A and B. The multiple regression equation

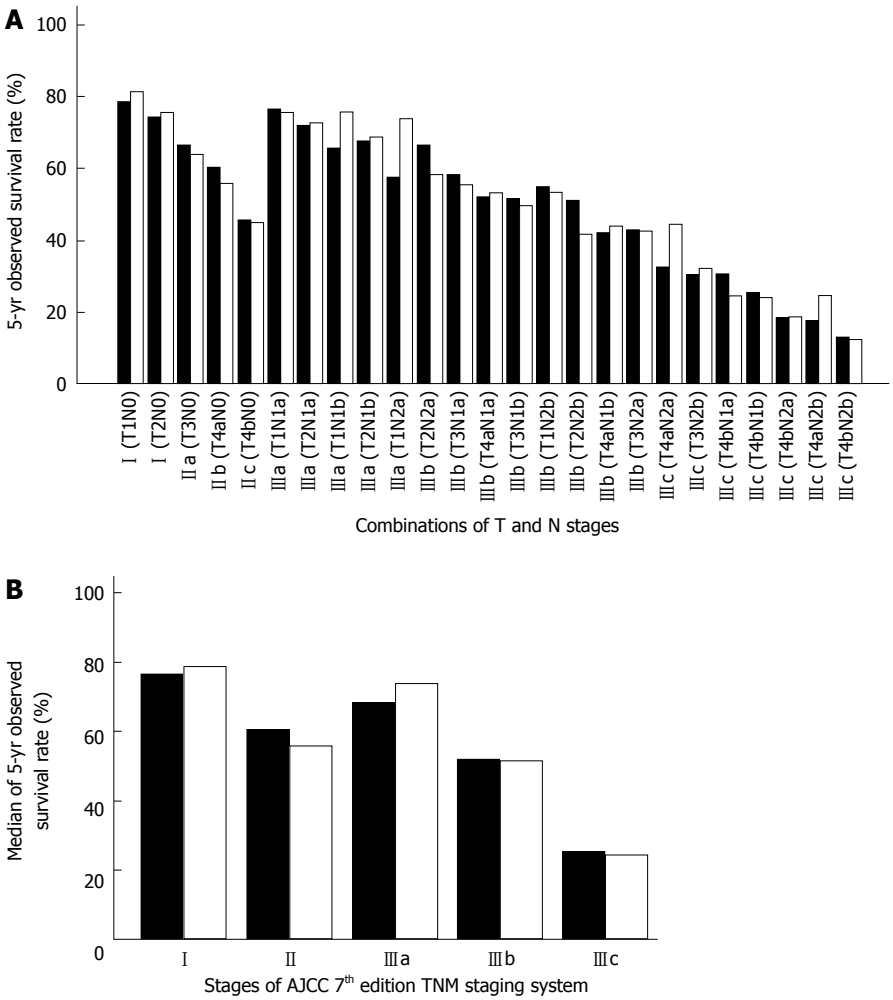

Figure 1 Relationship between survival of colorectal cancer patients and stages of the American Joint Committee on Cancer 7th edition tumor node metastasis staging system. A: The 5-year observed survival rate of colorectal cancer patients according to 25 combinations of T and N stages of the American Joint Committee on Cancer (AJCC) 7th edition tumor node metastasis (TNM) staging system; B: The median 5-year observed survival rate of colorectal cancer patients for overall stages (Ⅰ, Ⅱ, Ⅲa, Ⅲb and Ⅲc) according to the 7th edition of the AJCC TNM staging system. Colon cancer is represented by the solid black grids. Rectal cancer is represented by the white grids (Surveillance, Epidemiology, and End Results open data).

for colon cancer is: OS = (97.432 - 10.56T - 7.812N) × 100%. The relative weight of T = 0.58, and the relative weight of N = 0.42. These calculations indicate that the T stage affects colon cancer survival more significantly than the N stage.

The 3D scatter plots of T and N stages and 5-year OS for rectal cancer are shown in Figure 2C and D. The multiple regression equation for rectal cancer is: OS = (99.108 - 11.356T - 7.194N) × 100%. The relative weight of T = 0.61 and the relative weight of N = 0.39. Thus, the T stage appears to have greater weight in rectal cancer than in colon cancer.

TN scores and cluster analysis

The 25 combinations of T and N stages and the corresponding 5-year OS of colon cancer and rectal cancer are shown in Tables 1 and 2, respectively. According to the TN scores, the TNM staging system can be rearranged to stage Ⅰ (TN score ≤ 1.0), stage Ⅱ (1.0 < TN score ≤ 2.0), stage Ⅲa (2.0 < TN score ≤ 3.0), stage Ⅲb (3.0 < TN score ≤ 4.0), and stage Ⅲc (TN score > 4.0). The proposed TNM staging system according to these TN scores fits well with the 5-year OS of colorectal cancer patients after potentially curative surgery (Figures 3 and 4). The summary of our proposed TNM staging system is shown in Table 3.

DISCUSSION

The present study and Mori's report[4] both found that the 7th edition of the AJCC TNM staging system cannot accurately predict the survival of patients with colorectal cancer, especially for stages Ⅱc and Ⅲa[4]. It is easy to misinterpret the prognosis of Ⅱc as being better than that of Ⅲa. This defect of the current TNM staging system originates from the inherent notion that lymph node metastases (N stage) affect the prognosis more significantly than local invasion (T stage), and this opinion is reflected in the current classification of stages Ⅱ and Ⅲ colorectal cancer. Although patients with T4bN0 have a lower 5-year survival rate than many stage Ⅲa/b patients, they are still currently classified as stage Ⅱc. Recent data

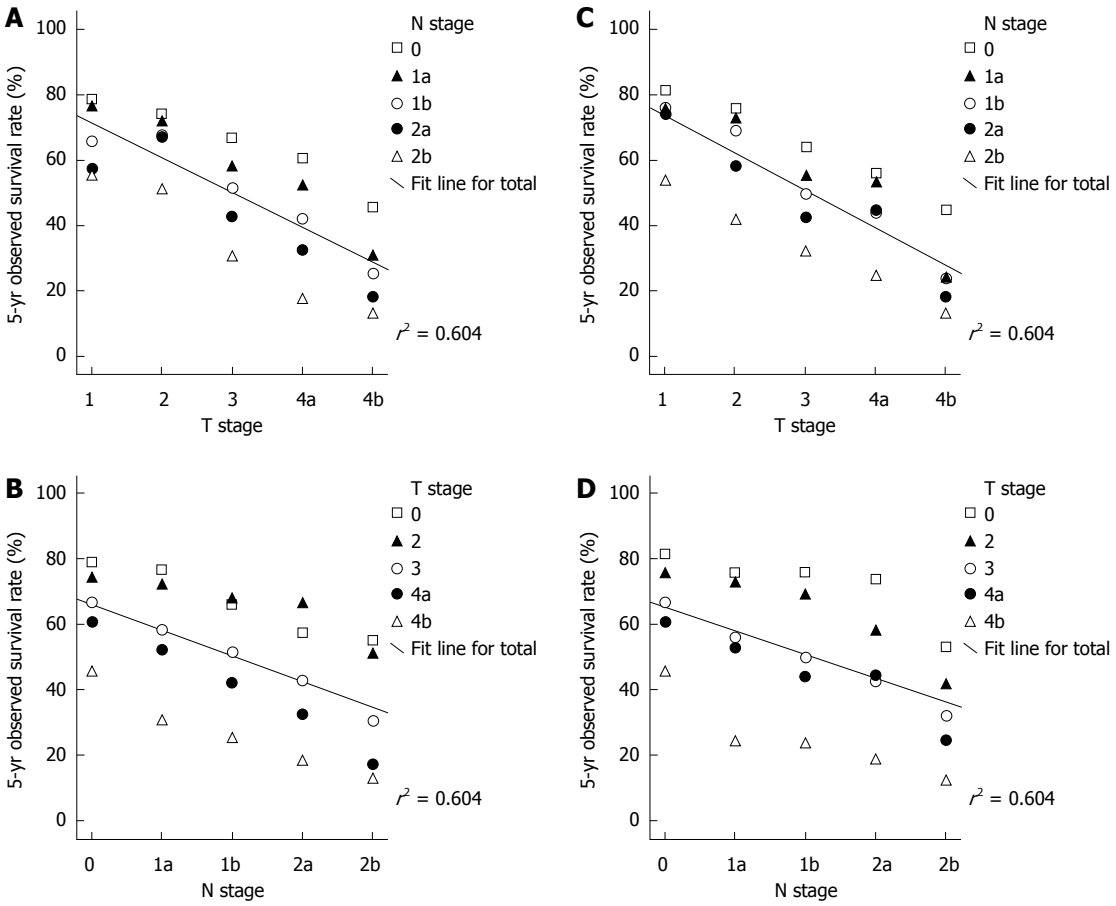

Figure 2 Scatter plots for T stage, N stage and the 5-year observed survival of colorectal cancer patients. A: Highlight of the T stage of colon cancer; B: Highlight of the N stage of colon cancer; C: Highlight of the T stage of rectal cancer; D: Highlight of the N stage of rectal cancer.

have shown that adjuvant chemotherapy improves both the progression-free survival and overall survival of patients with stage Ⅱ colon cancer[6], implying that there must be some problems with the existing staging system, especially in the identification of stages Ⅱ and Ⅲ.

There are 2 considerations that may be the root of the problem. Firstly, the TNM staging system traditionally relies on anatomical staging. The current system omits survival benefit because of the advances in surgery and adjuvant therapy in recent decades. As is well known, chemotherapy with oxaliplatin and fluorouracil and adjuvant radiotherapy have significantly improved the prognosis of patients with stage Ⅱ/Ⅲ colorectal cancer[7]. The data on which the 7th edition of the AJCC staging system for colorectal cancer were based were derived from the SEER outcome data of 1998-2002, well before the findings of adjuvant trials in stages Ⅱ and Ⅲ became available, and they certainly do not reflect current practice and prognosis[8,9].

Secondly and more importantly, our data indicate that the weight of the N stage has been over-estimated, and that this has been accompanied by an under-estimation of the T stage. This traditional concept needs to be reconsidered and correlated with contemporary survival data. The widespread application of complete mesocolic excision (CME) and total mesorectal excision (TME) standardized colorectal cancer surgery, with their greater lymph node yields, has reduced loco-regional recurrences and thereby improved survival rates[10,11]. On the other hand, patients with locally advanced (especially T4) tumors have a higher risk of local recurrence and peritoneal and distant metastases, resulting in poorer outcomes[12].

The findings of the present study support the hypothesis that the weight of T stage has been under-estimated in colorectal cancer patients. The relative weights of the T and N stages were 0.58 and 0.42, respectively, in colon cancer, and 0.61 and 0.39, respectively, in rectal cancer. To confirm that the T stage should carry more weight in the TNM staging system, our study calculated 25 categories of TN scores according to different T/N weightings. The survival rate decreased with increasing TN scores with good linear relationships. In addition, the proposed rearrangement of TNM stages according to the TN scores also showed good linear relationships with survival. Consequently, the traditional classification system, which relied more on the N stage, needs to be revised to place more emphasis on the T stage.

It is worth noting that the T stage has even greater weight in rectal cancer than in colon cancer, which probably indicates a higher risk and worse local recurrence consequences in rectal cancer. As a result, we propose that T4bN0 should be reclassified to stage Ⅲa for colon

Table 1 Tumor node scores and cluster analysis for colon cancer

AJCC 7th ed TNM stage	TN combinations	Patients (*n*)	TN score	5-yr OS	Proposed TNM stage	TN combinations	Patients (*n*)	TN score	5-yr OS
I	T1N0	10930	0.58	78.7%	I	T1N0	10930	0.58	78.7%
	T2N0	12931	1.16	74.3%		T1N1a	643	1.00	76.7%
ⅡA	T3N0	40338	1.74	66.7%	Ⅱ	T2N0	12931	1.16	74.3%
ⅡB	T4aN0	5020	2.32	60.6%		T1N1b	325	1.42	65.8%
ⅡC	T4bN0	3088	2.90	45.7%		T2N1a	1270	1.58	72.1%
ⅢA	T1N1a	643	1.00	76.7%		T3N0	40338	1.74	66.7%
	T2N1a	1270	1.58	72.1%		T1N2a	77	1.84	57.4%
	T1N1b	325	1.42	65.8%		T2N1b	896	2.00	67.7%
	T2N1b	896	2.00	67.7%	ⅢA	T3N1a	8759	2.16	58.2%
	T1N2a	77	1.84	57.4%		T1N2b	27	2.26	55.0%
ⅢB	T2N2a	300	2.41	66.6%		T4aN0	5020	2.32	60.6%
	T3N1a	8759	2.16	58.2%		T2N2a	300	2.41	66.6%
	T4aN1a	1311	2.74	52.2%		T3N1b	9107	2.58	51.7%
	T3N1b	9107	2.58	51.7%		T4aN1a	1311	2.74	52.2%
	T1N2b	27	2.26	55.0%		T2N2b	95	2.84	51.0%
	T2N2b	95	2.84	51.0%		T4bN0	3088	2.90	45.7%
	T4aN1b	1460	3.16	42.1%		T3N2a	5331	3.00	42.8%
	T3N2a	5331	3.00	42.8%	ⅢB	T4aN1b	1460	3.16	42.1%
ⅢC	T4aN2a	982	3.58	32.5%		T4bN1a	845	3.32	30.6%
	T3N2b	3235	3.42	30.4%		T3N2b	3235	3.42	30.4%
	T4bN1a	845	3.32	30.6%		T4aN2a	982	3.58	32.5%
	T4bN1b	929	3.74	25.4%		T4bN1b	929	3.74	25.4%
	T4bN2a	730	4.16	18.3%		T4aN2b	671	4.00	17.5%
	T4aN2b	671	4.00	17.5%	ⅢC	T4bN2a	730	4.16	18.3%
	T4bN2b	653	4.58	12.9%		T4bN2b	653	4.58	12.9%

OS: Observed survival. AJCC: American Joint Committee on Cancer; TNM: Tumor node metastasis.

Table 2 Tumor node scores and cluster analysis for rectal cancer

AJCC 7th ed TNM stage	TN combinations	Patients (*n*)	TN score	5-yr OS	Proposed TNM stage	TN combinations	Patients (*n*)	TN score	5-yr OS
I	T1N0	3348	0.61	81.4%	I	T1N0	3348	0.61	81.4%
	T2N0	6613	1.22	75.7%		T1N1a	274	1.00	75.7%
ⅡA	T3N0	10615	1.83	64.0%	Ⅱ	T2N0	6613	1.22	75.7%
ⅡB	T4aN0	818	2.44	55.7%		T1N1b	170	1.39	75.9%
ⅡC	T4bN0	769	3.05	44.7%		T2N1a	923	1.61	72.7%
ⅢA	T1N1a	274	1.00	75.7%		T1N2a	62	1.78	73.8%
	T2N1a	923	1.61	72.7%		T3N0	10615	1.83	64.0%
	T1N1b	170	1.39	75.9%		T2N1b	641	2.00	68.9%
	T2N1b	641	2.00	68.9%	ⅢA	T1N2b	24	2.17	53.2%
	T1N2a	62	1.78	73.8%		T3N1a	2758	2.22	55.4%
ⅢB	T2N2a	302	2.39	58.2%		T2N2a	302	2.39	58.2%
	T3N1a	2758	2.22	55.4%		T4aN0	818	2.44	55.7%
	T4aN1a	218	2.83	53.2%		T3N1b	3029	2.61	49.7%
	T3N1b	3029	2.61	49.7%		T2N2b	120	2.78	41.7%
	T1N2b	24	2.17	53.2%		T4aN1a	218	2.83	53.2%
	T2N2b	120	2.78	41.7%		T3N2a	1964	3.00	42.5%
	T4aN1b	262	3.22	43.9%		T4bN0	769	3.05	44.7%
	T3N2a	1964	3.00	42.5%	ⅢB	T4aN1b	262	3.22	43.9%
ⅢC	T4aN2a	199	3.61	44.3%		T3N2b	1791	3.39	32.0%
	T3N2b	1791	3.39	32.0%		T4bN1a	201	3.44	24.4%
	T4bN1a	201	3.44	24.4%		T4aN2a	199	3.61	44.3%
	T4bN1b	222	3.83	24.0%		T4bN1b	222	3.83	24.0%
	T4bN2a	156	4.22	18.5%		T4aN2b	198	4.00	24.5%
	T4aN2b	198	4.00	24.5%	ⅢC	T4bN2a	156	4.22	18.5%
	T4bN2b	152	4.61	12.3%		T4bN2b	152	4.61	12.3%

OS: Observed survival; AJCC: American Joint Committee on Cancer; TNM: Tumor node metastasis.

cancer, but to stage ⅢB for rectal cancer. In addition, T1N1a should be reclassified to stage Ⅰ, rather than stage ⅢA in the 7th edition of the AJCC TNM staging system, and T2N1a-1b and T1N1b-2a should be reclassified to stage Ⅱ, rather than stage ⅢA in the 7th edition of the AJCC TNM staging system. Our proposed rearrange-

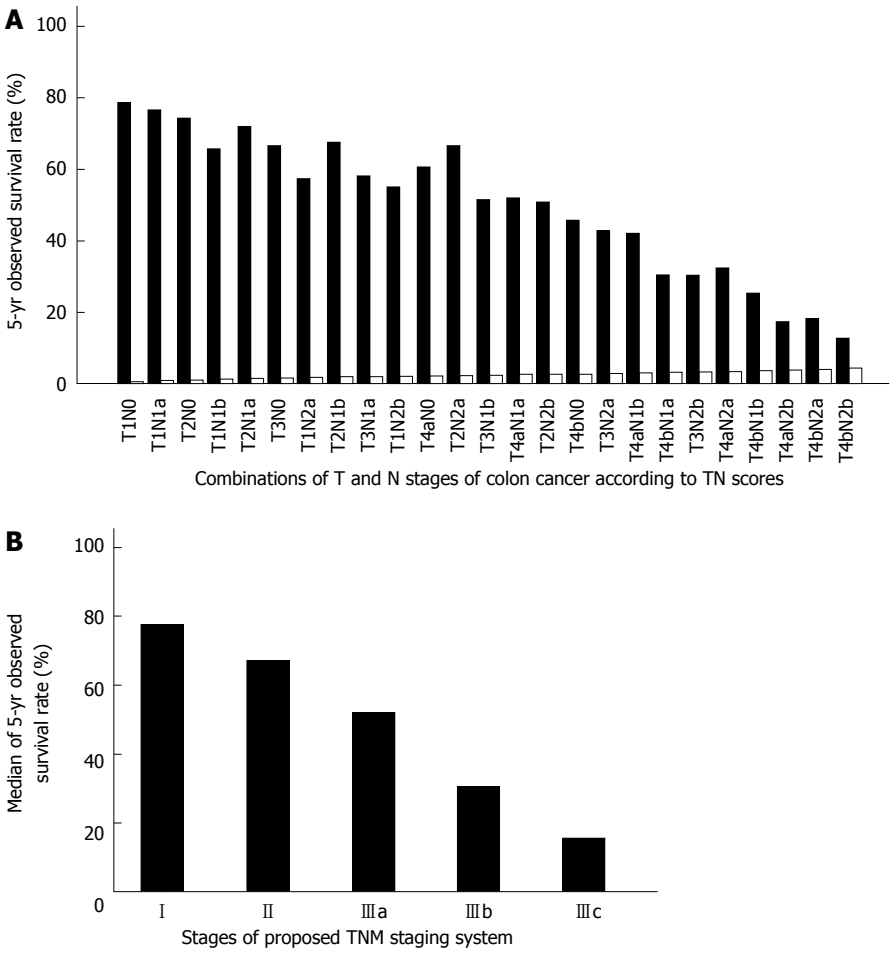

Figure 3 Relationship between survival of colon cancer patients and stages of the revised tumor node metastasis staging system. A: The 5-year observed survival rate of colon cancer patients for combinations of T and N stages according to TN scores. The observed survival rate is represented by the solid black grids. TN scores are represented by the white grids; B: The median 5-year observed survival rate of colon cancer patients for the proposed tumor node metastasis (TNM) staging system according to TN scores.

ment of the TNM staging system reflects the significance of the T stage in colorectal cancer and abandons the rigid classification by lymph node status, similar to the TNM staging system for gastric cancer[13]. It should be noted, however, that there may be potential biases arising from the SEER database, because the survival of patients can also be affected by factors such as surgical procedures, adjuvant therapies, and the number of lymph nodes, etc. Consequently, the reliability of our findings should be validated not only with regard to individual SEER data and other datasets, but also with regard to other staging systems, such as the 5th edition of the TNM staging system, which is used in Europe[4,14,15].

As mentioned above, patients who previously were classified as stage ⅢA (T1-2N1 and T1N2a) should now be reclassified as stage Ⅰ or Ⅱ. As the use of adjuvant therapy remains problematic for patients with colon cancer, information on treatment approaches was omitted in our study. In this regard, there are 2 factors to be considered. Firstly, stage ⅢA patients receive adjuvant therapy to improve their prognosis. Secondly, patients who have a good prognosis do not need either adjuvant therapy or intensive adjuvant therapy. Considering that patients with both stages Ⅱ and Ⅲ rectal cancer previously received adjuvant radiochemotherapy, some stage ⅢA patients still have a better prognosis than those in stage Ⅱ. It is speculated that some stage ⅢA colorectal cancer patients have a naturally good prognosis, no matter which treatment is offered. As stage Ⅲ patients traditionally receive adjuvant therapy, this issue should be clarified in future trials.

The question as to how to classify tumor deposits was not considered in this study. Peri-tumor deposits first emerged as prognostic indicators in the 5th edition of the AJCC TNM staging system in 1997[14]. A tumor nodule > 3 mm in diameter in the perirectal or pericolic adipose tissue, without histologic evidence of residual lymph node tissue, is classified as a regional lymph node metastasis (N category). A tumor nodule up to 3 mm in diameter is classified within the T category. These definitions are called the 3 mm rule. In the 6th edition of the AJCC TNM staging system published in 2002, the 3 mm rule was replaced by the contour rule[16]. A tumor nodule without histologic evidence of a residual lymph node is now classified within the N category if the nodule has the form and smooth contour of a lymph node. If the nodule has an irregular contour, it is classified within the

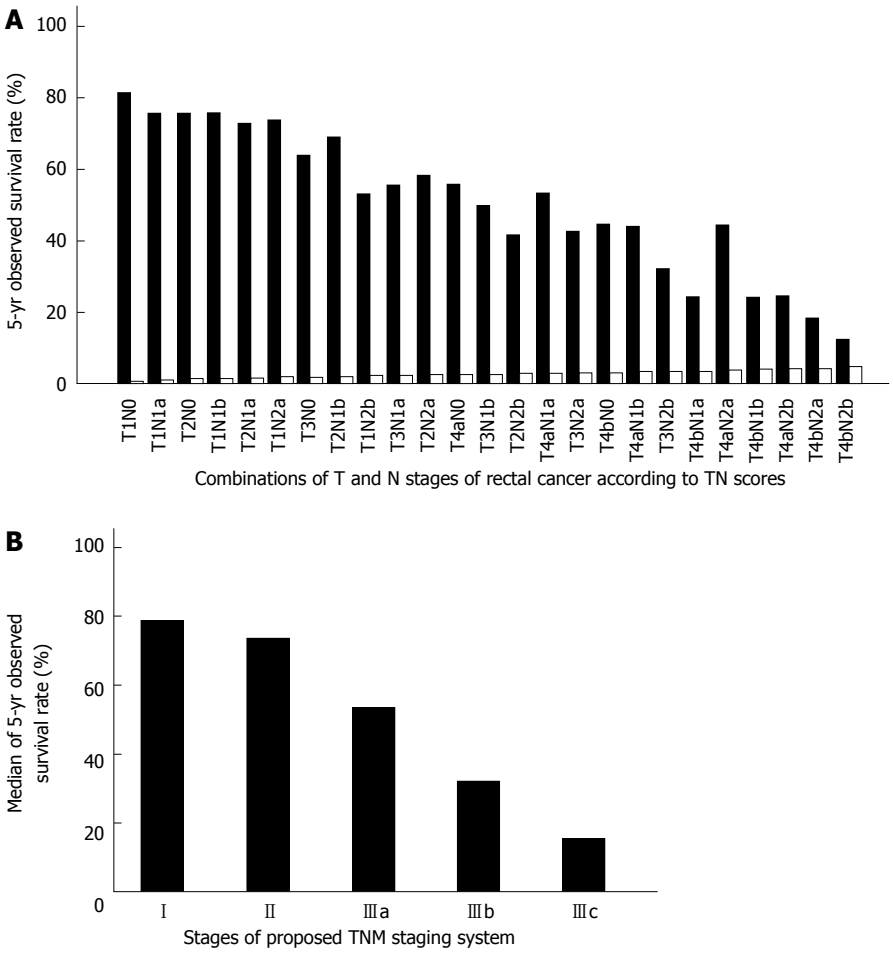

Figure 4 Relationship between survival of rectal cancer patients and stages of the revised tumor node metastasis staging system. A: The 5-year observed survival rate of rectal cancer patients for combinations of T and N stages according to TN scores. The observed survival rate is represented by the grey grids. TN scores are represented by the white grids; B: The median 5-year observed survival rate of rectal cancer patients for the proposed tumor node metastasis (TNM) staging system according to TN scores.

Table 3 Summary of proposed tumor node metastasis staging system

Proposed TNM stage for colon cancer	TN combinations	Proposed TNM stage for rectal cancer	TN combinations
I	T1N0-1a	I	T1N0-1a
II	T1N1b-2a	II	T1N1b-2a
	T2N0-1b		T2N0-1b
	T3N0		T3N0
IIIa	T1N2b	IIIa	T1N2b
	T2N2a-2b		T2N2a-2b
	T3N1a-2a		T3N1a-2a
	T4aN0-1a		T4aN0-1a
	T4bN0		
IIIb	T3N2b	IIIb	T3N2b
	T4aN1b-2b		T4aN1b-2b
	T4bN1a-1b		T4bN0-1b
IIIc	T4bN2a-2b	IIIc	T4bN2a-2b

TNM: Tumor node metastasis.

T category. In the 7th edition of the AJCC TNM staging system, the definition of tumor deposits is left to the discretion of the pathologist[1]. Colorectal cancer with an adjacent tumor deposit but no lymph node metastasis is now classified as N1c. The definition and classification of tumor deposits have kept changing in recent editions of TNM staging systems on the basis of expert consensus instead of high level evidence. There is some evidence to support the view that the 5th edition of TNM staging system is the best choice to define peri-tumoral tumor deposits in colorectal cancer[17,18].

The average number of lymph nodes within the SEER dataset was not well defined. Of the 109953 colon cancer cases in the SEER dataset, 13 or more lymph nodes were harvested in 37% of patients. As a result, it is easy to see how understaging can occur within the SEER dataset, especially for stage II patients. However, in terms of the average number of lymph nodes, the SEER dataset is equivalent to other databases. For example, in US hospital data, more than 60% of hospitals (792/1296) failed to archive a compliance benchmark for the 12-node measure[19]. A nationwide population-based study in the Netherlands showed that the median number of lymph nodes harvested in colon cancer was only 8[20]. However, the latest study based on SEER data

shows that the detection of fewer lymph nodes does not result in understaging. Although the proportion of patients with 12 or more lymph nodes has increased over the period 1988 to 2008 (from 34.6% in 1988-1990 to 73.6% in 2006-2008, $P < 0.001$), this has not resulted in a significant overall increase in the proportion of node-positive cases (40% in 1988-1990 *vs* 42% in 2006-2008, $P = 0.53$). Therefore the "upstaging" hypothesis as the primary basis for improved survival in patients with more lymph nodes is questionable[21].

It is difficult to evaluate the accurate stage for many patients with advanced rectal cancer who receive neoadjuvant chemoradiotherapy. While some cases of rectal cancer will respond and downstage, a stratified analysis was not possible in this study because adjuvant and palliative treatments are anfractuous between patients, and also because fewer patients received neoadjuvant treatment at the time the SEER data were collected (1998-2002). As a result, rectal cancer patients who did or did not receive neoadjuvant treatment were included together in this study without stratification. There are 3 reasons supporting the lack of stratification in this study. Firstly, the outcomes of rectal cancer after preoperative treatment are decided by the post-treatment pathologic stage rather than preoperative stage[22]. Secondly, pretreatment stages are not absolutely accurate, even when magnetic resonance imaging (MRI) or endorectal ultrasound have been employed[23]. Thirdly, the proposed TNM staging system for rectal cancer in this study is remarkably similar to that for colon cancer, but with no stratification for treatment. T4b is the exception in rectal cancer. Although our study showed that T4b has greater weight in rectal cancer than in colon cancer, it is not clear whether this is an intrinsic fact or a fault due to the lack of stratification for treatment. As mentioned above, the influence of treatment is difficult to assess considering the various treatments, especially after cancer recurrences. Prospective, randomized clinical trials comparing the prognosis of patients with high T stages but negative lymph nodes and patients with low T stages and low-positive N stages who received the same adjuvant therapy may be helpful to clarify this problem. It is worth noting that the MERCURY study found that postoperative ypT stage and circumferential resection margin (CRM), but not the post-treatment N status, were important predictors of the outcome of locally-advanced rectal cancer after neoadjuvant therapy[24,25]. This finding also implies that the T stage may affect the outcome of rectal cancer more significantly than the N stage. In the future, a separate staging system should be made for rectal cancer which emphasizes the T stage and the CRM status.

In conclusion, in the present study, we found that the T stage affects colorectal cancer survival more significantly than the N stage. Therefore, it is reasonable to stratify TNM stages according to relative T and N weightings. In our proposed rearrangement of the TNM staging system, T4bN0 should be reclassified as stage ⅢⅠa in colon cancer and stage ⅢⅠb in rectal cancer, while patients previously classified as ⅢⅠa (T1-2N1 and T1N2a) should be reassigned to stage Ⅰ or Ⅱ. Our proposed TNM staging system based on relative T and N weightings should be examined in future prospective, randomized controlled trials and stratified studies.

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COMMENTS

Background

The American Joint Committee on Cancer (AJCC) TNM staging system is widely used to predict the prognosis for patients with colorectal cancer and to guide adjuvant therapy after potentially curative surgery. The 7th edition of the AJCC TNM staging system for colorectal cancer, which was published in 2010, cannot predict survival linearly by stage. For example, the 5-year observed survival of stage ⅢⅠa patients (T1-2N1 and T1N2a) matches that of stage Ⅰ patients. On the other hand, stage ⅡⅠc patients have a poorer prognosis, equivalent to that of stage ⅢⅠb patients.

Research frontiers

In the 7th edition TNM staging system, stage T4 was stratified to T4a and T4b, and patients with T4bN0 lesions were reclassified from stage ⅡⅠb to ⅡⅠc. These changes reflect the fact that the T stage affects survival in colorectal cancer patients more significantly than previously believed.

Innovations and breakthroughs

Authors found that for colon cancer, the relative T and N stage weights were 0.58 and 0.42, respectively, and for rectal cancer, the relative T and N stage weights were 0.61 and 0.39, respectively. It appears that T stage has greater weight in rectal cancer than in colon cancer (which would be consistent with the greater risk of local recurrence seen with rectal cancer). Moreover, the authors propose a revised scheme for the 7th edition tumor node metastasis (TNM) staging system. Consequently, T4bN0 is classified to ⅢⅠa in colon cancer, but to ⅢⅠb in rectal cancer. It is the first try to revise established TNM staging system for colorectal cancer by shaking the keystone of lymph nodes status (N stage).

Applications

In the present study, authors found that the T stage affects colorectal cancer survival more significantly than the N stage. Therefore, it is reasonable to stratify TNM stages according to relative T and N weightings in future revision of the TNM staging system.

Terminology

Cluster analysis, also called group analysis, is a statistical analysis method for studying the classification of samples or indicators. In this study, the TNM staging system is rearranged according to the cluster analysis results of TN scores.

Peer review

The authors analyzed the relationship between the survival and stages of colorectal cancer using AJCC 7th edition TNM staging system. They found the 7th edition TNM staging system for colorectal cancer cannot predict survival linearly by stage, but the relative weight of T stage has more impact on patients survival based on multiple linear regression analysis. Even the criteria used in the TNM system have varied over time according to the different editions that AJCC and UICC have released, one aim for adopting a global standard is to give an indication of prognosis and assist in the evaluation of the results of treatment. To predict the survival of colorectal cancer more accurate, more and new factors should be introduced into the evaluation system. The authors found the relative weight of T/N stage is a factor that could predict survival effectively. It is novel for TNM staging system.

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PROSPECTIVE STUDY

Sedated *vs* unsedated colonoscopy: A prospective study

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Abstract

AIM: To compare sedated to unsedated colonoscopy in terms of duration, pain and the patient's willingness to repeat the procedure.

METHODS: Consecutive patients who underwent colonoscopies over a 2-year period were invited to participate. All patients who were to undergo our endoscopy unit were offered sedation with standard intravenous sedatives and analgesics, or an unsedated colonoscopy was attempted. Demographic details were recorded. The patient anxiety level prior to the procedure, time to reach the cecum, total discharge time, patient and endoscopist pain assessments, satisfaction after the examination and the patient's willingness to return for the same procedure in the future were recorded.

RESULTS: Among the 403 observed patients, more males were observed in the unsedated group (66.2% *vs* 55.2%, $P = 0.04$). Additionally, the unsedated group patients were less anxious prior to the procedure (5.1 *vs* 6.0, $P < 0.01$). The colonoscopy completion rates were comparable between the 2 groups (85.9% *vs* 84.2%, $P = 0.66$). The time to reach the cecum was also comparable (12.2 min *vs* 11.8 min); however, the total discharge times were shorter in the unsedated group (20.7 min *vs* 83.0 min, $P < 0.01$). Moreover, the average patient pain score (3.4 *vs* 5.7, $P < 0.01$) was lower in the sedated group, while the satisfaction score (8.8 *vs* 7.8, $P < 0.01$) was significantly higher. There was no significant difference, however, between the groups in terms of willingness to repeat the procedure if another was required in the future (83.3% *vs* 77.3%, $P = 0.17$).

CONCLUSION: Unsedated colonoscopy is feasible in willing patients. The option saves the endoscopy units up to one hour per patient and does not affect the patient willingness to return to the same physician again for additional colonoscopies if a repeated procedure is needed.

Key words: Sedation; Colonoscopy; Unsedated; Screening; Endoscopy

Core tip: Published information indicates that unsedated colonoscopies are acceptable in many countries; however, sedation is still a usual practice in many countries. Its burden includes escort requirement, time for recovery and activity restrictions. This study showed that unsedated colonoscopy is feasible in willing patients and it saves the endoscopy units up to one hour per patient. Contrary to some endoscopist's fears, patients were still willing to return to the same physician for colonoscopy if a repeat procedure was needed.

Aljebreen AM, Almadi MA, Leung FW. Sedated *vs* unsedated colonoscopy: A prospective study. *World J Gastroenterol* 2014; 20(17): 5113-5118 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5113.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5113

INTRODUCTION

Colonoscopy has become an indispensable gastroenterologist tool, and although the procedure has evolved over time, discomfort and pain remain one of the major concerns for patients undergoing colonoscopies[1]. To overcome these concerns, endoscopists commonly use conscious sedation (CS); however, their use does have some drawbacks, especially for elderly patients[2,3]. Additionally, sedation has been identified as a colorectal cancer screening barrier for colonoscopy use, whereby 14% of patients cited the need for an escort and time-off following sedation as the reasons for non-adherence to the recommended screening[4]. Moreover, the limited procedure numbers that can be performed due to the prolonged turnaround time for the recovery time after sedation have also been described as a barrier for colonoscopy use. A United States study, based on detailed patient diaries, revealed that a median of 39.5 h is spent for colonoscopies. After a colonoscopy, the median recovery time is 1.8 h and an additional 15.8 h is required to return to daily routines[5].

Published information indicates that unsedated colonoscopies are acceptable in many countries[6-11]. In the United States, unscheduled and unsedated colonoscopies have been offered to approximately 1% to 2%[12] of patients who are without an escort.

The main objective of this study was to compare sedated with unsedated colonoscopies in terms of time until discharge, willingness to repeat the procedure with the same endoscopist if future colonoscopies are required, pain levels and the patient and physician satisfaction levels.

MATERIALS AND METHODS

Patients and methods

Consecutive patients undergoing colonoscopy at our endoscopy unit from January 2010 to December 2012 were invited to participate in the study by a research assistant.

As is our practice, all patients who were to undergo colonoscopy in our endoscopy unit were offered sedation with standard intravenous sedatives and analgesics or an attempt at an unsedated colonoscopy. No attempt was made to pressure or coerce patients into having unsedated procedures. Patients were only excluded if they were undergoing both gastroscopy and colonoscopy at the same time, if interventional procedures were planned ahead of the colonoscopy or if they refused to participate in the study. All endoscopists, including supervised gastroenterology fellows, were invited to participate in the performance of sedated or unsedated colonoscopy examinations.

Demographic details, including age, gender, education level, prior endoscopic procedures, CS experience, weight and the procedure indications were recorded. Additionally, patient anxiety levels prior to the procedure were assessed using a 0-10 scale, whereby 0 indicated no anxiety at all, 5 indicated moderate anxiety and 10 indicated extreme anxiety.

Patients who choose to have sedation were given intravenous pethidine and midazolam in a ratio of 25:1 mg before the colonoscopy initiation. Additionally, dosages were adjusted according to the patient's age and weight. Patients who chose the non-sedated arm were given the option to ask for sedation if they felt it was necessary for procedure continuation.

The endoscopic findings and immediate complications (within 24 h of the procedure) were recorded in addition to documenting the quality of the preparation. All patients were monitored for cardiorespiratory depression with a pulse-oximeter.

Data recorded during the procedure included the time required to reach the cecum, patient discharge times from the endoscopy unit, any interventional procedures (such as biopsies or polypectomies), any medications given, the maximum drop in systolic blood pressure from baseline and the need for supplemental oxygen.

The time required to reach the cecum was the time from colonoscope insertion into the anus to the identification of all cecal landmarks. The discharge time was the time from colonoscope insertion until the patient was released from the endoscopy unit.

Just before releasing the patient from the endoscopy unit, a research assistant (non-blinded) asked the patient about their pain score and satisfaction level. Pain was evaluated by a visual analog scale from 0 to 10 (0: no pain, 10: worst pain). According to the scale in Table 1[10], the endoscopist was asked to rate the patient's pain level during the procedure, his procedure satisfaction level and the technical ease of the procedure just after procedure completion. Patients were also asked about their willingness to return to the same physician again, if a repeat colonoscopy procedure were required. The King Khalid University Hospital Institutional Review Board approved this study.

Statistical analysis

To estimate the sample size, we assumed that patients receiving routine sedation would find the procedure to be very acceptable and would have a mean pain score of 2. The smallest difference in pain score that was clinically important to detect was judged to be 2 units or a mean score of 4. Thus for mean pain score values of 2 and 4, estimated standard deviations of 2.5 and 3.5, respectively, α of 0.05, and power of 0.8, recruitment of 74 participants was required.

Data analyses included descriptive statistics computed for continuous variables, including means, standard deviations, minimum and maximum values as well as 95%CI. Frequencies were used for categorical variables. Univariate

Table 1 Pain and satisfaction scores

Pain and satisfaction scores	
Doctor's assessment of the patient's discomfort level	
5	Patient complained bitterly; asked to stop multiple times; numerous delays; pain very distracting for doctor
4	Patient complained multiple times; asked to stop; several delays; would not allow repeat examination with this level of discomfort
3	Few transient delays; overall pain reasonable; doctor would be comfortable repeating examination with this pain level
2	Minor transient pain on insertion only or withdrawal only; patient would do very well with a repeat examination with this level of sedation
1	No pain
Overall level of satisfaction with colonoscopy	
	Very satisfied with examination
	Somewhat satisfied with examination
	Somewhat unsatisfied with examination
	Very unsatisfied with examination

Table 2 Patient characteristics *n* (%)

Characteristics	Sedated	Unsedated	*P* value
Total	270	133	
Male	149 (55.2)	88 (66.2)	0.04
Female	121 (44.8)	45 (33.8)	
Average age (yr)	43.4 ± 17	48.4 ± 13.7	0.002
Outpatient	180 (66.7)	119 (89.5)	0.001
Weight (kg)	70.2 ± 18.2	77.9 ± 16.7	0.0004
Smoking	25 (9.3)	12 (9.1)	0.72
NSIADs use	50 (18.7)	20 (15)	0.4
Education level			
Less than high school	111 (41.1)	63 (47.4)	0.67
High school	44 (16.3)	22 (16.5)	
Some college	36 (13.3)	14 (10.5)	
College or more	70 (25.9)	32 (24.1)	
Physicians	9 (3.3)	2 (1.5)	
Abdominal pain	139 (51.5)	74 (55.6)	0.45
IBS	67 (24.8)	47 (35.3)	0.03
IBD	23 (8.5)	12 (9.1)	0.85
Previous pelvic surgery	35 (13)	29 (22)	0.03
Previous abdominal surgery	65 (24.1)	39 (29.3)	0.28
Anxiety level prior to colonoscopy	6.1 ± 3.7	5.2 ± 3.6	0.008
Previous sedated colonoscopy	50 (18.5)	11 (8.3)	0.0003
Previous sedated gastroscopy	55 (20.4)	8 (6)	0.0001
Preparation quality			
Good	139 (50.2)	64 (48.1)	0.72
Fair	92 (34.1)	51 (38.3)	
Poor	39 (14.1)	18 (13.5)	

Data are frequency counts (percentage of total) or mean ± SD. IBS: Irritable bowel syndrome; IBD: Inflammatory bowel disease.

and multivariate logistic regressions were used to examine associations between independent and dependent variables. The independent variables included: age, gender, anxiety level, patient pain score, patient satisfaction level, physician pain level assessment, physician satisfaction level, education level and prior pelvic surgery history. Additionally, odds ratios (OR) and 95%CI were calculated.

We used the STATA 11.2 software package (Stata Corp, TX, United States) for our analyses. A statistical significance threshold of $P = 0.05$ was adopted. No attempt at imputation was made for missing data.

RESULTS

The patient demographic details are shown in Table 2. A total of 403 patients were enrolled in the study with a mean age of 45.1 years (16) (range was from 9 to 85 years) and 58.8% of the subjects were male. 372 (92.3%) patients were Saudis, 37 (9.2%) were smokers, 35 (8.7%) were known to have inflammatory bowel disease (IBD) and 70 patients (17.5%) had a history of non-steroidal anti-inflammatory medication use. There were more males in the unsedated group (66.2% *vs* 55.2%, $P = 0.04$) and they were older (48.5 *vs* 43.4, $P = 0.002$), more likely to be outpatients (89.5% *vs* 66.7%, $P = 0.001$) and had fewer previous sedated colonoscopies and gastroscopies than the sedated group (8.3% *vs* 18.5%, $P = 0.0003$ and 6% *vs* 20.4%, $P = 0.0001$, respectively). Prior to the procedure, the sedated group patients were more anxious (6.1 ± 3.7, 95%CI: 5.7-6.6) than the unsedated group patients (5.2 ± 3.6, 95%CI: 4.6-5.8) ($P = 0.009$). Additionally, the education level of these patients was less than high school in 174 (43.2%), high school in 66 (16.4%), some college in 50 (12.4%), 102 (25.3%) had completed college or higher level of education and 11 (2.7%) were physicians. There were no significant differences between the groups.

The procedure outcomes are shown in Table 3. The colonoscopy completion rates between the 2 groups were comparable (85.9% *vs* 84.2%, $P = 0.66$), while the terminal ileum intubation rates were 41.8% in the sedated group compared with 27.7% in the unsedated group ($P = 0.01$).

The average midazolam and pethidine dosages used to achieve CS were 3.3 ± 1.3 mg and 43.4 ± 20.2 mg, respectively. Eight patients in the unsedated group required sedation after starting the procedure, with average midazolam and pethidine dosages of 2.5 and 40 mg, respectively.

Only 4 patients (1.5%) in the sedated group had transient oxygen desaturation, and no other complications were observed in either group.

Although the time required to reach the cecum was comparable between the sedated (12.2 ± 9.4 min, 95%CI: 11.1-13.4) and unsedated groups (11.8 ± 8.8 min 95%CI: 10.2-13.3, $P = 0.68$), the total discharge time for the sedated group was 82.9 ± 58.4 min (95%CI: 75.9-89.9) *vs* 20.7 ± 21.8 min (95%CI: 16.9-24.5) for the unsedated group ($P < 0.0001$). The average technical ease according to the

Table 3 Procedure outcomes *n* (%)

Outcomes	Sedated (*n* = 270)	Unsedated (*n* = 133)	*P* value
Completed	232 (85.9)	112 (84.2)	0.66
TI intubation	97 (35.9)	31 (23.3)	0.01
Findings			
Normal	126	55	0.34
Diverticulosis	14	13	0.09
Ulcerative colitis	18	9	1.00
Crohn's disease	22	1	0.001
Polyps	36	21	0.54
Tumor	16	3	0.13
Incomplete endoscopy reason			
Poor preparation	7	4	0.76
Pain	12	12	0.077
Technical difficulty	6	4	0.74
Obstruction	7	1	0.28
Others	6	0	0.18
Time to cecum (min)	12.2 ± 9.4	11.8 ± 8.8	0.68
Total time until discharge	83 ± 58	20.7 ± 21.8	< 0.0001
Average technical ease	8 ± 1.8	7.9 ± 2	0.45
Polypectomy	4	3	0.63
Complications			
Oxygen desaturation	4 (1.5)	0	NA

Data are frequency counts (percentage of total) or the mean ± SD. CD: Crohn's disease; TI: Terminal ileum; UC: Ulcerative colitis; NA: Not applicable.

Table 4 Pain score and anxiety level comparisons *n* (%)

Pain score and anxiety level	Sedated (*n* = 270)	Unsedated (*n* = 133)	*P* value
Average pain score by the patient	3.4 ± 3.4	5.7 ± 3.2	< 0.0001
Average pain score *via* physician assessment	3.3 ± 2.5	4.1 ± 3	0.007
Average patient satisfaction score	8.8 ± 2.2	7.7 ± 2.6	< 0.0001
Patient satisfaction			
Very satisfied	221 (81.9)	88 (66.2)	0.0007
Somewhat satisfied	36 (13.3)	27 (20.3)	0.08
Somewhat unsatisfied	5 (1.9)	7 (5.3)	0.07
Unsatisfied	8 (2.9)	11 (8.3)	0.02
Physician assessment of the patient discomfort level			
No pain	56 (20.8)	15 (11.3)	0.02
Minor transient pain	133 (49.4)	74 (55.6)	0.24
Few transient pain	46 (17.1)	19 (14.3)	0.56
Complained multiple times	28 (10.4)	16 (12)	0.01
Complained bitterly	6 (2.2)	9 (6.8)	0.007
Could not recall the procedure	124 (45.9)	5[1] (3.8)	< 0.0001
Patients willing to repeat the procedure	225 (83.3)	103 (78.6)	0.16
Average physician satisfaction	8.4	7.8 ± 1.9	0.001

Data are frequency counts (percentage of total) or mean ± SD. [1]All received sedation.

endoscopist was 8 ± 1.8 among the sedated group and 7.9 ± 2 in the unsedated group; however, no significant differences were observed between the groups ($P = 0.45$).

The average pain score reported by the patients was 3.4 ± 3.4 (95%CI: 3-3.8) in the sedated group *vs* 5.7 ± 3.2 (95%CI: 5.2-6.3) in the unsedated group ($P < 0.0001$), while the average pain score as assessed by the physician was 3.3 ± 2.6 (95%CI: 3-3.6) in the sedated group *vs* 4.1 ± 3 (95%CI: 3.6-4.6) in the unsedated group ($P = 0.007$) (Table 4). According to the endoscopists, 235 (87.3%) sedated group patients had no pain (20.8%) or complained of minor transient pain (49.4%) or little transient pain (17.1%), while only 34 (12.7%) patients complained multiple times (10.4%) or complained bitterly (2.3%). In contrast, 108 unsedated group patients (81.2%) had no pain (11.3%) or complained of minor transient pain (55.6%) or little transient pain (14.3%), while only 25 (18.8%) patients complained multiple times (12%) or complained bitterly (6.8%). A $P = 0.03$ was noted between the sedated and unsedated groups. According to the patients, 7 (2.6%) of the sedated group had a very bad experience compared with 10 (7.5%) of the unsedated group ($P = 0.0001$).

The average patient satisfaction score was 8.8 ± 2.2 (95%CI: 8.5-9) in the sedated groups *vs* 7.7 ± 2.6 (95%CI: 7.3-8.2) in the unsedated group ($P < 0.0001$). Moreover, 81.6% of the sedated group were very satisfied compared with 66.2% of the unsedated group ($P = 0.0001$), while only 3% of the sedated group were unsatisfied compared with 8.3% of the unsedated group ($P = 0.02$). In line with the patient satisfaction after the procedure, the average physician satisfaction after the procedure was significantly different between the sedated group (8.4 ± 1.6, 95%CI: 8.2-8.6) and unsedated group (7.8 ± 1.9, 95%CI: 7.6-8.3) ($P = 0.01$).

Additionally, 45.9% of the sedated group did not remember the procedure, compared with 3.7% of the unsedated group, and 8 patients (6%) of the unsedated group asked for sedation after the procedure began.

83.3% sedated group patients were willing to repeat the colonoscopy in the future if needed compared with 78.6% of the unsedated group. No significant difference was observed between the groups ($P = 0.27$).

Following univariate analyses (Table 5), male gender, lower anxiety scores prior to the procedure, high patient and physician satisfaction scores and a higher education level predicted the willingness to repeat the procedure in the future if required, while higher pain scores (whether assessed by the patient or the physician), higher anxiety level prior to the procedure, female gender, pelvic surgery and irritable bowel syndrome histories all predicted an unwillingness to repeat the procedure with the same sedation. With the multivariate analyses, however, only a higher satisfaction level (OR: 0.53, $P = 0.01$, 95%CI: 0.32-0.89) and a higher education level (OR: 1.37, $P = 0.01$, 95%CI: 1.05-1.79) predicted patient willingness to repeat the procedure by the same endoscopist if needed in the future.

DISCUSSION

Although sedation remains the dominant practice in the United States (US), unsedated colonoscopies have continued to be practiced in many parts of the world[13]. Twenty-eight percent of the US community[14] and 75%

Table 5 Univariate analysis of potential predictors of the willingness to repeat the colonoscopy

Variable	Odds ratio	*P* value	95%CI
Age	1.02	0.04	1.00-1.04
Gender (female)	0.44	0.002	0.26-0.74
Anxiety	0.85	0.003	0.79-0.92
Patient satisfaction score	1.43	0.0001	1.29-1.59
Physician satisfaction score	1.20	0.004	1.06-1.38
Prior pelvic surgery	0.45	0.01	0.25-0.83
Physician assessment of pain	0.84	0.001	0.77-0.92
Education level: college or more	3.04	0.003	1.45-6.37
Patient pain score	0.84	0.0001	0.78-0.90
Time to cecum	1.00	0.92	0.97-1.02
Ability to recall the procedure	0.82	0.49	0.47-1.40
Discharge time	1.00	0.20	0.99-1.00
Abdominal pain	0.78	0.35	0.47-1.31
IBS	0.56	0.03	0.33-0.96
Physician assessment of discomfort level			
Complained multiple times	0.16	0.001	0.06-0.46
Complained bitterly	0.11	0.001	0.03-0.39
Technical ease	1.14	0.03	1.01-1.29

IBS: Irritable bowel syndrome.

of Veterans Affairs[15] patients accept the on-demand sedation option. Amongst these, 77% to 81% were completed without sedation and reported minimal discomfort. With good bowel preparation, the cecal intubation success rate during unsedated colonoscopies, provided in as-needed or on-demand sedation forms, is > 90% when the attending staff performed the examinations[10,14-16].

Despite the significant difference between the groups in our study in terms of patient and physician pain assessments and satisfaction in favor of sedation, there was no significant difference found with regard to the willingness to repeat the procedure by the same physician if required in the future (83.3% *vs* 78.6%). One potential explanation for this finding is that the measured pain and satisfaction level differences, although significantly different, were not great enough to be clinically important. Among the 451 who underwent unsedated screening colonoscopies, Thiis-Evensen *et al*[7] found the rate of cecal intubation was 82%, 90% of these patients stated that they would undergo a repeat colonoscopy in 5 years. In another smaller study among 40 patients who underwent "sedation on demand" colonoscopy, 93% of these patients were willing to undergo another colonoscopy without prior sedation[17]. In a more recent US study, among 578 patients (27.6%) who chose to start the procedure without sedation, 81.1% of those completed the examination without medication and 97.4% were satisfied with their comfort level during the procedure and were willing to have their next colonoscopies performed without sedation[18]. Comparing sedated colonoscopy to "sedation on demand", Terruzzi *et al*[19] showed that the proportion of those stating they would not undergo a colonoscopy again in the future (22% *vs* 9.7%, $P < 0.005$) was significantly higher in the "on demand" sedation group.

The cecal intubation rate has been traditionally used as one of the benchmarks in studies investigating the use (or foregoing) of CS for colonoscopy procedures. In our study, the cecal intubation rate was the same between the sedated (85.9%) and the unsedated groups (84%). In general, the cecal intubation success rate during unsedated colonoscopy was > 90% when the attending staff performed the examinations[10,14,20], but was only 81% in the hands of supervised trainees[18], which was also the case in our study.

Although the discharge time was significantly shorter in the unsedated group, given no recovery time was needed, we found no differences between the groups when we compared cecum times. Consistent with our study, Petrini *et al*[14] demonstrated that the cecum times were comparable for both sedated and unsedated groups (9.71 min *vs* 9.87 min). When comparing sedated colonoscopy to "sedation on demand", Rex *et al*[10] observed that cecum times were significantly shorter in the sedated groups; however, the discharge times were significantly longer (55 min *vs* 10 min).

The factors that predicted willingness to attempt an unsedated colonoscopy, with a high performance success level and maintenance of satisfaction, included male gender, older age, abdominal pain absence, prior abdominal surgery, previous endoscopic procedure experience, the instruments used, endoscopist skill and higher education levels, particularly graduate level education[1,10,21-23].

In our study, male gender, lower anxiety score prior to the procedure, high patient and physician satisfaction scores and higher education levels all predicted the willingness to repeat the procedure in the future if required.

Despite the significant publications regarding sedation free colonoscopy, we think our study was closer to reality than what is observed in most endoscopy units, in terms of gastroenterology trainee involvement. Additionally, we demonstrated that unsedated colonoscopy can save up to 62 min per patient, which is a very important factor in most busy academic endoscopy units, and despite the pain scores, this finding was statistically significantly higher in the unsedated group. Moreover, the willingness to undergo the same procedure without sedation was similar in the sedated group, which is an important factor for endoscopists to consider if they consider performing unsedated colonoscopy on their patients. In contrast, the shortcomings of this study included the small study size, lack of documentation of the patient acceptance rate for unsedated colonoscopy, the non-randomized and unblinded design, and asking about the patient satisfaction level just after the procedure.

In conclusion, unsedated colonoscopy is feasible in patients who are willing to undergo this procedure without sedation and can save endoscopy units up to one hour per patient. Additionally, contrary to some endoscopists' fears, patients are still willing to undergo the same procedure in the future if required.

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COMMENTS

Background

The main objective of the study was to compare sedated with unsedated colonoscopies in terms of time until discharge, willingness to repeat future procedures with the same endoscopist, pain levels and patient and physician satisfaction levels.

Research frontiers

Published information indicates that unsedated colonoscopies are acceptable in many countries. There are many reasons why some patients prefer to undergo colonoscopy without sedation. No escort requirement, fear of the usual sedation-related complications and restrictions on activities for almost one full day are the common reasons why patients choose unsedated colonoscopy.

Innovations and breakthroughs

Despite involving gastroenterology trainees in this study and despite the observation that the pain score was statistically significantly higher in the unsedated group, this study showed that unsedated colonoscopy is feasible in willing patients. This option saves endoscopy units up to one hour per patient, and contrary to some of endoscopists fears, patients are still willing to return to the same physician again for future colonoscopies if a repeat procedure is needed.

Applications

Unsedated colonoscopy is a good option for some patients. It can saves endoscopy units up to one hour per patient. It does not decrease patient willingness to undergo future colonoscopies in future.

Peer review

This is a well-written and interesting study. This needed to be done as a sham controller randomized controlled trial giving saline versus conventional sedation. Instead the authors simply asked patients whether they wanted sedation or not making the results un-interpretable as the amount of bias introduced is enormous - those willing to consider not having sedation are a completely different cohort of patient in many ways

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META-ANALYSIS

Efficacy of ilaprazole in the treatment of duodenal ulcers: A meta-analysis

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Abstract

AIM: To compare the efficacy and tolerance of ilaprazole compared with other proton pump inhibitors (PPIs) in the treatment of duodenal ulcer.

METHODS: An electronic database search of Medline, Embase, the Cochrane controlled trials register, Web of Science, PubMed, and the Chinese Biomedical Literature Database (updated to July 2013), and manual searches were conducted. A meta-analysis of randomized controlled trials comparing the efficacy and tolerance of ilaprazole and other PPIs in the treatment of duodenal ulcers was performed.

RESULTS: Five articles involving 1481 patients were included. The meta-analysis showed no difference in the 4-wk healing rate between ilaprazole and other PPIs [89.7% *vs* 87.0%; relative risk (RR) = 1.02; 95%CI: 0.98-1.06; Z = 1.00; P = 0.32]. The results did not change in the sensitivity analyses. The meta-analysis indicated that the adverse effect rate in the ilaprazole group was lower than that in the control group, but the difference was not significant (9.7% *vs* 13.0%; RR = 0.81; 95%CI: 0.60-1.07; Z = 1.47; P = 0.14).

CONCLUSION: Ilaprazole is a highly effective and safe PPI in the treatment of duodenal ulcers. Ilaprazole can be recommended as a therapy for acid-related disorders, especially in Asian populations.

Key words: Ilaprazole; Proton pump inhibitor; Duodenal ulcer; Meta-analysis

Core tip: Ilaprazole, a proton pump inhibitor (PPI), is a newly developed medicine in the management of acid-related disorders. This meta-analysis showed that ilaprazole was a highly effective and safe PPI compared with other PPIs in the treatment of duodenal ulcer. Ilaprazole can be recommended as a therapy for acid-related disorders, especially in Asian populations.

Ji XQ, Du JF, Chen G, Chen G, Yu B. Efficacy of ilaprazole in the treatment of duodenal ulcers: A meta-analysis. *World J Gastroenterol* 2014; 20(17): 5119-5123 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5119.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5119

INTRODUCTION

Duodenal ulcer (DU) is a very common digestive disease with a high incidence all over the world[1-4]. As the first proton pump inhibitor (PPI), omeprazole has been used therapeutically for many years, and shown great efficacy in treating peptic ulcers[5-7]. Currently, research is focused on more effective PPIs with a lower dose and comparative safety[8-11].

Ilaprazole (also known as IY-81149), the latest pro-

ton pump inhibitor (PPI), has been less well reported in clinical practice, as a newly developed medicine in the management of acid related disorders[12,13]. Ilaprazole is synthesized by Il-Yang (South Korea) and presently developed by Livzon Pharmaceutical Group Inc. (China), and has been approved by the State Food and Drug Administration of China (license ID: CN 1121714A) with a recommended dose of 10 mg/d for peptic ulcers. The mechanism of ilaprazole's action to suppress gastric acid secretion is almost the same as omeprazole, in which the protonated substituted benzimidazoles suppress gastric acid secretion through inhibition of the H^+/K^+-ATPase at the secretory surfaces of gastric parietal cells[14,15].

Preclinical research found that ilaprazole had a more prolonged half-life and higher suppression of gastric acid secretion in a dose-dependent manner, and similar safety compared with omeprazole. A comparative pharmacodynamic study on patients with gastroesophageal reflux disease reported that ilaprazole, at a dose of 5 mg, provided gastric pH control comparable with the use of 20 mg omeprazole, and at doses of 10 and 20 mg it was found to have a more powerful and longer-lasting acid-suppressant effect than omeprazole at a dose 20 mg[16].

There have been several clinical trials comparing ilaprazole and other PPIs in the treatment of duodenal ulcer, which showed that ilaprazole had a high 4-wk healing rate[17-19]. The aim of the present study was to conduct a pooled meta-analysis of randomized controlled trials (RCT) comparing the efficacy and tolerance of ilaprazole with other PPIs in the treatment of duodenal ulcers.

MATERIALS AND METHODS

Literature search

Relevant studies were identified and selected by searching the databases, Medline (1990 to July 2013), Embase (1990 to July 2012), Cochrane controlled trials register (Cochrane Library Issue 2, 2013), Web of Science (1990 to July 2013), PubMed (updated to July 2013) and Chinese Biomedical Literature Database (1989 to July 2013) under the search term "ilaprazole". We also performed a full manual search from the bibliographies of each peer-reviewed paper selected. No language or date limitations were imposed. Furthermore, there was no limitation in publication form.

Study selection criteria

The selection criteria for inclusion in the meta-analysis were: (1) RCT comparing ilaprazole 10 mg/d and other PPIs in the treatment of duodenal ulcers; (2) duodenal ulcers must have been diagnosed by upper gastrointestinal endoscopy; (3) the patients should not receive other medical therapies before the trial, except the standard triple therapy for *Helicobacter pylori* (*H. pylori*) eradication; and (4) the duration of the trials should be 4 wk, and ulcer healing was also assessed by endoscopy after 4 wk of therapy. The decision to include or exclude any trial was made by 2 researchers separately. The 2 lists were compared and discrepancies were resolved.

Data extraction

Data were independently abstracted from each trial by 2 researchers, and disagreement was resolved by consensus. Data were extracted with a pre-designed review form. Data to be extracted were as follows: study design, number of patients in each treatment arm, duration of treatment, drug regimen, percentage of adverse effects, and quality score.

Quality of methodology

The methodological quality of studies included in the meta-analysis was scored with the Jadad composite scale (including items of randomization, double-blinding, and description of withdrawal/dropouts)[20,21]. This is a 5-point quality scale, with low quality studies having a score of $\leqslant$ 2 and high quality studies a score of $\geqslant$ 3[21,22]. Methodological quality assessment was independently performed by 2 of the present authors. Each study was given an overall quality score based on the above criteria, which was then used to rank studies.

Statistical analysis

The meta-analysis was performed using the Mantel-Haenszel method (fixed effects model) or the DerSimonian and Laird method (random effects model) with Review Manager Software (RevMan 5.1, Cochrane Collaboration, Oxford, England). The relative risk (RR) for each clinical event was presented with 95% confidence interval (CI). Heterogeneity was tested using the χ^2 test (with $P \leqslant 0.05$ indicating significant heterogeneity) and I^2 test (25%, 50%, and 75%, represent low, moderate, and high heterogeneity, respectively). The RR for each clinical event was pooled with the fixed effects model, and if the χ^2 test and I^2 test for heterogeneity were significant, the analysis was also done with random effects model.

RESULTS

Description of selected studies

The search strategy generated 32 studies. From these, we identified 5 trials involving 1481 patients comparing ilaprazole with other PPIs in the treatment of duodenal ulcer, which fulfilled the criteria for the meta-analysis. Four papers were published as peer-reviewed articles, and one as a meeting abstract[18]. Four were published in English and the other was published in Chinese[19]. The baseline characteristics of the 5 articles are listed in Table 1. All the trials were based on intention-to-treat analysis. All trials were of high quality except one[18] (Table 1). The results of the 5 trials are shown in Table 2[17-19,23,24].

Meta-analysis

We first compared ilaprazole at the standard dose 10 mg/d with other PPIs on the 4-wk healing rate and rate of adverse effects (Figure 1). There was no statistical heterogeneity in the 4-wk healing rate among the 5 trials and

Table 1 Baseline characteristics of trials included in the meta-analysis

Ref.	Language	Publication type	Time	Patients (*n*)	Duration (wk)	Jadad score
Ho *et al*[17], 2009	English	Full text	2002-2004	202	4	5
Zhou *et al*[19], 2009	Chinese	Full text	2005-2006	510	4	5
Song *et al*[18], 2010	English	Abstract	Not reported	156	4	2
Wang *et al*[23], 2011	English	Full text	2004-2005	117	4	5
Wang *et al*[24], 2012	English	Full text	2005-2006	496	4	5

Table 2 Results of the randomized controlled trials with intention-to-treat analysis

Ref.	Regimen	4-wk healing rate	Rate of adverse effects
Ho *et al*[17], 2009	I 10 mg/d	78.6% (77/98)	23.5% (23/98)
	O 20 mg/d	78.8% (82/104)	22.1% (23/104)
Zhou *et al*[19],2009	I 10 mg/d	90.3% (307/340)	8.2% (28/340)
	O 20 mg/d	87.6% (149/170)	11.2% (19/170)
Song *et al*[18], 2010	I 10 mg/d	85.9% (67/78)	6.4% (5/78)
	E 40 mg/d	87.2% (68/78)	7.5% (6/78)
Wang *et al*[23], 2011	I 10 mg/d	93.1% (54/58)	6.9% (4/58)
	O 20 mg/d	89.8% (53/59)	13.6% (8/59)
Wang *et al*[24], 2012	I 10 mg/d	95.0% (307/331)	8.5% (28/331)
	O 20 mg/d	90.0% (149/165)	11.6% (19/165)

I: Ilaprazole; O: Omeprazole; E: Esomeprazole.

the fixed effects model was used ($\chi^2 = 0.62$; $P = 0.96$; $I^2 = 0\%$) The meta-analysis showed no difference between the ilaprazole and other PPIs in 4-wk healing rate (89.7% *vs* 87.0%; RR = 1.02; 95%CI: 0.98-1.06; $Z = 1.00$; $P = 0.32$). Regarding adverse effects, there was no statistical heterogeneity found by the χ^2 test ($\chi^2 = 1.96$; $P = 0.74$) or I^2 test ($I^2 = 0\%$), and the fixed effects model was used. The meta-analysis indicated that the rate of adverse effects in the ilaprazole group was lower than that in the control group, but the difference was not significant (9.7% *vs* 13.0%; RR = 0.81; 95%CI: 0.60-1.07; $Z = 1.47$; $P = 0.14$).

Sensitivity analysis

The funnel plots for the 4-wk healing rate comparing ilaprazole at a dose of 10 mg/d with other PPIs showed some asymmetry, suggesting the possibility of publication bias (Figure 2). Thus, we further performed a sensitivity analysis to assess the stability and reliability of the results of the primary meta-analysis (Table 3). The sensitivity analysis only included the 4 trials of high quality (Jaded score $\geqslant$ 3). The analysis indicated no difference in the 4-wk healing rate between 10 mg/d ilaprazole and other PPIs (RR = 1.02; 95%CI: 0.98-1.07; $Z = 1.16$; $P = 0.25$).

Four trials were published in English and the other was published in Chinese. A further sensitivity analysis was made only including the studies published in the English. The analysis revealed no difference between the 10 mg/d ilaprazole and other PPIs in the trials published in English (RR = 1.01; 95%CI: 0.97-1.07; $Z = 0.61$; $P = 0.54$).

A final sensitivity analysis was performed only including trials using omeprazole as control. The analysis indicated no difference between the ilaprazole at a dose of 10 mg/d and omeprazole (RR = 1.02; 95%CI: 0.98-1.07; $Z = 1.16$; $P = 0.25$).

DISCUSSION

PPIs are highly effective medications widely used in the management of peptic diseases including gastric and duodenal ulcers, gastroesophageal reflux disease and Zollinger-Ellison syndrome[25]. Many new therapeutic drugs with similar structures and better therapeutic outcomes have been developed since omeprazole first entered the market, including rabeprazole, pantoprazole, lansoprazole, esomeprazole, and the new molecule we studied in this analysis, ilaprazole. Because ilaprazole was currently only approved in a number of Asian countries, the clinical studies on ilaprazole were not regularly reported in international journals, and most were conducted in China and published in Chinese. Thus, this study aimed to perform a systematic review and meta-analysis on the effect of ilaprazole on the healing of duodenal ulcers.

The current standard dose of ilaprazole recommended for the management of peptic diseases is 10 mg/d. The meta-analysis showed no difference between 10 mg/d ilaprazole and other PPIs with standard or higher doses. In addition, the sensitivity analyses also confirmed the results of the primary meta-analysis. The meta-analyses documented that ilaprazole was a highly effective PPI compared with other PPIs.

Ilaprazole shows major suppression of gastric acid secretion. As an inhibitor of acid output, ilaprazole is more powerful than omeprazole. An experimental study in a surgically-induced rat reflux esophagitis model showed that ilaprazole had a much lower ED_{50} than omeprazole[26]. Ilaprazole at a dose of 5 mg provided gastric pH control comparable with 20 mg omeprazole[16].

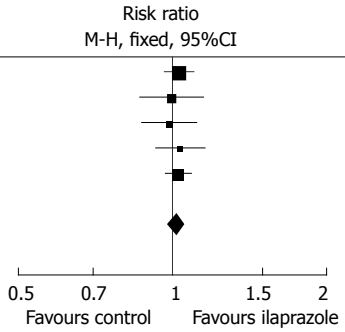
As for the safety and tolerability profile, the meta-analysis on adverse effects also revealed fewer adverse effects in the ilaprazole group, though the difference was not significant. Wang *et al*[23] reported that ilaprazole at a dose of 5, 10, or 20 mg/d is comparable to 20 mg/d omeprazole. Considering the rate of adverse effects of PPIs is low, and the adverse effects are usually mild, we may conclude that ilaprazole is a safe drug with minor adverse effects.

There were several limitations in this study. First, the low quality of 2 individual trials was a major limitation. Second, due to the fact that ilaprazole is only approved in Asian countries, the trials included in this study all come from Asian countries, and thus further trials are needed

A

Study or subgroup	Ilaprazole Events	Total	Control Events	Total	Weight	Risk ratio M-H, fixed, 95%CI	Year
Zhou 2009	307	340	149	170	33.2%	1.03 [0.96, 1.10]	2009
Ho 2009	77	98	82	104	13.3%	1.00 [0.86, 1.15]	2009
Song 2010	67	78	68	78	11.4%	0.99 [0.87, 1.12]	2010
Wang 2011	54	58	53	59	8.8%	1.04 [0.93, 1.16]	2011
Wang 2012	307	331	149	165	33.3%	1.03 [0.97, 1.09]	2012
Total (95%CI)		905		576	100.0%	1.02 [0.98, 1.06]	
Total events	812		501				

Heterogeneity: χ^2 = 0.62, df = 4 (P = 0.96); I^2 = 0%
Test for overall effect: Z = 1.00 (P = 0.32)



B

Study or subgroup	Ilaprazole Events	Total	Control Events	Total	Weight	Risk ratio M-H, fixed, 95%CI	Year
Ho 2009	23	98	23	104	25.7%	1.06 [0.64, 1.76]	2009
Zhou 2009	28	340	19	170	29.1%	0.74 [0.42, 1.28]	2009
Song 2010	5	78	6	78	6.9%	0.83 [0.27, 2.62]	2010
Wang 2011	4	58	8	59	9.1%	0.51 [0.16, 1.60]	2011
Wang 2012	28	331	19	165	29.2%	0.73 [0.42, 1.28]	2012
Total (95%CI)		905		576	100.0%	0.81 [0.60, 1.07]	
Total events	88		75				

Heterogeneity: χ^2 = 1.96, df = 4 (P = 0.74); I^2 = 0%
Test for overall effect: Z = 1.47 (P = 0.14)

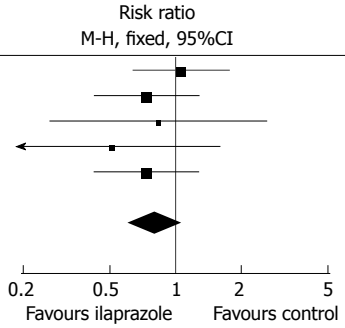


Figure 1 Meta-analysis chart. A: Meta-analysis of 4-wk healing rate comparing ilaprazole at 10 mg/d with other proton pump inhibitors (PPIs); B: Meta-analysis of adverse effects comparing 10 mg/d ilaprazole with other PPIs.

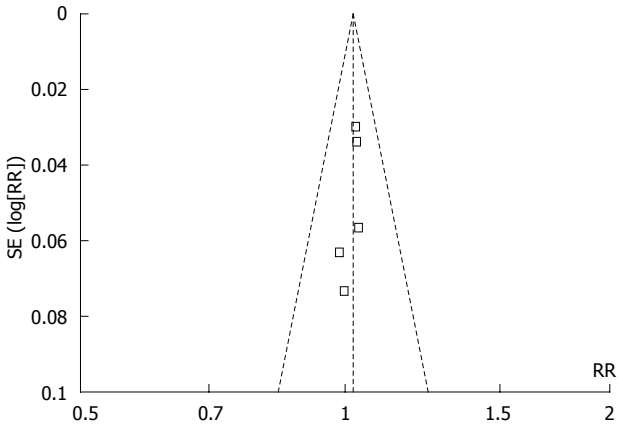


Figure 2 Funnel plot of the included trials comparing 10 mg/d ilaprazole with other proton pump inhibitors.

in Western populations. Third, there were few trials comparing ilaprazole at a dose of 5 mg/d with other PPIs.

In conclusion, ilaprazole is a highly effective and safe PPI in the treatment of duodenal ulcers. Ilaprazole can be recommended as a therapy for acid-related disorders, especially in Asian populations.

COMMENTS

Background

Ilaprazole, the latest proton pump inhibitor (PPI), is a newly developed medicine in the management of acid related disorders.

Research frontiers

There have been several clinical trials comparing ilaprazole and other PPIs in the treatment of duodenal ulcers which showed that ilaprazole had a high 4-wk healing rate.

Table 3 Sensitivity analysis of the included trials

Analysis	*n*	RR (95%CI)	*P* value
High quality studies	4	1.02 (0.98–1.07)	0.25
English studies	4	1.02 (0.97–1.07)	0.54
Studies using omeprazole as control	4	1.02 (0.98–1.07)	0.25

RR: Relative risk.

Innovations and breakthroughs

The authors conducted a meta-analysis of randomized controlled trials comparing the efficacy and tolerance of ilaprazole with other PPIs in the treatment of duodenal ulcer.

Applications

Ilaprazole is a highly effective and safe PPI in the treatment of duodenal ulcer. Ilaprazole can be recommended as a therapy for acid-related disorders, especially in Asian populations.

Peer review

This study evaluated the efficacy and tolerance of ilaprazole with other PPIs in the treatment of duodenal ulcer by conducting a meta-analysis. The findings are useful in the management of duodenal ulcer.

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META-ANALYSIS

Association between esophageal cancer risk and *EPHX1* polymorphisms: A meta-analysis

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Author contributions: Li QT, Kang W and Wang M contributed equally to this work; Su DK designed the meta-analysis; Li QT and Kang W performed the literature search and data extraction; Yang J, Zuo Y and Zhang W performed the data analysis; Li QT, Kang W and Wang M wrote the manuscript.

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Abstract

AIM: To summarize the relationship between p.Tyr113His and p.His139Arg polymorphisms in microsomal epoxide hydrolase (*EPHX1*) and risk for esophageal cancer (EC).

METHODS: The MEDLINE/PubMed and EMBASE databases were searched for studies of the association between *EPHX1* polymorphisms and EC risk that were published from the database inception date to April 2013. A total of seven case-control studies, including seven on p.Tyr113His (cases, *n* = 1118; controls, *n* = 1823) and six on p.His139Arg (cases, *n* = 861; controls, *n* = 1571), were included in the meta-analysis. After data extraction by two investigators working independently, the meta-analyses were carried out with STATA 11.0 software. Pooled odds ratios and 95%CI were calculated using a fixed-effects model or a random-effects model, as appropriate.

RESULTS: The pooled *EPHX1* p.Tyr113His polymorphism data showed no significant association with EC in any of the genetic models (OR = 1.00, 95%CI: 0.70-1.48 for Tyr/His *vs* Tyr/Tyr; OR = 1.10, 95%CI: 0.77-1.57 for His/His *vs* Tyr/Tyr; OR = 1.06, 95%CI: 0.75-1.49 for a dominant model; OR = 1.09, 95%CI: 0.89-1.34 for a recessive model). Similar results were obtained from the p.His139Arg polymorphism analysis (Arg/His *vs* His/His: OR = 1.02, 95%CI: 0.84-1.23; Arg/Arg *vs* His/His: OR = 0.96, 95%CI: 0.60-1.54; OR = 1.03, 95%CI: 0.78-1.37 for the dominant model; OR = 0.97, 95%CI: 0.61-1.56 for the recessive model). Subgroup analyses for ethnicity, subtype of EC, and source of controls (population-based or hospital-based) showed trends that were consistent with the pooled analysis (reported above), with no significant associations found.

CONCLUSION: This meta-analysis suggests that the p.Tyr113His and p.His139Arg polymorphisms in *EPHX1* may not be associated with EC development.

Key words: Esophageal cancer; Squamous cell carcinoma; Adenocarcinoma; *EPHX1*; Polymorphism; Meta-analysis

Core tip: A meta-analysis was performed to determine if the p.Tyr113His and p.His139Arg polymorphisms in microsomal epoxide hydrolase (*EPHX1*) are associated with an increased risk for esophageal cancer (EC). A total of seven studies of the association between EC risk and the *EPHX1* polymorphisms (p.Tyr113His in seven and p.His139Arg in six) were included in the analysis. No significant association was found in any of the genetic models for the p.Tyr113His polymorphism in *EPHX1* and EC. Similar results were obtained from the p.His139Arg polymorphism analysis. Subgroup analyses for ethnicity, subtype of EC, and source of controls also showed no significant association of *EPHX1* polymorphisms with EC risk.

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INTRODUCTION

Esophageal cancer (EC) is one of the most common fatal malignancies[1] and the sixth leading cause of cancer deaths worldwide[2]. Two histological subtypes of EC are characterized: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EADC). The pathogenesis of EC, however, remains poorly understood. Previous epidemiological studies have indicated that exposure to environmental carcinogens plays an important role in the development of EC[3,4].

Genetic susceptibility, in the form of phase I and phase Ⅱ metabolizing enzyme polymorphisms, may also be associated with an increased risk for EC[5]. Microsomal epoxide hydrolase (EPHX1) plays a dual role in the response to environmental carcinogens, in that EPHX1 both activates and detoxifies toxins. In response to environmental carcinogens, EPHX1 not only produces trans-dihydrodiols that can be metabolized to mutagenic, poisonous and carcinogenic polycyclic hydrocarbon diol epoxides, but also generates products necessary for the detoxification reaction. It has been shown previously that EPHX1 can catalyze hydrolysis of alkene and arene oxides to water-soluble trans-dihydrodiols[6,7].

The *EPHX1* gene, located on chromosome 1q42, is expressed in nearly all human tissues. EPHX1 activity varies widely among individuals, though the molecular basis of this variability is not fully understood. Genetic polymorphisms in exon 4 (A>G, p.His139Arg) and exon 3 (T>C, p.Tyr113His) of *EPHX1*, however, have been shown to alter the protein's function. In exon 4, the 139Arg polymorphism enhances EPHX1 activity by 25%. Alternatively, the 113His allele in exon 3 has a negative effect on enzymatic activity, reducing it by at least 50%[8]. Polymorphisms that alter EPHX1 enzyme activity may then lead to inter-individual differences in sensitivity to chemical carcinogens.

To date, a number of studies have investigated the association between *EPHX1* polymorphisms and EC risk in different populations[5,9-16]. The results, however, have been conflicting. In order to establish a comprehensive estimation of the association between *EPHX1* polymorphisms and EC risk, we conducted a meta-analysis of all available published studies.

MATERIALS AND METHODS

Data sources and search strategy

We searched the MEDLINE/PubMed and EMBASE databases for all articles published on the association between *EPHX1* polymorphisms and EC risk from the database inception date to April 2013. The following search terms were used: microsomal epoxide hydrolase, *EPHX1*, esophageal cancer, and polymorphism. No restrictions were applied. The references section of reviews and retrieved articles were searched in an effort to identify any additional eligible studies. If identified articles overlapped or were duplicated, only the most recent, largest or most complete study was selected.

Inclusion and exclusion criteria

We reviewed titles and abstracts of all citations and retrieved studies. The inclusion criteria were as follows: (1) case-control studies conducted to evaluate the association between *EPHX1* (p.Tyr113His and/or p.His139Arg) polymorphisms and EC risk; (2) sufficient genotype or allele data were presented to calculate the odds ratios (ORs) with 95% confidence intervals (CIs); and (3) the paper clearly described the sources of cases and controls. The following exclusion criteria were applied due to insufficient data for analysis: reviews, editorials, commentaries, and duplicated studies.

Data extraction

Two investigators (Li QT and Kang W) independently extracted relevant data from all eligible publications meeting the inclusion criteria. Disagreements were resolved by discussion. The characteristics of each included study were collected as follows: the first author's name, year of publication, the country of participants, participants' ethnicity, number of cases and controls, source of control group [*e.g.*, population-based (PB) or hospital-based (HB)], and genotypes of cases and controls. For our analysis, a PB case-control study was defined by the use of controls obtained from the general population, while an HB case-control study had obtained controls from a hospitalized patient population. In addition, we contacted the authors to collect further information when necessary.

Statistical analysis

The strength of the association between EC risk and *EPHX1* polymorphisms was estimated using ORs, with corresponding 95%CIs. For the *EPHX1* p.Tyr113His polymorphism, we assessed the association using a codominant model (His/His *vs* Tyr/Tyr and Tyr/His *vs* Tyr/Tyr), a dominant model (His/His + Tyr/His *vs* Tyr/Tyr), and a recessive model (His/His *vs* Tyr/His + Tyr/Tyr). The same models were used in the *EPHX1* p.His139Arg analysis.

Both the Cochran's Q test for heterogeneity[17] and the I^2 test to quantify the proportion of the total variation due to heterogeneity[18] were calculated. A P value less than 0.10 for the Q statistic indicated that heterogeneity was observed across studies and a random-effects model (the DerSimonian and Laird method) was used[19]; otherwise, a fixed-effects model (the Mantel-Haenszel method) was applied[20]. Subgroup analyses for ethnicity, subtype of EC, and source of controls (HB or PB) were conducted. Sensitivity analyses were conducted to estimate

Table 1 Characteristics of the studies included in the meta-analysis

Study	Year	Country	Ethnicity	Cases	Controls	Source of controls	Type of cancer	DNA source
Dura *et al*[9]	2012	The Netherlands	Caucasian	349	581	PB	ESCC and EADC	Blood and tissue samples
Ihsan *et al*[10]	2010	India	Asian	142	185	PB	ESCC	Blood samples
Jain *et al*[11]	2008	India	Asian	107	320	PB	ESCC	Blood samples
Casson *et al*[16]	2006	Canada	Caucasian	56	95	HB	EADC	Blood samples
Lin *et al*[13]	2006	China	Asian	145	352	PB	ESCC	Blood samples
Zhang *et al*[14]	2003	China	Asian	257	252	HB	ESCC	Blood samples
Wang *et al*[15]	2003	China	Asian	62	38	PB	ESCC	Tissue samples

EPHX1: Microsomal epoxide hydrolase; ESCC: Esophageal squamous cell carcinoma; EADC: Esophageal adenocarcinoma; PB: Population-based controls; HB: Hospital-based controls.

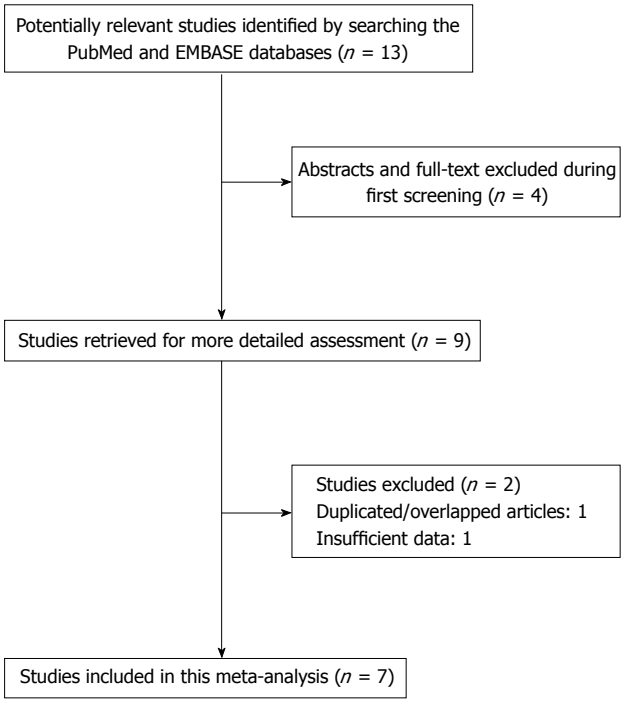

Figure 1 Flow diagram of the study selection strategy for this meta-analysis.

the stability of the results such that each study was omitted one at a time to reflect the influence of the individual data set on the pooled OR.

Potential publication bias was assessed by visual inspection of funnel plot asymmetry, the Begg's rank correlation method[21] and the Egger's weighted regression method[22]. All statistical analyses were conducted using the STATA software, version 11.0 (STATA Corp., College Station, TX, United States). All *P* values were two-sided.

RESULTS

Study selection for the meta-analysis

We preliminarily identified 13 studies based on the search terms applied. After the abstracts were screened and the full text of each article was assessed, a total of nine articles met the inclusion criteria. However, one article by Wang *et al*[12] was excluded due to insufficient data and another study conducted by Casson *et al*[16] was excluded because of the use of overlapping subjects. As a result, seven case-control studies[8-10,12-15] were included in this meta-analysis (Figure 1).

Characteristics of the included studies

The included studies were all reported in English. Five articles utilized Asian populations and two utilized Caucasian populations. Five of the studies obtained DNA for genotyping from blood samples, while two studies obtained DNA from EC tissue samples. To analyze the ESCC subtype, six studies were eligible with a total sample size of 804 cases and 1147 controls. To analyze the EADC subtype, two studies were pooled for analysis and included 314 cases and 676 controls. The main characteristics of all included studies are presented in Table 1.

EPHX1 p.Tyr113His polymorphism and EC risk

To analyze the *EPHX1* p.Tyr113His polymorphism in relation to EC risk, seven studies with a total of 1118 cases and 1823 controls were pooled for analysis. The combined results showed that no significant association was observed in any of the genetic models applied (Figure 2; Table 2). The subgroup analysis for ethnicity, EC subtype, and source of controls also showed no evidence of association in any of the genetic models (Table 2).

EPHX1 p.His139Arg polymorphism and EC risk

Six studies with 861 cases and 1571 controls were eligible to analyze the association of the *EPHX1* p.His139Arg polymorphism and EC risk. The *EPHX1* p.His139Arg polymorphism analysis showed that the Arg allele had no significant association with EC susceptibility when compared to the His wild-type allele (Table 3). Similar results were obtained from the subgroup analysis for ethnicity, EC subtype, and source of controls; the results are summarized in Table 3.

Sensitivity analysis

We conducted a sensitivity analysis to assess the stability of this meta-analysis. When any one study was omitted, the results were not altered (data not shown). These data suggest that our results are stable and credible.

Publication bias

After performing the Begg's test and the Egger's test for publication bias, we observed no obvious bias in this me-

Table 2 Distribution of epoxide hydrolase p.Tyr113His genotypes in controls and esophageal cancer patients

Variable	Studies	Cases/controls	OR (95%CI)	*P* value	*P* for heterogeneity	I^2	Model
Tyr/His *vs* Tyr/Tyr							
Total	7	1118/1823	1.00 (0.70-1.48)	0.928	0.001	72.8	R
Caucasian	2	405/676	0.92 (0.71-1.20)	0.555	0.192	41.2	F
Asian	5	713/1147	1.14 (0.65-2.00)	0.638	0.001	79.9	R
ESCC	6	804/1147	1.08 (0.69-1.69)	0.746	0.001	75.6	R
EADC	2	314/676	0.95 (0.71-1.26)	0.729	0.160	49.2	F
PB	5	805/1476	1.04 (0.66-1.66)	0.857	0.001	78.3	R
HB	2	313/347	0.95 (0.39-2.28)	0.902	0.057	72.4	R
His/His *vs* Tyr/Tyr							
Total	7	1118/1823	1.10 (0.77-1.57)	0.592	0.046	53.3	R
Caucasian	2	405/676	0.74 (0.48-1.13)	0.165	0.172	46.5	F
Asian	5	713/1147	1.23 (0.94-1.60)	0.137	0.120	45.3	F
ESCC	6	804/1147	1.18 (0.92-1.52)	0.196	0.158	37.2	F
EADC	2	314/676	0.72 (0.45-1.15)	0.163	0.183	43.7	F
PB	5	805/1476	1.18 (0.78-1.78)	0.423	0.079	52.1	R
HB	2	313/347	0.78 (0.24-2.58)	0.688	0.034	77.7	R
His/His + Tyr/His *vs* Tyr/Tyr							
Total	7	1118/1823	1.06 (0.75-1.49)	0.753	0.001	74.0	R
Caucasian	2	405/676	0.76 (0.42-1.38)	0.367	0.088	65.6	R
Asian	5	713/1147	1.23 (0.77-1.98)	0.382	0.001	77.8	R
ESCC	6	804/1147	1.15 (0.78-1.70)	0.484	0.002	74.0	R
EADC	2	314/676	0.77 (0.41-1.46)	0.421	0.074	68.6	R
PB	5	805/1476	1.13 (0.74-1.70)	0.575	0.002	77.0	R
HB	2	313/347	0.87 (0.33-2.26)	0.769	0.017	82.4	R
His/His *vs* Tyr/His + Tyr/Tyr							
Total	7	1118/1823	1.09 (0.89-1.34)	0.416	0.188	31.4	F
Caucasian	2	405/676	0.77 (0.51-1.16)	0.213	0.281	13.9	F
Asian	5	713/1147	1.23 (0.97-1.55)	0.093	0.388	3.3	F
ESCC	6	804/1147	1.20 (0.95-1.50)	0.121	0.469	0.0	F
EADC	2	314/676	0.91 (0.29-2.84)	0.878	0.041	76.1	R
PB	5	805/1476	1.14 (0.89-1.45)	0.294	0.210	31.8	F
HB	2	313/347	0.97 (0.66-1.43)	0.888	0.111	60.6	F

EPHX1: Microsomal epoxide hydrolase; OR: Odds ratio; R: Random-effects model; F: Fixed-effects model; ESCC: Esophageal squamous cell carcinoma; EADC: Esophageal adenocarcinoma; PB: Population-based controls; HB: Hospital-based controls.

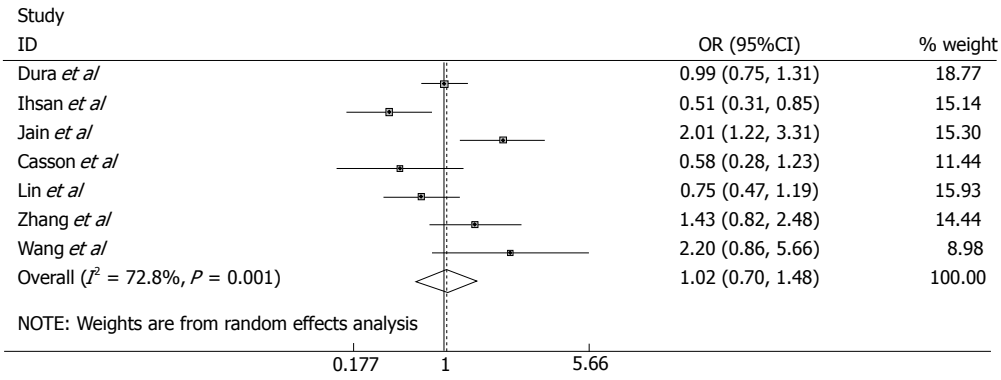

Figure 2 Odds ratios with 95%CI for the microsomal epoxide hydrolase p.Tyr113His polymorphism and risk of esophageal cancer (Tyr/His *vs* Tyr/Tyr).

ta-analysis (Begg's test, $P = 1.00$; Egger's test, $P = 0.852$) (Figure 3).

DISCUSSION

Summary of results

In recent years, there has been increased interest in investigating the potential association of *EPHX1* polymorphisms and susceptibility to EC. Because the results of these studies have been inconsistent, it is necessary to perform a meta-analysis of the studies performed to date. In the meta-analysis presented herein, seven studies on *EPHX1* polymorphisms were analyzed to provide the most comprehensive assessment to date of the association between *EPHX1* polymorphisms and EC risk. We observed no significant association of the p.Tyr113His and p.His139Arg polymorphisms in *EPHX1* with EC risk, even when a subgroup analysis for ethnicity, EC

Table 3 Distribution of the microsomal epoxide hydrolase p.His139Arg genotypes in controls and esophageal cancer patients

Variable	Studies	Cases/controls	OR (95%CI)	*P* value	*P* for heterogeneity	I^2	Model
Arg/His *vs* His/His							
Total	6	861/1571	1.02 (0.84-1.23)	0.869	0.176	34.8	F
Caucasian	2	405/676	0.97 (0.74-1.27)	0.824	0.955	0.0	F
Asian	4	456/895	1.09 (0.69-1.73)	0.709	0.060	59.4	R
ESCC	5	547/1319	0.99 (0.67-1.46)	0.954	0.052	57.5	R
EADC	2	314/676	1.05 (0.79-1.40)	0.718	0.755	0.0	F
PB	5	805/1476	1.02 (0.84-1.24)	0.833	0.106	47.5	F
HB	1	56/95	0.95 (0.47-1.91)	NA	NA	NA	NA
Arg/Arg *vs* His/His							
Total	6	861/1571	0.96 (0.60-1.54)	0.855	0.206	30.6	F
Caucasian	2	405/676	0.61 (0.31-1.21)	0.158	0.308	3.9	F
Asian	4	456/895	1.59 (0.80-3.16)	0.184	0.324	13.6	F
ESCC	5	547/1319	1.28 (0.72-2.30)	0.402	0.346	10.6	F
EADC	2	314/676	0.60 (0.28-1.27)	0.178	0.313	1.8	F
PB	5	805/1476	1.08 (0.66-1.76)	0.765	0.251	25.5	F
HB	1	56/95	0.22 (0.03-1.90)	NA	NA	NA	NA
Arg/Arg + Arg/His *vs* His/His							
Total	6	861/1571	1.03 (0.78-1.37)	0.810	0.095	46.6	R
Caucasian	2	405/676	0.92 (0.71-1.20)	0.555	0.719	0.0	F
Asian	4	456/895	1.14 (0.71-1.81)	0.593	0.042	63.5	R
ESCC	5	547/1319	1.02 (0.68-1.53)	0.918	0.028	63.1	R
EADC	2	314/676	1.00 (0.75-1.31)	0.976	0.550	0.0	F
PB	5	805/1476	1.07 (0.78-1.48)	0.676	0.062	55.5	R
HB	1	56/95	0.82 (0.42-1.62)	NA	NA	NA	NA
Arg/Arg *vs* Arg/His + His/His							
Total	6	861/1571	0.97 (0.61-1.56)	0.911	0.247	25.0	F
Caucasian	2	405/676	0.62 (0.31-1.21)	0.163	0.307	4.0	F
Asian	4	456/895	1.66 (0.84-3.30)	0.148	0.454	0.0	F
ESCC	5	547/1319	1.36 (0.76-2.45)	0.297	0.480	0.0	F
EADC	2	314/676	0.58 (0.28-1.24)	0.160	0.328	0.0	F
PB	5	805/1476	1.10 (0.67-1.80)	0.704	0.312	16.1	F
HB	1	56/95	0.23 (0.03-1.91)	NA	NA	NA	NA

EPHX1: Microsomal epoxide hydrolase; OR: Odds ratio; R: Random-effects model; F: Fixed-effects model; ESCC: Esophageal squamous cell carcinoma; EADC: Esophageal adenocarcinoma; PB: Population-based controls; HB: Hospital-based controls; NA: Not available.

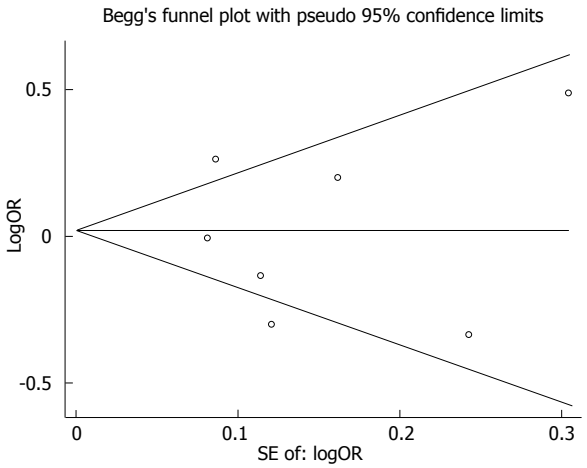

Figure 3 Funnel plot of the *EPHX1* p.Tyr113His polymorphism to determine if publication bias was present (Tyr/His *vs* Tyr/Tyr).

subtype, or source of controls was performed.

Explanations for absence of EC association with the EPHX1 polymorphisms

The ESCC and EADC EC subtypes are reflected histologically by the progression from metaplasia to dysplasia to carcinoma. The recent identification of molecular markers lends further insight into the molecular pathogenesis of the different EC subtypes and there is no distinction between ESCC and EADC.

We found that the *EPHX1* p.Tyr113His and p.His139Arg polymorphisms were not associated with EC risk. Previous studies have also found no association between EC and polymorphisms in the cytochrome oxidase genes of *CYP1A1*, *CYP1B1*, *CYP2A6* and *CYP2E1*, the glutathione S transferase genes of *GSTM1* and *GSTP1* or *EPHX*[4]. Concurrently, other studies have demonstrated that polymorphisms in *EPHX1* are risk factors for hepatocellular carcinoma, colorectal cancer, lung cancer, and cervical cancer. Zhong *et al*[23], however, demonstrated that the p.His139Arg microsomal epoxide hydrolase genotype may not be associated with hepatocellular carcinoma, while Liu *et al*[24] provided data that the *EPHX1* p.Tyr113His polymorphism had no association with colorectal cancer development.

We performed a subgroup analysis for ethnicity[5,25,26] and observed no difference in the association between the polymorphisms and EC risk in Caucasians or Asians. The lack of an observed association may be due to the limited number of studies included in this meta-analysis.

The allele and genotype frequencies of polymorphisms and their effects on EC risk varied in different ethnicities. Larger and well-designed multi-center studies using Caucasian and Asian populations are needed to re-evaluate such an association. Moreover, different sources of controls may be a confounding factor that influenced the conclusion of our study.

Some studies in this meta-analysis used PB controls as the reference group, while others used HB controls as the reference group. In order to eliminate the potential bias from this confounding factor, subgroup analysis by source of controls was conducted. The pooled results indicated that no significant association between *EPHX1* (p.His139Arg and p.Arg139His) polymorphisms and EC risk was observed in PB or HB studies. HB studies are prone to selection biases because some of the controls may actually be ill (so that they are more similar to the cases). HB controls are not representative of the general population, especially when the investigated genotypes were patient controls. A proper PB control subject may be the superior choice to reduce potential biases in genetic association studies.

Potential confounders

In the current study, a Begg's funnel plot and the Egger's test were performed to assess potential publication bias. The funnel plots were symmetric in shape and the statistical results did not reveal any publication bias. Moreover, the results were consistent when the sensitivity analysis was performed, which implied that the results were reliable.

Limitations

Several limitations should be acknowledged for this analysis. Heterogeneity is a potential problem when interpreting the results of a meta-analysis and the sources of heterogeneity are usually explored in most meta-analyses. EC is a multi-factorial disease and potential gene-gene and gene-environment interactions should be considered. Different ethnicities have diverse genetic backgrounds and varied environmental exposures. In the present study, significant heterogeneity was detected in overall comparisons for the *EPHX1* polymorphisms. Although we performed a careful database search for published studies, used strict criteria for study inclusion, and performed precise data extraction and original data analysis, significant heterogeneity still existed in some of our comparisons. However, in the subgroup analysis for ethnicity, the significant heterogeneity persisted in some genetic models in both the European and Asian populations. Small sample size may have contributed to limited statistical power to estimate the possible EC risk with *EPHX1* polymorphisms. A consortium based on thousands of individuals would be more ideal for this type of association study.

This meta-analysis suggests that the *EPHX1* p.Tyr113His and p.His139Arg polymorphisms may be not associated with EC development. Further studies with a larger sample size are needed to further assess the presence of an association.

COMMENTS

Background

Esophageal cancer (EC) is the sixth leading cause of cancer death, but the underlying pathogenic mechanism is not yet fully elucidated. Several studies have investigated the potential association between polymorphisms in the microsomal epoxide hydrolase (*EPHX1*) gene and susceptibility to EC development. Polymorphisms in *EPHX1* alter its enzymatic activity and may thus lead to inter-individual differences in sensitivity to chemical carcinogens. However, the association of these polymorphisms with genetic susceptibility is currently unclear.

Research frontiers

Over the past two decades, many studies have been performed in diverse populations to determine if associations exist between *EPHX1* polymorphisms and risk for EC. The results, however, have been conflicting and no consistent conclusion has been reached.

Innovations and breakthroughs

The findings in this meta-analysis are of great value. The *EPHX1* p.Tyr113His and p.His139Arg polymorphisms may be not associated with EC development. Subgroup analyses for ethnicity, subtype of EC, and source of controls [hospital-based (HB) or population-based (PB)] were conducted; yet, no significant association was observed for any of these subgroups. No evidence of publication bias was found.

Applications

EPHX1 p.Tyr113His and p.His139Arg polymorphisms, ethnicity, subtype of EC, and source of controls (HB/PB) may be not associated with EC development. An exploration of this association may not be relevant to EC development.

Terminology

EPHX1 is a critical biotransformation enzyme that converts epoxides to trans-dihydrodiols during aromatic compound degradation. The epoxides can be conjugated and excreted from the body. EPHX1 functions in both the activation and detoxification of epoxides.

Peer review

This manuscript presents a well-performed meta-analysis that assesses the association of two *EPHX1* polymorphisms with esophageal cancer risk. The authors show clearly that neither of the *EPHX1* polymorphisms, p.Tyr113His or p.His139Arg, is associated with an increased risk for EC.

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CASE REPORT

One case of intrahepatic cholangiocarcinoma amenable to resection after radioembolization

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Abstract

We report the case of a 57-year-old man who was diagnosed with a large unresectable cholangiocarcinoma associated with 2 satellite nodules and without clear margins with the right hepatic vein. Despite 4 cycles of GEMOX (stopped due to a hypertransaminasemia believed to be due to gemcitabine) and 4 cycles of FOLFIRINOX, the tumor remained stable and continued to be considered unresectable. Radioembolization (resin microspheres, SIRS-spheres®) targeting the left liver (474 MBq) and segment IV (440 MBq) was performed. This injection was very well tolerated, and 4 more cycles of FOLFIRINOX were given while waiting for radioembolization efficacy. On computed tomography scan, a partial response was observed; the tumor was far less hypervascularized, and a margin was observed between the tumor and the right hepatic vein. A left hepatectomy enlarged to segment Ⅷ was performed. On pathological exam, most of the tumor was acellular, with dense fibrosis around visible microspheres. Viable cells were observed only at a distance from beads. Radioembolization can be useful in the treatment of cholangiocarcinoma, allowing in some cases a secondary resection.

Key words: Intrahepatic cholangiocarcinomal; Radioembolization; Surgery; Combined treatments

Core tip: A 57-year-old man with abdominal pain was diagnosed with a large unresectable hepatic tumor. On liver biopsy, this intrahepatic cholangiocarcinoma was observed within the normal liver parenchyma. After 2 systemic chemotherapy regimens, the tumor remained stable. A radioembolization (SIRS-Spheres®) delivering 120 Gy to the tumor, 7 Gy to the normal liver and 4 Gy to the lungs was performed. Three months later, the tumor was less vascularized and had shrunk, and a resection could be performed. On pathological examination, most of the tumor was acellular with fibrosis centered on microspheres, and only a few viable cells were noticed.

Servajean C, Gilabert M, Piana G, Monges G, Delpero JR, Brenot I, Raoul JL. One case of intrahepatic cholangiocarcinoma amenable to resection after radioembolization. *World J Gastroenterol* 2014; 20(17): 5131-5134 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5131.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5131

INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer[1,2] after hepatocellular carcinoma, with approximately 10000 new cases/year in Europe[3], and exhibits a dismal prognosis. The incidence of ICC is growing in many countries[4]. Cure can be expected only from surgical resection[5] and in the early stages. However, the vast majority of patients presents with advanced disease or experience tumor recurrence after initial resection. In locally advanced or metastatic patients, systemic chemotherapy combining cisplatin and gemcitabine is the current gold standard[6], but tumor response and overall survival remain poor. Intra-arterial treatment in locally advanced unresectable cases seems promising in cases of liver-confined disease. Radioembolization with 90Y-loaded beads has been reported in a few studies as efficient in ICC. We present the case of a locally advanced ICC receiving systemic chemotherapy without major efficacy, followed by treatment with 90Y radioembolization (resin microspheres) that permitted resection with major (near complete) histologic response related histologically to the radioembolization.

CASE REPORT

A 57-year-old man without any past history presented with abdominal pain on his right side in December 2011. Ultrasound (US) and computed tomography (CT) scan demonstrated a large tumor on the median part of the liver without any abdominal lymph nodes or extrahepatic tumors. Alpha-fetoprotein levels were 29 ng/mL (ULN = 5 mg/mL), and carcinoembryonic antigen and carbohydrate antigen 19-9 serum levels were normal. On magnetic resonance imaging, an 11-cm nodule with two satellite tumors was identified. The main tumor was invading the left portal pedicle and the left and median supra-hepatic veins and exhibited no security margin with the right supra-hepatic vein (Figure 1A) and the right hepatic artery. Colonoscopy and gastroscopy were normal. Pathological analysis of the liver biopsy confirmed an ICC; the surrounding liver parenchyma was normal.

The patient was treated with a GEMOX regimen (gemcitabine 1000 mg/m² D1 and oxaliplatin 100 mg/m² D2) every 2 wk. After the fourth cycle, the appearance of hepatic cytolysis likely due to gemcitabine led us to stop this treatment and to shift to a FOLFIRINOX regimen. After 4 cycles, the CT scan revealed a stable disease, and resection was considered impossible.

Local hepatic treatment with radioembolization was subsequently attempted. A biodistribution analysis of 99 mTc macroaggregated albumin injected in the target arteries did not reveal any lung shunting of extrahepatic uptake. The therapeutic injection was performed on 23rd July 2012 with two selective injections: one in the left hepatic artery of 474 Mbq of 90Y-resin microspheres (SIRS-Spheres®, Sirtex Medical, Lane Cove, Australia) and the other of 440 Mbq in the segment IV artery arising from the right hepatic artery. Dosimetry calculations

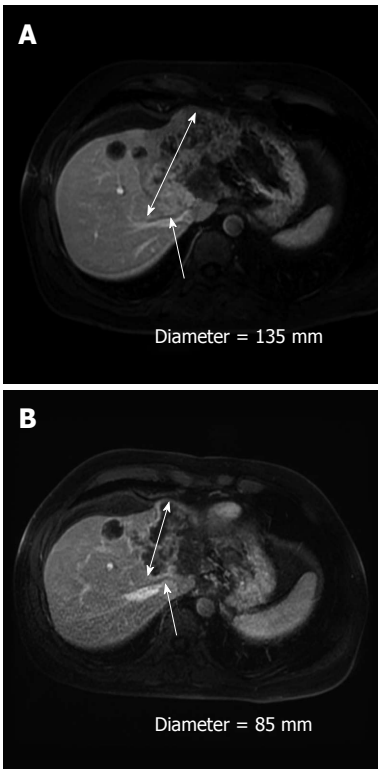

Figure 1 Magnetic resonance imaging. A: MRI before treatment revealing a large intrahepatic cholangiocarcinoma and 2 small satellites nodules; there was no clear margin with the right hepatic vein (arrows); B: After systemic chemotherapy and one intra-arterial injection of 90Y-resin microspheres, the tumor size decreased, the tumor appeared less vascularized, and the margin between the tumor and hepatic vein appeared free (arrows).

(BSA method) corresponded to 120 Gy delivered to the tumor, 7 Gy to the non-tumorous liver and 4 Gy to the lungs. No side effects were noted, and 4 more cycles of FOLFIRINOX were administered. In September 2012 (2 mo after the radioembolization), the CT scan revealed a partial response (Figure 1B), but at the arterial phase, the hypervascular component of the tumor had clearly declined, and a margin between the tumor and the right hepatic vein could be observed. The volume of the left lobe only slightly increased after radioembolization from 1323 mL up to 1420 mL.

A left hepatectomy enlarged to segment Ⅷ was performed on October 30th, 2012 without major difficulty. The pathological examination revealed that most of the tumor was composed of acellular, dense, collagen fibrosis with many beads included. The center of the tumor was entirely fibrotic; at the periphery of the tumor (Figure 2), some neoplastic cords could be identified in the fibrosis at a distance from beads; these cords were largely unicellular and sometimes organized around a glandular cavity. This response was classified as a major tumoral regression with a R0 resection. The patient was alive without evidence of recurrence 1 year after the surgery.

DISCUSSION

This case of partial radiologic tumor response allowing

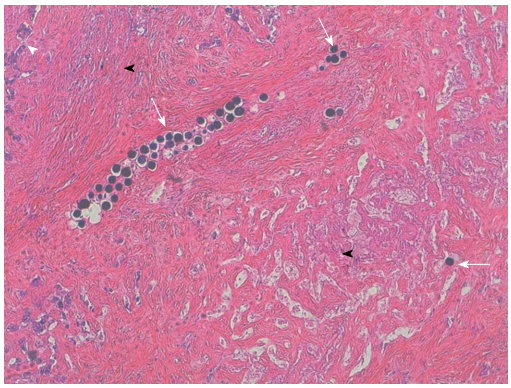

Figure 2 Pathologic examination. Pathologic examination of the resected specimen exhibiting multiple microspheres mainly in the vessels (arrows), the microspheres were surrounded by intense fibrosis (black arrow heads) with tumor cells only on the tumor periphery (white arrow head).

a complete R0 tumor resection and major histological tumor response after chemotherapy and one single radioembolization illustrates the usefulness of multidisciplinary approaches in locally advanced liver tumors, particularly ICC, and the efficacy of radioembolization. In this case, pathological examination revealed a close relationship between the presence of beads and severe necrosis/fibrosis; conversely viable tumor cells were only observed at the periphery of the tumor, at considerable distances from beads.

Radioembolization involves the injection of microspheres loaded with 90Y into the feeding artery. These spheres had a diameter ranging from 25 to 60 μm. Currently, two different types of microspheres are available, glass (TheraSphere®, Nordion, Canada) and resin (SIR Sphere, SIRTEX, Australia), and these differ in size and activity per sphere, which is important for glass spheres but less important for resin microspheres. This treatment achieves the microembolization of tumorous vessels and delivers local irradiation; 90Y is a very energetic isotope with a cytotoxic range of several millimeters (median 2.5 mm) and a short half-life of 64.2 h. This isotope is only a beta emitter, and patients can be discharged the same day. This treatment is a therapeutic option in hepatocellular carcinoma and in hepatic colorectal metastases. Large-scale randomized trials are ongoing to determine the best place for these loco-regional treatments.

Few data are available on 90Y radioembolization in advanced cholangiocarcinoma[7-9]; both resin and glass microspheres have been used. All series are retrospective and have confirmed tolerance to this therapeutic option. The response rate is difficult to summarize, as some series used the classical Response Evaluation Criteria In Solid Tumors (RECIST)/WHO criteria, whereas others used EASL criteria or mRECIST more "logically" with this approach to measure the vascularized part of the tumor. The response rate was approximately 25%-30% using WHO or RECIST and higher (73%) with EASL. In one series, 5 of 46 patients benefited from downstaging from an R0 surgery[10], as in our case. In most cases, the future remnant liver volume increased after radioembolization of the contralateral lobe[11]; this increase was not obvious in our case.

Therefore, in unresectable but localized ICC, radioembolization can be considered a useful tool that results in curative resection in some cases. This option should be considered in some borderline cases for surgical resection.

COMMENTS

Case characteristics

Authors report the case of a 57-year-old man who was diagnosed with a large unresectable cholangiocarcinoma associated with 2 satellite nodules and without clear margins with the right hepatic vein.

Clinical diagnosis

Despite 4 cycles of GEMOX (stopped due to a hypertransaminasemia believed to be due to gemcitabine) and 4 cycles of FOLFIRINOX, the tumor remained stable and continued to be considered unresectable. Radioembolization (resin microspheres, SIRS-spheres®) targeting the left liver (474 MBq) and segment IV (440 MBq) was performed.

Laboratory diagnosis

On computed tomography scan, a partial response was observed; the tumor was far less hypervascularized, and a margin was observed between the tumor and the right hepatic vein. A left hepatectomy enlarged to segment VIII was performed.

Treatment

Radioembolization can be useful in the treatment of cholangiocarcinoma, allowing in some cases a secondary resection.

Peer review

Interesting case report dealing with the value of radioembolization in the treatment of initially unresectable CCC. Useful aspect of a multimodal pathway.

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CASE REPORT

Ulcerative colitis worsened after *Clostridium difficile* infection: Efficacy of infliximab

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Abstract

The incidence of *Clostridium difficile* (*C. difficile*) infection (CDI) is 1.8%-5.7% in admitted patients with ulcerative colitis (UC). CDI can worsen UC and increase the risk for colectomy or even death, thus necessitating therapy escalation, such as increasing the corticoid therapy or starting a biologic treatment. Several reported cases with infliximab therapy have provided favorable outcomes in UC patients with CDI, suggesting that infliximab treatment may be protective; however, the optimal infliximab treatment regimen for UC patients with CDI remains to be established. Here, we report a case of worsening UC in the presence of recurrent CDI. The patient had received prior ciprofloxacin and immunosuppressive therapy during a prolonged hospital stay. The deterioration in the patient's condition likely resulted from the ability of *C. difficile* to promote relapsing of UC by activating the immune response. Ultimately, the patient was treated with a high dose of infliximab after a low trough level of infliximab at week 8 was identified, yielding better clinical results. Infliximab was found to be safe after repetitive episodes of CDI. The trough level of infliximab was therefore a useful indicator to guide therapy and correlated well with the patient's outcome.

Key words: *Clostridium difficile*; Ulcerative colitis; Inflammatory bowel disease; Trough level; Infliximab

Core tip: *Clostridium difficile* infection (CDI) in patients with ulcerative colitis (UC) can worsen the disease and increase the risk of colectomy and death, thus requiring an escalation of treatment. This report reveals a case of UC worsened by recurrent CDI, which likely activated the patient's immune response and stimulated the relapse of UC. Lack of response to infliximab therapy was indicated by low trough levels, and a high-dose infliximab regimen yielded better clinical results. Infliximab trough levels were found to correspond with patient outcome, and thus may serve to guide treatment in similar cases of UC with CDI.

Seicean A, Moldovan-Pop A, Seicean R. Ulcerative colitis worsened after *Clostridium difficile* infection: Efficacy of infliximab. *World J Gastroenterol* 2014; 20(17): 5135-5140 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5135.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5135

INTRODUCTION

Clostridium difficile (*C. difficile*) infection (CDI) represents an important cause of morbidity and mortality in patients with inflammatory bowel disease (IBD), resulting in longer hospital stays and higher healthcare expenses[1,2]. CDI has been reported to occur in 0.9%-2.2% of admissions for Crohn's disease and 1.8%-5.7% for ulcerative colitis (UC); these incidence rates are higher than in the general

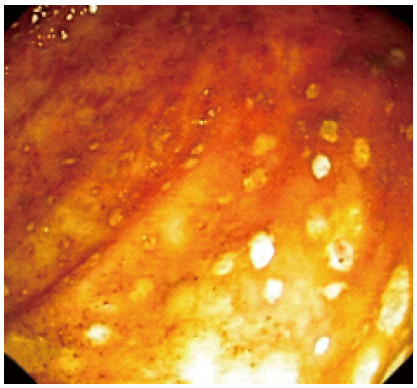

Figure 1 Pseudomembranous colitis in the ulcerative colitis patient. Colonoscopy image showing pseudomembranes in the descending colon, with a loss mucosal vascular pattern.

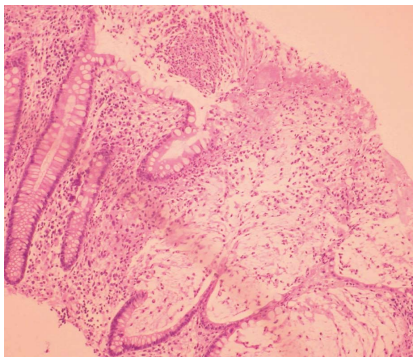

Figure 2 Colonic histology of *Clostridium difficile* infection superimposed on ulcerative colitis. Necrosis of superficial crypts with a dense infiltrate of neutrophils, fibrin, and cellular debris covering the mucosal surface.

population (0.48%)[1,3] and have shown an increasing trend in recent years[2]. It has also been reported that 8.2% of UC patients and 1% of those with Crohn's disease are silent carriers, without distinct episodes of CDI[4]. The major risk factors for CDI in IBD are drug-related, including the use of antibiotics such as fluoroquinolones, clindamycin, cephalosporins, and penicillins[2,4,5], as well as proton pump inhibitors or immunosuppressive therapies[6]. Baseline or newly initiated therapies with corticoids triple the risk of CDI and double the mortality rate, in a dose- and duration-independent manner[7,8]; furthermore, immunomodulators, such as azathioprine, methotrexate and 6-mercaptopurine, increase the risk of CDI, especially in UC patients[2,4]. Other CDI risk factors are host-related, such as a patient age over 65 years, prolonged hospital stay, and history of gastrointestinal surgery[4].

CASE REPORT

A 37-year-old male patient with a history of UC in the last 2 years, with frequent flare-ups (more than six per years) treated with steroids and mesalamine, was admitted to our hospital. One month before admission, the patient had experienced a new relapse with rectal bleeding, diarrhea (9-10 stools/d) and fever (38 ℃), which was treated with mesalamine and steroids accompanied by ciprofloxacin to address a concomitant urinary infection. Despite the therapy, the status worsened, with the patient experiencing fever of 38.4 ℃, 20 stools/d, and abdominal distension without tenderness. A colonoscopy performed in a different hospital revealed the presence of pseudomembranes, supporting the diagnosis of CDI superimposed on UC. Metronidazole and vancomycin were administrated for 5 d.

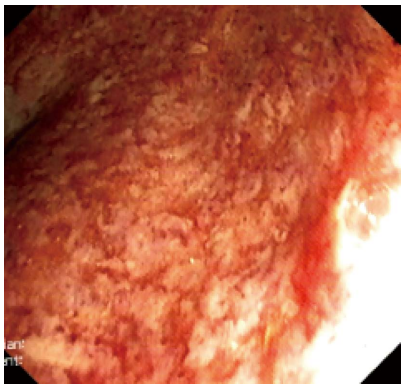

Figure 3 Severe endoscopic aspect of ulcerative colitis. Ulcerations, loss of vascular pattern and edema of the mucosae were noted in the rectum.

On admission to our hospital, the patient had more than 20 stools/d, fever, and leukocytosis (24000/mm^3), with 87% neutrophilia, normal C-reactive protein (CRP) level, no anemia, hypoproteinemia (5 g/dL), and hypoalbuminemia (2.6 g/dL). A stool culture, including *C. difficile* toxins, was negative. The presence of pseudomembranes with a diminished vascular pattern of the mucosa from the cecum to the sigmoid colon was noted on a repeat colonoscopy; however, the rectum appeared normal (Figure 1). Colonic biopsy showed leukocytes, fibrin, mucus, and epithelial cells adherent to the surface of the underlying inflamed and necrotic mucosa, supporting the diagnosis of pseudomembranous colitis (Figure 2). X-ray radiography revealed no distension of the transverse or right colon, but the transabdominal ultrasound showed the presence of ascites. With respect to the potential diagnosis of relapsing CDI, the patient was started on oral vancomycin (125 mg *qid*, continued for 14 d); three days later, azathioprine and a higher dose of steroids was administered, which resulted in clinical improvement. Within 15 d of discharge, the patient reported a relapse with 8 stools/d, at which time he self-administered oral metronidazole for 10 d without consulting the doctor, but which provided a favorable outcome.

Three months after the initial admission to our hospital, the patient presented again with 6 stools/d, bleeding, and urgency. His temperature was normal, CRP level was slightly elevated, and *C. difficile* toxins were again negative. Proctosigmoidoscopy revealed multiple ulcerations, friability, mucosal edema, and loss of vascular pattern. Histopathologic examination with hematoxylin and eo-

sin staining and immunohistochemistry indicated severe UC and no cytomegalovirus (CMV)-induced cytopathic damage ("inclusion bodies"). After an infliximab (5 mg/kg per day) induction regimen at 0, 2 and 6 wk, the patient was still experiencing 6 stools/d, showed signs of severe colitis in endoscopy (Figure 3), and had a two-fold increase in CRP level. The trough level of infliximab measured at 8 wk after initiation was 0.062 μg/mL, and the anti-infliximab antibodies were negative (ELISA kit, Immundiagnostik, Bensheim, Germany). The low trough level suggested a partial response, and the dose of infliximab was consequently increased to 10 mg/kg per day; the patient showed a rapid clinical remission after the first administration, as evidenced by 1 stool/d, without blood. Remission was confirmed endoscopically after the administration of the second 10 mg/kg per day dose, and the patient was returned to 5 mg/kg per day, with a detectable trough infliximab level of 3 μg/mL. After a 12-mo follow-up, the patient remained in steroid-free remission.

DISCUSSION

C. difficile is a gram-positive, spore-forming anaerobic bacterium that is revealed when the normal colonic flora is disrupted[9]. The bacteria produce enterotoxin A and cytotoxin B, which bind to specific receptors in colonic mucosal cells and gain entry to the intracellular space, leading to a systemic inflammatory response (fever, multi-organ failure), toxic megacolon, and perforation. The capability of bacterial adherence to the mucosa is genetically determined, influenced by polymorphisms of the host *TNFRSF14* gene[10]. Colonic infection is common[2], but small intestinal involvement or pouchitis have been reported with CDI[11,12]. Although IBD patients with CDI acquire their infection in an outpatient setting in 47%-79% of cases[2,4,13], the number of in-hospital infections is increasing.

The clinical manifestations of CDI-associated IBD are usually indistinguishable from those of IBD alone, such as watery diarrhea or bloody stools, with systemic signs of severity (fever, tachycardia, hypotension), abdominal distention, or signs of complications (fulminant colitis, toxic megacolon, or bowel perforation)[6]. Leukocytosis sometimes occurs, even before diarrhea[14], indicating the need to test for CDI[11], as high numbers of leukocytes and increased serum levels of creatinine are associated with the development of severe-complicated CDI[15]. Hypoalbuminemia is related to severe diarrhea as a result of protein-losing enteropathy and negative acute-phase proteins[16], which may explain the ascites in our patient. Though not observed in the present case, ascites associated with the distention of the transverse colon can also suggest toxic megacolon and bowel perforation. The diagnosis of CDI is based on toxin detection in stool samples, with low sensitivity, or on colonic histology, which has only been reported as positive in 5% of CDI-IBD patients[6]. Pseudomembranes containing mucus, protein, and inflammatory cells are usually detected on colonoscopy in isolated CDI, but they may be absent if the patient is taking immunomodulators[2,17], though their presence does not influence the clinical outcome[18].

The long-term outcome of the patient in the present case was very good after only two high doses of infliximab, with complete remission one year later. This is in agreement with several other reported CDI-UC cases treated with infliximab[19]. It has been postulated that infliximab therapy may even protect against CDI[10]. The initiation of infliximab therapy is not associated with CDI[7], based on a pooled analysis of three UC studies and seven Crohn's disease studies[20]. One study reported that despite similar short-term outcomes for CDI-IBD and IBD patients in terms of cyclosporine use and length of hospitalization, the patients with CDI-IBD may have worse long-term outcomes[21], with 37%-50% requiring hospitalization for treatment of flare-ups[2,22]. Furthermore, intensification of treatment for IBD occurs more often after CDI[22,23], either by initiation or escalation of biologic therapy or immunosuppressants[24]. However, the escalation of IBD treatment should be avoided in the first 72 h after starting CDI therapy[16], as demonstrated in the present case. The mortality in CDI-IBD patients is 3.2-6 times greater than in patients with IBD alone[6,18,25,26], with a mortality rate as high as 25%[5]. It has been reported that 10%-35% of CDI patients ultimately require a colectomy[2,4,18,21,22,24]. Patients with CDI-IBD should be followed up carefully and their IBD aggressively managed.

CDI recurs in about 11%-30% of patients after the initial course of treatment[6,14,27] by reinfection with the initial strain or with a new strain, which usually occurs 1-3 wk after antibiotic discontinuation. Treatment with metronidazole is not recommended beyond the first recurrence due to concerns for peripheral neuropathy following extended use, especially in the elderly. Moreover, oral administration of metronidazole has a poor treatment outcome, with a reported failure rate of up to 50%[28]. As a result, the direct use of oral vancomycin has been recommended as an initial therapy for CDI-IBD[2], with prolonged, tapered and pulsed-dose vancomycin as the preferred approach for multiple recurrences of CDI[29]. Alternative treatments for recurrence include fecal microbiota transplantation, which has shown a high rate of success[30]. 5-aminosalicylic acid (5-ASA) therapy is considered a protective option for patients with CDI-IBD[4]; although, the prescribed mesalamine was ineffective in the current case. Combination treatment with antibiotics and immunosuppressants has been associated with colectomy or death in 12% of cases at the 3-mo follow-up[6], though this was not confirmed in other small studies[13]. Newer antibiotics, probiotics, and immunotherapy may be beneficial, but more investigation is needed[16].

In the case reported here, CDI worsened the short-term outcome of UC, most probably because *C. difficile*

alters the natural history of UC by activating an innate immune response to the organism that activates the UC[19]. This case highlights the clinical challenges that can be encountered during UC therapy, with the potential benefit of escalating infliximab dosage to a high level in order to resolve a recurrent CDI. This approach led to a good long-term (12-mo) outcome, but no guidelines for the use of infliximab trough level to guide modification of therapy are yet available for such situations. It is important to note that our patient had a prolonged hospital stay and previous ciprofloxacin and corticosteroid therapy, which represent risk factors for CDI.

The decision for the escalation of infliximab in this case rather than a colectomy after the induction period was based on clinical findings and the trough level of infliximab. A level below 0.5 μg/mL indicates the need for dose escalation or a shortening of the interval between infusions[31]. Detectable trough serum infliximab after an induction period predicts clinical remission, endoscopic improvement, and a lower risk for colectomy in UC patients[32], which corresponds to the improved long-term outcome in our patient. However, a recent study has shown that a low level of infliximab (< 2.2 μg/mL) at week 14 leads to the formation of anti-infliximab antibodies and infliximab discontinuation[33]. However, in a retrospective study of IBD patients who were no longer responding to infliximab, the clinical improvement upon intensification of infliximab therapy occurred irrespective of infliximab serum trough levels or antibodies[34]. Though not detected in our patient, particular attention must be paid to concomitant CMV infection, which can be diagnosed by hematoxylin and eosin staining for evidence of cytopathic damage in epithelial cells, or by immunohistochemistry, PCR DNA detection, or serum IgM detection[35].

In conclusion, this case highlights some of the difficulties in treating CDI-UC. The short-term outcome of the patient was worsened by CDI, possibly resulting from *C. difficile* activation of an innate immune response that stimulated the UC[21]. As demonstrated here, low trough levels of infliximab prompted the administration of a higher dose with a favorable treatment result, however no guidelines for the use of trough levels are yet available for such situations. Thus, trough levels of infliximab may be useful and safe for guiding the treatment of UC worsened by CDI.

COMMENTS

Case characteristics

A 37-year-old male patient with a history of ulcerative colitis (UC) diagnosis 2 years prior and subsequent treatment with steroids and mesalamine but with frequent flare-ups over that period, was admitted for a current flare-up with symptoms of diarrhea, fever, rectal bleeding, abdominal distention but no tenderness.

Clinical diagnosis

Severe flare-up of UC.

Differential diagnosis

Severe flare-up of UC with or without associated cytomegalovirus (CMV) infection, tuberculosis or *Clostridium difficile* (*C. difficile*) infection (CDI).

Laboratory diagnosis

Leukocytosis, hypoalbuminemia, negative stool toxins for *C. difficile*.

Imaging diagnosis

The initial colonoscopy performed showed pseudomembranes with diminished vascular pattern. A second colonoscopy showed multiple ulcerations, friability, mucosal edema, loss of vascular pattern with histopathological appearance suggesting severe UC.

Pathological diagnosis

The initial pathological examination showed leukocytes, fibrin, mucus, and epithelial cells adherent to the surface of the underlying inflamed and necrotic mucosa, which sustained the diagnosis of pseudomembranous colitis on UC. The second examination using hematoxylin-eosin staining and immunohistochemistry showed no CMV-induced cytopathic damage ("inclusion bodies").

Treatment

For severe flare-up of UC after recurrent CDI, an infliximab induction regimen (5 mg/kg per day) was administered at weeks 0, 2 and 6. After a low trough level of infliximab was detected at week 8, a high-dose infliximab regimen (10 mg/kg per day) was given at weeks 14-22. When the trough level increased, the dose was reduced to 5 mg/kg per day and the patient was found to be in clinical remission at the 1-year follow-up appointment.

Term explanation

Detectable but low trough level of infliximab represents an indication for increasing the dose or shortening the interval. The optimal trough level has not yet been established.

Experiences and lessons

Trough level of infliximab is a useful tool in guiding treatment of UC worsening associated with CDI.

Peer review

Infliximab therapy was safe in the patient with worsened UC associated with recurrent CDI. Measurement of the trough level of infliximab was helpful in guiding an increase in infliximab therapy. The presence of ascites, in this case, was a sign of hypoalbuminemia correlated with severe diarrhea as a result of protein-losing enteropathy; however, ascites associated with distention of the transverse colon can sugest toxic megacolon and bowel perforation, which was not the case for this particular patient.

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CASE REPORT

^{18}F-FDG PET/CT imaging for a gastrointestinal mantle cell lymphoma with multiple lymphomatous polyposis

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Abstract

Multiple lymphomatous polyposis (MLP) is an uncommon type of gastrointestinal lymphoma characterized by the presence of multiple polyps along the gastrointestinal tract. Most of this entity is in fact considered the counterpart of gastrointestinal tract involvement for mantle cell lymphoma (MCL). To our knowledge, there have been no reports on [fluorine-18]-fluorodeoxy-glucose (^{18}F-FDG)-positron emission tomography (PET)/computed tomography (CT) imaging for gastrointestinal MCL with MLP. We present the results of ^{18}F-FDG PET/CT imaging in a patient with gastrointestinal tract involvement of MCL showing continuous MLP from the stomach to the rectum and intestinal intussusception. FDG-PET/CT findings were false negative in typical MLP spreading widely over the gastrointestinal tract, but uptake was noted in large lesions with deep infiltration considered atypical as MLP. On FDG-PET/CT imaging, the Ki-67 proliferative index, which is a cell proliferation marker, showed neither correlation with the presence of uptake nor the maximum standardized uptake value.

Key words: ^{18}F-fluorodeoxy-glucose-positron emission tomography/computed tomography imaging; Mantle cell lymphoma; Multiple lymphomatous polyposis; Gastrointestinal tract; Ki-67 proliferative index

Core tip: To the present, there have been no reports on [fluorine-18]-fluorodeoxy-glucose (^{18}F-FDG)-positron emission tomography (PET)/computed tomography (CT) imaging of gastrointestinal mantle cell lymphoma (MCL) with multiple lymphomatous polyposis (MLP). In this report, we present the results of ^{18}F-FDG PET/CT imaging in such a MCL patient showing continuous MLP from the stomach to the rectum. We also compared FDG-PET/CT imaging with the Ki-67 proliferative index, an index of cell proliferation, and found that their relationship was inconsistent. The findings of FDG-PET/CT were false negative in typical MLP, but uptake was noted in larger lesions with deep infiltration considered atypical MLP, regardless of the Ki-67 proliferative index.

Saito M, Miyazaki M, Tanino M, Tanaka S, Miyashita K, Izumiyama K, Mori A, Irie T, Tanaka M, Morioka M, Tsukamoto E. ^{18}F-FDG PET/CT imaging for a gastrointestinal mantle cell lymphoma with multiple lymphomatous polyposis. *World J Gastroenterol* 2014; 20(17): 5141-5146 Available from: URL:

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INTRODUCTION

Multiple lymphomatous polyposis (MLP) is a term applied to a specific lymphoma characterized by a distinctive pattern of gastrointestinal tract involvement in which long segments of the intestine are superficially infiltrated by multiple white nodular or polypoid tumors, ranging in size from 0.2 to 2 cm[1,2]. In most of MLP, lymphoma cells originate in the mantle zone of the lymphoid follicle, so this disease is actually considered a subtype of malignant lymphoma called mantle cell lymphoma (MCL)[3,4]. Since Cornes' report in 1961, various documented cases of MLP have been reported in the literature[5,6]. However, to the present, there have been no reports on [fluorine-18]-fluorodeoxy-glucose (^{18}F-FDG)-positron emission tomography (PET)/computed tomography (CT) of gastrointestinal MCL cases with MLP. We encountered a rare case of gastrointestinal MCL showing continuous MLP from the stomach to the rectum and intestinal intussusception, and present the results of ^{18}F-FDG PET/CT imaging in this report. Additionally, we closely evaluated its relationship with FDG-PET/CT imaging and the Ki-67 proliferative index, a cell proliferation marker. This is the world's first report studying the causes of the low sensitivity of FDG-PET/CT for the detection of MCL with gastrointestinal tract involvement.

CASE REPORT

Clinical presentation of the patient

A 49-years-old Japanese female who had noted lumps in the cervical and inguinal regions for about half a year to 1 year developed right abdominal discomfort and constipation for 2 wk. She was suspected to have malignant lymphoma on contrast-enhanced CT and was referred to our hospital. Examinations on admission revealed mild anemia (hemoglobin: 10.9 g/dL), hypoproteinemia (total protein: 5.4 g/dL), and hypoalbuminemia (albumin: 3.1 g/dL), and the soluble interleukin 2 receptor level was elevated at 4460 U/mL (normal range: 124-466 U/mL). Inguinal lymph node biopsy was performed, immunophenotype with double-color flow cytometry showed strong positivity (80%-90% ≤) for CD5, CD19, and CD20, and fluorescence *in situ* hybridization showed IgH/CCND1 (bcl1) in 97.0%. As below mentioned, a histopathological diagnosis of MCL was made. She received hyper-CVAD chemotherapy (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone combined with high dose cytarabine and methotrexate) with rituximab for 8 courses. Lymphoma lesion in the duodenal bulb was perforated after chemotherapy for 3 courses, however, a favorable response to hyper-CVAD chemotherapy was obtained. She is alive at present for more than 2.6 years after initial therapy, with no recurrence.

Table 1 Correlation between fluorodeoxy-glucose-positron emission tomography/computed tomography and the Ki-67 proliferative index

PET (-)		PET (+)		
Portion	Ki-67	Portion	SUV_{max}	Ki-67
Stomach	43.8%	Duodenum (bulb)	5.1	50.2%
Duodenum (descending)	32.7%	Ascending colon	7.7	30.1%
Transverse colon	34.7%	Rectum	6.9	32.1%

SUV_{max}: Maximum standardized uptake value; Ki-67: Ki-67 proliferative index.

Radiological and endoscopic features

CT showed enlargement of systemic lymph nodes, and also revealed tumorous lesions in the gastrointestinal tract, as well as clear thickening of the gastric wall (Figure 1A).

Whole-body ^{18}F-FDG PET/CT was performed using a PET/CT system (Gemini-GXL 16; Philips Medical Systems, Inc., Cleveland, Ohio). She was instructed to fast for 6 h before the injection of ^{18}F-FDG. The serum glucose level before injection was 85 mg/dL. The injected dose of ^{18}F-FDG was 199.6 MBq. A scan was performed 70 minutes after injection. An expert nuclear medicine physician (Tsukamoto E) interpreted the PET/CT imaging. The maximum standardized uptake value (SUV_{max}) was measured semi-quantitatively. On FDG-PET/CT, as well as in various lymph nodes, uptake of SUV_{max} 5-7 was observed in the tumorous lesions in the duodenal bulb, ileocecal region, ascending colon, and rectum (Figure 2A, Table 1), which were also observed on CT. Except for these lesions, no uptake was noted in the other gastrointestinal tract.

Gastrointestinal endoscopy showed MLP spreading over a wide area of the stomach (Figure 1B). Three large tumorous lesions with central depressions were observed in the duodenal bulb (→PET positive, Figure 2B and C), and MLP extended continuously on its anal side (Figure 1C).

Before chemotherapy, colonoscopy showed MLP consisting of large nodules in the rectum (→PET positive, Figure 2D and E). The ascending colon was intussuscepted due to a large tumor (→PET positive, Figure 2F and G), and MLP spread from the anal side of this site to the rectum (Figure 1D).

X-ray imaging of the small intestine yielded findings compatible with MLP mainly in the ileum (not shown). X-ray imaging of the colon showed large tumors in the ascending colon and cecum (→both were PET positive, Figure 2F, H and I).

After chemotherapy, insertion of the colonoscope to the ileocecal region became possible and MLP was also confirmed in the terminal ileum (Figure 1E). It was found that there were intussuscepted at tumors in the ascending colon arising at and around the ileocecal valve and the cecal tumor arose near the appendiceal orifice (not shown).

Pathological features

Duodenal bulb was perforated and surgically resected

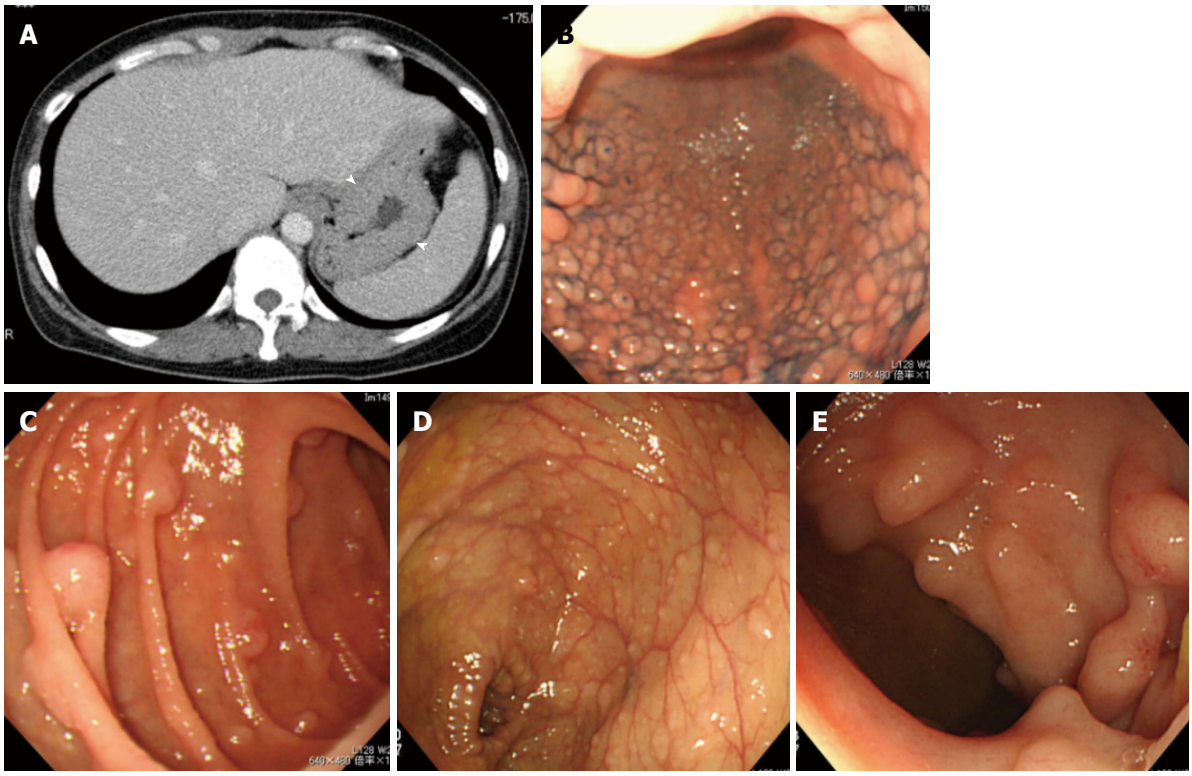

Figure 1 Images of typical type multiple lymphomatous polyposis. A: Abdominal CT: thickening of the gastric wall (arrow head) was clearly observed; B, C: Gastrointestinal endoscopy: stomach (dye spraying) (B), descending portion of the duodenum (C); D, E: Colonoscopy: transverse colon (D), terminal ileum (E). B-E detected typical multiple lymphomatous polyposis, but none showed uptake on fluorodeoxy-glucose-positron emission tomography/computed tomography.

(Figure 3A). Histopathological examination showed that lymphoma lesion invaded deep into the muscularis propria (Figure 3B), and tumor cells varying in size from small to middle-sized proliferated densely (Figure 3C). Lymphoma cells were positive for CD20, CD5, and Cyclin D1 on immunohistochemical staining (Figure 3D-F). These features were compatible with MCL.

Proliferation indices were assessed by staining representative slides with the monoclonal MIB1 antibody (DAKO, Tokyo, Japan) directed against the Ki-67 antigen. Before chemotherapy, biopsy was performed from each of the 6 gastrointestinal lesions shown in the figure, and the Ki-67 proliferative index was evaluated (Figure 3G). It was 43.8%, 32.5%, and 34.7% in the stomach, duodenum (descending portion), and transverse colon, respectively, which were compatible with typical type MLP negative on PET. On the other hand, Ki-67 proliferative index was 50.2%, 30.1%, and 32.1%, and SUV_{max} was 5.1, 7.7, and 6.9, respectively, in the lesions of the duodenum (bulb), ascending colon, and rectum, which were positive on PET and appeared atypical as MLP (Table 1). The Ki-67 proliferative index was neither related to the presence or absence of uptake nor the SUV_{max} on FDG-PET/CT imaging.

DISCUSSION

This report presented a rare case of gastrointestinal MCL showing continuous MLP from the stomach to the rectum. Gastrointestinal tract involvement for MCL presents a variety of lesions[7], ranging from the characteristic MLP to mucosal changes that are too vague to be identified endoscopically and can only be diagnosed through biopsy[8,9]. We previously advocated that lymphoma cells of MCL had invaded the lamina propria to submucosal layer before MLP developed[10].

FDG-PET is a non-invasive imaging technique, commonly performed on patients with malignant lymphoma and recommended for initial staging in diffuse large B-cell and Hodgkin lymphoma[11]. The usefulness of FDG-PET in MCL was also reported, with pretreatment PET scans being positive in 94%-100%[12,13], however, PET scans have too low of a sensitivity (11%-20%) to detect gastrointestinal tract involvement for MCL[13,14]. These reports did not discuss the causes of the low sensitivity of FDG-PET for the detection of MCL with gastrointestinal tract involvement, and the morphological characteristics of a picked up lesion as those of MLP. Our case was the first report to present PET/CT imaging of MCL case with multiple MLP from the stomach to the rectum. The findings of FDG-PET/CT were false negative in typical MLP, particularly, gastric lesions were detected by CT, and the sensitivity of FDG-PET/CT was lower than that of CT. Meanwhile, we do not deny the possible contribution of not administering a H_2-blocker or proton pump inhibitor, the duodenal bulb had been perforated in the

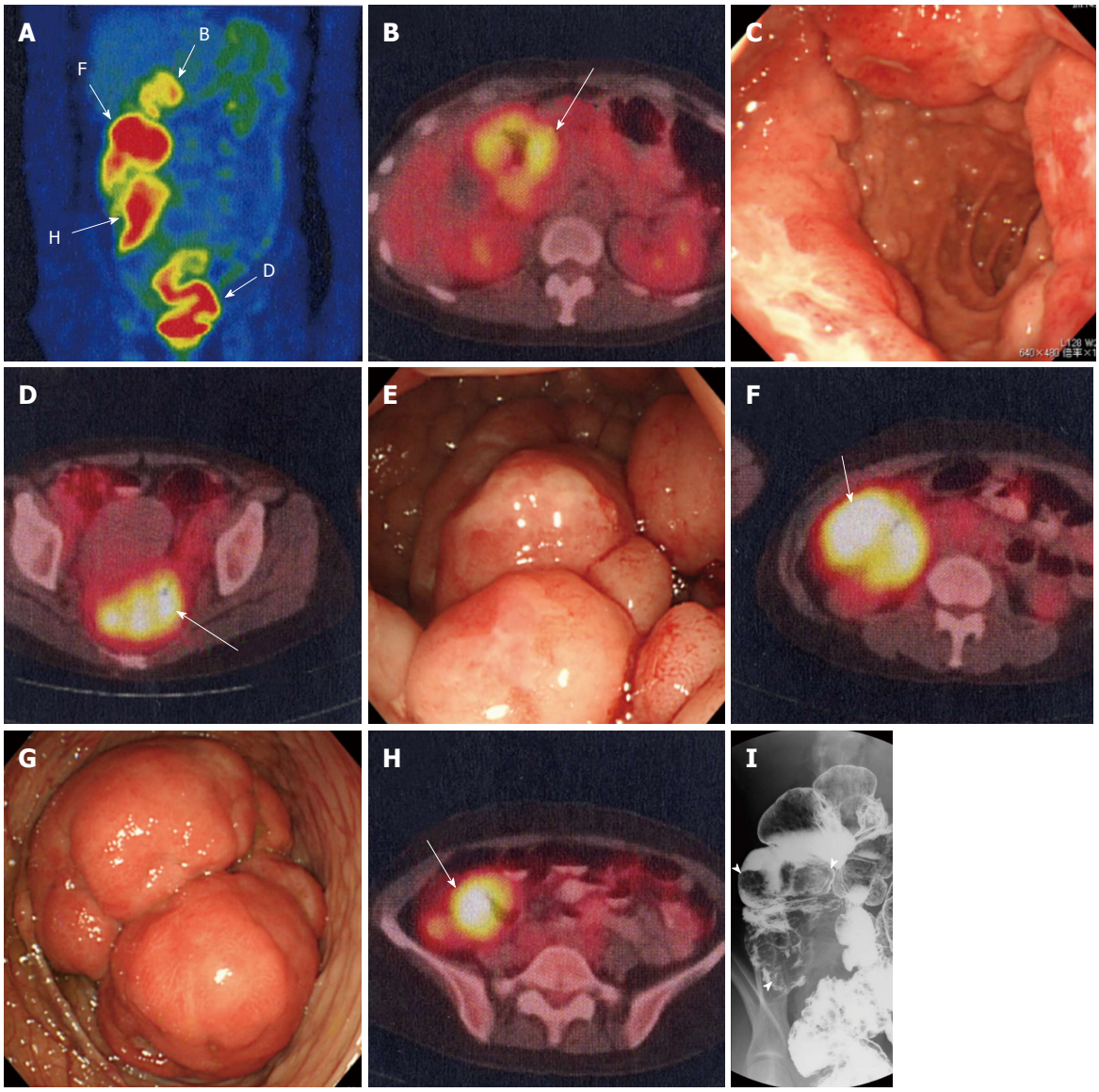

Figure 2 Images of atypical type multiple lymphomatous polyposis. A, B, D, F, H: [fluorine-18]-fluorodeoxy-glucose -positron emission tomography/computed tomography: (A) Uptake in the gastrointestinal tract was noted at the 4 sites shown in longitudinal images. The SUVmax was 5.1, 6.9, 7.7, and 6.5 in the duodenal bulb (B), rectum (D), ascending colon (F), and cecum (H), respectively; C: Gastrointestinal endoscopy: Three large tumorous lesions circumferentially surrounding the duodenal bulb were observed; E, G: Colonoscopy: Multiple larger nodules than usual type MLP were observed in the rectum (E). The ascending colon was intussuscepted due to a large tumor (G); I: X-ray imaging of the ascending colon and cecum: Large tumorous lesions were observed in the ascending colon and cecum (arrowheads).

present case because lymphoma cells had deeply invaded the muscularis propria. Uptake was noted on FDG-PET/CT imaging in atypical type MLP, probably because the lesions were larger and infiltrated deeper than in typical type MLP regardless of the histologic malignant potential, the Ki-67 proliferative index of lymphoma cells. Gastrointestinal MCL lesions are generally shallow[1,10]. This may reduce the sensitivity of FDG-PET/CT for gastrointestinal MCL.

It has also recently been reported that a relationship between SUV_{max} in ^{18}F-FDG PET/CT imaging and Ki-67-positive tumor cells was revealed in untreated patients with non-Hodgkin lymphoma including MCL[15]. On the other hand, there have been no reports of evaluation of the Ki-67 proliferative index in gastrointestinal MCL showing MLP. In this study, the lack of statistical relationship between SUV_{max} and the percentage of Ki-67-positive cells may be explained by the fact that the highest SUV_{max} is determined by analysis on the whole body, while Ki-67 immunostaining is restricted to tissue biopsies in which sampling errors are possible.

Gastrointestinal lesions, including MLP, frequently reflect the pathogenesis of MCL. However, ^{18}F-FDG PET/CT is not useful for the accurate initial staging of MCL before treatment because of the low detection rate of gastrointestinal lesions. In the future, more gastrointestinal MCL patients should be examined the findings of ^{18}F-FDG PET/CT uptake, which is observed exclusively in large and deeply infiltrating lesions as shown in the present study.

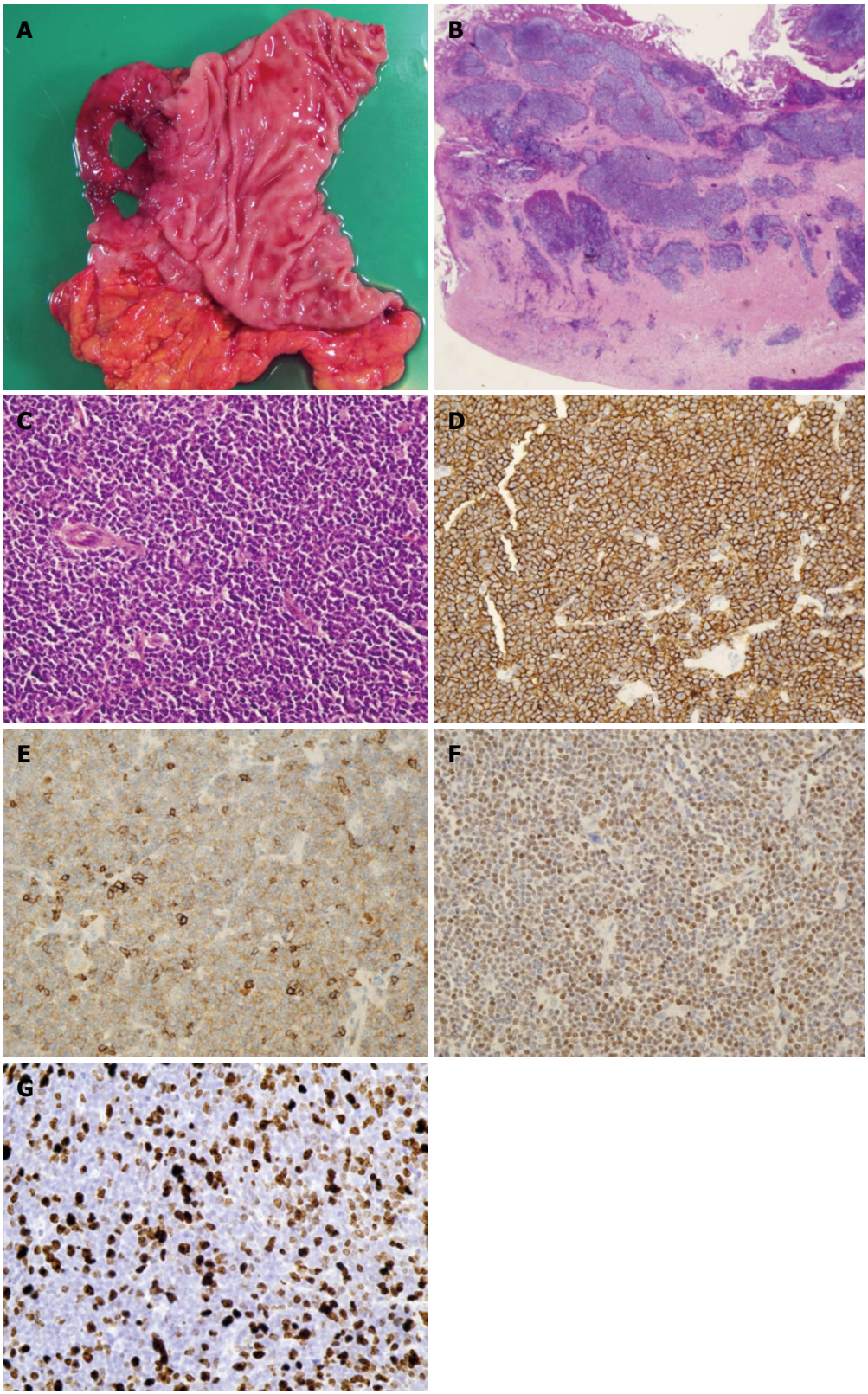

Figure 3 Pathological findings. A: Macroscopic specimen showed perforation in the duodenal bulb; B: Histopathologically, lymphoma lesion in the duodenal bulb invaded deep into the muscularis propria (HE stain, x 20); C: Tumor cells varying in size from small to middle-sized densely proliferated (HE stain, x 200); D-F: These tumor cells were positive on immunohistochemical staining for CD20 (D), CD5 (E), and Cyclin D1 (F), findings compatible with mantle cell lymphoma (x 200); G: Ki-67 proliferative (MIB-1) index in the duodenal bulb was 50.2% (1397 cells/2781 cells) (x 200).

In conclusion, the findings of FDG-PET/CT were false negative in typical MLP, but uptake was noted in larger lesions with deep infiltration considered atypical MLP, regardless of the Ki-67 proliferative index.

COMMENTS

Case characteristics

A 49-years-old female with lumps in the cervical and inguinal regions developed right abdominal discomfort and constipation.

Clinical diagnosis

Bilateral cervical and inguinal lymph nodes were palpable and mild tenderness was found in the right-sided abdomen.

Differential diagnosis

Colon cancer with lymph nodes metastases.

Laboratory diagnosis

Hemoglobin 10.9 g/dL; total protein 5.4 g/dL; albumin 3.1 g/dL; soluble interleukin 2 receptor 4460 U/mL; other laboratory data were within normal limits.

Imaging diagnosis

(fluorine-18)-fluorodeoxy-glucose (^{18}F-FDG)-positron emission tomography (PET)/computed tomography (CT) showed tumorous lesions in the duodenal bulb, ileocecal region, ascending colon, and rectum with SUV_{max} of 5-7, as well as in various lymph nodes, in addition to multiple lymphomatous polyposis (MLP) in the other gastrointestinal tract with no FDG activity.

Pathological Diagnosis

Gastrointestinal biopsy and resected duodenal bulb revealed mantle cell lymphoma (MCL), positive for CD20, CD5, and Cyclin D1.

Treatment

The patient was treated with hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone combined with high dose cytarabine and methotrexate) with rituximab for 8 courses.

Related reports

^{18}F-FDG PET/CT have too low of a sensitivity (11%-20%) to detect gastrointestinal tract involvement for MCL and there have been no reports on ^{18}F-FDG PET/CT imaging for gastrointestinal MCL with MLP. On the other hand, there have been no reports of evaluation of the Ki-67 proliferative index in gastrointestinal MCL showing MLP.

Term explanation

Ki-67 proliferative index is a cell proliferation marker. According to recently report, a relationship between SUV_{max} in ^{18}F-FDG PET/CT imaging and Ki-67-positive tumor cells was revealed in untreated patients with non-Hodgkin lymphoma including MCL

Experiences and lessons

^{18}F-FDG PET/CT findings were false negative in typical MLP spreading shallow, but uptake was noted in large lesions with deep infiltration considered atypical as MLP regardless of the Ki-67 proliferative index of lymphoma cells.

Peer review

This report presented a rare case of gastrointestinal MCL with MLP, and the presentation on ^{18}F-FDG PET/CT imaging was shown and well discussed.

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CASE REPORT

Intrathoracic caudate lobe of the liver: A case report and literature review

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Abstract

Heterotopic supradiaphragmatic livers are rare. A total of 23 cases of primary supradiaphragmatic livers have been reported in the literature. The clinical presentations of heterotopic supradiaphragmatic liver are variable. The simultaneous detection of intrathoracic accessory liver and pulmonary sequestration is extremely rare, and only one case has previously been reported. It is difficult to make a correct diagnosis preoperatively. We presented a 53-year-old woman with complaints of an intermittent, productive cough and dyspnea for two months that was refractory to medical treatment. She had no previous history of trauma or surgery. A chest radiograph only showed a widening of the mediastinum. Contrast-enhanced computed tomography of the chest revealed a well-circumscribed homogenous soft-tissue mass, approximately 4.35 cm × 2.5 cm × 6.14 cm in size, protruding through the right diaphragmatic crura to the right pleural cavity, attached to the inferior vena cava, esophagus and liver. There was no conclusive diagnosis before surgery. After the operation, we discovered that this patient was the first case of a supradiaphragmatic heterotopic liver, which passed through the inferior vena cava foramen and was coincidentally combined with an intralobar pulmonary sequestration that was found intraoperatively. We discussed its successful management with surgical resection *via* a thoracic approach and reviewed the published literature.

Key words: Intrathoracic liver; Pulmonary sequestration; Supradiaphragmatic ectopic liver

Core tip: Heterotopic supradiaphragmatic livers are rare. A total of 23 cases of primary supradiaphragmatic liver have been reported in the literature. The simultaneous detection of intrathoracic accessory liver and pulmonary sequestration is extremely rare. It is difficult to make a correct diagnosis preoperatively. We reported the first case of a supradiaphragmatic heterotopic liver passing through the inferior vena cava foramen that coincidentally combined with intralobar pulmonary sequestration. We discussed the successful operation *via* a thoracic approach and reviewed the published literature.

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INTRODUCTION

Heterotopic supradiaphragmatic livers are exceedingly rare. Abnormally positioned liver tissues have been described, but these are more commonly observed in the abdominal cavity than in the thoracic cavity. This is one

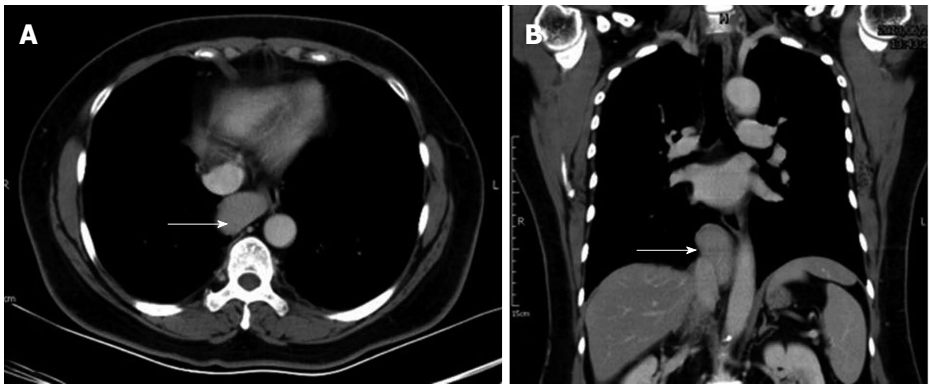

Figure 1 Computed tomography of the patient's chest showing a well-circumscribed soft-tissue mass (white arrow), approximately 4.35 cm × 2.5 cm × 6.14 cm in size, over the middle mediastinum, with mild compression on the esophagus. A: Sagittal view; B: Transverse view.

of the first examples of a supradiaphragmatic heterotopic liver in the literature[1]. The clinical presentations of heterotopic supradiaphragmatic livers are variable. The simultaneous detection of an intrathoracic accessory liver and pulmonary sequestration was reported as the first and only one case in 2008[2]. The etiology of an intrathoracic liver coinciding with pulmonary sequestration is not well known. Herein, we describe the first case of a supradiaphragmatic heterotopic liver passing through the inferior vena cava foramen and coincidentally combined with intralobar pulmonary sequestration.

CASE REPORT

A 53-year-old woman was referred to our center from an outside clinic complaining of intermittent productive cough and dyspnea over the previous 2 mo that was refractory to medical treatment. She denied any previous history of trauma or surgery. There were no positive findings on the physical examination. A chest roentgenogram only showed a widening of the mediastinum. Despite adequate medical treatment, her symptoms persisted. Contrast-enhanced computed tomography (CT) of the chest revealed a well-circumscribed homogenous soft-tissue mass, approximately 4.35 cm × 2.5 cm × 6.14 cm in size, protruding through the right diaphragmatic crura to the right pleural cavity attached to inferior vena cava (IVC), esophagus and liver (Figure 1). There was no pulmonary consolidation or other abnormalities of the bilateral lungs in imaging studies. In addition, the esophagram showed no submucosal lesions. Based upon these imaging findings, the tumor at middle mediastinum and right pleural cavity provoked discussion of a surgical resection.

Video-assisted thoracoscopic surgery (VATS) through the right chest wall was performed initially, and the operative findings revealed hypoplasia and regional hyperemic changes at the pleural surface of the right lower lobe (RLL) of the lung, with two obvious aberrant vessels from the right hemidiaphragm. After dissecting the visceral pleura and the tense capsule, the inferior pulmonary vein was identified, and a well-defined, red-brown, 7 cm × 4.5 cm × 2 cm solid mass abutting the IVC from the cardiophrenic angle of the right hemidiaphragm was found (Figure 2). Due to the limited area of the sequestrated lung of RLL, a wedge resection with an Endo-GIA staple instrument was performed. Due to the indeterminate nature of the mass and the location close to the IVC, we converted the operation to a right limited thoracotomy. The tumor was biopsied, and the frozen section showed reactive liver tissue with cirrhotic change. Therefore, we resected the tumor, which was extruding from the right pleural cavity, and then repaired the foramen of the IVC. The pathologic report showed liver tissue with chronic inflammation and cirrhotic changes (Figure 3). The patient had a good recovery after the surgery and was discharged home one week later. CT-angiography of the chest was conducted after the surgery to retrospectively evaluate the pulmonary sequestration (Figure 4). The aberrant arteries were identified from the abdominal aorta.

DISCUSSION

Supradiaphragmatic, intrathoracic liver tissue is very rare. A total of 23 cases of intrathoracic liver have been reported in the literature[1-23]. We introduced a rare case of an accessory liver lobe herniation from the IVC foramen to the right pleural cavity, combined with an intralobar sequestration of the RLL. This is the second case of a simultaneous detection of intrathoracic liver and pulmonary sequestration. The first case was reported in 2008, and the multiple cystic lesions in the right upper lobe, which were thought to constitute a congenital cystic malformation, were in fact an intralobar pulmonary sequestration[2]. However, the sequestrated lung of the above case was not typically located at the lower lobe, and the aberrant vessels were not visible. We found the sequestrated lung during the operation and then followed up with CT angiography to identify the feeding arteries of the sequestrated lung. Table 1 showed that seven cases (7/24, 29%) had diaphragmatic defects, and only the present supradiaphragmatic liver passed through the IVC foramen. There were 18 cases (18/24, 75%) associated with right-side intrathoracic livers. The majority of the intrathoracic

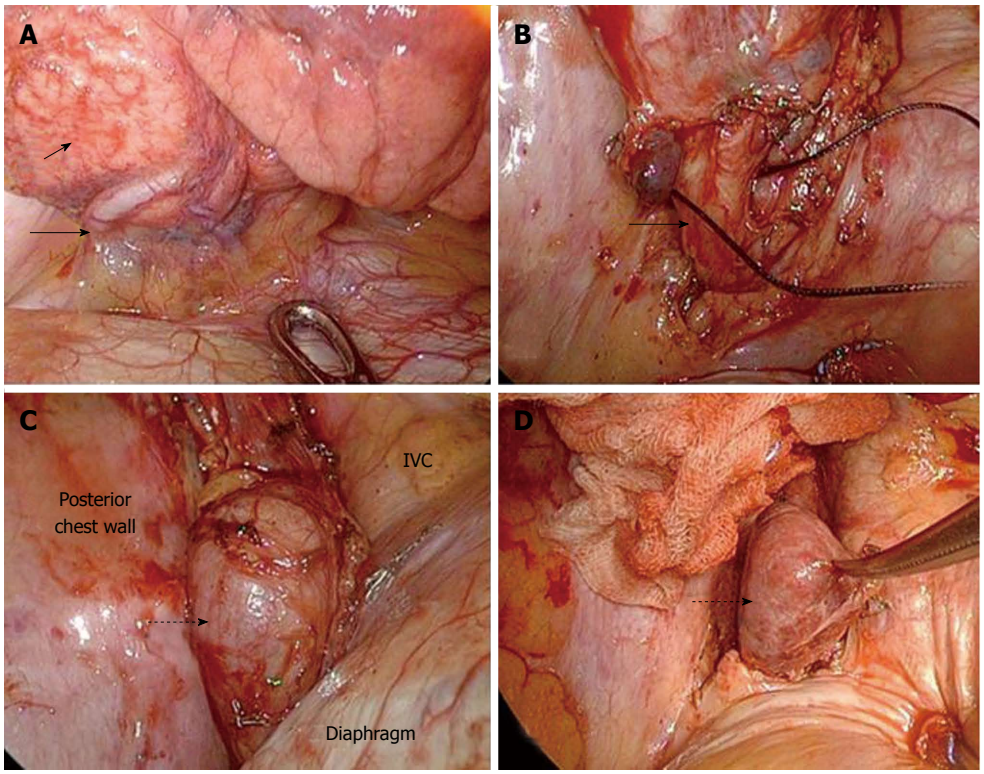

Figure 2 Intraoperative picture. Intraoperative picture showing the exposure of the abnormal lung with hypervascularity at the posterior basal segment (A) (short arrow) and aberrant vessels (B) (long arrow) passing through diaphragm. The mass (C) (dotted arrow), covered by the sac, abutted the inferior vena cava (IVC), posterior chest wall and diaphragm. After dissecting the covering sac, a herniated liver (D) (dotted arrow) was impressed.

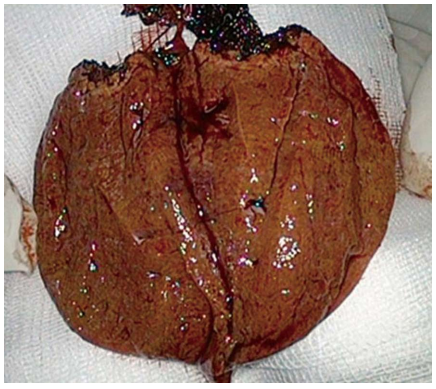

Figure 3 Cutting surface of the resected specimen showed liver tissue with cirrhotic changes.

ectopic or accessory liver lobes were connected to the orthotopic liver by means of a small pedicle that pierced the diaphragm or passed through a small hiatus[1,9,12,15-17,19,21]. In the presenting case, the caudate liver connected with the main portion of the liver just beneath the IVC. However, the pre-operative CT scan of the chest was not able to demonstrate this clearly, and it was difficult to make appropriate diagnosis before the operation.

Such events are most often found in the vicinity of the liver, such as in the gallbladder, spleen, pancreas, umbilicus, adrenal gland, or omentum, and usually some connective tissue or mesenteric attachment to the liver remains[10]. Very rare cases involving the thoracic cavity can be found. Of those cases reported in the literature, almost all were misdiagnosed, most often as a pulmonary tumor and sometimes as pulmonary sequestration or hydatid cyst[12,21]. The misdiagnosis of such cases often leads to potentially unnecessary or inappropriate thoracotomies and the resection of the ectopic liver tissue. Although heterotopic liver tissue may be acquired secondary to trauma or diaphragmatic hernia repair, previous reports have speculated that this condition mostly represents a developmental defect of the septum transversum[10]. The most probable explanation is the development of an accessory liver lobule, with atrophy or regression of the original connection to the abdominal liver[10]. Retrospectively re-examining the clinical evidence, we found at least two signs implying the possibility of an accessory liver lobe in the thoracic cavity. First, the mass was homogeneous, with a density similar to that of a normal liver. Second, the mass was adjacent to the hemidiaphragm and connected to caudal lobe of liver through a defect of the right crus. An intrathoracic ectopic or accessory liver lobe is rarely of clinical significance[12,16]. Surgery is not always required for such a condition. However, the cases reported were almost always respected because of their inappropriate diagnoses. There were a few cases that did have symptoms, such as chest pain, hemoptysis, or dry cough[12,21], that may or may not have been related to the underlying condition. Such abnormal tissue could lead to further liver pathologies, such as cancer, hepatitis, and tissue ischemia secondary to torsion[21]. To avoid malignant

Table 1 Literature review of published cases associated with primary intrathoracic supradiaphragmatic livers

Ref.	Sex/age	Location of mass	Symptoms	Associated anomalies	Preoperative diagnosis	Surgical procedure	Diaphragm	Histology
Hansbrough *et al*[1]	M/26 yr	Right CP angle	Abdominal pain	None	Mesothelioma	Right thoracotomy	Intact	Atypical cirrhosis
Kaufman *et al*[3]	F/48 yr	Right cardiophrenic angle	None	None	None	Right thoracotomy	Intact	Normal liver
Le Roux *et al*[4]	M/18 yr	Right CP angle	None	None	None	Right thoracotomy	Right diaphragmatic defect	Normal liver
Hudson *et al*[5]	M/21 yr	Right diaphragmatic dome	None	None	Diaphragmatic tumor	Right thoracotomy	Intact	Abundant bile ducts
Caron *et al*[6]	F/26 yr	Right CP angle	Asthenia	None	Benign tumor of the pleura or diaphragm	Right thoracotomy	Intact	Chronic inflammation
Sehdeva *et al*[7]	F/21 yr	Left diaphragmatic dome	Chest pain	None	Pleural or diaphragmatic tumor	Left thoracotomy	Intact	Centrilobular congestion
Desvignes *et al*[8]	F/7 yr	Right cardiophrenic angle	None	Right mirror lung, no scissure	Pulmonary sequestration	Right thoracotomy	Right diaphragmatic defect	Signs of pycnosis
Desvignes *et al*[8]	F/20 yr	Parenchyma of right lower lobe	Hemoptysis	Bochdaleck hernia	Hydatid cyst	Right thoracotomy	Right diaphragmatic defect	Normal liver
Lasser *et al*[9]	M/51 yr	Right thoracic cavity	Chest pain	None	Pleural tumor	Right thoracotomy	-	Hyperplasia
Mendoza *et al*[10]	F/6 mo	Parenchyma of right lower lobe	Respiratory distress	-	Solitary pulmonary mass	Autopsy	Intact	Normal liver
Shah *et al*[11]	F/1 d (36w GA)	Left thoracic cavity	Respiratory distress	-	-	Autopsy	Left diaphragmatic defect	Normal liver
Rendina *et al*[12]	F/19 yr	Right CP angle	None	None	Benign pleural tumor	Right thoracotomy	Intact	Normal liver
Shapiro *et al*[13]	F/1 d (26w GA)	Right diaphragmatic dome	Respiratory distress	-	-	Autopsy	-	Normal liver
Iber *et al*[14]	M/6 yr	Right thoracic cavity	Mild asymmetry of chest	None	Benign pleural tumor	Right thoracotomy	-	-
Babu *et al*[15]	M/17 mo	Right CP angle	Recurrent pneumonia	None	Diaphragmatic hernia with a sequestrated lung or herniated liver	Right thoracotomy	Intact	Normal liver
Beiler *et al*[16]	M/1 d (39w GA)	Left diaphragmatic dome	Respiratory distress	Bochdaleck hernia	Diaphragmatic hernia	Laparotomy	Left diaphragmatic defect	Normal liver
Bedii Salman *et al*[17]	F/6yr	Left thoracic cavity	-	Left diaphragmatic hernia	Left diaphragmatic hernia	Laparotomy	Left diaphragmatic defect	Normal liver
Luoma *et al*[18]	F/full term	Left thoracic cavity	Respiratory distress	Left hydrothorax	Left paramediastinal mass with massive hydrothorax	Left thoracotomy	Intact	Normal liver
Chen *et al*[20]	M/13 mo	Right diaphragmatic dome	Respiratory distress	Repeated pneumonia	Pleural or diaphragmatic tumor	Right thoracotomy	Intact	Normal liver
Choi *et al*[2]	M/3 yr	Right cardiophrenic angle	Cough and fever	Intralobar pulmonary sequestration of right upper lobe	Pulmonary sequestration	Right thoracotomy	Intact	Normal liver
Han *et al*[21]	F/26 yr	Left CP angle	Dry cough	None	Pulmonary sequestration	Left thoracotomy	Intact	Normal liver
Wang *et al*[22]	M/39 yr	Right thoracic cavity	Chest pain	None	A benign tumor of right lung	Right thoracotomy	Intact	Normal liver
An *et al*[23]	F/48 yr	Right cardiophrenic angle	Cough and dyspnea	None	Benign fibrous tumor of the pleura or peripheral lung carcinoid tumor	Right thoracotomy	Intact	Normal liver

F: Female; M: Male.

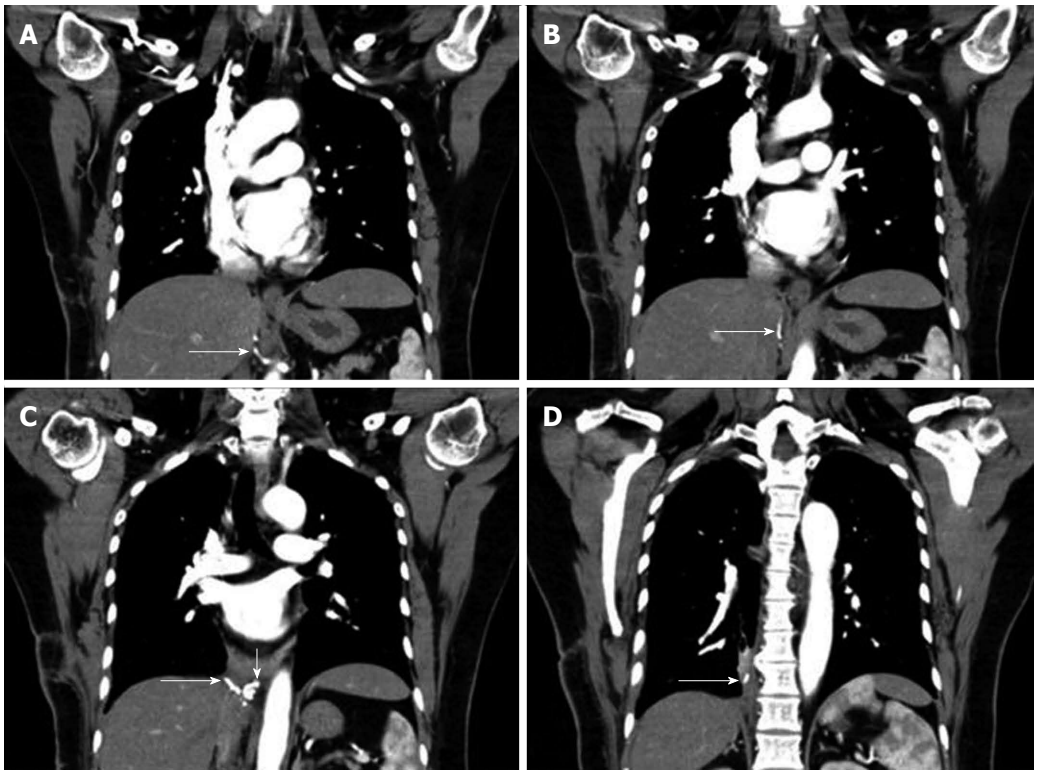

Figure 4 Postoperative computed tomography angiography confirmed the cutting end of the aberrant vessels. A-D: Showed the trend of the aberrant vessel. The remnant aberrant vessel from abdominal aorta (long arrow), engorged aberrant vessels (short arrow).

changes in this loco-regional cirrhosis of the liver[24], surgical resection was the first choice for treatment.

Because heterotopic supradiaphragmatic livers are extremely rare and occur at tricky anatomic locations, it is difficult to make an appropriate diagnosis preoperatively. In our case, we also misdiagnosed the liver as a pulmonary sequestration before surgery. The intralobar pulmonary sequestration was found incidentally at the time of the operation because of the engorged aberrant vessels from systemic circulation; it was then divided by stapled Endo-GIA due to the small area of the sequestrated lung of the RLL. The pathological report of the sequestrated lung showed inflammation, mucus accumulation, microcystic changes and dilated lymphatic channels. Pulmonary sequestration is a rare congenital malformation of the lower respiratory tract[25] that consists of a nonfunctioning mass of lung tissue that lacks normal communication with the tracheobronchial tree and receives its arterial blood supply from the systemic circulation[26]. There are intralobar and extralobar forms, the former of which is embedded in a normal lung and the latter, separated from the adjacent lung by its own visceral pleural investment[27]. Misdiagnosis or delayed diagnosis of such pulmonary malformations results in unnecessary treatments and hospitalizations, as well as in frequent, recurrent infectious complications. The definitive treatment for sequestrated lung diseases is surgical resection, which is curative and has low morbidity and mortality rates[28].

In addition, a multi-detector spiral CT scan with multi-planar reconstruction and magnetic resonance imaging can also reveal the location of the mass and its relation to the normal liver parenchyma[19]. Furthermore, with enhanced scanning and hepatic angiography, if the hepatic vessels extend to the mass, the diagnosis of an accessory liver lobe is more definitive[19]. Thoracoscopy is also effective in the diagnosis of the intrathoracic accessory liver lobes in suspected patients, and conveniently, the lesion might be resected with minimally invasive procedures under thoracoscopy, if the connecting pedicle is not too large in size[22]. If an intrathoracic mass is suspected to be a pulmonary or chest wall benign tumor, abnormal liver tissue should enter the scope of a differential diagnosis to avoid potentially unnecessary or inappropriate surgical interventions[22]. Table 1 revealed that 19 cases underwent thoracotomy, and only two had laparotomy. We attempted to use VATS with the removal of the mediastinal tumor at first, but we finally converted to thoracotomy because of the unclear anatomic view and our diminished confidence.

We presented a very interesting case of supradiaphragmatic heterotopic liver coincidentally combined with intralobar pulmonary sequestration. The ectopic liver was the first case to pass through IVC foramen. To our knowledge, these two anatomic abnormalities are congenital and developmental problems and may constitute a specific type of syndrome.

COMMENTS

Case characteristics

A 53-year-old woman was referred to the authors' center from an outside clinic complaining of intermittent productive cough and dyspnea over the previous 2 mo that was refractory to medical treatment.

Clinical diagnosis
A middle mediastinal tumor was impressed initially.

Differential diagnosis
Differential diagnoses included an esophageal cyst, pulmonary tumor or metastatic mediastinal lesions by computed tomography (CT) imaging before surgery.

Laboratory diagnosis
No laboratory data could help to diagnosis in this case.

Imaging diagnosis
Contrast-enhanced CT of the chest revealed a well-circumscribed homogenous soft-tissue mass, approximately 4.35 cm × 2.5 cm × 6.14 cm in size, protruding through the right diaphragmatic crura to the right pleural cavity attached to inferior vena cava, esophagus and liver.

Pathological diagnosis
The pathologic report showed liver tissue with chronic inflammation and cirrhotic changes.

Treatment
Video-assisted thoracoscopic surgery with wedge resection of right lower lobe of lung through the right chest wall was performed initially, and then converted right limited thoracotomy with resection of intrathoracic liver.

Experiences and lessons
Intrathoracic liver or pulmonary sequestration should be included in differential diagnoses of patient with unknown etiology of mediastinal lesions.

Peer review
This strengths included the first case of a supradiaphragmatic heterotopic liver passing through the inferior vena cava foramen that coincidentally combined with intralobar pulmonary sequestration and literature review in intrathoracic ectopic liver.

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CASE REPORT

Solitary plexiform neurofibroma of the stomach: A case report

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Author contributions: Shi L conceived and coordinated the study, and participated in data collection and manuscript writing; Liu FJ participated in the study design, data collection, production of histopathological figures, and manuscript writing; Jia QH, Guan H and Lu ZJ participated in data collection and manuscript writing.

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Abstract

Plexiform neurofibroma (PN) of the digestive tract is very rare and usually part of the generalized syndrome of neurofibromatosis type 1 (von Recklinghausen disease). Solitary PN of the stomach is extremely rare and has not been reported in the literatures. Here we present a case of solitary PN of the stomach, which was not associated with von Recklinghausen disease. A 38-year-old male presented abdominal pain and distention for 7 d. The patient underwent endoscopy of the upper gastrointestinal tract, which revealed a 3.5 cm protruding and cauliflower-shaped mass with a shallow 1 cm central ulcer in the greater curvature of the stomach. The lesion was removed by laparoscopic surgery. Histological examination demonstrated characteristic histological findings of spindle-shaped cells. Immunohistochemical analysis showed that the tumor cells were positive for S-100 protein, but negative for CD34, KI-67, CD117, and actin. Based on histological findings, gastrointestinal stromal tumor could be excluded, and thus the case was confirmed as PN. We described the clinical features, physical examination, endoscopic findings, and histopathological examination of this case.

Key words: Plexiform neurofibroma; Neurofibromatosis; Von Recklinghausen disease; Abdominal pain

Core tip: Solitary plexiform neurofibroma (PN) is extremely rare. We report a case of solitary PN of the stomach in a 38-year-old male who underwent laparoscopic surgery. The case was not associated with von Recklinghausen disease, and the patient was in good condition 6 mo after surgery, with no tumor recurrence. To our knowledge, this is the first reported case of isolated stomach PN undergoing laparoscopic surgery. Endoscopic treatment is technically feasible and may be considered as the procedure of choice for solitary PN treatment. A long-term follow-up endoscopy of the upper gastrointestinal tract is greatly needed.

Shi L, Liu FJ, Jia QH, Guan H, Lu ZJ. Solitary plexiform neurofibroma of the stomach: A case report. *World J Gastroenterol* 2014; 20(17): 5153-5156 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5153.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5153

INTRODUCTION

Plexiform neurofibroma (PN) is a rare benign peripheral nerve sheath tumor mainly associated with von Recklinghausen disease. PN is rarely presented as a sporadic lesion in a patient without features of generalized neurofibromatosis[1]. Up until now, there were no reports of solitary PN of the stomach without evidence of neurofibromatosis type 1 (NF1) in the literature. Here we describe a rare case of a solitary PN of the stomach in a patient without

features of NF1.

CASE REPORT

A 38-year-old man was admitted to our department due to abdominal pain and distention for 7 d. Informed consent was obtained from the patient. No other signs of von Recklinghausen disease were found in this patient or his family. Physical examination revealed moderate tenderness on palpation of the upper abdominal region. Physical examination of the lungs, heart, and other systems revealed no abnormal features. The patient had no significant medical background or family history. No abnormal findings were revealed by laboratory tests. Chest X-ray, electrocardiography, and abdominal sonogram showed no abnormal features. For diagnostic purposes, an endoscopy of the upper gastrointestinal tract was performed, which revealed a 3.5 cm protruding and cauliflower-shaped mass with a shallow 1 cm central ulcer in the greater curvature of the stomach (Figure 1). Further endoscopic examination revealed no lesions in the small bowel or colon. Computed tomography (CT) imaging with and without contrast medium revealed a soft tissue mass-like lesion about 2.5 cm × 3.5 cm in the greater curvature of the stomach (Figure 2). Upper gastrointestinal radiography showed a mass in the greater curvature of the stomach (Figure 3). The patient underwent an endoscopic submucosal dissection for the en-bloc resection of the lesion. Injection of saline into the submucosa during gastroscopy did not completely elevate the tumor. Therefore, local gastroscopic resection was not indicated. Laparoscopic surgery was successfully performed.

The tumor was diagnosed as sporadic PN, which rarely occurs in the digestive tract. The diagnosis was confirmed by laparoscopic surgery with complete resection. Histological examination demonstrated characteristic histological findings of spindle-shaped cells. Immunohistochemical analysis showed that the tumor cells were positive for S-100 protein (Figure 4), but negative for CD34, KI-67, CD117, and actin. Gastrointestinal stromal tumor (GIST) could thus be excluded in our case and confirmed as PN. Furthermore, we performed PCR and sequencing on a blood sample from the patient and found no any mutations in the *NF1* gene (data not shown). We performed upper gastrointestinal endoscopy six months post-operatively for follow-up, and found no evidence of recurrence.

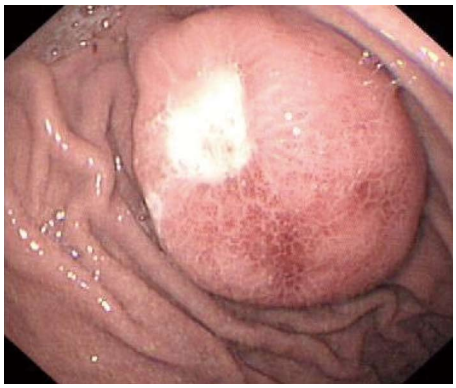

Figure 1 Endoscopic examination revealed a 3.5 cm protruding and cauliflower-shaped mass with a shallow 1 cm central ulcer in the greater curvature of the stomach.

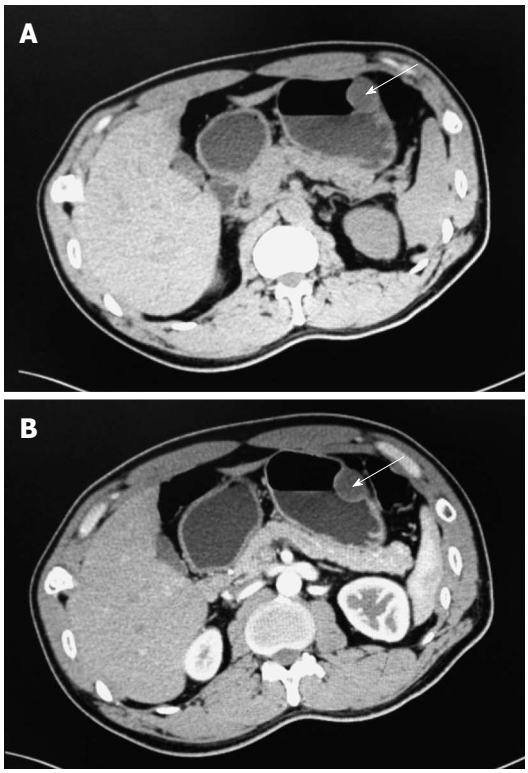

Figure 2 Radiological images of the abdomen. A: Computed tomography (CT) showed a soft tissue mass-like lesion about 2.5 cm × 3.5 cm in the greater curvature of the stomach (arrow); B: Post-contrast abdominal CT shows the same area not representing the contrast-enhanced appearance (arrow).

DISCUSSION

Neurofibromatosis is a benign peripheral nerve sheath tumor. It is a neurocutaneous disorder with two clinical forms: (1) NF1, described by von Recklinghausen; and (2) central type 2, described as a central neurofibromatosis that mainly affects the central nervous system[2]. NF1 is the most common type of neurofibromatosis and accounts for about 90% of all cases. An autosomal-dominant mutation of the neurofibromin (tumor suppressor) gene on chromosome 17 causes tumors of the peripheral nerves known as neurofibromas. NF2 is an autosomal-dominant disorder that induces tumor development. NF-1 is a systemic disorder that may affect any organ in the body, with clinical presentation depending upon the body system involved. Diagnosis of NF1 is based upon a series of clinical criteria as defined by the National Institutes of Health Consensus Statement[3]: (1) at least six so-called "café-au-lait" spots in post-pubertal patients; (2) at least two neurofibromas of any type or one PN; (3) axillary or groin freckling; (4) at least two so-called "Lisch

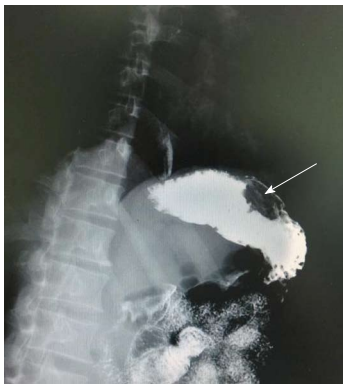

Figure 3 Upper gastrointestinal radiography showed a mass in the greater curvature of the stomach (arrow).

nodules"; (5) optic glioma; (6) distinctive osseous bony dysplasia (such as thinning of the long bone cortex or sphenoid wing dysplasia; and (7) a close family member with NF1. The patient should have at least two of the previous criteria to be considered as NF1.

PN has a network-like growth involving multiple fascicles of a nerve, and may include multiple branches of a large nerve trunk. PN is a rare benign tumor developing from Schwann cells of the peripheral nervous system, often associated with NF1. Isolated PN in other locations without NF1 features has been very rarely encountered in clinical practice. The few cases have been reported so far have been in the oral cavity[4], submandibular salivary gland[1], lesser omentum[5], penis[6], cheek mucosa[7], and intraparotid facial nerve[8]. The specific cause and development of solitary PN are not known, but the possibility of mosaicism of NF-1 or a related gene has been considered. It is supposed that solitary PN may be caused by a sporadic localized mutation of the NF1 tumor suppressor gene[9]. However, in this case, we found no direct evidence of NF1 mutation.

To our knowledge, this is the first report on the rare case of PN in the stomach. The 38-year-old male patient was diagnosed as PN based on clinical appearance, physical examination, and histological features. Our case should be distinguished from GIST, which usually occurs in the small intestine as multiple and clinically indolent tumors. GIST is consistently CD117 (KIT)-positive, generally CD34-positive, occasionally actin-positive, and rarely S-100-positive[10]. According to immunohistochemical analysis, the tumor from this case was positive for S-100 protein, but negative for CD34, KI-67, CD117, and actin. Thus, GIST could be excluded in this case.

Although the cause and development of this rare case remain speculative, due to the malignancy potential, surgical resection, such as open surgery, combined endoscopic/laparoscopic intragastric resection, and laparoscopically gastroesophageal resection, is advocated[11]. The pre-operative diagnosis of PN is often difficult. As a result, PN usually needs to be resected surgically. The application of fine needle aspiration (FNA), including computed tomography guided, transabdominal ultrasonography guided, and endoscopic ultrasonography guided FNA, facilitates the diagnosis of GIST[12]. However, FNA evaluation should not replace subsequent examination of resected specimens[13].

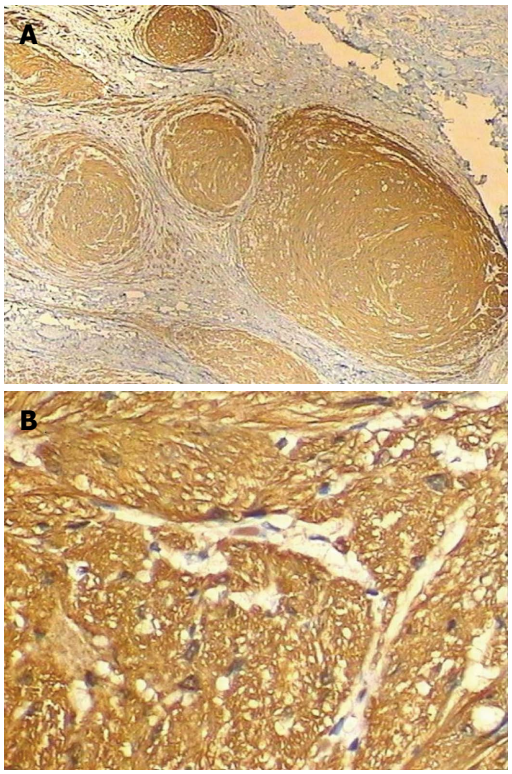

Figure 4 Histological examination of the gastric lesion. A: Histological findings showing irregularly distorted, enlarged nerve bundles (× 40); B: Histopathological examination revealed nuclear and cytoplasmic reactivity with S-100 protein immunostain (× 400).

With the wide application of novel techniques, including endoscopic mucosal resection and endoscopic submucosal dissection, endoscopic resection has recently been performed as a curative treatment for adenoma and early colorectal cancer[14]. Endoscopic resection provides a better quality of life for the patient than conventional open surgery, as it is minimally invasive, brings complete resection, and supports detailed histopathologic evaluation of the specimens.

Although PN is generally benign, prompt resection is recommended because of a risk of malignant transformation. We advocated that endoscopic treatment is technically feasible and may be considered as the procedure of choice for solitary PN. Furthermore, a long-term follow-up endoscopy of the upper gastrointestinal tract is greatly needed.

COMMENTS

Case characteristics

A 38-year-old male was admitted to their department on an emergency basis because of abdominal pain and distention for 7 d.

Clinical diagnosis

Physical examination revealed moderate tenderness on palpation of the upper abdominal region.

Differential diagnosis
Immunohistochemical analysis showed that this case could be excluded as gastrointestinal stromal tumor.

Laboratory diagnosis
No abnormal findings were revealed by routine laboratory tests.

Imaging diagnosis
Computed tomography and upper gastrointestinal radiography showed a mass in the greater curvature of the stomach.

Pathological diagnosis
Histological examination demonstrated characteristic histological findings of spindle-shaped cells.

Treatment
Laparoscopic surgery was successfully performed.

Related reports
Isolated plexiform neurofibroma (PN) in other body organs without features of NF1 has been very rarely encountered in clinical practice. The few cases of isolated PN that have been reported so far have been located in the oral cavity, submandibular salivary gland, lesser omentum, penis, cheek mucosa, and intraparotid facial nerve.

Term explanation
Plexiform neurofibroma is a rare benign peripheral nerve sheath tumor mostly associated with von Recklinghausen disease and rarely presenting as a sporadic lesion in a patient without features of generalized neurofibromatosis.

Experiences and lessons
Immunohistochemical analysis should be performed to distinguish plexiform neurofibroma from gastrointestinal stromal tumors.

Peer review
This is the first report on a rare case of plexiform neurofibroma in the stomach. The authors described the clinical features, physical examination, endoscopic findings, and histopathological examination of this case. However, further information on the expression of the *NF1* gene and histological diagnosis will help us further understand the pathogenesis of this case.

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CASE REPORT

Clinical and computed tomography features of adult abdominopelvic desmoplastic small round cell tumor

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Abstract

To investigate the clinical and computed tomography (CT) features of desmoplastic small round cell tumor (DSRCT), we retrospectively analyzed the clinical presentations, treatment and outcome, as well as CT manifestations of four cases of DSRCT confirmed by surgery and pathology. The CT manifestations of DSRCT were as follows: (1) multiple soft-tissue masses or diffuse peritoneal thickening in the abdomen and pelvis, with the dominant mass usually located in the pelvic cavity; (2) masses without an apparent organ-based primary site; (3) mild to moderate homogeneous or heterogeneous enhancement in solid area on enhanced CT; and (4) secondary manifestations, such as ascites, hepatic metastases, lymphadenopathy, hydronephrosis and hydroureter. The prognosis and overall survival rates were generally poor. Commonly used treatment strategies including aggressive tumor resection, polychemotherapy, and radiotherapy, showed various therapeutic effects. CT of DSRCT shows characteristic features that are helpful in diagnosis. Early discovery and complete resection, coupled with postoperative adjuvant chemotherapy, are important for prognosis of DSRCT. Whole abdominopelvic rather than locoregional radiotherapy is more effective for unresectable DSRCT.

Key words: Desmoplastic small round cell tumor; Peritoneum; Pathology; Computed tomography; Clinical features

Core tip: Desmoplastic small round cell tumor (DSRCT) is a rare abdominopelvic malignancy with multicentric growth. The third decade may be a peak period of incidence, and the disease can also occur in older people. Computed tomography can display characteristic features that are helpful in diagnosis of DSRCT. Prognosis and overall survival rates are generally poor. Early discovery and complete resection, coupled with postoperative adjuvant chemotherapy, are important for prognosis of DSRCT. Whole abdominopelvic rather than locoregional radiotherapy is more effective for unresectable DSRCT.

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INTRODUCTION

Desmoplastic small round cell tumor (DSRCT) is a rare and highly aggressive malignancy with poor prognosis, which was first described by Gerald *et al*[1] in 1989. Since then, more then 200 cases have been reported in the literature, and nearly one-third of them have been reported in the radiological literature. DSRCT primarily occurs in adolescents and young adults aged 15-25 years and develops in the abdominopelvic cavity with metastases commonly found in the peritoneum, liver, and lymphoid tissues. There is a strong male predilection, with male/female ratios ranging from 3:1 to 9:1[2-5]. Clinical presentations are notoriously nonspecific. Vague abdominal pain or discomfort, abdominal distension, change in bowel habits, and palpable abdominal mass are common[3-7]. Most patients are diagnosed in the advanced stages and have a poor prognosis. Bulky peritoneal soft-tissue masses without an apparent organ-based primary site are the imaging characteristics of abdominopelvic DSRCT.

Here, we report the appearance on computed tomography (CT) and the clinical features of four patients (two men and two women; age range, 24-64 years; mean, 35.5 years) with abdominopelvic DSRCT. This retrospective study was approved by the institutional review board of our hospital, which waived the need for informed consent.

CASE REPORT

Case 1

A 26-year-old man had a hypoechoic mass with an echoless area in the left mid-abdomen upon ultrasound (US) examination. On subsequent abdominal and pelvic CT, a 40 mm × 60 mm, well-defined, solid cystic tumor was discovered in the corresponding area (Figure 1A and B). A 24 mm × 27 mm soft tissue mass located on the left side of the pelvic cavity near the rectum was also found (Figure 1C). The pelvic mass, as well as the solid area of the left mid-abdominal mass, showed slight enhancement on contrast-enhanced CT (Figure 1B). The patient underwent resection of the abdominal and pelvic tumors, which were found to have arisen from the greater omentum and peritoneum, respectively. The two masses were subjected to histopathological examination. Microscopic evaluation showed that the tumor cells were small and round with hyperchromatic nuclei and scant cytoplasm (Figure 1D), and immunohistochemical staining was positive for CD99, epithelial membrane antigen (EMA), chromogranin A (CgA), neuron-specific enolase (NSE) and vimentin (Vim). The above features were compatible with DSRCT.

During the subsequent postoperative 6 mo, chemotherapy consisting of ifosfamide, pegylated liposomal doxorubicin, and diamminedichloroplatinum (IEP) was administered for six cycles. The patient was discharged from hospital after recovery.

Thirty-three months after surgery, follow-up CT showed a solitary, slightly enlarged pelvic lymph node and multiple homogeneous tumor nodules reappearing in the retrovesical and pararectal regions (Figure 1E). Six months later, CT showed that the pelvic tumor nodules had enlarged rapidly, forming a multinodular confluent tumor (Figure 1F). The patient died of serious complications caused by recurrence and metastases 42 mo after surgery.

Case 2

A 28-year-old woman presented to our emergency department complaining of persistent pain in the right lower abdomen for 2 d, and the pain became worse for 1 d and slight fever developed. Physical examination revealed a large pelvic non-tender mass. US and CT showed a 150 mm × 120 mm solid-cystic mass located in the pelvis and the right lower abdomen, with a small amount of ascites. The solid area of the tumor showed slight enhancement on contrast-enhanced CT. The tumor was considered to be ovarian cancer by US and CT, and total hysterectomy and bilateral adnexectomy were performed. During the operation, a large mass was found originating from the right broad ligament, and several 20 mm × 20 mm nodules were discovered in the rectouterine space and greater omentum. Acute appendicitis with yellow to tan exudate and hyperemia was seen at the same time. Intraperitoneal perfusion chemotherapy with 20 mg nitrogen mustard was administered before closing the abdomen.

Histopathological evaluation of the excised tumor and nodules showed sheets and clusters of well-demarcated nests of tumor cells with areas of necrosis and hemorrhage. The cells were small and monomorphic with hyperchromatic nuclei, and the immunohistochemical stain was positive for cytokeratin (CK), CD99, EMA, NSE, and desmin. The disease was ultimately diagnosed as DSRCT.

After surgery, she was treated with CAP chemotherapy (cyclophosphamide, adriamycin and cisplatin) for four cycles, then with paclitaxel and cisplatin for one cycle. However, the tumor recurred only 4 mo postoperatively, with symptoms of bowel obstruction and uroschesis. She then received transcatheter arterial chemoembolization and γ ray stereotactic radiotherapy. The recurrent mass volume reduced slightly shortly after γ-knife therapy, but soon it continuously enlarged again (Figure 2A). She died of abdominal widespread tumor implantation and metastases (Figure 2B and C) and uncontrolled recurrence 13 mo after initial surgery.

Case 3

A 64-year-old woman was admitted to hospital with frequent urination and low back pain lasting for 2 wk. On physical examination, a large, medium hardness, poorly defined, non-tender palpable mass was seen predominantly in the mid-right lower abdomen. She also had positive percussion pain in the right kidney area. On US, the tumor was heterogeneous and hypoechoic with surrounding blood flow signals (Figure 3A). CT showed a

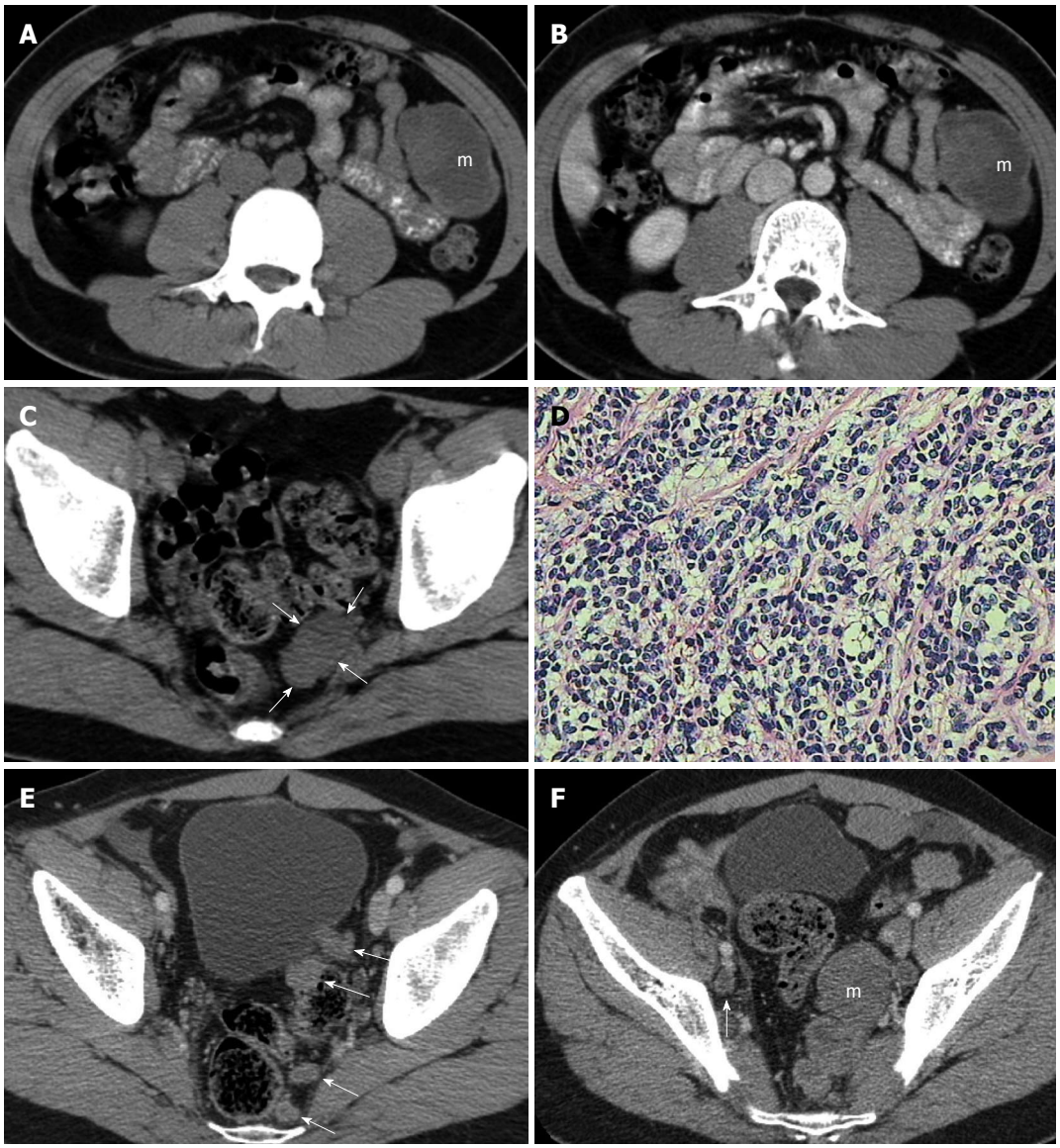

Figure 1 A 26-year-old man without any clinical symptoms. A-C: Preoperative plain and contrast-enhanced computed tomography (CT) showed a solid-cystic mass (m) in the left mid-abdomen and a homogeneous soft-tissue mass (arrows) in the pelvis (A and C: Plain scan, B: Enhanced scan). The solid area of the abdominal tumor showed slight enhancement; D: Photomicrograph of surgical specimen showed nests or clusters of small tumor cells outlined by characteristic desmoplastic stromal bands (hematoxylin and eosin staining, × 400); E: CT re-examination 33 mo after surgery showed multiple homogeneous tumor nodules (arrows) reappearing in the retrovesical and pararectal regions; F: Six months later, CT showed that pelvic tumor nodules had enlarged rapidly forming a multinodular confluent tumor (m), as well as a slightly enlarged lymph node (arrow).

114 mm × 175 mm confluent solid mass in the lower abdomen and pelvic cavity with moderate, heterogeneous enhancement, and several other soft-tissue masses in the pelvic cavity (Figure 3B), as well as lymphadenopathy within the retroperitoneum (Figure 3C). The right mid-lower ureter was invaded with moderate hydronephrosis. In addition, mild ascites on the surface of the liver and spleen and in the rectouterine fossa was seen on US and CT. One week later, an incisional biopsy was taken and subjected to histopathological evaluation. Immunohistochemical staining was positive for CK, EMA, NSE, CgA, synaptophysin (Syn), CD3 and CD56. Two weeks after exploratory laparotomy, the patient commenced palliative radiotherapy.

For nearly a month, she received a total dose of 54 Gy. Follow-up CT at the later stage of radiotherapy showed that all of the masses were significantly reduced in size (Figure 3D and E). However, extensive peritoneal and hepatic metastatic tumors, as well as lymphatic metastasis appeared shortly thereafter, although the largest mass regressed to 35 mm × 36 mm (Figure 3F). The patient only survived for 4 mo after initial presentation.

Case 4

A 24-year-old man presented with a 2-mo history of fatigue and faint abdominal pain and low-grade fever for 2 wk. On physical examination, abdominal distension with shifting dullness was discovered. The patient underwent abdominopelvic CT, which showed diffuse, wavy, omental soft-tissue masses and mesenteric nodules, diffuse

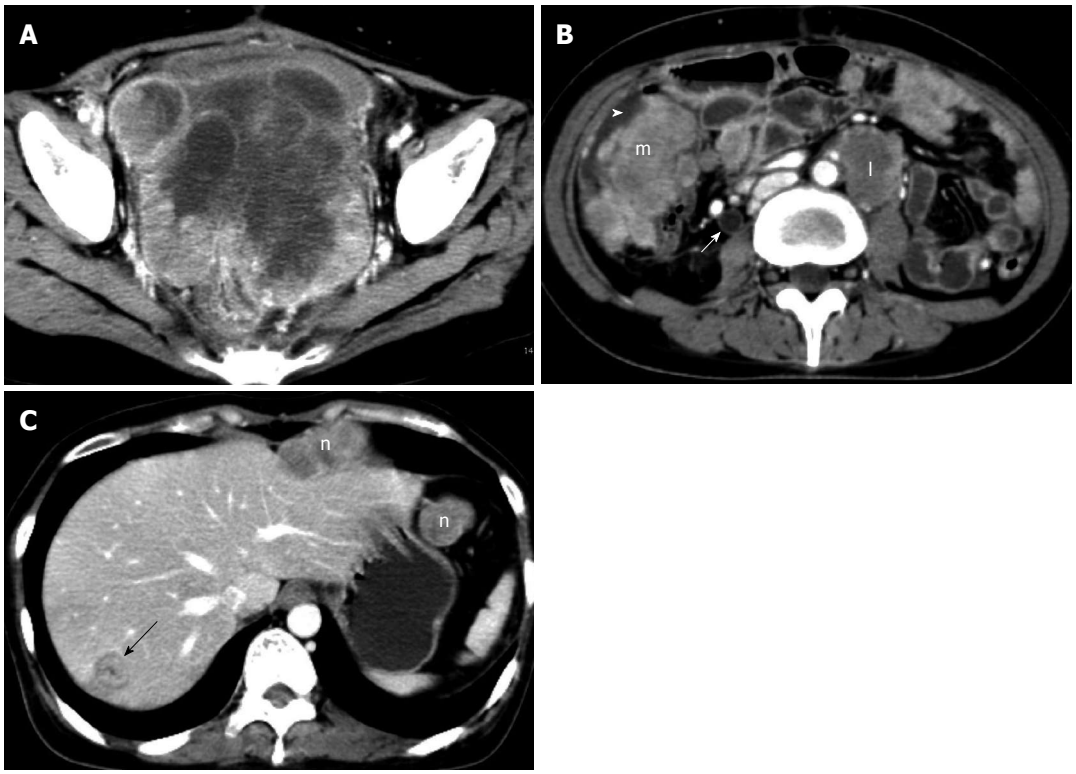

Figure 2 A 28-year-old woman with a large abdominopelvic desmoplastic small round cell tumor. A: Enhanced computed tomography (CT) 4 mo after surgery showed a bulky heterogeneous pelvic mass with areas of low attenuation, and the solid portion of the mass showed obvious enhancement; B, C: Enhanced CT scan 1 mo later showed well-enhanced, lobulated mesenteric masses (m) in the right lower quadrant. A small amount of peritoneal effusion (arrowhead), enlarged para-aortic lymph node (l), right hydroureter (white arrow), omentum nodules (n), and liver metastases (black arrow) were also present.

Table 1 Abdominopelvic findings on computed tomography, *n* (%)

CT findings	Patients	
	Initial diagnosis (*n* = 4)	Follow-up period (*n* = 3)
Omental/mesenteric/serosal masses	4 (100)	3 (100)
Pelvic dominant mass	2 (50)	3 (100)
Tumor calcification	1 (25)	0 (0)
Liver metastases/infiltration	1 (25)	2 (67)
Abdominal/pelvic lymphadenopathy	1 (25)	3 (100)
Ascites	2 (50)	2 (67)
Urinary tract obstruction	1 (25)	2 (67)
Bowel obstruction	1 (25)	1 (33)

The fourth patient did not undergo computed tomography (CT) examination during follow-up period.

peritoneal thickening scalloping the liver edges with liver infiltration, along with massive ascites. The density of the masses was homogeneous on plain scanning, except for punctate calcification, and showed slight to moderate enhancement on contrast-enhanced CT (Figure 4A and B). Laparoscopic exploration was undertaken, and the resultant biopsy revealed a DSRCT. The patient refused further treatment, and died 3 mo after initial presentation.

Abdominopelvic CT findings of all the four patients are summarized in Table 1.

DISCUSSION

DSRCT is a small round blue cell tumor similar to other tumors such as Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, and Wilm's tumor. Typical pathological findings include abundant desmoplastic stroma and poorly differentiated small cells. The tumor is uniquely different from other tumors in that it expresses epithelial, neural, myogenic, and mesenchymal markers. Also, DSRCT generally contains a specific chromosomal abnormality (t11; 21)(p13; q12)[3,7-10]. Most DSRCTs arise in the peritoneal cavity without a primary visceral site of origin, and most investigators believe that the tumor originates from the mesothelium (or from submesothelial or subserosal mesenchyme), which is most extensive in the peritoneum[2,3].

Previous studies have indicated that DSRCT most commonly affects male adolescents and young adults. In our study, distribution by sex did not confirm this male preponderance. The typical age range at diagnosis is 18-25 years[2,11]. In our series, the mean age at diagnosis was 35.5 years (range, 24-64 years), and three (75%) patients were in the third decade of life, and the other was in her 60s. We suppose that the third decade may be a peak period of incidence for DSRCT, and this disease can also occur in older people.

The presenting symptoms of DSRCT are nonspecific, and usually related to the site of involvement. One

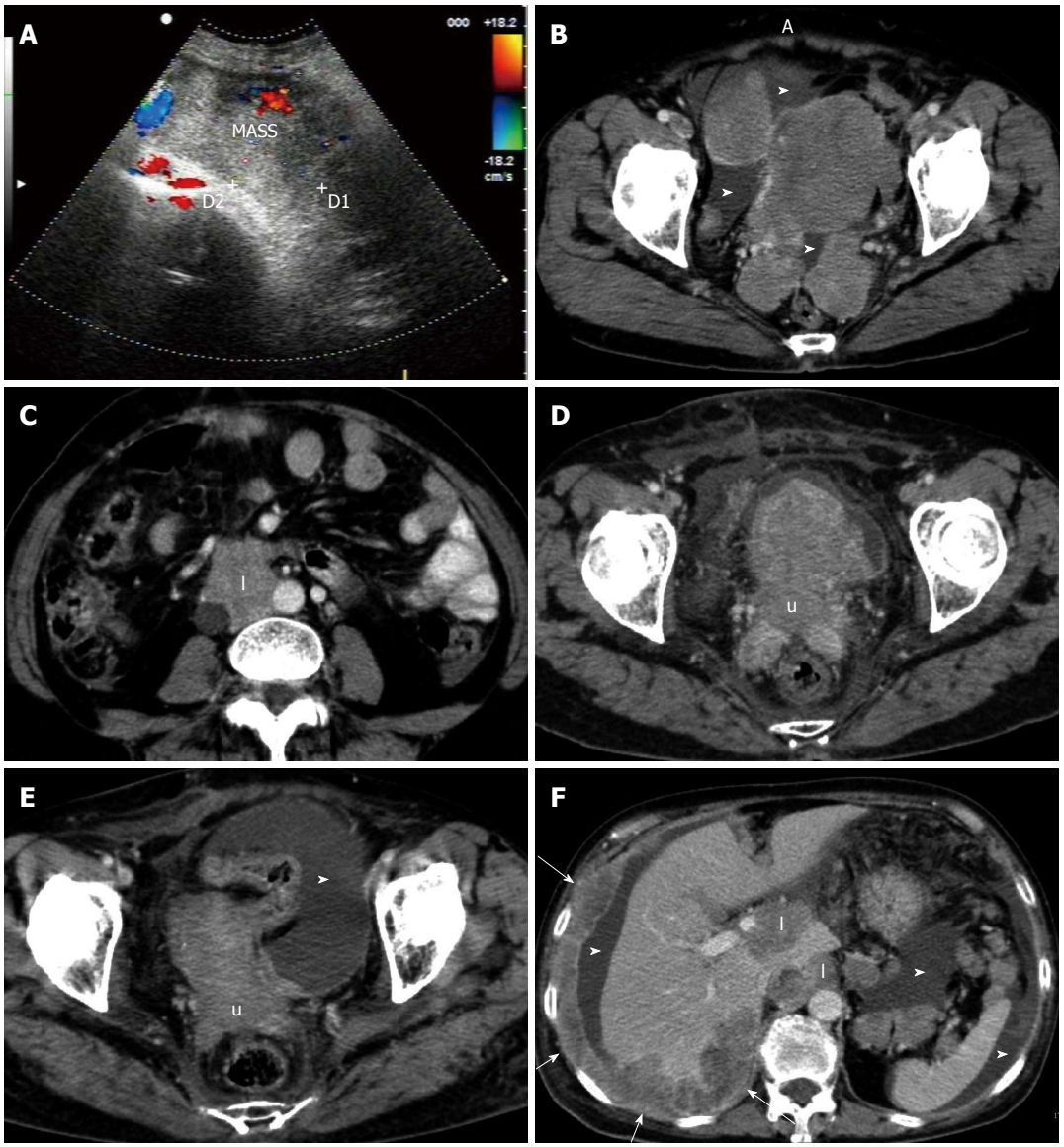

Figure 3 A 64-year-old woman with frequent urination and low back pain. A: Abdominal US demonstrated a large heterogeneous hypoechoic mass with surrounding blood flow; B, C: Contrast-enhanced computed tomography (CT) before treatment showed multiple well-enhanced masses with variable sizes in the pelvic cavity, as well as a small amount of ascites (arrowheads) and lymphadenopathy within the retroperitoneum (I); D: Enhanced CT at the later stage of radiotherapy, showed marked shrinkage of the pelvic tumors ("u" for uterus), along with a small volume of ascites (arrowhead); E, F: Enhanced CT 3 wk after radiotherapy showed further reduced volume of the masses ("u" for uterus). At the same time, diffuse and nodular serous membrane thickening (arrows) with liver infiltration and innumerable mesenteric masses of variable size in the left upper quadrant, and lymphadenopathy (I) within the retroperitoneum and hepatic portal region, along with a moderate volume of ascites (arrowhead), were demonstrated. All of the tumor tissues presented with heterogeneous moderate enhancement.

of our patients complained of frequent urination, and we speculate that tumors compressing the bladder might contribute to this clinical manifestation by sharply reducing bladder capacity.

Patients often initially present with abdominal pain or large, palpable abdominal masses, therefore, CT is most often used for initial diagnosis. Moreover, at the time of initial diagnosis, disseminated tumor with multiple abdominopelvic masses and metastases often exists, and CT is most often used for staging and follow-up[3]. Some studies have reported that the most common anatomical site for this disease is the pelvis, and the second most common site is the peritoneum, with widespread surface masses and nodules[2-7]. Among our cases, three had one or more pelvic masses and two had peritoneal surface masses at the time of initial diagnosis. As the volume of pelvis is far less than the abdomen, pelvic masses always merge into a bulky lobulated mass as they grow, resulting in the presence of a dominant mass in the pelvis for patients with DSRCT. On CT, the hallmark imaging feature is multiple, lobulated, low-attenuation, heterogeneous soft-tissue masses in the omentum or mesentery or along the abdominopelvic peritoneal surfaces, without a distinct organ of origin[3,12-14]. Punctate calcification may be present within tumors in a few cases.

In our cases, solitary peritoneal tumors were found in one patient who did not have any clinical symptoms at initial diagnosis, and with lesions located in the omen-

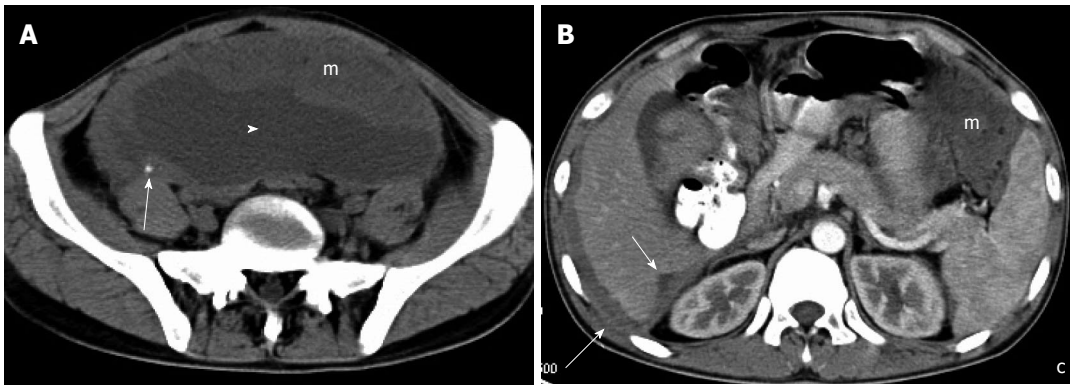

Figure 4 A 24-year-old man with faint abdominal pain. A: Plain computed tomography (CT) demonstrated a large, wavy, omental soft-tissue mass (m) with foci of calcification (arrow), and massive ascites (arrowhead); B: Enhanced CT showed diffuse thickening of the perihepatic parietal peritoneum with liver infiltration (arrows), and omental mass (m). All of the tumor tissues presented with slight uniform enhancement.

tum and pararectal region. However, when re-examined 33 mo after surgery, multiple recurrent irregular nodules were seen in the retrovesical space. Thus, we conclude that DSRCT is multicentric in origin, even if it occasionally appears solitary at the time of early detection.

On enhanced CT, large masses always show heterogeneous enhancement after intravenous administration of contrast medium, and the degree of enhancement is mild to moderate. Focal areas of non-enhancement or low attenuation on contrast-enhanced abdominopelvic CT possibly represent high fibrotic content, in addition to necrosis and intratumoral stale hemorrhage[3,7,12-14]. We found that most smaller masses and peritoneal nodules were almost homogeneous whether on plain or enhanced scanning, such as the first case of ours, the large mass in the mid-abdomen appeared with inhomogeneous cyst, but the small nodule in the pelvis appeared with uniform soft-tissue density.

Apart from multiple peritoneal masses, ascites, lymphadenopathy or liver metastases are often found, and most patients may be asymptomatic for a long period of time and diagnosis is only made when tumor burden is large. Pattern of disease spread includes direct seeding along the peritoneal and serosal surfaces and lymphatic and hematogenous spread[15]. Ascites occurs when the tumor is so extensive that little peritoneal surface remains for absorption of physiological intraperitoneal fluid, and massive ascites implies dismal prognosis. In our cases, ascites, enlarged lymph nodes, and hematogenous dissemination or direct invasion to the liver, as well as hydronephrosis and hydroureter, were also common manifestations at initial or follow-up CT. Hepatic parenchyma is the most common site of extraperitoneal involvement in DSRCT, followed by lung, bone, splenic parenchyma, pleura, and soft tissue[6,15-17]. As for our cases, no metastases to the lungs or other organs, except for the liver, were seen on initial diagnosis or during postoperative follow-up. Urinary tract and bowel obstruction can also be present in the progressive stage secondary to obstruction by tumor.

Ultrasound may be helpful in guiding percutaneous biopsy of relatively superficial lesions but it does not help characterize lesions further, typically demonstrating lobulated, heterogeneous hypo-echoic lesions[18]. ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG PET-CT) may play an important role in patient management, allowing detection of early recurrence of disease and consequent change in treatment strategy[7,19]. None of our cases had undergone PET-CT examination.

A diagnosis of DSRCT usually can be favored by a combination of factors. The radiologic differential diagnosis for multiple solid peritoneal masses is extensive and includes various neoplastic, inflammatory, and miscellaneous processes. Leiomyomatosis peritonealis disseminata, a rare condition affecting premenopausal women, can appear similar to desmoplastic small round cell tumor on imaging. Malignant mesothelioma of the peritoneum is usually infiltrative, but may also manifest as discrete tumors, which are usually accompanied by a variable amount of ascites. Rhabdomyosarcoma is most common in younger children, generally < 10 years of age. Desmoid fibromatosis, peritoneal tuberculosis, fibrosing mesenteritis, splenosis, and amyloidosis are other disorders whose infiltrative and/or tumefactive manifestations overlap with the appearance of DSRCT[12,14].

DSRCT is an aggressive disease with a poor prognosis and a mean survival time of 23 mo and an overall 5-year survival rate of 15%[3,20]. Timely diagnosis is therefore critical to the management of these patients. Combination chemotherapy, radiotherapy, and gross total tumor resection or debulking surgery have been advocated to treat patients with DSRCT. Only one of our patients had a relatively good prognosis and survived 42 mo, who had solitary peritoneal tumors discovered incidentally by health examination. The rest who were in advanced stage at initial diagnosis all had extremely poor prognosis and only survived 3-13 mo, even when comprehensive treatment was given. We suggest that early discovery and complete resection, in addition to postoperative adjuvant chemotherapy, should be key to good prognosis of DSRCT.

There is no standard chemotherapy regimen for DSRCT.

Similar to other small round-cell tumors, DSRCT is alkylator-sensitive and dose-responsive. Kushner *et al*[21] reported a 100% response rate using a chemotherapy regimen (P6 protocol) consisting of vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide in 10 patients with DSRCT. Hayes-Jordan *et al*[6] and Mrabti *et al*[22] thought anthracycline-based therapy regimens (doxorubicin, etoposide, cisplatin, cyclophosphamide) may be best used for recurrent disease[6,22]. Some patients have been reported to achieve complete radiological remission after chemotherapy [21,23,24]. Goodman *et al*[24] reported whole abdominopelvic irradiation in 21 patients who had received chemotherapy followed by debulking operation, and the median time to relapse was 19 mo and median overall survival was 32 mo. In one of our cases, pelvic palliative radiotherapy was administered which resulted in a favorable response by shrinking the local tumor, but a large number of new lesions still emerged in the abdomen at the same time. Therefore, whole abdominopelvic irradiation, rather than locoregional radiotherapy, is a more effective treatment strategy for unresectable DSRCT, because the tumor has the property of multicentric growth in the abdominopelvic cavity.

In conclusion, DSRCT is a rare abdominopelvic malignancy with multicentric growth. The third decade may be a peak period of incidence, and the disease can also occur in older people. CT can display characteristic features that are helpful in diagnosis of DSRCT. The presence of a pelvic dominant, lobulated, low-attenuation, heterogeneous soft-tissue mass with mild to moderate enhancement after intravenous contrast medium administration, along with omental, mesenteric, peritoneum or serosal surface masses, is a characteristic feature of DSRCT. Ascites, hepatic metastases, lymphadenopathy, hydronephrosis and hydroureter are also commonly encountered in patients with DSRCT. Prognosis and overall survival rates are generally poor. Early diagnosis and complete resection, in addition to postoperative combination chemotherapy, are important for prognosis of DSRCT. Whole abdominopelvic radiotherapy, rather than locoregional radiotherapy, is more feasible for unresectable DSRCT.

COMMENTS

Case characteristics

The tumors with multicentric growth arised in the peritoneal cavity without a primary visceral site of origin.

Clinical diagnosis

The presenting symptoms of desmoplastic small round cell tumor (DSRCT) are nonspecific, and usually related to the site of involvement.

Differential diagnosis

The radiologic differential diagnosis for multiple solid peritoneal masses is broad and includes various neoplastic, inflammatory, and miscellaneous processes.

Laboratory diagnosis

Laboratory examination makes no contribution to diagnosis.

Imaging diagnosis

The presence of a pelvic dominant, lobulated, low-attenuation, heterogeneous soft-tissue mass with mild to moderate enhancement after intravenous contrast medium administration, along with omental, mesenteric, peritoneum or serosal surface masses, is a characteristic computed tomography (CT) feature of DSRCT.

Pathological diagnosis

Typical pathological findings include abundant desmoplastic stroma and poorly differentiated small cells.

Treatment

Early diagnosis and complete resection, in addition to postoperative combination chemotherapy, are important for prognosis of DSRCT. Whole abdominopelvic radiotherapy, rather then locoregional radiotherapy, is more feasible for unresectable DSRCT.

Related reports

Hepatic parenchyma is the most common site of extraperitoneal involvement in DSRCT, followed by lung, bone, splenic parenchyma, pleura, and soft tissue.

Term explanation

No uncommon terms are present in the case report.

Peer review

These are well described and the CT scans nicely illustrate the tumors.

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CASE REPORT

Hepatitis B surface antigen seroconversion after HBV reactivation in non-Hodgkin's lymphoma

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Abstract

Reactivation of hepatitis B virus (HBV) can occur in lymphoma patients infected with HBV when they receive chemotherapy or immunotherapy. Prophylactic administration of lamivudine (LAM) reduces the morbidity and mortality associated with HBV reactivation. However, what defines HBV reactivation and the optimal duration of treatment with LAM have not yet been clearly established. HBV reactivation may occur due to the cessation of prophylactic LAM, although re-treatment with nucleoside analogs may sometimes result in hepatitis B surface antigen (HBsAg) seroconversion, which is a satisfactory endpoint for the management of HBV infection. We report a case of HBV reactivation in a 68-year-old HBsAg-positive patient who received rituximab-based immunochemotherapy for follicular lymphoma. HBV reactivation developed following cessation of prophylactic LAM therapy. The patient subsequently received treatment with entecavir (ETV), which led to a rapid and sustained suppression of HBV replication and HBsAg seroconversion. We also appraised the literature concerning HBV reactivation and the role of ETV in the management of HBV reactivation in lymphoma patients. A total of 28 cases of HBV reactivation have been reported as having been treated with ETV during or after immunosuppressive chemotherapy in lymphoma patients. We conclude that ETV is an efficacious and safe treatment for HBV reactivation following LAM cessation in lymphoma patients treated with rituximab-based immunochemotherapy.

Key words: Hepatitis B surface antigen; Seroconversion; Non-Hodgkin's lymphoma; Rituximab; Entecavir

Core tip: We describe the case of a 68-year-old hepatitis B surface antigen (HBsAg)-positive male patient who received rituximab-based immunochemotherapy for follicular lymphoma, and experienced hepatitis B virus (HBV) reactivation following cessation of lamivudine prophylaxis. Subsequent entecavir treatment produced rapid, sustained viral suppression and HBsAg seroconversion. Lamivudine prevents HBV reactivation but resistance rates may be as high as 17% in lymphoma patients. Available data suggest that entecavir is effective and safe for the treatment of HBV reactivation in lymphoma patients. Prophylactic antiviral therapy is recommended for patients with active or occult HBV infection following chemotherapy or immunochemotherapy. Potent antiviral drugs with a high genetic barrier to resistance should be considered in these cases.

Liu WP, Zheng W, Song YQ, Ping LY, Wang GQ, Zhu J. Hepatitis B surface antigen seroconversion after HBV reactivation

in non-Hodgkin's lymphoma. *World J Gastroenterol* 2014; 20(17): 5165-5170 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i17/5165.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i17.5165

INTRODUCTION

Hepatitis B virus (HBV) is highly prevalent in many malignancies, such as hepatocellular carcinoma and non-Hodgkin's lymphoma (NHL)[1,2]. In recent years, rituximab, a chimeric monoclonal antibody directed against the CD20 antigen on B cells, has greatly improved the prognosis and outcome of patients with NHL[3,4]. However, rituximab induced profound and persistent depletion of the circulating population of B cells, leading to dysregulation of host immunity to HBV and increased risk of HBV reactivation[5,6]. Consequently, viral reactivation is an area of concern whenever an HBV-positive patient receives chemotherapy or immunochemotherapy[7]. Prophylactic administration of antiviral agents may reduce the incidence of HBV reactivation but flares do occur in 60% of patients following discontinuation of antiviral treatment[8]. We report a case of HBV reactivation following cessation of prophylactic lamivudine (LAM) in a patient with NHL who received rituximab-based treatment. Early administration of entecavir (ETV) successfully prevented further progression of HBV infection, leading to hepatitis B surface antigen (HBsAg) seroconversion.

CASE REPORT

A 68-year-old male was admitted to our hospital for follicular lymphoma in March 2009. He had chronic HBV infection for more than two decades. On admission, his alanine aminotransferase (ALT) levels were within the upper limit of normal (< 40 U/L). The patient's serology was found to be positive for HBsAg, hepatitis B surface antibody (anti-HBs), hepatitis B envelope antibody (anti-HBe), and hepatitis B core antibody (anti-HBc). However, he was HBeAg-negative. His HBsAg and anti-HBs titers were > 250 IU/mL and 45.81 mIU/mL, respectively, as measured by chemiluminescence microparticle immunoassays. His serum HBV DNA concentration was undetectable (limit of detection by polymerase chain reaction: 1000 copies/mL). The time course of the levels of liver enzymes and HBV DNA is shown in Figure 1.

From March 2009 to July 2009, administration of 5 cycles of immunochemotherapy (rituximab, fludarabine, cyclophosphamide) led to partial remission of the patient's lymphoma. No additional treatment with anticancer drugs or corticosteroids followed. Prophylactic LAM (100 mg daily) was administered on the first day of immunochemotherapy and continued for 4 mo after completion of immunochemotherapy (total: 8 mo). In February 2010, 3 mo following cessation of LAM therapy, the patient's HBV DNA level rose to 8.15×10^4 copies/mL. His ALT, HBsAg, and anti-HBs levels were 24 U/L, 121 IU/mL and 0.18 mIU/mL, respectively. Reactivation of HBV infection was considered and antiviral treatment with ETV 0.5 mg daily was administered immediately. In March 2010, 1 mo after ETV initiation and while still receiving ETV therapy, the patient's HBV DNA concentration fell below detectable levels, while his ALT level increased to 62 U/L. In April 2010, 2 mo after ETV initiation, the patient achieved clearance of HBsAg and normalization of ALT levels. In July 2010, 4 mo after ETV initiation, the patient became anti-HBs-positive (titer: 13.5 mIU/mL), indicating HBsAg seroconversion. In December 2010, 7 mo after HBsAg seroconversion, ETV treatment was stopped (total: 10 mo). In March 2011 (4 mo after discontinuing ETV treatment), his HBsAg level was still negative and the patient's anti-HBs titer had increased to 93.6 mIU/mL. The patient's ALT levels remained normal and his HBV DNA level was undetectable. Until September 2012, 21 mo after ETV discontinuation, his HBsAg level remained negative and the patient's anti-HBs titer had increased to 112.3 mIU/mL (Figure 2). His ALT levels also remained normal, while the HBV DNA concentration was undetectable at the patient's last two visits (Figure 1). Administration of ETV was well tolerated throughout the treatment period.

Other reported cases

The nucleoside analog ETV provides the advantage of a higher genetic barrier to resistance than LAM for the treatment of chronic hepatitis B[9]. ETV has also been used to prevent HBV reactivation during chemotherapy or immunosuppressive therapy, although this experience is limited[10]. Several studies have examined the use of ETV in the treatment of HBV reactivation in lymphoma patients and suggest its effectiveness and safety[11-13]. A total of 28 cases of HBV reactivation reported in the literature involved ETV administration during or after immunosuppressive chemotherapy in patients with lymphoma (Table 1). Nine cases of HBV reactivation developed during chemotherapy or immunochemotherapy[11-17], while the remaining cases occurred after chemotherapy or immunochemotherapy[11,14,18-22]. Twenty-four patients received rituximab-based immunochemotherapy regimens[12-22]. Five patients died of hepatic failure following HBV reactivation[11,15,17,20,22], 4 of whom received rituximab-based regimens[15,17,20,22]. Clearance of HBsAg was observed in only 5 patients[11,19,21]. LAM was administered with the intention of preventing HBV reactivation in 4 patients from 2 different studies[11,18]. Of these, 3[18] developed HBV reactivation-related hepatitis 2-4 mo after discontinuation of LAM while the remaining case of HBV reactivation occurred 8 mo after cessation of LAM treatment[11].

DISCUSSION

Malignant lymphoma is a leading cause of cancer-related

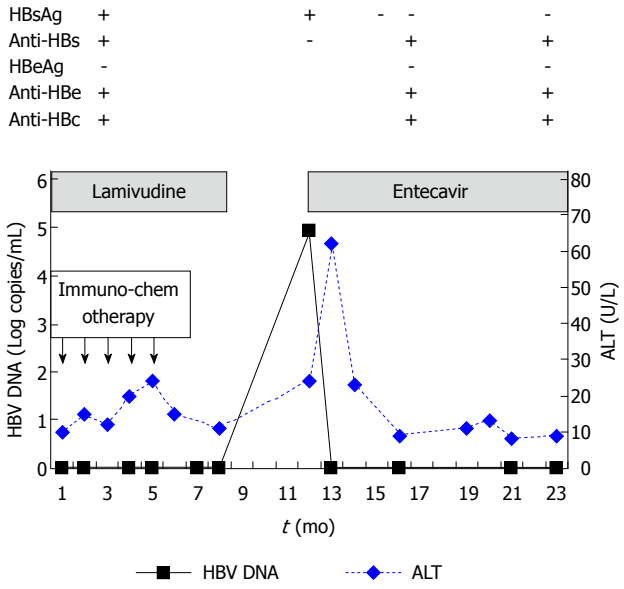

Figure 1 Time course of serum hepatitis B virus DNA and alanine aminotransferase levels during and after immunochemotherapy. HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase.

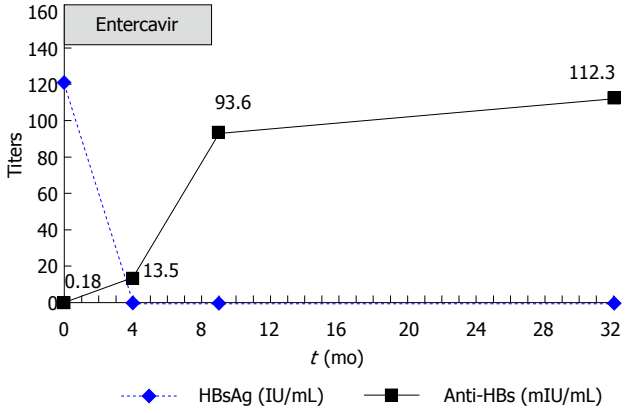

Figure 2 Time course of quantitative titers of hepatitis B surface antigen and hepatitis B surface antibody antibodies after entecavir treatment. Anti-HBs: Hepatitis B surface antibody; HBsAg: Hepatitis B surface antigen.

mortality, despite the fact that the long-term prognosis of patients with diffuse large B cell lymphoma has improved following the introduction of immunochemotherapeutic agents, such as rituximab[23]. The occurrence of HBV infection has been associated with lymphoma and hepatocellular carcinoma[24]. In addition, reactivation of HBV may be a fatal complication in patients with HBV infection who receive immunochemotherapy for lymphoma, especially rituximab-based regimens. While the exact definition of HBV reactivation differs among investigators[25], reactivation of HBV is deemed to occur in both HBsAg-positive or -negative patients. Among patients who only present with anti-HBc antibody-positive serology, the risk factors for HBV reactivation include male gender and low anti-HBs titer[14].

Given the substantial morbidity and mortality associated with HBV reactivation and hepatitis flares, prophylactic antiviral therapy should be administered to HBsAg-positive cancer patients if they receive immunochemotherapy. LAM has been shown to be clinically effective in reducing the incidence and severity of HBV reactivation, but treatment guidelines differ in their recommendations for prophylactic antiviral therapy[26-28]. In addition, the optimal duration of prophylactic LAM therapy has not yet been clearly established. For instance, the incidence of YMDD mutation and HBV reactivation following withdrawal of LAM in patients with NHL were similar to that for patients with chronic hepatitis B. In a long-term study, 17% of HBsAg-positive NHL patients developed YMDD mutation during LAM therapy (median duration: 11.5 mo), and 4% developed HBV reactivation following LAM withdrawal[29]. In one prospective study, 23.9% of 46 patients with hematological malignancies developed HBV reactivation after withdrawal of LAM prophylaxis[30]. HBV reactivation was more likely to develop in patients with elevated HBV DNA levels prior to chemotherapy. A prolonged administration of antiviral therapy may be necessary in these patients; however, drug resistance must be considered. ETV may be the preferred drug because of its high antiviral potency and high barrier to resistance. In a retrospective study, ETV showed a very low rate of prophylaxis failure. HBV reactivation was not detected in 31 HBsAg-positive patients treated with ETV prophylaxis (median duration: 17 mo)[31]. A randomized controlled trial confirmed that ETV prophylaxis until 3 mo after completion of chemotherapy was insufficient even in patients with undetectable hepatitis B. One of the 41 patients in the ETV prophylaxis group had delayed HBV reactivation, almost 7 mo after discontinuing ETV prophylaxis. Therefore, it is important to routinely monitor HBV DNA levels after discontinuation of ETV[32].

In a community-based follow-up study, spontaneous clearance of HBsAg from serum occurred in 562 chronic hepatitis B patients during 24829 person-years of follow-up evaluation, resulting in an overall annual seroclearance rate of 2.26%[33]. The levels of HBV DNA at baseline and follow-up evaluation were the most significant predictor of HBsAg seroclearance[34]. To our knowledge, there has been no report to date of spontaneous HBsAg seroclearance following HBV reactivation. It is therefore unclear whether the HBsAg seroconversion observed in our patient could be attributed to the ETV treatment or considered spontaneous.

In conclusion, prophylactic antiviral therapy is highly recommended in patients with active or occult HBV infection who receive chemotherapy or immunochemotherapy. In the event of HBV reactivation at the time HBV prophylaxis is stopped, administration of a potent antiviral agent with a high genetic barrier to resistance should be considered.

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Table 1 Hepatitis B virus reactivation in patients with lymphoma and treated with entecavir

Author	Patients (*n*)	Hematologic malignancy	Pre-treatment HBV markers	Prophylaxis with anti-viral drugs	Rituximab-based regimen	Time of HBV reactivation (during/after anti-tumor therapy)	Outcome (alive/died)	HBsAg clearance (yes/no)
Ferreira *et al*[22]	1	DLBCL	HBsAg$^-$, anti-HBs$^+$, anti-HBc$^-$	No	Yes	0/1	0/1	NR
Niitsu *et al*[14]	6	DLBCL	HBsAg$^-$	No	Yes	2/4	5/1[1]	NR
Chung *et al*[15]	1	DLBCL	HBsAg$^-$, anti-HBs$^+$, anti-HBc$^+$	No	Yes	1/0	0/1	0/1
Lee *et al*[16]	1	FL	HBsAg$^-$, anti-HBs$^+$	No	Yes	1/0	1/0	NR
Stange *et al*[17]	2	B cell lymphoma	unknown	No	Yes	2/0	1/1	NR
Brost *et al*[11]	4	AML and lymphoma	Unknown for all	1 Yes, 3 No	No	2/2	3/1	3/1
Sanchez *et al*[12]	1	CLL	HBsAg$^-$	No	Yes	0/1	1/0	NR
Colson *et al*[13]	1	B cell lymphoma	HBsAg$^-$, anti-HBs$^-$, anti-HBc$^+$	No	Yes	1/0	1/0	NR
Mimura *et al*[18]	3	B cell lymphoma	HBsAg$^+$	Yes	Yes	0/3	3/0	NR
Matsue *et al*[19]	5	B cell lymphoma	HBsAg$^-$	No	Yes	0/5	5/0	1/4
Wu *et al*[20]	1	DLBCL	HBsAg$^-$, anti-HBc$^+$	No	Yes	0/1	0/1	0/1
Fukushima *et al*[21]	2	NHL, DLBCL	HBsAg$^-$	No	Yes	0/2	2/0	1/1

DLBCL: Diffuse large B cell lymphoma; FL: Follicular lymphoma; AML: Acute myeloid leukemia; CLL: Chronic lymphocytic leukemia; NHL: Non-Hodgkin's lymphoma; NR: Not reported. [1]Patient died of lymphoma-related causes.

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COMMENTS

Case characteristics

A 68-year-old patient was admitted to hospital for follicular lymphoma, with a 2-decade history of chronic hepatitis B virus (HBV) infection. He received rituximab-based immunochemotherapy for follicular lymphoma and experienced HBV reactivation following cessation of lamivudine prophylaxis.

Clinical diagnosis

The patient had no symptoms or signs when HBV reactivation occurred.

Differential diagnosis

Hepatitis due to drugs, alcoholic hepatitis, and other factors were excluded.

Laboratory diagnosis

After cessation of lamivudine (LAM) prophylaxis, the patient's HBV DNA (by polymerase chain reaction) rose to 8.15 × 10^4 copies/mL from an undetectable baseline level, followed by elevated alanine aminotransferase.

Treatment

The patient received entecavir (ETV) 0.5 mg/d for 10 mo and experienced sustained viral suppression with HBsAg seroconversion.

Related reports

ETV provides the advantage of a higher genetic barrier to resistance than LAM for the treatment of chronic hepatitis B. Several studies examining the use of ETV for treating HBV reactivation in lymphoma patients suggest that it is a safe and effective therapy.

Term explanation

HBV reactivation was defined as a tenfold increase in HBV DNA level or the reappearance of detectable HBV DNA.

Experience and lessons

Prophylactic antiviral therapy is highly recommended in patients with active or occult HBV infection who receive chemotherapy or immunochemotherapy; a potent antiviral agent with a high genetic barrier to resistance should be considered.

Peer review

This case reports the relatively uncommon finding of hepatitis B surface antigen seroconversion following ETV therapy in a lymphoma patient with chronic HBV who experienced HBV reactivation following rituximab-based immunochemotherapy. It is well written.

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The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

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