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REVIEW

Less common etiologies of exocrine pancreatic insufficiency

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Abstract

Exocrine pancreatic insufficiency (EPI), an important cause of maldigestion and malabsorption, results from primary pancreatic diseases or secondarily impaired exocrine pancreatic function. Besides cystic fibrosis and chronic pancreatitis, the most common etiologies of EPI, other causes of EPI include unresectable pancreatic cancer, metabolic diseases (diabetes); impaired hormonal stimulation of exocrine pancreatic secretion by cholecystokinin (CCK); celiac or inflammatory bowel disease (IBD) due to loss of intestinal brush border proteins; and gastrointestinal surgery (asynchrony between motor and secretory functions, impaired enteropancreatic feedback, and inadequate mixing of pancreatic secretions with food). This paper reviews such conditions that have less straightforward associations with EPI and examines the role of pancreatic enzyme replacement therapy (PERT). Relevant literature was identified by database searches. Most patients with inoperable pancreatic cancer develop EPI (66%-92%). EPI occurs in patients with type 1 (26%-57%) or type 2 diabetes (20%-36%) and is typically mild to moderate; by definition, all patients with type 3c (pancreatogenic) diabetes have EPI. EPI occurs in untreated celiac disease (4%-80%), but typically resolves on a gluten-free diet. EPI manifests in patients with IBD (14%-74%) and up to 100% of gastrointestinal surgery patients (47%-100%; dependent on surgical site). With the paucity of published studies on PERT use for these

conditions, recommendations for or against PERT use remain ambiguous. The authors conclude that there is an urgent need to conduct robust clinical studies to understand the validity and nature of associations between EPI and medical conditions beyond those with proven mechanisms, and examine the potential role for PERT.

Key words: Celiac disease; Inflammatory bowel disease; Exocrine pancreatic insufficiency; Malabsorption; Epidemiology; Pancreas; Pancreatic cancer; Secretion/absorption; Surgery

Core tip: Exocrine pancreatic insufficiency (EPI) results from primary pancreatic diseases or secondarily impaired exocrine pancreatic function. Pancreatic enzyme replacement therapy (PERT) may prevent serious nutritional complications when such patients have symptomatic EPI. However, EPI may be more prevalent in patients with non-pancreatic diseases, diabetes, and pancreatic cancer than has generally been appreciated. Scant published evidence on EPI in these less common etiologies precludes firm recommendations on management. Robust clinical studies are urgently needed to understand the relationships between EPI and medical conditions beyond those with proven mechanisms, and examine the potential role for PERT.

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INTRODUCTION

The pancreas is a dual function organ that possesses both exocrine and endocrine components that are critical for the digestion, absorption, and metabolism of nutrients. Normal digestion requires the exocrine function of the pancreas for macronutrient digestion. This occurs primarily *via* enzymatic hydrolysis by pancreatic enzymes, in particular lipase, amylase, and proteases (trypsin and chymotrypsin)[1]. Exocrine pancreatic insufficiency (EPI) refers to the presence of maldigestion and malabsorption of nutrients and is a consequence of primary loss of functional parenchyma and/or secondarily impaired exocrine pancreatic function and insufficient pancreatic enzyme activity. EPI is sometimes defined simply as an enzyme output less than 10% of that necessary to sustain normal digestion; however, there is no consensus in the literature on the definition of EPI. Furthermore, the clinical presentation of EPI can vary widely depending on the underlying cause, as well as disease stage, diet, and other factors.

EPI is most commonly caused by diseases that destroy the pancreatic parenchyma, such as chronic pancreatitis and cystic fibrosis, as well as pancreatic resection[1,2]. The incidence of EPI in chronic pancreatitis ranges from 30% of patients with mild disease to 85% with severe disease[3]. Approximately 85% of infants with cystic fibrosis have EPI at birth[4]. However, EPI is also observed in other conditions that include unresectable pancreatic cancer; metabolic diseases (diabetes mellitus)[1,2]; impaired hormonal stimulation of exocrine pancreatic secretion by cholecystokinin (CCK); celiac disease or inflammatory bowel disease (IBD) due to loss of intestinal brush border proteins[1,2]; small intestinal bacterial overgrowth[5,6], although not all investigators have found a clear association[7]; impaired coordination between motor and secretory functions (asynchrony); impaired enteropancreatic feedback, and/or inadequate mixing of pancreatic juices with ingested food after gastrointestinal surgery; and other diseases that affect the pancreas, such as hemochromatosis[8,9] (Tables 1 and 2)[1,2].

In this publication, we first briefly review the diagnosis of EPI and management with pancreatic enzyme replacement therapy (PERT). Most knowledge about EPI comes from studies in patients with chronic pancreatitis or cystic fibrosis, and has already been thoroughly explained in previous reviews. Therefore, our article focuses on several other disease states for which the association with EPI is less straightforward, such as inoperable pancreatic cancer, diabetes mellitus, celiac disease, IBD, and gastrointestinal surgery. Finally, we summarize the limited available data on PERT for the treatment of patients with EPI due to these less common etiologies.

LITERATURE SEARCHES

Searches of BIOSIS Previews, Derwent Drug File, Embase, Embase Alert, International Pharmaceutical Abstracts, MEDLINE, and SciSearch were performed to identify eligible literature from the earliest available date to December 5, 2016. The search terms for EPI were "EPI" or "exocrine pancreatic insufficiency" or "maldigestion" or "malabsorption" or "nutritional deficiency" or "steatorrhea" or "(fat* or oil* or elastase) pre/2 (stool* or fece* or fecal)" or "pancreatic near/3 (function or test)" with the terms NOT "chronic pancreatitis" or "cystic fibrosis". For pancreatic cancer, the search included the following terms: "pancreatic cancer" or "pancreatic adenocarcinoma" or "pancreatic tumor". For diabetes mellitus, the search strategy included "diabetes" and "type 1" or "IDDM" or "insulin-dependent" or "type 2" or "NIDDM" or "noninsulin-dependent" or "type 3" or "type 3c" or "type Ⅲ" or "pancreatogenic". For celiac disease, the search included "celiac disease" or "celiac*". For IBD, the search strategy included "inflammatory bowel disease" or "ibd" or "Ulcerative Colitis" or "Crohn*"

Table 1 Causes of exocrine pancreatic insufficiency

Definite association with EPI
Chronic pancreatitis
Pancreatic tumor/cancer
Cystic fibrosis
Pancreatic resection
Pancreatic hemochromatosis
Mechanisms associated with EPI not fully identified
Type 1 and 2 diabetes
Type 3c (pancreatogenic) diabetes
Gastrointestinal diseases
Celiac disease
Inflammatory bowel disease
Crohn's disease
Ulcerative colitis
Gastrointestinal surgery
Aging

EPI: Exocrine pancreatic insufficiency.

or "Crohn Disease". For gastrointestinal surgery, the search strategy included "gastrointestinal surgery" or "digestive system surgical procedures" or [(post or surg*) near/5 ("gastr*" or "bariatric" or "duodenal switch" or "biliopancreatic diversion")] or "bariatric surger*" or "gastrectom*" or "gastric bypass" or "stomach bypass". Reviews, practical guidelines, letters, editorials, and articles were evaluated. The searches returned 582 hits, from which 163 published articles were initially selected. Subsequently, articles were selected based on their clinical relevance, and additional papers were found after a review of the reference lists of these articles. Only a few were designed as prospective controlled studies with clearly defined methodology; this underscores the lack of data to support associations and mechanisms relevant to the conditions explored.

DIAGNOSIS OF EPI

Patients with EPI may exhibit a wide variety of clinical symptoms and nutritional deficiencies (Table 3). Clinical symptoms associated with EPI include steatorrhea (large-volume, foul-smelling stools), diarrhea, weight loss, flatulence, and abdominal pain. EPI may be diagnosed when fecal fat excretion is > 7 g/d on a 100-g fat/d diet. In EPI, fat malabsorption often develops prior to protein and carbohydrate malabsorption because lipase has a higher susceptibility to intraluminal denaturation and proteolytic destruction compared with other enzymes[10,11]. Furthermore, the deficiency in pancreatic lipase cannot be compensated by gastric lipase, the only other lipolytic enzyme in adult humans[12]. Because the exocrine pancreas has a large functional reserve capacity, clinical symptoms may not manifest until exocrine pancreatic function is < 10% of normal[13]. Untreated malabsorption places patients at high risk for developing nutritional deficiencies[14], which can manifest as other health problems, including decreased bone mineral density resulting in osteoporosis or osteomalacia[15-17]; bone metabolism deficiencies and muscle spasms; impaired night vision and decreased immune competence[16,18,19]; coagulation problems[16]; and ataxia and peripheral neuropathy[16]. Additionally, EPI has been associated with high morbidity and mortality secondary to malnutrition-related complications and an increased risk of cardiovascular events[20].

In routine clinical practice, EPI may be difficult to diagnose, particularly in the early stages when patients are less symptomatic. Often patients make dietary modifications to reduce symptoms. Patients may have low serum levels of fat-soluble vitamins, micronutrients, and lipoproteins[21]. Severe symptomatic EPI can be diagnosed by the presence of steatorrhea, diarrhea, flatulence, or weight loss[22], which often manifest when fecal fat excretion is > 7 g/d (Table 4). Early diagnostic studies relied on direct pancreatic function tests (*i.e.*, those involving collection and analysis of secretions directly from the duodenum or pancreatic duct, including the secretin-pancreozymin and Lundh tests[2]), which remain the most sensitive and specific methods for assessing exocrine pancreatic function. Direct tests, however, are limited by their cost, duration, and invasive nature, which involve endoscopic aspiration or tube aspiration of secretions from the duodenum for several hours. During the past 20 years, the use of non-invasive indirect methods has become more common. These tests are more readily performed in multiple settings and are based on the measurement of fecal elastase and fecal fat[2,23,24].

The coefficient of fat absorption is the gold standard for diagnosing fat maldigestion; however, it is poorly accepted by patients and laboratory personnel because it requires a strict diet containing 100 g of fat daily for 5 d and collection of all feces for the last 3 d (classical Van de Kamer test)[2]. In addition, fat excretion > 7 g/d indicates steatorrhea but is not informative about whether this is due to EPI or extrapancreatic causes. Fecal elastase is a pancreatic enzyme that is stable during passage through the gastrointestinal tract; some consider its measurement as the new gold standard for EPI diagnosis[2]. However, the current cutoffs that are used to define EPI might be improved if the cutoff were reduced to 128 μg/g stool[25] or 84 μg/g stool[26]. For better sensitivity, formed stool samples are best, as loose samples may spuriously dilute and lower the elastase levels and give a false positive result[16,24]. Fecal elastase, measured by enzyme-linked immunosorbent assay, has a good sensitivity for moderate EPI (75%) and high sensitivity for severe EPI (95%), and has a higher specificity (79%-96%) compared with the direct tests[27]. It should be noted that decreased fecal elastase values have been reported in patients with conditions not typically associated with EPI, such as HIV infection (23%-54%), advanced renal disease (10%-48%), and irritable bowel syndrome (6%)[28]. It is commonly accepted

Table 2 Factors involved with exocrine pancreatic insufficiency in different medical conditions[1,16,77,78,107,129,131,133,143,144,152-154,164]

Mechanism involved	Pancreatic cancer	Diabetes mellitus	Celiac disease	IBD	GI surgery
Normal pancreas		√	√	√	√
Abnormal pancreas	√	√	√	√	
Low or absent pancreatic enzyme production	√	√	√	√	√
Lack of stimulus for pancreatic enzyme production			√	√	√
Postcibal asynchrony	√	√	√	√	√
Pancreatic or biliary tract abnormalities	√	√		√	
GI malabsorption			√	√	√

EPI: Exocrine pancreatic insufficiency; GI: Gastrointestinal; IBD: Inflammatory bowel disease.

Table 3 Common signs and symptoms of exocrine pancreatic insufficiency[1,14-16,18,19,22]

Sign/symptom	Associated findings
Excessive flatulence	Abdominal bloating or distension, cramps, belching
Steatorrhea	Fatty, bulky stools; increased bowel movements
Malnutrition	Weight loss, anorexia, fatigue
Vitamin D deficiency	Deficient bone mineralization, osteomalacia, osteoporosis
Vitamin K deficiency	Coagulation abnormalities, ecchymoses, bone metabolism deficiencies
Vitamin A deficiency	Night blindness, decreased immune competence
Vitamin E deficiency	Ataxia and peripheral neuropathy
Hypocalcemia	Muscle spasms, osteomalacia, osteoporosis
Hypoalbuminemia	Nail leukonychia

that a fecal elastase-1 level ⩽ 200 μg/g stool indicates EPI, with levels of 100 to 200 μg/g typically indicating mild to moderate impairment and levels < 100 μg/g reflecting severe impairment[29-31]. Fecal elastase testing is considerably more sensitive than the fecal chymotrypsin or PABA test and is the standard clinical marker for moderate to severe EPI[32-34]. However, there is poor correlation between fecal elastase levels and coefficient of fat absorption, making fecal elastase less attractive for clinical research and regulatory purposes[35]. Additionally, because fecal elastase values are unaffected by PERT, enzymes do not need to be stopped before testing; unfortunately, this also means that fecal elastase testing is ineffective for monitoring response to PERT, unlike direct measurement of fat absorption[3].

Although not widely available, other tests for the diagnosis of EPI include the ^{13}C-mixed triglyceride (^{13}C-MTG) breath test and secretin-enhanced diffusion-weighted magnetic resonance cholangiopancreatography imaging (MRCP). In the ^{13}C-MTG test, the patient ingests a small amount of ^{13}C-marked triglycerides which are degraded by lipases in the intestine to ^{13}C-marked fatty acids. The absorbed ^{13}C fatty acids are metabolized by the liver, and $^{13}CO_2$ is exhaled[36]. Lower lipase activity is associated with less $^{13}CO_2$ in the exhaled breath. This test can also be used to assess the effects of PERT[37]. Pancreatic exocrine function can also be assessed by changes in duodenal filling, pancreatic duct caliber, and accumulation of fluid in the pancreatic parenchyma, as monitored by MRCP following stimulation with exogenous secretin[38,39].

PERT

PERT is the backbone of EPI treatment. Patients with abnormal fecal fat excretion, steatorrhea, and/or weight loss are generally considered candidates for PERT[20]. The aims of PERT are to compensate for deficiencies in endogenous enzyme secretion, correct maldigestion and malabsorption, and ameliorate symptoms resulting from a loss of exocrine function. To achieve this, the enzymatic activity delivered into the duodenum in conjunction with gastric emptying must be sufficient to optimize digestion and nutrient absorption[21]. A main goal of PERT is to restore sufficient intestinal lipase levels[11]. Unprotected lipase is irreversibly inactivated in the acidic environment of the stomach (pH ⩽ 4). Consequently, inhibition of gastric acid secretion has been used to prevent lipase inactivation. Modern preparations consist of pancreatic enzymes encapsulated in microspheres or microgranules, with an enteric coating designed to release the enzymes into the pH-neutral environment of the intestinal lumen[40]. A number of porcine lipase preparations are approved for PERT[41], and the reader is referred to publications from national and professional organizations for recommended dosages[16,42-44] Replacement of protease and amylase is also important in EPI, where some of its symptoms relate to deficiency of these two enzymes, as well. Pancrelipase of porcine origin contains the three enzymes (lipase, protease, and amylase) in adequate ratios to treat EPI.

In randomized controlled trials, PERT improved the coefficient of fat absorption, clinical symptoms,

Table 4 Symptoms and tests used in the diagnosis of exocrine pancreatic insufficiency[2,16,23,24]

Clinical symptoms
Steatorrhea
Diarrhea
Flatulence
Weight loss
Laboratory findings
Fecal fat > 7 g/d on a 100-g fat/d diet
Inconvenient; special high-fat diet and prolonged collection of feces
Considered gold standard
An abnormal coefficient of fat absorption is not specific for EPI
Fecal elastase-1 level ⩽ 200 μg/g stool; < 100 μg/g stool = severe EPI
Simple, convenient, and widely available
Measured on a random stool sample
Liquid stools may lead to falsely low results due to dilution
Less accurate in mild stages of disease
Positive qualitative fecal fat (Sudan Ⅲ) staining
Special high-fat diet
Less accurate; semi-quantitative microscopic method
Insensitive for mild disease
Fecal chymotrypsin ⩽ 6 U/g stool
Less sensitive than fecal elastase for mild EPI
Fluorescein dilaurate (pancreolauryl test)
Easy to perform
Not widely available
^{13}C-mixed triglyceride breath test
Well established
Not widely available
Imaging/endoscopy
Pancreatic duct dilatation
Main pancreatic duct calculi
Endosonographic criteria of chronic pancreatitis
Secretin-enhanced diffusion-weighted magnetic resonance cholangiopancreatography imaging
New
Not widely available

EPI: Exocrine pancreatic insufficiency.

and quality of life (QoL) of patients with EPI and significantly slowed gastric emptying[45-48]. Patients with EPI experienced a reduction in stool frequency and fat/water content, as well as abdominal pain and flatulence[47]. PERT is generally well tolerated; treatment-emergent adverse events include headache, infection, abdominal pain, flatulence, diarrhea, and dyspepsia[45-47,49]. However, because only porcine PERT products are currently available, allergic reactions, including anaphylactic shock, could potentially occur. Furthermore, fibrosing colonopathy, a rare but serious complication, has been reported in children[50] and adults[51] with cystic fibrosis receiving high-dose PERT, but there have been no reports in subjects with chronic pancreatitis.

PANCREATIC CANCER AND EPI

Pancreatic cancer ranks fourth among cancer-related deaths in the United States and has a 5-year survival rate of 7.2%[52,53]. This review focuses on inoperable pancreatic cancer, as the relationship between pancreatectomy and EPI is already well-recognized. EPI in patients with pancreatic cancer is related to the loss of pancreatic parenchyma and/or obstruction of the main duct, which impedes the production of pancreatic enzymes or their transportation into the duodenum. The most important predictors for EPI are localization of the tumor to the pancreatic head, ⩾ 90% destruction of normal tissue, degree of ductal obstruction, and surgical loss of pancreatic tissue[1,16,54]. The severity of ductal obstruction is proportional to the length of the obstructed duct; hence, enzyme secretion decreases as the cancer spreads distally, from head to body to tail[1,54,55].

The reported occurrence of malabsorption and exocrine dysfunction varies between 66% and 92% of patients with pancreatic cancer[30,56-59], with 65% to 75% of patients experiencing fat malabsorption and 50% of patients experiencing some degree of protein malabsorption[60,61]. In a prospective study of patients with an inoperable tumor of the pancreatic head region, 66% had EPI at diagnosis and 92% had a fecal elastase level < 200 μg/g by the 6-mo follow-up; 77% of patients were being treated with PERT[59]. In a systematic review, the prevalence of EPI was 25% to 50% in patients with advanced pancreatic cancer who did not undergo resection[62]. Although EPI is usually moderate in severity[61], in a prospective study, Partelli *et al*[31] detected extremely reduced (fecal elastase ⩽ 20 μg/g) in 25%, severely reduced (> 20 to < 100 μg/g) in 14%, and moderately reduced exocrine pancreatic secretion (⩾ 100-200 μg/g) in 11% of patients with advanced pancreatic cancer[31]. Pancreatic function abnormality seems to be higher in patients with tumors located in the pancreatic head versus in the body or tail[31,63]. Furthermore, in a prospective study, significantly more patients with a pancreatic head tumor had extremely reduced exocrine pancreatic secretion (fecal elastase ⩽ 20 μg/g) versus patients with a body or tail tumor; notably, a significant correlation was found between extremely reduced exocrine pancreatic secretion and poor survival.

Several studies have also reported inadequate enzyme secretion (trypsin, lipase, amylase, elastase, and chymotrypsin) in patients with pancreatic cancer compared with healthy controls[55,58,64]. Elastase production may be reduced earlier and to a greater extent compared with the output of other enzymes, for unknown reasons[64]. Additionally, fecal amylase activity was significantly decreased in pancreatic cancer patients compared with healthy controls[65].

PANCREATIC CANCER AND PERT

Approximately 80% to 90% of patients with pancreatic cancer have unresectable or advanced metastatic disease, leaving only palliative treatment options to manage symptoms[66]. Gastrointestinal and diet management problems negatively impact patients' QoL[67]; consequently, early treatment of EPI has been suggested to reduce symptoms[59] and to improve weight gain and fat absorption in patients with

pancreatic cancer[60,66]. The National Comprehensive Cancer Network has advised that PERT be given to patients with pancreatic cancer who show symptoms of EPI[68]. Other organizations have noted that PERT may help maintain weight and promote QoL in patients with pancreatic cancer[40,69].

The recommendations for PERT use in pancreatic cancer patients were made despite a paucity of data to support them. Only two randomized placebo-controlled trials have investigated the use of PERT in pancreatic cancer (Table 5). In a double-blind trial of 21 patients with unresectable cancer of the pancreatic head, patients treated with 50000 units of lipase/meal gained 1.2% in body weight in 8 wk, while those receiving placebo lost 3.7%[66]. Fat absorption also improved by 25% with PERT, whereas it dropped by 25% with placebo. Steatorrhea did not significantly differ between groups; however, there was a trend for lower stool frequency in patients receiving PERT. When patients receiving placebo were switched to open-label PERT, they demonstrated weight stabilization and improvements in steatorrhea-related symptoms. In a double-blind, placebo-controlled study in patients with unresectable pancreatic cancer (43% had severe EPI, defined as fecal elastase-1 < 100 μg/g stool), mean weight loss after 8 wk of PERT (-1.49%) was not significantly different compared with placebo (-2.99%)[70]. However, PERT did improve nutritional status in a subset of patients with unresectable cancer of the pancreatic head region. Additionally, in an uncontrolled study of patients with unresectable pancreatic cancer, patients with moderate to severe fat or protein malabsorption showed improved nutrient absorption with PERT[60].

DIABETES MELLITUS AND EPI

Type 1 diabetes is considered a primary autoimmune process characterized by typically early onset, an eventual absolute lack of insulin, and islet cell antibodies[71]. Type 2 diabetes is a metabolic disorder characterized by hyperglycemia in the context of insulin resistance and a relative lack of insulin[71]. A third type of diabetes, type 3c or pancreatogenic diabetes[71-73], occurs secondary to parenchymal pancreatic disease and is characterized by an absent pancreatic polypeptide response to nutrients and loss of islet cells by inflammatory destruction and fibrosis[74,75]. The relationship between EPI and diabetes is complex due to the close anatomic and physiologic linkages between the exocrine and endocrine pancreas; pathological conditions of the endocrine tissue can cause impairment of exocrine function and vice versa. Furthermore, depending on the particular diagnostic tests that are used, there is the chance of inadvertently classifying type 3c diabetes as type 1 or 2, confounding understanding of their relative prevalence and relationship to EPI.

Type 1 and type 2 diabetes

Marked alterations in the exocrine pancreas are observed in patients with diabetes, including changes in size, morphology, and histology[76]. No studies have examined at what point during the course of diabetes these pancreatic abnormalities develop. Diabetic pancreata are often atrophic and can have prominent fatty involutions and calcification[77,78]. Atrophy is more pronounced in type 1 *vs* type 2 diabetes[79,80]. Moreover, the pancreata of diabetic patients are significantly smaller and have higher lobulation compared with healthy controls[79,80]. In a cadaveric study, the mean weight of pancreata in type 1 diabetic patients weighed about a half of that of controls[81], while magnetic resonance imaging studies in adults with recent-onset diabetes found only a 26% to 31% reduction in pancreatic volume index after adjustment for body weight compared with healthy controls[82,83]. Additionally, pancreatic volume in diabetic patients was significantly lower when elastase and/or chymotrypsin levels were low[77]. Atrophy of the gland and acini, lymphocytic infiltration, moderate to severe fibrosis, and fatty changes were noted on autopsy in the exocrine pancreas of Japanese patients with diabetes[84].

EPI associated with diabetes is typically mild to moderate and not associated with overt steatorrhea. The prevalence of EPI is higher in type 1 diabetes (26%-57%)[85-89] compared with type 2 diabetes (20%-36%)[78,85,86,88], significantly so in a pooled literature analysis of 3662 patients with diabetes (39% *vs* 28%, respectively, using a cutoff of fecal elastase 200 μg/g stool; $P < 0.00001$)[76]. Severe reductions in fecal elastase levels (< 100 μg/g) have been observed in 11% to 30% of patients with type 1 diabetes[85-88,90] and 3% to 20% of patients with type 2 diabetes[85,86,88,91,92]. Notably, in a large screening study of diabetic patients, correlations between exocrine insufficiency and early onset/longer duration of diabetes, insulin use, and lower body mass index (BMI) have been demonstrated[86]. Fecal elastase levels have also been found to correlate with worse glycemic control, less residual β-cell function, and higher BMI[93,94].

Fecal fat excretion inversely correlates with fecal elastase levels in type 1 diabetes; however, excessive fecal fat excretion occurred in 22% of patients with normal fecal elastase levels[87]. In a cohort of diabetics with fecal elastase levels < 100 μg/g, 59% of patients excreted ⩾ 7 g of fat per day[86]. Interestingly, 45% of type 1 diabetics with pathological fat excretion were asymptomatic in one prospective study[89]. Fecal fat excretion did not correlate with the type or duration of diabetes, age at onset, glycemic control, or BMI[89,95]. The prevalence of EPI was 33% using the direct secretin-cerulein test in patients with type 1 diabetes; among patients with an abnormal secretin-cerulein test result and steatorrhea ($n = 8$), 50% had decreased lipase but none had an enzyme secretion level

Table 5 Pancreatic enzyme replacement therapy clinical trials

Study	Study design, duration (when given), and number of patients	Disease	Results	Adverse effects
Bruno *et al*[66]	DBRPC, 8 wk, 24 adults (21 analyzed)	Pancreatic cancer	The mean absolute difference for PERT *vs* placebo in percentage change in body weight was 4.9% (P = 0.02); other outcomes were numerically improved with PERT *vs* placebo [fat absorption coefficient, 12% increase *vs* 8% decrease (P = 0.13); stool frequency, decrease of 1/d *vs* increase of 2/d (P = 0.07)]	No treatment-related AEs
Woo *et al*[70]	DBRPC, 8 wk, 67 adults	Pancreatic cancer	The mean change in body weight at 8 wk was similar with PERT *vs* placebo (-1.49% *vs* -2.99%; P = 0.381), but the mean change in nutritional status was superior with PERT *vs* placebo in the subset of patients with cancer of the pancreatic head (PG-SGA score, -42.65% *vs* 32.93%; P = 0.039)	Three patients died [PERT group, 2/34 (6%); placebo group, 1/33 (3%)] There were no PERT-related serious AEs
Perez *et al*[60]	Open-label, 12 adults	Pancreatic cancer	Most patients with moderate to severe fat (6/7) or protein (3/3) malabsorption improved, but no patients with mild fat or protein (0/8) malabsorption improved	No descriptions regarding TEAEs
Ewald *et al*[49]	DBRPC, 16 wk, 80 adults	Type 1 diabetes	No significant change in HbA_{1c}, fasting glucose, or postprandial glucose; increase in mean vitamin D from baseline to week 16 (PERT, from 54.1 to 59.4 nmol/L; placebo, 60.2 to 62.7 nmol/L)	TEAEs occurred in 33 patients (84.6%) in PERT group and in 35 (85.4%) in PBO group; most frequent AEs were headache, infection, pain, diarrhea, and dyspepsia
Carroccio *et al*[150]	DBRPC, 2 mo, 40 children	Celiac disease	Significant mean ± SD weight gain in first 30 d (1131 ± 461 g with PERT *vs* 732 ± 399 g with placebo; P < 0.006), not significant at 60 d	No undesired side effects were reported
Evans *et al*[141]	Open-label, up to 4 yr, 20 adults	Celiac disease	Significant increase in fecal elastase from median of 90 μg/g to 365 μg/g (P < 0.0001) and improvement in chronic diarrhea with reduction in median stool frequency from 4/d to 1/d ($P \leqslant 0.0001$), but no weight increase (P = 0.3)	No descriptions regarding TEAEs
Leeds *et al*[135]	Open-label, up to 2 yr, 20 adults	Celiac disease	Significant improvement in chronic diarrhea with reduction in median stool frequency from 4/d to 1/d ($P \leqslant 0.0001$), but no weight increase (P = 0.3)	No descriptions regarding TEAEs
Huddy *et al*[181]	Open-label, 10 adults	Esophagectomy	Improvement in diarrhea and steatorrhea (9/10), increased weight (7/10)	Nausea in 1 patient
Armbrecht *et al*[183]	DBRPC crossover trial, 2 wk (plus 1-wk washout), 15 adults	Total gastrectomy	Improved stool consistency (score, 7.6 with PERT *vs* 9.3 with placebo; P < 0.05), but not the number of bowel movements or abdominal symptoms	No descriptions regarding TEAEs
Hillman *et al*[166]	Open-label, 6 mo, 30 adults	Partial gastrectomy	Mean ± SE weight gain of 6.73 ± 0.77 (P < 0.001), mean ± SE decrease in steatorrhea of 49.7% ± 7.7% (P < 0.001)	No descriptions regarding TEAEs
Brägelmann *et al*[184]	DBRPC, 14 d, 52 adults	Total gastrectomy	Improvement of overall well-being (15/23 with PERT *vs* 6/24 with placebo; P = 0.006), but no improvement of specific symptom	No descriptions regarding TEAEs

AE: Adverse event; DBRPC: Double-blind, randomized, placebo-controlled; GIP: Gastric inhibitory polypeptide; GLP-1: Glucagon-like peptide-1; HbA_{1c}: Glycated hemoglobin A_{1c}; PBO: Placebo; PERT: Pancreatic enzyme replacement therapy; PG-SGA: Patient-generated subjective global assessment; TEAE: Treatment-emergent AE.

< 10% of normal, which is typically when steatorrhea manifests[89]. In the same study, the fecal elastase test had low sensitivity (36%-55%) and specificity (59%-77%) to reproduce the secretin-cerulein test results; the authors concluded that low fecal elastase levels do not reliably indicate EPI in type 1 diabetes.

Secretory abnormalities have been noted in diabetics[96-100]. Frier *et al*[96] observed reductions in exogenously stimulated secretion of amylase (66%) and trypsin (54%) in type 1 diabetics, and the degree of dysfunction correlated with disease duration in a small controlled study. Bicarbonate output was also significantly reduced and showed a significant inverse correlation with the daily insulin dosage in patients with a disease duration < 10 years. Furthermore, hyperglucagonemia, which is observed in some type 2 diabetic patients, is associated with a marked inhibition of pancreatic enzyme output, including lipase, amylase, and trypsin[97]. Increased somatostatin, also found in some diabetic patients, dose-dependently inhibits secretion of pancreatic bicarbonate, amylase, and trypsin[98-100].

Several theories have been proposed to explain the reduced exocrine function in diabetes, including

imbalances between stimulatory and inhibitory islet hormones, pancreatic atrophy or fibrosis, autonomic neuropathy, altered release of gastrointestinal regulatory mediators, and autoimmunity[79,80,84,101,102]. Disturbances in acinar-islet interactions with imbalances between stimulatory (insulin) and inhibitory (glucagon, somatostatin) islet hormones are linked to EPI in some diabetic patients[101]. Insulin has a trophic effect on the acinar cells and a stimulatory effect on exocrine enzyme secretion in animal models and cell cultures, suggesting that insulin deficiency may play a role in pancreatic atrophy[101,103]; insulin deficiency in diabetic patients may lead to pancreatic atrophy[79,80,84]. If insulin deficiency were the primary reason for exocrine dysfunction, however, then all patients with type 1 diabetes would be expected to have EPI.

Regulation of pancreatic enzyme elaboration and secretion depends on gastrointestinal hormones and local neuronal signals[101]. Unsurprisingly, therefore, autonomic diabetic neuropathy and secondary gastroparesis can impair enteropancreatic reflexes, such as changes in gut peptides, that may mediate as much as 50% of the postprandial exocrine pancreatic response[75,101,104]. Diabetic microangiopathy can reduce pancreatic perfusion and cause arterial lesions that can lead to pancreatic fibrosis[105,106]. Patients with type 2 diabetes are also at an increased risk for biliary disease, which can diminish secretions from the pancreas[107]. Finally, autoimmune diseases can involve both the exocrine and endocrine glands, as antibodies against islet cells can cross-react with acinar cells[34]. Autoantibodies against exocrine pancreatic antigens were detected in 77% of patients with type 1 diabetes, but were not detected in any patients with type 2 diabetes[108]. In summary, screening for EPI in patients with type 1 or type 2 diabetes is appropriate when symptoms suggest pancreatic insufficiency.

Type 3c diabetes

Pancreatogenic or type 3c diabetes occurs secondary to pancreatic disease, injury, or resection and accounts for 5% to 10% of the Western diabetic population[8,9,109,110]. Despite the prevalence of type 3c diabetes, the American Association of Clinical Endocrinologists and American College of Endocrinology have not formally included it in their guidelines[73]. The etiologies of type 3c diabetes include chronic pancreatitis (76%-79%), pancreatic cancer (8%-9%), hereditary hemochromatosis (7%-8%), cystic fibrosis (4%), and post-pancreatic resection (2%-3%)[8,9]. Furthermore, the prevalence of type 3c diabetes in chronic pancreatitis is correlated with the degree of exocrine dysfunction (with a prevalence of 63%, 32%, and 13% with severe, moderate, and mild dysfunction, respectively)[111]. Per diagnostic criteria, all patients with type 3c diabetes display signs of EPI[112], and this EPI is more severe compared with that of patients with type 1 and type 2 diabetes, as demonstrated by lower stimulated bicarbonate and trypsin output[96] and lower fecal elastase levels[94].

Because of the close anatomical relationship between exocrine and endocrine cells, type 3c diabetes may result from progression of the primary pancreatic exocrine disease that destroys islet cells by pancreatic inflammation or fibrosis[111,113]. Indeed, the impairment of pancreatic endocrine function in chronic pancreatitis proceeds in parallel with the destruction and spread of fibrosis inside islet cells[111,114,115]. Additionally, α- and β-cell responses were reduced in patients with autoimmune pancreatitis[115]. Mechanisms besides simple islet cell destruction may also be involved, as even small adenocarcinomas are associated with type 3c diabetes[116].

DIABETES MELLITUS AND PERT

Despite a paucity of clinical data for PERT use in patients with diabetes, position statements have stated that symptomatic patients with fecal elastase levels <100 μg/g may be treated with PERT[16,117] but should be carefully monitored because of the risk of disturbances in glucose homeostasis[118]. Of course, increased glucose uptake may reduce the risk of hypoglycemia[34]. There is some evidence that PERT can improve glucose metabolism by augmenting the effects of incretins and increasing postprandial insulin secretion[48,119]; however, no significant differences in hemoglobin A1c, fasting glucose, or oral glucose tolerance test results were observed between patients with type 1 diabetes treated with PERT and placebo (Table 5)[49].

In summary, there are different gastrointestinal motility and comorbid conditions in patients with diabetes mellitus that may result in EPI or decreased digestion or absorption of fat and protein. Early EPI is very difficult to diagnose in diabetic patients, where the condition appears and progresses insidiously across years. Although endocrinologists have not formally recognized type 3c diabetes, most of the conditions that lead to type 3c diabetes have a well-known association with EPI and the need for PERT. An interdisciplinary approach is needed to better define the possible association of EPI with diabetes and potential mechanisms, and to separate them from pancreatic processes that may or may not be related to diabetes. Furthermore, guidelines are needed to help clinicians decide when to test diabetic patients for EPI, and when use of PERT is beneficial.

AGING AND EPI

There are few studies on the effects of aging on exocrine pancreas function, and most[120-122] but not all studies[123] have found that EPI appears to increase with age. For example, in a study of older individuals (age 60 to > 79 years) without gastrointestinal

diseases or diabetes, fecal elastase-1 levels correlated negatively with age and were significantly lower in individuals > 70 years of age compared with a control group (age 20-28 years)[122]. Among subjects over 60 years of age, 21.7% had fecal elastase-1 levels below 200 μg/g stool.

CELIAC DISEASE AND EPI

Celiac disease is an inappropriate T-cell–mediated reaction to gluten that causes inflammatory injury to the small intestine; the estimated worldwide prevalence is 1% to 2%[124,125]. Diarrhea is common with celiac disease and is typically attributed to gluten-related indigestion, malabsorption, and fluid secretion. The primary treatment is a gluten-free diet, which usually improves gastrointestinal function, diarrhea, and weight gain. Nonetheless, 17% to 61% of patients with treated celiac disease have persistent diarrhea[126,127]. Diagnostic testing of celiac patients with chronic diarrhea on a gluten-free diet determined that EPI was present in 12% (based on pancreatic test or trial of PERT) to 18% (based on steatorrhea and trial of PERT)[126,128].

Pancreatic dysfunction occurs in some patients with celiac disease but is typically transient and normalizes with a gluten-free diet[129,130]. However, some patients do have substantially impaired exocrine pancreatic function, leading to maldigestion, malabsorption, and malnutrition. Comorbid chronic pancreatitis has been one possible explanation for these severe cases, with several publications reporting a higher incidence of chronic pancreatitis in patients with celiac disease[131,132]. In patients with untreated celiac disease, the prevalence of EPI (measured and defined by the fecal elastase test) ranges from 4% to 80%[133-138]. Subnormal secretion of at least 1 pancreatic enzyme was observed in 22% to 33% of patients with untreated celiac disease[130,139,140]. In a small controlled study, a significant inverse correlation was demonstrated between the severity of intestinal damage and fecal elastase levels in patients with celiac disease[133]. Carroccio *et al*[140]. reported normalization of fecal chymotrypsin in almost all patients with celiac disease on a strict gluten-free diet[140],which speaks to the functional aspect of EPI due to lack of stimulus rather than to structural damage. It has been suggested that impairment of exocrine pancreatic dysfunction is related to mucosal villous atrophy[129,133], and thus can improve when mucosal regeneration occurs with a gluten-free diet and other treatments[137,141].

In a single-center study by Rana *et al*[142], 36 patients with celiac disease serologically and histopathologically diagnosed were studied with fecal elastase, endoscopic ultrasound (EUS), and elastography. At study entry, 10 of the patients (28%) were diagnosed with EPI based on abnormal fecal elastase levels; 9 (90%) of these patients had villous atrophy of the duodenum, and 1 patient had a history of several episodes of acute pancreatitis. The 10 patients were subjected to a gluten-free diet, and after 3 mo 7 patients had a repeat fecal elastase test that had normalized in all cases, except for the patient with prior acute pancreatitis events. Elastography results were normal in all 8 patients who consented to EUS, except for the patient with prior acute pancreatitis events. The authors concluded that EPI, identified based on fecal elastase levels in adult patients with celiac disease, may be unrelated to structural changes in the pancreatic parenchyma and might be reversible by a gluten-free diet in most patients.

The pathophysiological mechanisms of EPI in celiac disease may be multifactorial. A primary mechanism could be a defective postprandial response to intraluminal contents by an atrophic upper intestinal mucosa with altered synthesis, storage, and/or secretion of secretin and CCK, which are potent stimulators of pancreatic secretion. Postprandial plasma CCK levels were significantly lower in patients with untreated celiac disease compared with controls and were significantly correlated with fecal elastase levels[133]. Impaired CCK release leads to reduced pancreatic stimulation and secretion, postcibal asynchrony between gastric emptying and gallbladder contraction, and fat maldigestion[143,144]. Decreased secretin release by the extensively damaged jejunal mucosa has also been reported[145]. General malnutrition is associated with defects in pancreatic secretion[146]; consequently, it is not unexpected that protein malnutrition in celiac disease is associated with a decrease in pancreatic enzyme output, as well as structural changes in the pancreas, including atrophy of acinar cells with fewer secretory granules, pancreatic fibrosis, and a smaller pancreatic head[147,148]. One study, however, reported that EPI in celiac disease may be independent of nutritional status[139]. There is some evidence for malabsorption of amino acids in patients with untreated celiac disease[149], which might contribute to EPI by restricting the substrates for synthesis of digestive enzymes.

CELIAC DISEASE AND PERT

Pancreatic function tests are usually not performed on newly diagnosed patients or patients with uncomplicated celiac disease; these tests should be considered if there is persistent diarrhea or steatorrhea despite a gluten-free diet or if there are signs of overt malnutrition. Patients on a gluten-free diet with low fecal elastase levels should receive PERT[16]. Data from a double-blind randomized trial of children with celiac disease on a gluten-free diet demonstrated that PERT increased body weight versus placebo during the first 30 days after diagnosis (Table 5)[150]. Similarly, PERT reduced chronic diarrhea from 4 to 1 stools/day in 90% of patients with celiac disease in 2 other uncontrolled studies[135,141].

Gastroenterologists specializing in celiac disease have not recognized a definite association between

celiac disease and EPI and are silent on the possible association and the need for treatment with PERT. Further studies are required to demonstrate whether there is any direct association between celiac disease and EPI.

IBD AND EPI

Crohn's disease (CD) and ulcerative colitis (UC) are chronic relapsing immune-mediated disorders of the gastrointestinal tract, characterized by chronic gastrointestinal inflammation. It is suggested that these disorders result from an aberrant immune response and loss of tolerance to the normal intestinal flora. Patients with IBD are at an increased risk for developing EPI, particularly if they have $\geqslant$ 3 daily bowel movements (BMs), loose stools, and a history of surgery[34,151]. Autopsy studies have found pancreatic lesions in 38% of patients with CD and 53% of patients with UC without prior evidence of pancreatitis[152]. Although still widely used, the fecal elastase test has poor diagnostic accuracy in patients with diarrhea[12]. In a cross-sectional study of 237 unselected patients with IBD, 21% demonstrated exocrine dysfunction as measured by the PABA test, and 19% exhibited abnormally low bicarbonate secretion in response to a secretin test; the frequency of abnormal results was similar in patients with CD and UC[153]. Furthermore, 8.4% of patients had a pancreatic duct abnormality[153].

CD

As a group, patients with CD have significantly decreased lipase, amylase, and trypsin activity compared with controls; these changes are not correlated with disease duration or location or extent of a previous bowel resection[154,155]. Factors related to impaired pancreatic function were disease activity, localization, and extent of bowel involvement[154]. The prevalence of EPI based on low fecal elastase levels varies between 14% and 30% of patients with CD[151,156]. Angelini *et al*[157] determined that 35% of patients with CD have impaired bicarbonate and/or enzyme secretion[157]. Depending on the involvement of the gastrointestinal tract (ileum, colon, or both), abnormal fat excretion varies between 17% and 35% in patients with CD[158].

Possible mechanisms for the development of EPI in CD include pancreatic autoantibodies, duodenal reflux, and reduced secretory hormone secretion. About one third of patients with CD have autoantibodies against pancreatic components[159-161], suggesting that EPI could result from immunologic induction of pathways that impair exocrine function. These antibodies appear specific for CD, as opposed to an individual with UC or without IBD[159,160]. Other possible mechanisms for pancreatitis in patients with CD include duodenal reflux into the pancreatic duct through an inflamed and incompetent ampulla of Vater and fistula formation between the pancreatic duct and the duodenum[162]. These processes could play a role in CD-associated EPI by damaging the pancreatic duct. Indeed, pathological changes in the pancreatic duct that may impede flow have been reported in patients with CD and UC[153]. Finally, scarring or inflammation may reduce intestinal hormone secretion, thus insufficiently stimulating the pancreas[154].

UC

In unselected patients with IBD, 22% of patients with UC had fecal elastase levels $\leqslant$ 200 μg/g, and 9% had severe EPI (fecal elastase $\leqslant$ 100 μg/g)[151]. Additionally, using a secretin-cerulein test, 50% of patients with UC demonstrated bicarbonate and/or enzyme insufficiency, while 74% had an abnormal PABA test[153,157]. By magnetic resonance cholangiopancreatography, 16.5% of patients with UC had a pancreatic duct abnormality, compared with no individuals in the control group[163].

IBD AND PERT

Despite the high prevalence of EPI in patients with IBD, we identified no studies that assessed whether PERT can improve maldigestion or malabsorption in patients with either CD or UC, nor any guidelines for the use of PERT in these populations.

GASTROINTESTINAL SURGERY AND EPI

Upper gastrointestinal surgery can distort the normal anatomy and physiology of digestion, thus disrupting the intricate sequence of events that control normal digestion and absorption. Maldigestion occurs in as many as 80% of patients following such procedures, and EPI may contribute to the pathogenesis[164]. Pancreatectomy results in bulk loss of enzyme-producing cells and is already an indication for PERT, so it will not be discussed here.

Post-gastrectomy diarrhea and/or steatorrhea occur in > 47% of gastrectomy patients, and significant weight loss is common[165-167]. In one study, all patients (n = 15) developed severe EPI 3 mo after total gastrectomy[168]. Steatorrhea was also observed in all patients (n = 30) who underwent a partial gastrectomy[166]. Two additional studies reported pathological fecal fat excretion in 92% and 67% of patients after total gastrectomy[167,169]. Additionally, using the ^{13}C-mixed triglyceride breath test, 82% of patients exhibited fat maldigestion after a Whipple procedure[164]. Finally, using the same diagnostic test, Perez Aisa *et al*[170] recently reported that 38% of patients developed fat malabsorption following partial or total gastrectomy.

EPI and altered pancreatic enzymes and gastrointestinal hormone levels were reported after both total and partial gastrectomies[168,171-173]. Luminal pancreatic enzyme and bile salt concentrations were

markedly reduced after subtotal gastrectomies[173], and significant reductions in the stimulated secretion of pancreatic juice (76%), trypsin (89%), chymotrypsin (91%), and amylase (72%) were observed after total gastrectomy compared with preoperative levels[168]. In another study, total gastrectomy significantly decreased bicarbonate (48%), lipase (39%), and chymotrypsin (24%) output in comparison with non-operated controls[167]. In a third study, only 30% of patients had EPI following subtotal or total gastric resection as measured by the fecal chymotrypsin test[171]. Low levels of gastrin and pancreatic polypeptide and high levels of postprandial plasma CCK have also been reported following total gastrectomy[168].

Gastrectomy disrupts several of the normal digestive processes; different factors may contribute to the postoperative changes, including deficient trituration of nutrients, altered gastric emptying, pancreatic denervation, postcibal asynchrony between gastric emptying and gallbladder contraction, and/or decreased absorptive surface and enzyme contact[16]. When the duodenum is also resected (gastroduodenal resection), a reduction in CCK secretion from the duodenum decreases pancreatic stimulation and contributes to EPI[164]. Likewise, Roux-en-Y gastric bypass surgery to treat obesity disrupts the normal digestive process, and almost a third of patients develop EPI post-operatively[174]. However, since the purpose of the procedure is to effect weight loss, it is unlikely that EPI in this situation would be treated.

The vagus nerve plays an important role in the regulation of exocrine pancreatic secretions, as vago-vagal enteropancreatic reflexes mediate responses in the intestinal phase of exocrine pancreatic secretion[175]. Vagotomies, which reduce gastric acid secretion by severing the vagal nerve supply to the stomach, also cause dysfunction of the exocrine pancreas; during extensive gastric surgery, severing of the vagus nerve (truncal vagotomy) can contribute to postoperative EPI, and a vagotomy by itself is sufficient to cause EPI[176]. In 2 studies, patients had decreased pancreatic juice, lipase, trypsin, and bicarbonate secretion following vagotomy[177,178]. In a similar study, fecal fat excretion was significantly increased after vagotomy and 45% of patients developed steatorrhea[179].

Extensive small bowel resections leading to short bowel syndrome can also reduce endogenous exocrine pancreatic secretion. Short bowel syndrome is characterized by malabsorption, with contributing factors including a reduction in gastrointestinal hormones (particularly CCK), postcibal asynchrony, gastric acid hypersecretion, loss of intestinal regulatory feedback, massive loss of absorptive surface, and rapid transit through the small intestine[1,180]. Additionally, total parenteral nutrition and anti-diarrheal agents used to treat short bowel syndrome are associated with pancreatic and gastric hyposecretion[180]. Some of these mechanisms, though not all, involve the pancreas, suggesting a role for EPI. There is wide variability depending upon the individual and the specific region resected. In patients undergoing ileal resection for CD, fecal fat excretion showed a highly significant correlation to the ileal length resected; for patients with only a 30-cm resection or less, the prevalence of abnormal fat excretion was 37%, whereas 100% of patients who underwent a 90-cm resection or greater displayed abnormal fecal fat excretion[158].

Esophagectomy has also been associated with EPI in one study (n = 63); 10 patients (16%) who underwent an esophagectomy had weight loss and fecal elastase levels < 200 μg/g stool and had symptomatic EPI with diarrhea and/or steatorrhea[181]. Potential mechanisms include decreased gastric reservoir, vagal denervation, and the presence of pyloroplasty that may be part of the procedure and cause dumping syndrome.

GASTROINTESTINAL SURGERY AND PERT

Despite a paucity of evidence regarding PERT use following gastrointestinal surgery, PERT is often recommended for post-surgical patients with steatorrhea, diarrhea, weight loss, or maldigestion-related symptoms[164,182]. In patients with EPI post-esophagectomy, 9 of 10 patients with fecal elastase levels < 200 μg/g stool had symptomatic improvement (no diarrhea or steatorrhea) with PERT and 70% experienced weight gain (Table 5)[181]. PERT may also be appropriate for asymptomatic patients with fat excretion > 15 g/d, as these patients are at high risk for developing nutritional deficiencies[164,182]. It has been suggested that PERT in combination with a high-energy diet over 6 to 8 meals/d may improve nutritional status and symptoms in these patients[11]. Because each patient and surgery is unique and patients have different degrees of EPI, PERT dosing must be tailored to the individual symptoms of a patient.

Data regarding the overall benefits of PERT in total or partial gastrectomy patients are conflicting; while some evidence suggests improved stool consistency[183], weight gain[166], quality of life[184], and reduced steatorrhea and fecal fat excretion[166,185], the same benefits were not observed in all studies. For example, in the double-blind crossover study that showed improvements in stool consistency following a total gastrectomy, there were no beneficial effects of PERT on fecal fat output; however, in the subset of patients with massive steatorrhea, there was a significant reduction in fecal fat excretion following treatment with PERT[183]. The variable trial results

prevent definitive conclusions about the benefits of PERT in fecal fat excretion and steatorrhea following gastric surgery.

CONCLUSION

The prevalence of EPI may be higher in patients with diverse non-pancreatic diseases or pancreatic cancer (Table 2) than has generally been appreciated. EPI should be considered as a possible etiology for any patient with diabetes, celiac disease, IBD, gastrointestinal surgery, or pancreatic cancer who presents with malnutrition, weight loss, and/or abnormal fatty stools (Table 4). In patients with symptomatic EPI, dietary modifications should be implemented and PERT may be initiated and doses should be titrated to achieve the optimal response.

Evidence from clinical research on EPI in less common etiologies is scanty and precludes firm recommendations on management. The lack of studies and evidence-based practices on the association of EPI with the medical conditions discussed herein makes conclusions difficult and needs to be substituted with consensus and clinical practice guidelines derived from future prospective, controlled studies, to confirm or refute these associations. EPI is a serious condition that, once confirmed and regardless of the cause, requires PERT treatment to prevent devastating, sometimes fatal, nutritional complications associated with untreated maldigestion and malabsorption. Further studies are needed to define the association of EPI with these conditions and to support recommendations on the timing of diagnostic testing and initiation of PERT.

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MINIREVIEWS

Radiofrequency ablation for hepatic hemangiomas: A consensus from a Chinese panel of experts

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Abstract

Recent studies have shown that radiofrequency (RF) ablation therapy is a safe, feasible, and effective procedure for hepatic hemangiomas, even huge hepatic hemangiomas. RF ablation has the following advantages in the treatment of hepatic hemangiomas: minimal invasiveness, definite efficacy, high safety, fast recovery, relatively simple operation, and wide applicability. It is necessary to formulate a widely accepted consensus among the experts in China who have extensive expertise and experience in the treatment of hepatic hemangiomas using RF ablation, which is important to standardize the application of RF ablation for the management of hepatic hemangiomas, regarding the selection of patients with suitable indications to receive RF ablation treatment, the technical details of the techniques, therapeutic effect evaluations, management of complications, *etc*. A final consensus by a Chinese panel of experts who have the expertise of using RF ablation to treat hepatic hemangiomas was reached by means of literature review, comprehensive discussion, and draft approval.

Key words: Hepatic hemangiomas; Radiofrequency ablation; Consensus

Core tip: Recent studies have shown that radiofrequency (RF) ablation therapy is a safe, feasible, and effective procedure for hepatic hemangiomas. It is necessary to formulate a widely accepted consensus among the experts in China who have extensive expertise and experience in the treatment of hepatic hemangiomas using RF ablation, which is important to standardize the application of RF ablation for the management of hepatic hemangiomas.

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INTRODUCTION

Hepatic hemangioma is the most common benign tumor of the human liver, with an incidence of 0.4% to 20% in the general population and a prevalence of 0.4% to 7.3% incidentally found in autopsy[1-5]. Most incidentally identified and asymptomatic hepatic hemangiomas do not need medical interventions. However, therapies are warranted for giant hepatic hemangiomas (⩾5 cm) that cause significant symptoms or the peripherally located hemangiomas posing the risk of life-threatening spontaneous rupture and hemorrhage[6-10].

Hepatic hemangiomas have been treated with a wide spectrum of therapies. Traditionally, surgical resection and surgical enucleation are the mostly used treatments of choice[6-10]. Minimally invasive therapies for hepatic hemangioma include transcatheter arterial embolization (TAE)[11-14], radiation therapy[15,16], and radiofrequency (RF) ablation[17-37]. Orthotopic liver transplantation has been performed as the treatment choice in rare circumstances[38]. In recent years, RF ablation has been increasingly used for managing hepatic hemangiomas due to its unique advantages compared with other therapies, such as minimal invasiveness, low cost, low incidence of complications, short duration of hospital stay, and increased patient compliance[17-37]. So far, 501 patients with hemangiomas treated by RF ablation have been reported in the literature[17-37]. A widely accepted consensus or guideline, embracing the selection of patients with suitable indications to receive RF ablation treatment, the application of the techniques, therapeutic effect evaluations, and management of complications, is needed to standardize the management of hepatic hemangiomas using RF ablation.

CONSENSUS DEVELOPMENT

The literature search was performed with an inclusion of published articles during the period from 2003 to 2017. The searched database is Medline. Medical subject headings and free-text words were used for searches, including radiofrequency ablation and hepatic hemangioma. Twenty-one original research papers pertinent to RF ablation treatment for hepatic hemangiomas were included. Of the 21 included papers (501 patients), 13 (465 patients) came from China, including ten in English and three in Chinese[17-37]; two came from South Korea, in which 25 patients with hepatic hemangiomas underwent ultrasound-guided percutaneous RF ablation[21,25]; and Western experience contributed six case reports including 11 patients on the RF ablation for hepatic hemangiomas[18,23,24,26,30,36]. Studies were reviewed and selected for further screening analyses and for subsequent consensus studies.

Base on a comprehensive appraisal of the published original research articles related to RF ablation for hepatic hemangiomas, a Chinese panel of experts was contacted and convened by electronic mails and/or phone. The recruitment of the experts was determined based on the following comprehensive criteria: the number of reported cases using RF ablation for hepatic hemangiomas and the impact of the published articles. All the experts work in well-known, high-volume centers, and their clinical experiences are well documented in scientific papers. They reviewed both the literature and their institutional experience. The draft was completed by the corresponding author and his team and then was circulated to all participants for comments. The revised version was redistributed to the experts for approval or further comments. After more than nine months of electronic mail or, on occasion, face-to-face discussions, the final version of consensus was formulated.

OVERVIEW OF HEPATIC HEMANGIOMAS

Pathology and clinical features

Hepatic hemangioma is usually a solitary tumor mass, although multiple lesions may be present in both hepatic lobes in up to 40% of the patients. Hepatic hemangiomas include cavernous hemangioma, sclerosing hemangioma, hemangioendothelioma, and capillary hemangioma. It consists of blood-filled cavities fed by the hepatic arterial circulation, with walls lined by a single layer of endothelial cells, and manifests as a veritable chaotic entanglement of distorted blood vessels confined to a region as small as a few milliliters and as large as 10 cm, 20 cm, and even 40 cm[1-3].

According to the diameter of hepatic hemangioma, it could be divided into three categories: small (<5 cm), giant (5-9.9 cm), and huge (≥10 cm). Giant hemangiomas are also defined by a diameter larger than 4 cm in the literature[1-5].

Diagnosis

In most situations, hepatic hemangiomas (especially when smaller than 4 cm) do not show any signs and/or symptoms, most likely being discovered incidentally during imaging investigations for other unrelated conditions. However, a few patients may present a wide variety of non-specific clinical symptoms such as pain in the right upper abdomen, decreased appetite, premature satiation sensation, nausea, vomiting, and abdominal discomfort such as sense of fullness and postprandial bloating (early or late). Spontaneous or traumatic rupture is the most severe complication. This has a catastrophic outcome if not promptly managed, and is the reason why correct diagnosis and management are extremely important[1-3].

Hepatic hemangiomas can be specifically diagnosed by ultrasonography (US) or contrast-enhanced US, contrast-enhanced computed tomography (CT), and magnetic resonance imaging (MRI) because of their typical imaging characteristics[4]. On US images, hepatic hemangiomas present as a homogeneous, round or oval lesion with well-defined hyperechogenicity, and likelihood of posterior acoustic enhancement. Other imaging techniques, such as contrast-enhanced CT or MRI, are recommended for confirmation in case of inconclusive ultrasonographic results, or if a giant hemangioma requires treatment[4,5]. The typical hemangioma appears on CT or MRI scans as a hypointense, well-defined lesion, which after contrast injection shows peripheral nodular enhancement with progressive homogeneous centripetal filling.

TRADITIONAL TREATMENT OF HEPATIC HEMANGIOMAS

Surgical procedure used to be the first choice of treatment for hepatic hemangiomas, including hemangioma enucleation, lobectomy (segmentectomy) or partial hepatectomy, and tumor suture or ligation[6-10]. Liver transplantation is used for rare cases which need medical intervention but the tumor is too large to undergo surgical resection, ruptures, or has hemorrhage combined with Kasabach-Merritt syndrome[38]. Surgical resection is rather invasive and associated with relatively high risks of perioperative morbidity (27%), mortality (3%) and long hospitalization[6-10].

Radiation therapy can destroy the endothelial cells and smooth muscle cells of hepatic hemangiomas, consequently leading to thrombosis, necrosis, and fibrosis inside the tumors, which provides partial reduction in hemangioma size and relief of symptoms. Currently, radiation therapy is used rarely in hepatic hemangioma because it is not considered as a

curative therapy. Moveover, it may have the risks of radiation hepatitis, veno-occlusive disease, and hepatic neoplasia[15,16].

TAE has been undertaken as an effective alternative treatment for managing hepatic hemangioma. Before TAE, hepatic arterial angiography is carried out to assess the blood supply to the tumor, tumor size, tumor number, tumor position, and intrahepatic vascular variants. The superselective catheterization of the hepatic arteries leading to the tumor body is followed by embolizing the tumor vessels using an embolization agent mixed with pingyangmycin or bleomycin. With the destruction of endothelial cells lining the blood sinus, thrombosis is formed to block the blood supply to the body of hemangioma. However, TAE is not considered as curative because recurrence is common due to vascular recanalization[34]. Moreover, the treatment may have disastrous complications, such as systemic embolization, biliary damage, and hepatic rupture as the size of tumors increases[11,12]. Several reports have advocated TAE as a temporizing and auxiliary preoperative procedure for huge hepatic hemangioma or spontaneously ruptured hemangiomas to decrease the risk of surgery[11-14,34].

HISTORY OF RF ABLATION FOR HEPATIC HEMANGIOMAS

RF ablation is performed by using RF-induced thermal energy to damage the endothelial lining vascular structures as a result of promoting thrombosis[18], to induce necrotic coagulation[19], as well as to destruct erythrocytes and cause vascular smooth muscle cell disappearance and fibrosis in the ablated zone[19].

In 2003, Cui *et al*[17] reported the first cohort of 12 patients who received the percutaneous RF ablation treatment for 15 hepatic hemangiomas (2.5-9.5 cm) under US guidance. In 2004, Zagoria *et al*[18] reported the successful treatment of a symptomatic hepatic hemangioma (5.0 cm) using percutaneous RF ablation under CT guidance. A few more studies show that percutaneous RF ablation therapy is a safe, minimally invasive, and effective locoregional treatment for selected patients with hepatic hemangiomas[21,23]. In 2006, Fan *et al*[20] reported the use of laparoscopic RF ablation therapy for treating 21 patients with 50 hepatic hemangiomas (5.5 cm ± 2.0 cm) located on the surface of the liver or adjacent to the gallbladder. The results showed that laparoscopic RF ablation therapy is a safe, feasible, and effective treatment option for patients with extrorse hemangiomas. In 2016, a prospective study demonstrated the benefits and disadvantages of laparoscopic RF ablation as compared with surgical resection for managing hepatic hemangioma[35]. Sixty-six patients with symptomatic-enlarging hepatic hemangiomas (4 cm $\leqslant$ diameter < 10 cm) either underwent laparoscopic RF ablation (n = 32) or open resection (n = 34). Laparoscopic RF ablation was associated with significantly shorter operative time (138 min *vs* 201 min, P < 0.001) and less blood loss than open resection. Patients after laparoscopic RF ablation experienced significantly less pain and required less analgesia use, significantly shorter length of hospital stay, and lower hospital cost. The study showed that laparoscopic RF ablation, as a minimal invasive treatment option, is as safe and effective a procedure as open resection for patients with symptomatic-enlarging hepatic hemangiomas smaller than 10 cm.

During the initial period of RF ablation for hepatic hemangiomas, authors always selected and treated hepatic hemangiomas < 10 cm using RF ablation because of the lack of experience. Sporadic cases with huge hepatic hemangiomas were treated by RF ablation. Whether RF ablation should be accepted for treating huge hemangiomas is still in debate because of the requirement of long ablation time. Lengthy ablation time is prone to cause hemolysis, which can lead to the complications of hemoglobinuria, hemolytic jaundice, anemia, or even acute kidney injury (AKI)[25-36]. The incidence of complications post RF ablation is proportionally associated with the size of hemangiomas and the ablation time[28-36]. Park *et al*[25] described the 100% ablation of 10 hepatic hemangiomas larger than 5 cm but less than 10 cm and an ablation in 60% (3/5) of hepatic hemangiomas $\geqslant$ 10 cm. Hence, they drew a conclusion that the best indication for RF ablation was giant hepatic hemangiomas, but huge hepatic hemangiomas were the comparative contraindication. Gao *et al*[28] reported the same technical difficulties when they treated 17 huge hemangiomas $\geqslant$ 10 cm in 16 patients with RF ablation using cluster electrodes. In their study a high rate of complete ablation (82.4%, 14/17) was achieved, but ablation-related complications were seen in all the 16 patients with hemangiomas $\geqslant$ 10 cm, including significant systemic inflammatory responses and acute respiratory distress syndrome (ARDS, grade Ⅳ)[39]. Gao *et al*[31] adopted two approaches to treat 21 large hemangiomas in 21 patients with the expectation of lessening the incidence of complications and achieving a higher success rate: (1) using cool-tip cluster electrodes; and (2) closely monitoring the patient's temperature and hemoglobinuria to warrant a termination of the procedure if the temperature exceeds 39 ℃ or signs of hemoglobinuria appeared. Complete ablation was achieved in 90.5% (19/21) of cases and ablation-related complications reduced to 47.6% (10/21). According to the Dindo-Clavien classification for complications[39], all the complications were grade Ⅰ (including hemoglobinuria in ten cases,

Table 1 Indications and contraindications of radiofrequency ablation for hepatic hemangiomas

Indications
The maximum diameter of hemangiomas > 5 cm
Tumor gaining an enlargement of more than 1 cm within 2 yr
Persistent hemangioma-related abdominal pain or discomfort
Consent to receive the RF ablation
Contraindications
Severe bleeding tendency, platelets < 50 × 10^9/L, international normalized ratio > 1.5, severe platelet function disorders (prothrombin time >18 s and prothrombin activity < 40%)
Malignant tumors
Kasabach-Merritt syndrome
Infection, especially biliary system inflammation
Low immune function
Severe primary organ failure such as the liver, kidney, heart, lung and/or brain

fever in four cases, hemolytic jaundice in three cases, anemia in one case, and elevated serum transaminase in four cases). Eighteen patients required one session and three patients with hemangiomas ⩾ 14.0 cm required two sessions of RF ablation. This study by Gao *et al*[31] showed that RF ablation for huge hepatic hemangiomas is minimally invasive, safe, and effective. Other studies reported the similar results[27,30].

INDICATIONS AND CONTRAINDICATIONS FOR RF ABLATION FOR HEPATIC HEMANGIOMAS

Indications

Indications include: the maximum diameter of hemangiomas > 5 cm; on regular imaging follow-up, tumor gaining an enlargement of more than 1 cm within 2 years; persistent hemangioma-related abdominal pain or discomfort with the definite exclusion of other gastrointestinal diseases which cause the epigastric pain *via* gastroscopy examinations; patients' decline to receive surgical treatment but with the consent to receive the RF ablation (Table 1)[25-34].

Contraindications

Contraindications include: patients with severe bleeding tendency, platelets < 50 × 10^9/L, severe platelet function disorders (prothrombin time > 18 s and prothrombin activity < 40%), international normalized ratio > 1.5, malignant tumors, Kasabach-Merritt syndrome, infection, especially biliary system inflammation, low immune function, and severe primary organ failure such as the liver, kidney, heart, lung and/or brain[28-34]. Anticoagulation and/or antiplatelet drugs should be discontinued at least 5-7 d prior to ablation (Table 1).

ANESTHESIA PROTOCOLS

For percutaneous procedures, general anesthesia is recommended to prevent pain and discomfort during RF procedure. Controlled ventilation would reduce ablation attempts and increase the success rate while RF ablation is performed for patients under general anesthesia[31-35].

PROCEDURES OF RF ABLATION

RF ablation for hepatic hemangiomas can be performed *via* a percutaneous, laparotomy or laparoscopic approach. The diversity of approaches helps extend the scope of treatment indications[25-34].

Hepatic hemangiomas deeply located in liver parenchyma are suited to be treated by percutaneous CT-guided RF ablation. After induction of general anesthesia, patients are placed in a supine position. Grounding is achieved by attaching two pads to the patient's thighs. The skin entry point of the RF electrodes is determined by the guidance of CT imaging. Under the monitoring and guidance of CT, the RF electrodes are percutaneously inserted through the liver to target the tumor. After CT images confirm the acting tip of the RF electrode is located in the tumor center, RF procedure is performed[28].

Subcapsular hepatic hemangioma is suitable to be treated *via* a laparoscopic approach using US guidance[20,33,35]. Under general anesthesia, patients were placed in a supine position. After a pneumoperitoneum (CO_2 at 12 mmHg) is established, a thorough intraperitoneal exploration with a 30° laparoscope through a 10-mm umbilical port is performed. Another 10 mm subxiphoid port is created at the midline of abdomen and an additional 10 mm right or left lateral subcostal port is placed if needed, depending on the location of the hepatic hemangiomas. Under US guidance, the RF electrodes are introduced into the peritoneal cavity through the subcostal abdominal wall under the direct laparoscopic view and deployed into the tumor. The RF procedure is monitored by intraoperative US, which can increase the ability to guide the RF electrode placement and evaluate the efficacy of ablation. After ablation, the ablated lesion became hyperechoic because of outgassing from heated tissues. Laparoscopic biopsy of liver lesions before the ablation is not needed with the consideration of avoiding unnecessary bleeding. For the patients with gallbladder stones or simple hepatic cysts, laparoscopic cholecystectomy (LC) or deroofing of the hepatic cysts may be performed during ablation. LC had to be performed beforehand if the lesions are encroaching on the gallbladder fossa.

Laparotomy is more invasive than percutaneous and laparoscopic approaches. With the increasing

experiences in RF ablation and the improvement of laparoscopic techniques, laparotomy is only used for an alternative approach. In case of unexpected incidence of uncontrollable bleeding during the procedure *via* percutaneous and laparoscopic approaches, laparotomy needs to be performed for controlling the bleeding efficiently and preventing the occurrence of severe complications.

TECHNICAL DETAILS TO BE NOTED

Common strategies of ablation for hepatic hemangiomas

A transhepatic route for RF electrode placement to target the tumor is recommended to prevent bleeding from the electrode-poking site of hemangiomas. RF ablation is initiated at the treatment point close to the margin of the tumor to minimize the risk of bleeding and the heat sink effect. The heat sink effect refers to cooling by adjacent visible (>1 mm diameter) blood vessels when ablated tissues are heated. Overlapped ablation zones are warranted by repositioning the RF electrodes in the tumor mass repeatedly under the guidance and monitoring of CT or US imaging, ensuring a complete ablation of the tumors. An intratumoral ablation is necessary to minimize injuries to normal liver parenchyma and the needle tract ablation can prevent the incidence of needle puncture-induced bleeding[31].

In the laparoscopic approach, the Pringle maneuver can be used to decrease the heat sink effect[28]. Furthermore, the laparoscopic approach also offers a direct vision of the entire RF procedure, which is helpful to identify and manage bleeding from the puncture site or tumor rupture[31-33].

Based on our experience on treating 76 huge hepatic hemangiomas[31-33], we prefer to use internally cooled cluster electrodes to treat the tumors by taking their advantage of achieving an efficient and much concentrated thermal energy in the tumor tissue, which is expected to reduce the incidence of ablation-related complications. Internally cooled cluster electrodes, for example Cool-tip ones, are straight electrodes without an array of prongs, and the tip is internally cooled by continuous infusion of cold saline. Thus, the temperature of the electrode itself is not extremely high, which is helpful to avoid the instant charring at the tissue around the probe and reduce tissue impedance. The efficient heat deposition creates a larger ablated zone within a shorter period of time. Another advantage of internally cooled electrodes is that they induce a sustaining high temperature in the tumor with limited "heat sink effect" caused by blood flow in the adjacent vessels, which can enhance the ablation effectiveness for the tumor tissue abutting the vessel. Moreover, a specially-designed Cool-tip electrode is more visible under the guidance of CT or US imaging, which allows the accurate placement of the electrodes in the tumor without injuring the adjacent organs[31].

Special strategies of ablation for huge hepatic hemangiomas

Larger tumor size is a risk factor for ablation-induced complications. For huge hemangiomas, a few precautions should be taken to lessen the risk of severe complications and enable a successful treatment.

It is unnecessary to achieve a complete ablation of a large tumor using one ablation session if the patient shows the sign of elevating body temperature and hemolysis, considering the severe risk of hemolysis-induced AKI[36]. A repeat RF ablation session can be rescheduled to obtain the expected treatment effect[30].

RF ablation combined with TAE could be an option to achieve a synergistic treatment effect for huge hepatic hemangiomas because the blockade of tumor blood supply by TAE can decrease the lesion size to some extent, facilitating the subsequent RF ablation and minimizing the risk of ablation-related complications. Additionally, the hyper-attenuation of iodized oil deposited in the tumor on CT images can facilitate the targeting placement of the RF electrodes in the tumor and thus increase the success rate of complete ablation[34].

Laparoscopic resection of hemangioma boosted by intratumoral coagulation by RF ablation is a safe, effective option for treating huge hepatic subcapsular hemangiomas with low loss of blood and minimal complications. This technique lies in the advantage that a completely coagulated zone created by sequential RF ablation along the dissection margin warrants the successful removal of the tumor tissue without occluding the hepatic vessels before the tumor dissection. Compared with treating the tumor using RF ablation alone, this technique involves the ablation of the tumor tissue at the resection margin rather than the total hepatic hemangioma, thus shortens the ablation time and avoids the incidence of severe ablation-related complications[37].

COMPLICATIONS

Hemorrhage of hepatic hemangioma

Bleeding at the electrode entry site: Due to the hypervascular nature of hepatic hemangioma and intratumoral high blood pressure, there is high risk of bleeding from the puncture site of the tumor. To prevent bleeding, the electrode needs to be advanced *via* a transhepatic approach to target the tumor and a needle tract ablation needs to be performed while withdrawing the electrode. When the RF electrode

placement is performed *via* laparotomy or laparoscopic approach, RF ablation should be launched from the exterior margin of the tumor from the beginning of a lower RF power[31-34].

Rupture of hepatic hemangioma: Abrupt rupture of hepatic hemangioma could result in massive hemorrhage. For hemangiomas located in the surface of the liver, an improper advancement of the RF electrode directly through the tumor will lose the protection of liver parenchyma. In this context, the RF electrode puncture could induce massive bleeding from the electrode puncture site. On the other hand, when an extra-high RF output power is applied at the beginning of RF ablation, a rapid increase of intratumoral temperature and pressure will induce the burst force in the tumor, which will cause an abrupt rupture of the tumor and life-threatening bleeding. Under the direct view of laparoscopy, hemostasis procedure can be applied to the bleeding site such as applying ablation to stop bleeding. In case the bleeding is not controlled by these measures, a conversion to open surgery is advocated to achieve hemostasis.

Puncture injury to adjacent organs

The accidental injuries to the adjacent organs including the gallbladder, gastrointestinal organs, kidney, diaphragm, lungs, and heart need to avoid. While using multipolar expandable electrodes, the deployment of each prong of the electrodes need to be verified carefully[28,40]. The high visibility of Cool-tip electrodes on CT or US images facilitates the insertion of the electrodes in the tumor without causing accidental injury to adjacent organs[31].

Thermal injury to the pleura and diaphragm

For hepatic hemangiomas situated in the subdiaphragm hepatic dome area, thermal injuries to the diaphragm frequently occur during RF ablation *via* the percutaneous approach[32]. The thermal injury to the diaphragm may manifest as the shoulder pain because the diaphragm and the shoulder skin are innervated by the same phrenic nerves arising from nerve roots C3, C4, and C5[32,41]. Diaphragmatic perforation and herniation were reported as major complications of RF ablation for hepatic tumors abutting the diaphragm in nine cases[41]. RF ablation injuries more frequently occur in cases with hepatic hemangiomas than hepatic cancers due to the high thermal energy and duration needed for large-sized hemangiomas. RF ablation *via* a laparoscopic approach can minimize the risk of diaphragm injury because the pneumoperitoneum can elevate the diaphragm to increases the operation space. Therefore, laparoscopic RF ablation therapy should be used as the first-line treatment for hepatic hemangiomas abutting the diaphragm[32].

Thermal injury to the lung

A patient with two huge hemangiomas in the right lobe (16.0 cm and 11.0 cm), treated with percutaneous RF ablation, developed ARDS immediately after an ablative time of total 250 min[28]. The major complication was resolved by conservative treatment[28]. The pathogenesis of ARDS needs to be investigated, and it is speculated that RF ablation of large quantity of tumor tissues induces significant systemic inflammatory responses[42-45]. Pre-ablation TAE as an adjuvant therapy or multiple ablation sessions is recommended to prevent the risk of ARDS[31,34].

Hemolysis

Hemolysis and anemia: RF ablation poses a risk of predisposing huge hypervascular hepatic hemangiomas to the severe complication of hemolysis. Hemolysis can lead to various degrees of hemoglobinuria, hemolytic jaundice, anemia, or even AKI[28-31,36].

AKI: AKI caused by ablation-induced hemolysis in patients with huge hepatic hemangiomas has been reported[36]. Hemoglobin is released upon erythrocyte destruction and is filtered by the glomerulus into the urinary space. In the urinary space, hemoglobin is degraded and releases heme pigments which can cause tubular injury. Furthermore, volume depletion enhances both vasoconstriction and the formation of obstructing casts, and also is of critical importance for the development of heme-induced AKI[36].

Patients with huge hepatic hemangiomas should be sufficiently hydrated before RF ablation and during the procedure. When any signs or symptoms indicating hemolysis emerge in the course of RF ablation, such as rising body temperature and hemoglobinuria, the RF procedure should be terminated and a repeat RF ablation treatment may need to be rescheduled based on a comprehensive evaluation of the tumor[31].

Other complications

Except for relatively severe RF ablation-related complications, some minor complications may take place, such as liver damage, fever, and skin burn injury at the site where the grounding pad is attached. The ablation-induced self-limiting liver injury can resolve within a short period of time without the need of any medication. The necrotic tissue of tumors can cause unspecific inflammatory reaction and mild hyperthermia. In case that the body temperature is higher than 39 ℃, physical cooling can be used to alleviate the discomfort of hyperthermia. To prevent the burn injury to the skin in patients receiving long time RF ablation, multiple grounding pads can be

Table 2 Ablation-related complications and preventive measures

Complication	Preventive measures
Bleeding at the electrode entry site	The electrode needs to be advanced *via* a transhepatic approach to target the tumor and a needle tract ablation needs to be performed while withdrawing the electrode. Radiofrequency (RF) ablation should be launched from the exterior margin of the tumor from the beginning of a lower RF power.
Rupture of hepatic hemangioma	Under the direct view of laparoscopy, hemostasis procedure can be applied to the bleeding site such as applying ablation to stop the bleeding. If it fails, a conversion to open surgery is advocated to achieve the hemostasis.
Puncture injury to adjacent organs	The high visibility of Cool-tip electrodes on computed tomography or ultrasonography images facilitates the insertion of the electrodes in the tumor without causing accidental injury to adjacent organs.
Thermal injury to the pleura and diaphragm	Laparoscopic RF ablation should be used as the first-line treatment for hepatic hemangiomas abutting the diaphragm.
Thermal injury to the lung	Pre-ablation transcatheter arterial embolization as an adjuvant therapy or multiple ablation sessions is recommended to prevent the risk of acute respiratory distress syndrome.
Hemolysis	The patients should be sufficiently hydrated before RF ablation and during the procedure. When any signs or symptoms indicating the hemolysis emerge in the course of ablation, the RF procedure should be terminated and a repeat RF ablation treatment may need to be rescheduled based on a comprehensive evaluation of the tumor.
Liver damage	The ablation-induced liver injury can resolve spontaneously without the need of any medication.
Fever	Physical cooling can be used to alleviate the discomfort of hyperthermia.
Skin burn injury	Multiple grounding pads can be applied or ice pad can be used to cool the skin with the contact of grounding pad.

applied or ice pad can be used to cool the skin with the contact of grounding pad[31-33]. The ablation-related complications and preventive measures are listed in Table 2.

POST-TREATMENT EVALUATION

Contrast-enhanced CT or MRI can be used to evaluate the therapeutic effect of RF ablation for tumors one month after the treatment. On contrast-enhanced CT or MRI, complete ablation is defined as no nodular or irregular enhancement adjacent to the ablation zone and incomplete ablation is defined as irregular peripheral-enhanced foci in the ablation zone. In cases of complete ablation, subsequent CT or MRI examinations are repeated at a 6-mo interval[31-33]. Repeated RF ablation procedures are not needed unless the residual tumor is growing significantly or posing a risk of spontaneous rupture.

CONCLUSION

RF ablation therapy is a safe, feasible, and effective procedure for hepatic hemangiomas, even for huge hepatic hemangiomas. RF ablation has the following advantages in the treatment of hepatic hemangiomas: minimal invasiveness, definite efficacy, high safety, fast recovery, relatively simple operation, and wide applicability.

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ORIGINAL ARTICLE

Basic Study

Detection of *KRAS* G12D in colorectal cancer stool by droplet digital PCR

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Abstract

AIM

To assess *KRAS* G12D mutation detection by droplet digital PCR (ddPCR) in stool-derived DNA from colorectal cancer (CRC) patients.

METHODS

In this study, tumor tissue and stool samples were collected from 70 patients with stage Ⅰ-Ⅳ CRC diagnosed by preoperative biopsy. *KRAS* mutational status was determined by pyrosequencing analysis of DNA obtained from formalin-fixed paraffin-embedded (FFPE) tumor tissues. The *KRAS* G12D mutation was then analyzed by ddPCR in FFPE tumors and stool-derived DNA from patients with this point mutation. Wild-type (WT) tumors, as determined by pyrosequencing, were included as controls; analysis of FFPE tissue and stool-derived DNA by ddPCR was performed for these patients as well.

RESULTS

Among the total 70 patients included, *KRAS* mutations were detected by pyrosequencing in 32 (45.71%), whereas 38 (54.29%) had WT tumors. The frequency of *KRAS* mutations was higher in left-sided tumors (11 located in the right colon, 15 in the left, and 6 in the rectum). The predominant point mutation was *KRAS* G12D (14.29%, n = 10), which was more frequent in early-stage tumors (I-IIA, n = 7). In agreement with pyrosequencing results, the *KRAS* G12D mutation was detected by ddPCR in FFPE tumor-derived DNA, and only a residual number of mutated copies was found in WT controls. The *KRAS* G12D mutation was also detected in stool-derived DNA in 80% of all fecal samples from CRC patients with this point mutation.

CONCLUSION

ddPCR is a reliable and sensitive method to analyze *KRAS* G12D mutation in stool-derived DNA from CRC patients, especially at early stages. This non-invasive approach is potentially applicable to other relevant biomarkers for CRC management.

Key words: Droplet digital PCR; *KRAS*; Stool; Formalin-fixed paraffin-embedded; Pyrosequencing; Colorectal cancer

Core tip: The potential of droplet digital PCR (ddPCR) to detect *KRAS* G12D mutation in stool DNA from colorectal cancer (CRC) patients was examined as a proof-of-concept for the applicability of this technology to study DNA biomarkers in stool-derived DNA. It was shown that *KRAS* G12D detection in stool-derived DNA from CRC patients by ddPCR is feasible and provides comparable results to the analysis of formalin-fixed paraffin-embedded tissue by pyrosequencing. These results suggest that analysis of *KRAS* mutations and other molecular biomarkers in stool by ddPCR could represent a complementary non-invasive approach to standard screening tests for CRC.

Olmedillas-López S, Lévano-Linares DC, Aúz Alexandre CL, Vega-Clemente L, León Sánchez E, Villagrasa A, Ruíz-Tovar J, García-Arranz M, García-Olmo D. Detection of *KRAS* G12D in colorectal cancer stool by droplet digital PCR. *World J Gastroenterol* 2017; 23(39): 7087-7097 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7087.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7087

INTRODUCTION

Colorectal cancer (CRC) is the second and third most common cancer in women and men, respectively, with more than one million cases diagnosed each year worldwide[1]. Current therapeutic options have increased overall survival (OS), but have also made clinical decisions more complex, especially in patients with an initial diagnosis of metastatic colorectal cancer (mCRC)[2]. Biological agents targeting the epidermal growth factor receptor (EGFR), such as cetuximab and panitumumab, used either as monotherapy or in combination with standard chemotherapy, are indicated in mCRC patients with *RAS* (*KRAS* and *NRAS*) or *KRAS* wild-type (WT) tumors, respectively[3,4]. These strategies significantly improve progression-free survival (PFS) and OS in *KRAS* WT mCRC patients depending on the therapeutic regimen applied (chemotherapy and line of treatment)[2,5].

KRAS oncogene mutations, mostly found in codons 12 and 13[6], have been described in approximately 30%-40% of CRC tumors[7-9]. These mutations are associated with absence of response to therapy with biological agents[10,11] and have been correlated with worse prognosis[12,13]. In fact, in Europe and the United States, monoclonal antibody-based therapy has been restricted to patients with WT tumors[14], as when administered in association with standard chemotherapy, this treatment may result in an increased cost and toxicity[10]. Drug resistance can occur months after start of combined therapy, likely due to intratumoral heterogeneity and proliferation of small sub-groups of clonal cells carrying resistance mutations that are difficult to identify by most of the currently applied methods[14,15]. Therefore, highly sensitive and specific methodologies are needed to detect and quantify molecular markers, including *KRAS* mutations, which play a pivotal role in early detection and clinical management of CRC patients.

Droplet digital PCR (ddPCR) is increasingly seen as one of the most powerful techniques to accurately detect a wide variety of genetic alterations in many cancer types. These molecular biomarkers have been analyzed by ddPCR in different body fluids such as blood, urine, cerebrospinal fluid, pleural effusions, ascites and sputum (reviewed in[16]).

Stool-derived DNA is a potential non-invasive alternative source of DNA for tumor genotyping in CRC due to the high rate of exfoliation of tumor cells into the bowel lumen[17]. Digital PCR was first described in 1999 by Vogelstein and Kinzler in a study aimed at identifying *KRAS* in DNA obtained from fecal samples of CRC patients[18]. Based on the isolation of single molecules by limiting dilution of DNA samples and individual amplification by PCR, mutations were detected using fluorescent probes. However, this methodology was found to be quite laborious and difficult to translate into clinical practice[19]. The introduction of new instrumentation involving nanofluidic devices and improved emulsion chemistries has allowed for more widespread use of digital PCR, giving way to the current commercially available platforms[19]; of these, emulsion-based ddPCR has undergone huge growth in cancer research. In fact, a recent study has investigated the application of ddPCR to quantify mRNA biomarkers in stool from patients with CRC as a potential non-invasive screening test[17]. Thus, analysis of DNA obtained from fecal samples in patients with CRC may complement currently used procedures for diagnosis and disease follow-up.

In our experience, ddPCR has shown high sensitivity for detection of mutated *KRAS* alleles in circulating cell-free DNA (cfDNA) in plasma from CRC patients[20]. However, early-stage patients sometimes have undetectable levels of circulating tumor DNA (ctDNA)[20,21]. The aim of this study was to evaluate the feasibility of *KRAS* G12D mutation detection in stool-derived DNA from CRC patients by ddPCR, including early-stage patients.

MATERIALS AND METHODS

Patients

Seventy CRC patients were consecutively included in this study from 2014 to 2015 in the Department of General Surgery at Fundación Jiménez Díaz University Hospital (Madrid, Spain). Inclusion criteria were endoscopic histological diagnosis of CRC and eligibility for primary tumor resection with curative intent. Patients with primary tumors located in the rectum who had received prior neoadjuvant treatment were excluded. All subjects signed an informed consent in accordance with a protocol approved by the Ethics Committee for Clinical Research of this institution (PIC 63/2016_FJD).

Fecal sample collection

All fecal samples were collected during hospitalization before surgery without any bowel preparation (with the exception of patient 17, who was subjected to cathartic preparation due to an oversight). Stool samples were collected in sterile containers and stored at -20 ℃ until analysis.

DNA extraction

A total amount of 200-500 mg of fecal sample was used for DNA extraction. DNA was isolated using the QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA from the LS-174T cell line (kindly provided by the Translational Oncology Division, OncoHealth Institute, IIS-FJD, which had previously purchased this cell line from the American Type Culture Collection, ATCC in Manassas, VA, United States) and DNA from peripheral blood mononuclear cells of a healthy donor were extracted with the QIAamp DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol. DNA from formalin-fixed paraffin-embedded (FFPE) tumors was extracted using the Cobas DNA Sample Preparation Kit (Roche Molecular Systems, Inc., Branchburg, NJ, United States) following the manufacturer's instructions. The quantity and purity of the DNA obtained was estimated by NanoDrop (ND-2000 UV-Vis Spectrophotometer; Nanodrop Technologies Inc., Waltham, MA, United States).

Mutation detection in FFPE tumor samples by pyrosequencing

Quantitative analysis of *KRAS* mutations was performed by pyrosequencing in accordance with routine practice at the Department of Pathology at Fundación Jiménez Díaz University Hospital, using the CE-IVD marked Therascreen *KRAS* Pyro kit (Qiagen), according to the manufacturer's protocols. Each PCR product was analyzed by pyrosequencing using the Therascreen *KRAS* Pyro reagents (Qiagen), Streptavidin Sepharose High Performance (GE Healthcare Bio-Science AB, Uppsala, Sweden), and a PyroMark Q24 instrument (Qiagen).

Mutation detection in FFPE tumor tissues and stool samples by ddPCR

ddPCR assays were performed using the QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, United States) with the Prime-PCR™ ddPCR™ Mutation Detection Assay Kit (Bio-Rad); amplicon size was 57 bp. DNA from LS-174T, a human colon adenocarcinoma *KRAS* G12D heterozygous cell line, was used as a positive control. *KRAS* WT control DNA was obtained from peripheral blood mononuclear cells of a healthy donor. Background was measured by adding water to the reaction mixture instead of

DNA. The PCR reaction mixture (20 μL) contained 10 μL of ddPCR Supermix (no dUTP) for probes, 1 μL of each primer/probe mix (target and reference, labeled with HEX and FAM fluorophores, respectively), and 2-8 μL of stool-extracted DNA. Different amounts of stool-derived DNA were assayed per sample, as the proportion of human DNA with respect to bacterial DNA was unknown and could vary among patients. A total amount of 100 ng of cell-derived control DNA was added per well. In case of DNA from FFPE tumors, 50 ng per well was used. Thermal cycling consisted of 10 min at 95 ℃, 40 cycles of 94 ℃ for 30 s, and 55 ℃ for 60 s. Results were analyzed using QuantaSoft v.1.7 software (Bio-Rad) and reported as number of copies per 20 μL reaction as well as copies per ng of DNA. Three to four replicates of each stool sample were analyzed. FFPE tumors and controls were assayed in duplicate.

Statistical analysis

A non-parametric Mann-Whitney test for significance was performed using R software.

RESULTS

Patient characteristics

A total of 70 stool and tissue samples from CRC patients were collected and included in the study. All patients were classified according to the distance between the primary tumor and anal margin (cm) reported in the preoperative colonoscopy. Forty-one (58.57%) were male, with a median age of 73 years. Only 5 (7.14%) had a diagnosis of mCRC at baseline.

The patients included in this study were representative of all tumor locations, with 28 (40%) located in the right colon, 34 (48.57%) in the left colon, and 8 (11.43%) in the rectum. Interestingly, 45 (64.29%) patients were diagnosed at an early stage (I-IIA). Clinical and pathological features are summarized in Table 1.

KRAS mutations in FFPE tumor samples analyzed by pyrosequencing

KRAS mutations were found in the tumors of 32 patients (45.71%), including 11 in the right colon, 15 in the left, and 6 in the rectum. Most mutations were located at codon 12 (n = 17, 53.12%) or codon 13 (n = 6, 18.75%). The most prevalent mutation was G12D (n = 10, 14.29%). Two were located in the right colon, 6 in the left colon, and 2 in the rectum. This mutation was found more frequently in early-stage tumors (I-IIA, n = 7). The incidence of the different types of mutations is shown in Table 2.

KRAS G12D detection in FFPE tumor samples by ddPCR

FFPE tumors from patients found to have a *KRAS* G12D mutation by pyrosequencing were analyzed using ddPCR. Five CRC patients with WT *KRAS* exon 2 tumors were selected as controls. One control carried a *KRAS* Q61L mutation (exon 3) that did not interfere with our assays. Results obtained from ddPCR analysis of FFPE tumor DNA from these 15 CRC patients were in agreement with pyrosequencing results. A residual number of *KRAS* G12D copies was found in WT tumors. Due to this level of unspecific background signal, mean copies/ng DNA from control patients plus 2 standard deviation (SD) was considered as a threshold for positivity (0.41 copies/ng DNA). All samples from patients with G12D-positive tumors were above this threshold and showed a significantly higher number of mutant copies/ng DNA than patients with WT *KRAS* tumors (median, 106 and 0.19 copies/ng DNA, respectively; P = 0.001). However, the difference in number of WT *KRAS* copies/ng DNA between both groups was not statistically significant (210.00 copies/ng *vs* 208.40 copies/ng DNA, median; P = 0.699).

KRAS G12D detection in stool samples by ddPCR

Subsequently, we analyzed the presence of the *KRAS* G12D mutation by ddPCR analysis of fecal samples from the 10 patients with mutated tumors by pyrosequencing. Stool DNA from 5 patients with tumors carrying the WT *KRAS* exon 2 were also included as controls. A limited number of *KRAS* G12D-positive events were detected in stool DNA from control WT *KRAS* exon 2 samples. Consequently, mean control value plus 2 SD was established as the positivity threshold. Thus, in our study, stool samples were required to contain more than 1.9 copies/20 μL of reaction to be considered positive for the mutation. According to this threshold, which was equivalent to > 3 positive events per sample, the *KRAS* G12D mutation was detected in 8 of 10 patients. Of these 8 positive samples, 6 were from early-stage tumors. Samples from Patients 46 and 64 were considered negative because they had values less than or equal to the positivity threshold. *KRAS* G12D mutation levels in stool samples are shown in Tables 3 and 4. The median number of copies of *KRAS* G12D/20 μL of reaction as well as copies/ng of stool DNA in control patients differed significantly from those with mutated tumors (P = 0.017). The *KRAS* WT sequence was also detected in stool samples of all CRC patients, though there were no significant differences in the median number of *KRAS* WT copies/ng of stool DNA between both groups (P = 0.129).

In summary, the results of pyrosequencing were in 100% agreement with ddPCR analysis in FFPE tissues, whereas ddPCR detected the *KRAS* mutation in 8 out of 10 stool samples.

DISCUSSION

Despite the advances made in CRC research, the

Table 1 Patient characteristics

		Colorectal cancer patients, *n* = 70		
		Right colon, *n* = 28	Left colon, *n* = 34	Rectum, *n* = 8
Sex	Female	13	13	3
	Male	15	21	5
Age (mean)		76.89	72.26	70.63
pT	T1	3	4	2
	T2	6	9	2
	T3	16	18	3
	T4	3	3	1
pN	N0	20	22	8
	N+	8	12	-
Stage	I	9	10	4
	IIa	9	10	3
	IIb	1	1	1
	IIIa	1	3	-
	IIIb	4	5	-
	IIIc	2	2	-
	IV	2	3	-
KRAS status	Mutant	11	15	6
	Wild-type	17	19	2

disease remains a major cause of death worldwide. Recently, the analysis of *KRAS* oncogene mutations has taken on a major prognostic role in CRC clinical management[22] owing to the fact that the presence of these mutations, which have been described in approximately 30%-40% of cases[7,9,23], could determine the absence of response to anti-EGFR therapies and worse outcome in cases of metastatic disease[10-13]. The most frequent *KRAS* mutations are located at codons 12 and 13; of these, G12D and G13D are particularly relevant, representing around 13%-14% and 6%-7% of all cases, respectively[7,23]. Moreover, worse prognosis has been documented among patients with tumors with the G12D mutation[8,23]. In agreement with previous observations, the incidence of *KRAS* G12D mutation in our study population was 14.29%.

Molecular biomarkers in blood and stool may be used as a complementary screening strategy and prognostic tool for the prediction of clinical outcome in patients with or at high risk for CRC[24]. Not only in CRC, but also in many other human malignancies, the analysis of molecular biomarkers in plasma and other body fluids is attracting increasing interest as a highly valuable, non-invasive predictive tool for monitoring disease progression and response to treatment[16]. In CRC, *KRAS* mutations have been analyzed in blood, both in DNA obtained from circulating tumor cells[25] and in cfDNA[26]. *KRAS*-mutation detection by digital PCR has been described using several commercially available platforms[14,27-32], most of which are focused on detecting mutations in plasma. One of these strategies is ddPCR, which has shown a remarkably high sensitivity when detecting these minority *KRAS* alleles present at low levels in plasma DNA.

In a previous study, using ddPCR, we detected the *KRAS* G12V mutation in plasma cfDNA from 9 of 10 patients whose tumors were also mutated[20]. In this study, we found that metastatic patients had a significantly higher number of mutated copies in circulating cfDNA than M0 patients. The only negative sample was obtained from a T1N0M0 patient. These results are in line with other studies: Bettegowda *et al*[21] also reported that ctDNA in plasma increases with disease stage, and only 47% of early-stage patients with a wide variety of cancers had detectable levels of ctDNA. Similarly, Galanopoulos *et al*[26] recently described that the *KRAS* codon 12 mutation rate in cfDNA is significantly higher in CRC patients compared to healthy subjects, though this methodology seems to have limited potential for predicting the existence of premalignant lesions (neoplastic colonic polyps). Taken together, these findings suggest that at early disease stages, levels of mutated copies in circulating cfDNA may be, in some cases, too low for detection. Thus, alternative non-invasive methods are still needed.

Interestingly, in a very recent study, ddPCR was also used to quantify an mRNA biomarker, *ITGA6*, in stool from patients with CRC[17]. Tumor-derived nucleic acids present in stool samples come from the exfoliation of tumor cells of the intestinal mucosa and are a non-invasive, alternative source of genetic material for *KRAS* oncogene mutation screening in CRC patients[33]. Exfoliation of colonocytes into the large bowel lumen is a continuous, naturally-occurring phenomenon that seems to be exacerbated in tumors[34,35]. Thus, colonocyte shedding from malignant lesions is more frequent than from healthy mucosa[36,37]. Hypothetically, DNA from colorectal tumors should be shed into the bowel fecal content before reaching the bloodstream. This would make testing stool DNA for CRC screening more time-sensitive than plasma or other biological fluids[38].

The proof-of-concept study for stool DNA analysis for CRC detection screened 15 point mutations in several genes, including *KRAS*[39]. Subsequently, several case-control and prospective studies[40-42] led to the 2014 approval by the United States Food and Drug Administration of a fecal DNA analysis system called Cologuard™ (Exact Sciences Corporation, Madison, WI, United States) for CRC detection. This system includes an immunochemical assay for human hemoglobin and molecular biomarkers associated with CRC, such as methylation markers (*BMP3* and *NFRG4* gene promoter regions), *KRAS* mutations, and β-actin. The test is based on amplification and detection by Quantitative Allele-specific Real-time Target and Signal

Table 2 *KRAS* mutational status by pyrosequencing

	KRAS wild-type	*KRAS* mutations											
		Codon 12				Codon 13		Others					
		G12D	G12V	G12R	G12S	G13D	G13R	A146V	A146T	A59T	Q61R	Q61H	Q61L
Right colon	17	2	0	1	0	3	1	1	1	0	1	1	0
Left colon	19	6	5	0	0	1	0	1	1	1	0	0	0
Rectum	2	2	0	0	1	1	0	0	0	0	0	1	1
		10	5	1	1	5	1	2	2	1	1	2	1
Total	38 (54.29%)	32 (45.71%)											

Amplification (QuARTS™) technology. However, this system still has limited application in clinical practice due to its elevated cost. Additionally, the technical difficulties of this test include the need for a large volume of stool sample and the high rate of false-positive results, creating a need for more confirmative colonoscopies and additional costs[38]. Further studies evaluating the cost-effectiveness of this test for large-scale population screening are needed[43].

Fecal DNA analysis for mutation detection has also been reported using several digital PCR systems[18,27,44-48], the first of which were the studies carried out by Vogelstein and Kinzler[18,44], which led to the development of BEAMing (named for 'beads, emulsions, amplification, and magnetics')[27]. Other examples are target-enriched multiplex PCR (Tem-PCR)[47], MDHB (multiplex digital PCR coupled with hydrogel bead-array)[46], and MLPA-DABA (multiplex ligation-dependent probe amplification-digital amplification coupled with hydrogel bead-array)[48]. It is worth mentioning that, to date, none of these systems has been further developed and subjected to clinical validation for stool DNA screening.

In our study, DNA from fecal samples of CRC patients was successfully obtained in all cases. Presence of the *KRAS* G12D mutation was determined by pyrosequencing of FFPE tissue as a reference standard. Results of *KRAS* G12D mutation detection in FFPE tumors using ddPCR were in total agreement with pyrosequencing analysis. This was expected, given the fact that ddPCR has been proven to achieve higher sensitivity than pyrosequencing[49]. Once the *KRAS* G12D mutation had been screened in tumor tissues, DNA from stool samples obtained from the same patients prior to surgery was also analyzed. Thus, we were able to detect the *KRAS* G12D mutation in 8 out of 10 stool samples from patients known to carry this mutation in their tumors using both methods. It is noteworthy that 6 of these 8 samples were from early-stage patients (Ⅰ-ⅡA), highlighting the potential of this approach to identify *KRAS* mutations at the initial stages of the disease.

Absorbance at a wavelength of 230 nm has been reported as an indicator of the level of potential PCR inhibitors in fecal samples[50], and it should be noted that the two negative samples showed peak of absorbance at this same wavelength. Thus, the sensitivity of detection in our assay could have been greatly reduced by the presence of PCR inhibitors in these samples.

The sample from Patient 17 is noteworthy for its remarkably high concentration of both mutated and WT copies. It is worth mentioning that this patient was subjected to cathartic preparation prior to sample collection due to an oversight. The rest of the samples were collected without any bowel preparation. We hypothesize that purging could have increased the exfoliation of tumor cells into the bowel lumen. This unexpected observation raises the question of whether bowel preparation could be advisable prior to sample collection to increase the sensitivity of detection in stool screening of *KRAS* mutations by ddPCR.

To our knowledge, this is the first study to evaluate the feasibility of detection of the *KRAS* G12D mutation in stool DNA from CRC patients using this particular ddPCR platform. Our results are in line with the above-mentioned recently published study by Herring *et al*[17], reporting the detection and accurate quantification of an mRNA biomarker in stool from CRC patients using the same ddPCR system. In light of these results, the analysis of CRC biomarkers in stool using ddPCR merits further study in larger cohorts of patients to evaluate the clinical utility of this approach.

We analyzed only the most prevalent *KRAS* mutation (G12D) in our population, as it was the only one with a sufficient number of samples available for analysis (n = 10). Another reason for choosing G12D as a target was that it has the highest incidence in CRC patients worldwide and is associated with poor clinical outcome[8,23]. For lower-incidence mutations, such as G12V, there were too few samples in our study population to provide conclusive results. The analysis of *KRAS* G12D performed in this study represents a proof-of-concept of the feasibility of this strategy as a first step prior to the screening of other relevant hotspot mutations.

This preliminary study demonstrates the capability of ddPCR to detect *KRAS* mutations in stool-derived DNA, acting as a complementary approach to tissue biopsy for tumor genotyping. These results pave the

Table 3 ***KRAS*** **G12D mutation levels in DNA from stool samples of wild-type control patients**

Patient	Tumor location	Tumor stage	G12D							Wild-type						
			Single			Merged				Single			Merged			
			Positive events	Copies/μL	Copies/20 μL reaction	Positive events	Copies/μL	Copies/20 μL reaction	Copies/ng DNA	Positive events	Copies/μL	Copies/20 μL reaction	Positive events	Copies/μL	Copies/20 μL reaction	Copies/ng DNA
53	Left	I	1	0.1	2	3	0.07	1.40	0.011	94	9	180	469	10.50	210.00	1.615
			2	0.17	3.4					12	10.8	216				
			0	0	0					116	9.9	198				
			0	0	0					136	12.1	242				
56	Left	II A	1	0.08	1.6	1	0.02	0.40	0.003	17	1.4	28	76	1.56	31.20	0.240
			0	0	0					25	2.1	42				
			0	0	0					16	1.3	26				
			0	0	0					18	1.4	28				
63	Right	II A	1	0.08	1.6	1	0.02	0.42	0.003	258	20.4	408	957	20.30	406.00	3.123
			0	0	0					222	22.1	442				
			0	0	0					217	19	380				
			0	0	0					260	19.9	398				
71	Right	III B	1	0.08	1.6	3	0.06	1.20	0.009	89	6.7	134	377	7.20	144.00	1.108
			2	0.15	3					104	7.9	158				
			0	0	0					93	6.9	138				
			0	0	0					91	7.3	146				
96	Left	IV	1	0.09	1.8	3	0.06	1.20	0.009	32	3	60	133	2.84	56.80	0.437
			1	0.08	1.6					33	2.6	52				
			1	0.09	1.8					34	3	60				
			0	0	0					34	2.9	58				

way for the ddPCR analysis of other molecular biomarkers of CRC in stool, including other *KRAS*, *NRAS* and *BRAF* mutations. A multiplex assay simultaneously covering all *KRAS* mutations relevant for anti-EGFR therapy decision-making would maximize the benefits and optimize the cost-effectiveness of this approach. Further studies involving larger cohorts of patients and samples collected at different time points throughout the progression of the disease should be performed in order to confirm the prognostic value and economic viability of this tool before implementation in clinical practice.

This study is the first to describe the detection of *KRAS* G12D mutation in stool-derived DNA from CRC patients using a commercially available ddPCR platform, including individuals at early stages of the disease. We hypothesized that ddPCR could be a reliable and sensitive method to analyze *KRAS* mutations in stool-derived DNA providing reproducible and accurate results. Our findings suggest this approach, which is fast, simple and affordable, could be adaptable to the detection of other clinically relevant molecular biomarkers for CRC management. These advantages with respect to other previously described stool-based strategies, together with instrumentation and protocols easily adoptable by any lab, make our approach more feasible for implementation into routine clinical practice. In light of our results, it could be proposed that biomarker analysis by ddPCR of stool samples may complement current CRC screening methods; stool-derived nucleic acid testing by ddPCR offers an alternative tool to tissue genotyping and blood-based biomarker quantification, being less invasive than the former and, probably, more time-sensitive than

Table 4 ***KRAS*** **G12D mutation levels in DNA from stool samples of patients with** ***KRAS*** **G12D mutated tumors**

			G12D							Wild-type						
			Single			Merged				single			Merged			
Patient	Tumor location	Tumor stage	Positive events	Copies/μL	Copies/20 μL reaction	Positive events	Copies/μL	Copies/20 μL reaction	Copies/ng DNA	Positive events	Copies/μL	Copies/20 μL reaction	Positive events	Copies/μL	Copies/20 μL reaction	Copies/ng DNA
12	Rectum	I	4	0.32	6.4	14	0.37	7.40	0.051	155	12.6	252	493	13.10	262.00	1.819
			5	0.39	7.8					158	12.5	250				
			5	0.39	7.8					180	14.1	282				
17	Left	I	1147	95	1900	4849	97.20	1944.00	41.362	4684	446	8920	19664	456.00	9120.00	194.043
			1243	98	1960					4996	458	9160				
			1269	99	1980					5094	460	9200				
			1190	97	1940					4890	461	9220				
29	Left	ⅢB	5	0.41	8.2	11	0.30	6.00	0.024	94	7.7	154	257	7.10	142.00	0.577
			3	0.24	4.8					90	7.4	148				
			3	0.25	5					73	6.2	124				
30	Right	I	6	0.57	11.4	18	0.54	10.80	0.017	238	22.8	456	757	23.10	462.00	0.728
			7	0.64	12.8					268	24.8	496				
			5	0.43	8.6					251	21.8	436				
43	Right	ⅢC	6	0.59	11.8	19	0.59	11.80	0.035	418	42	840	1393	44.10	882.00	2.602
			8	0.7	14					492	45.5	910				
			5	0.46	9.2					483	44.8	896				
461	Left	ⅡC	1	0.09	1.8	3	0.09	1.80	0.014	81	7	140	265	7.80	156.00	1.238
			1	0.09	1.8					97	8.3	166				
			1	0.09	1.8					87	8.2	164				
51	Rectum	I	13	1	20	47	0.90	18.00	0.295	1292	103	2060	5110	102.60	2052.00	33.639
			12	0.9	18					1291	102	2040				
			9	0.68	13.6					1297	102	2040				
			13	1	20					1230	103	2060				
641	Left	I	0	0	0	0	0.00	0.00	0.000	141	12	240	653	12.20	244.00	1.877
			0	0	0					163	12.1	242				
			0	0	0					154	11.4	228				
			0	0	0					195	13.3	266				
70	Left	I	11	1.1	22	45	1.37	27.40	0.036	2515	282	5640	8516	294.00	5880.00	7.656
			24	1.9	38					3313	305	6100				
			10	1	20					2688	291	5820				
75	Left	ⅡA	0	0	0	11	0.33	6.60	0.032	304	27.2	544	964	29.00	580.00	2.788
			2	0.18	3.6					326	29.8	596				
			9	0.8	16					334	30.1	602				

[1]Patients 46 and 64 were considered negative because they had values less than or equal to the positivity threshold.

the later, especially at early stages, as tumor DNA will reasonably reach the fecal content more quickly than the bloodstream, at least during the initial phases of cancer development.

ARTICLE HIGHLIGHTS

Research background

Clinical management of colorectal cancer (CRC) requires analysis of molecular biomarkers, such as *KRAS* or *NRAS* mutations, which are associated with the emergence of resistance to therapy with biological agents. Tumor genotyping is usually performed using DNA from tissue biopsies, and, in recent years, from blood as well. However, at early disease stages, levels of mutated copies in circulating cell-free DNA may be, in some cases, too low for detection. Thus, extremely sensitive and non-invasive alternative methods are still needed to improve detection and achieve accurate quantification of these biomarkers.

Research motivation

Stool is an alternative and non-invasive source of genetic material for tumor genotyping in CRC. To date, several strategies based on analysis of molecular markers in fecal samples have been proposed, though their application in clinical practice remains limited due to their elevated cost and reduced sensitivity at early stages of disease.

Research objectives

The aim of this study was to assess the potential of droplet digital PCR (ddPCR) to detect the *KRAS* G12D mutation in stool-derived DNA from CRC patients as a proof-of-concept for the applicability of this technology as a non-invasive method of studying clinically relevant DNA biomarkers in stool.

Research methods

KRAS mutations were determined by pyrosequencing in DNA obtained from formalin-fixed paraffin-embedded (FFPE) tumor tissues. Then, *KRAS* G12D mutation was analyzed by ddPCR in FFPE tumors and stool-derived DNA in samples obtained from patients carrying this point mutation.

Research results

The *KRAS* G12D mutation was detected by ddPCR in FFPE tumor-derived DNA and in stool-derived DNA in 80% of all fecal samples from CRC patients with this mutation.

Research conclusions

This is the first study to describe the detection of the *KRAS* G12D mutation in stool-derived DNA from CRC patients using a commercially available ddPCR platform, including in individuals with early stages of the disease. dPCR served as a reliable tool for detecting this clinically relevant mutation in stool-derived DNA from CRC patients. Several stool-based strategies involving digital PCR have been investigated to analyze relevant mutations for CRC management. However, none of these approaches has been further developed and subjected to clinical validation for stool DNA screening to date.

The advantages of ddPCR technology, together with instrumentation and protocols easily adoptable by any lab, support a potential translation of this approach to clinical scenarios. Our results show that *KRAS* G12D detection in stool-derived DNA from CRC patients by ddPCR is feasible and suggests this technology might be useful for the analysis of other molecular markers in stool. The authors hypothesized that ddPCR could be a reliable and sensitive method of analyzing *KRAS* mutations in stool-derived DNA, providing reproducible and accurate results.

This study proposed a new strategy based on detecting *KRAS* mutations in stool-derived DNA using a commercially available ddPCR platform. ddPCR is an emulsion-based amplification technology with fluorescently labelled probes. *KRAS* G12D mutation detection in stool-derived DNA by ddPCR is a fast, simple, and affordable approach which could be adapted to detect other clinically relevant molecular biomarkers for CRC management. This technique is more feasible for implementation into routine clinical practice than other previously described stool-based strategies.

ddPCR provided sensitive, accurate, and reproducible results for detection of the *KRAS* G12D mutation in stool-derived DNA from CRC patients, especially at early stages of the disease. In light of our results, it could be proposed that biomarker analysis by ddPCR in stool samples may complement current CRC screening methods; stool-derived nucleic acid testing by ddPCR offers an alternative to tissue genotyping and blood-based biomarker quantification, is a less invasive tool than the former and is likely more time-sensitive than the latter, especially at early stages, as tumor DNA will reasonably reach the fecal content more quickly than the bloodstream, at least during the initial phases of cancer development.

Research perspectives

KRAS mutations are analyzable by ddPCR in stool-derived DNA from CRC patients, including early-stage patients. This observation merits further studies aimed at evaluating and improving the efficiency of this approach prior to its clinical application. These results pave the way for ddPCR analysis of other molecular biomarkers of CRC in stool. Further studies involving larger cohorts of patients and samples collected at different time points throughout the progression of the disease should be performed in order to confirm the prognostic value and economic viability of this tool before implementation in clinical practice. A multiplex assay simultaneously covering all *KRAS* mutations relevant for anti-EGFR-therapy decision-making would maximize the benefits and optimize the cost-effectiveness of this approach. This strategy should be further investigated as a complementary screening test for early detection of CRC.

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ORIGINAL ARTICLE

Basic Study

Optimal timing for the oral administration of Da-Cheng-Qi decoction based on the pharmacokinetic and pharmacodynamic targeting of the pancreas in rats with acute pancreatitis

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Abstract

AIM

To identify the optimal oral dosing time of Da-Cheng-Qi decoction (DCQD) in rats with acute pancreatitis (AP) based on the pharmacokinetic and pharmacodynamic parameters.

METHODS

First, 24 male Sprague-Dawley rats were divided into a sham-operated group [NG(a)] and three model groups [4hG(a), 12hG(a) and 24hG(a)]. The NG(a) and model groups were administered DCQD (10 g/kg.BW) intragastrically at 4 h, 4 h, 12 h and 24 h, respectively, after AP models induced by 3% sodium taurocholate. Plasma samples were collected from the tails at 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h after a single dosing with DCQD. Plasma and pancreatic tissue concentrations of the major components of DCQD were determined by high-performance liquid chromatography tandem mass spectroscopy. The pharmacokinetic parameters and serum amylase were detected and compared. Second, rats were divided into a sham-operated group [NG(b)] and three treatment groups [4hG(b), 12hG(b) and 24hG(b)] with three corresponding control groups [MG(b)s]. Blood and pancreatic tissues were collected 24 h after a single dosing with DCQD. Serum amylase, inflammatory cytokines and pathological scores of pancreatic tissues were detected and compared.

RESULTS

The concentrations of emodin, naringin, honokiol, naringenin, aloe-emodin, chrysophanol and rhe-ochrysidin in the 12hG(a) group were higher than those in the 4hG(a) group in the pancreatic tissues (P < 0.05). The area under the plasma concentration-time curve from time 0 to the time of the last measurable concentration values ($AUC_{0\rightarrow t}$) for rhein, chrysophanol, magnolol and naringin in the 12hG(a) group were larger than those in the 4hG(a) or 24hG(a) groups. The 12hG(a) group had a higher C_{max} than the other two model groups. The IL-10 levels in the 12hG(b) and 24hG(b) groups were higher than in the MG(b)s (96.55 ± 7.84 *vs* 77.46 ± 7.42, 251.22 ± 16.15 *vs* 99.72 ± 4.7 respectively, P < 0.05), while in the 24hG(b) group, the IL-10 level was higher than in the other two treatment groups (251.22 ± 16.15 *vs* 154.41 ± 12.09/96.55 ± 7.84, P < 0.05). The IL-6 levels displayed a decrease in the 4hG(b) and 12hG(b) groups compared to the MG(b)s (89.99 ± 4.61 *vs* 147.91 ± 4.36, 90.82 ± 5.34 *vs* 171.44 ± 13.43, P < 0.05).

CONCLUSION

Late-time dosing may have higher concentrations of the most major components of DCQD, with better pharmacokinetics and pharmacodynamics of anti-inflammation than early-time dosing, which showed the late time to be the optimal dosing time of DCQD for AP.

Key words: Da-Cheng-Qi decoction; Acute pancreatitis; Pharmacokinetics; Oral dosing time; Pharmacodynamics

Core tip: Our study group had raised the hypothesis of tissue pharmacology of an herbal recipe, which assumed the effect of an herb formula is related to its target tissue distribution or concentration of effective components in target tissues. This study was then designed to screen the optimal oral dosing time of Da-Cheng-Qi decoction (DCQD) in rats with acute pancreatitis based on the pharmacokinetics of the main absorbed components and the pharmacodynamics of DCQD targeting of the pancreas.

Zhang YM, Zhu L, Zhao XL, Chen H, Kang HX, Zhao JL, Wan MH, Li J, Zhu L, Tang WF. Optimal timing for the oral administration of Da-Cheng-Qi decoction based on the pharmacokinetic and pharmacodynamic targeting of the pancreas in rats with acute pancreatitis. *World J Gastroenterol* 2017; 23(39): 7098-7109 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7098.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7098

INTRODUCTION

Da-Cheng-Qi decoction (DCQD) was first described in Shang-Han-Lun, a classical work of Traditional Chinese Medicine (TCM). DCQD consists of four Chinese herbs: dahuang (*Rheum palmatum* L.), mangxiao (*Mirabilite*, $Na_2SO_4 \cdot 10H_2O$), houpu (*Magnolia officinalis* Rehd. et Wils.), and zhishi (*Citrus aurantium* L.). It has been widely used as a purgative to treat diseases with constipation and to clear away internal heat[1], such as in acute pancreatitis (AP), which has a consensus in the relative treatment guidelines in China. The efficacy of DCQD, administered orally or by coloclyster to patients with AP, is obvious. According to clinical observation, it can reduce intra-abdominal hypertension[2] and decrease the risk of developing acute respiratory distress syndrome in severe AP (SAP) patients with systemic inflammatory response syndrome, and shorten their length of hospitalization[3].

In animal experiments, it can ameliorate acute pancreatic, intestinal, lung and liver injury complicated with SAP[4,5]. It can also reduce the generation of reactive oxygen species in AR42J cells and regulate the apoptosis/necrosis switch, to ameliorate the pancreatic inflammation and pathological damage[6]. Other therapeutic activities of DCQD for AP, such as its antioxidant, anti-inflammatory and anti-ulcer properties[7] are important. Its numerous roles indicate the wide applicability of DCQD in AP.

However, early oral dosing with DCQD is contradictory to the conventional therapy that considers fasting and water deprivation are necessary for pancreatic rest in the early stage of AP. Conventionally, any stimulation of the exocrine function of the pancreas by fluid or solid nutrients would promote the release of proteolytic enzymes and affect the disease course negatively[8]. Early oral dosing with DCQD may increase gastric contents, worsen stomachache and abdominal distension, and even aggravate the disease severity. However, it is not clear whether early oral administration of DCQD increases pancreatic secretion and worsens the disease severity. One clinical observation revealed that the best time for gastrointestinal unblocking by herbs was within 48 h after the onset of AP[9]. At the same time, releasing excessive turbidity should be done sooner rather than later to prevent intestinal function failure and disease deterioration[9]. Some studies have indicated that AP patients must pass feces within 24 h after the onset of AP, as one method to support intestinal function to control systemic inflammatory response syndrome and protect organ functions[9]. It is still unclear if the optimal oral dosing time of DCQD should be earlier or later. Therefore, it is important to find the optimal dosing time of DCQD at which DCQD will not worsen the disease severity.

The necrosis of pancreatic acinar cells would worsen the disease, and the induction of apoptosis would decrease the disease severity[6]. Thus, based on the effect of DCQD on regulation of the apoptosis/necrosis switch of pancreatic acinar cells to ameliorate pancreatic inflammation and pathological damage[6], the study aimed to identify the optimal oral dosing time of DCQD in rats with AP according to the pharmacokinetics of the absorbed components and the pharmacodynamics of DCQD targeting of the pancreas.

MATERIALS AND METHODS

Animals

Sprague-Dawley male rats (n = 66) aged 90 ± 5 d, with body weights of 280-300 g, were purchased from Chengdu Dashuo Bio-Technique Co. Ltd. (Chengdu, China). The animals were maintained as previously described[10]. Before AP induction, rats were fasted for 12 h. This study was performed according to the Guide for the Care and Use of Laboratory Animals of Sichuan University (Chengdu, China) and the Animal Ethics Committee Guidelines of the Animal Facility of the West China Hospital (Chengdu, China).

Preparation of drugs

Sodium taurocholate was supplied by Sigma (St. Louis, MO, United States). Spray dried particles of dahuang, mangxiao, houpu, and zhishi were purchased from Chengdu Green Herbal Pharmaceutical Co. Ltd. (Chengdu, China). Chloral hydrate (10%), paraformaldehyde (4%) and methanol were obtained from Tedia Co. Ltd. (Nos. 509221 and 609144; Fairfield, OH, United States,). Glacial acetic acid (No. 20030911) and ethyl acetate (No. 20070116) were purchased from Chengdu Kelon Chemical Reagent Factory (Chengdu, China). Reference standards of the 10 components of DCQD were purchased from the same companies. According to the proportion of crude drugs (dahuang 12 g, houpu 24 g, zhishi 12 g, and mangxiao 9 g), the granules of the four drugs were stirred with ultra-pure water by magnetic stirrers with a speed setting of grade 5 for 1 h in a water bath at a temperature of 37 ℃ for 30 min. According to the Method of Pharmacology, the least dosage of DCQD is 0.6 g/100 g.BW for rats. In this study, the dosage was 1 g/100 g.BW (10 g/kg.BW) with the concentration of 1 g/mL.

Equipment and conditions

The magnetic stirrer (C-MAG, MS 7) was provided by the IKA Company (Breisgau, Germany). The analytical balance (BSA-224S-CW) was provided by the Sartorius Company (Goettingen, Germany). The micro-infusion pump was obtained from KD Scientific (Holliston, MA, United States). Conventional operation instruments, fixation-machines for rats, and the 1.5 mL and 10 mL centrifuge tubes were purchased from Shimadzu (Kyoto, Japan). The high-performance liquid chromatography tandem mass spectroscopy (HPLC-MS/MS) system consisted of a SIL-HTc autosampler (Shimadzu), a LC-10ADvp pump (Shimadzu), and an API3000 triple-quadrupole LC-MS system (Applied Biosystems, Foster City, CA, United States). This system was controlled by Analyst 1.4. Software (Chinese Pharmacological Society, Beijing, China). The chromatographic column was an Ultimate XB-C_{18} (5 μm, 50 mm × 4.6 mm). The mobile phase consisted of methanol-water (92:8, v/v) delivered at a flow rate of 0.3 mL/min. The column was maintained at ambient temperature, and the injection volume was 80 μL. The Anke centrifuge TGL-16B was supplied by Shanghai An-Ting Science Technology Instrument Factory (Shanghai, China). All aqueous solutions and buffers were prepared with ultra-pure water from a Millipore Rios™-16 water purifier (Millipore, Billerica, MA, United States). Standard stock solutions were prepared

by dissolving the reference standards (100 μg/mL for emodin, aloe-emodin, chrysophanol, naringin, naringenin, hesperidin, magnolol and honokiol; 20 μg/mL for rhein and rheochrysidin) and internal standard (40 μg/mL for ibuprofen) in methanol[1]. Stock solutions were stored at -20 ℃. Working standard solutions were prepared freshly by diluting stock solutions in sodium hydroxide solution (0.1 mol/L). Internal standard working solution (200 ng/mL) was prepared by diluting the stock solution in methanol-water (1:1, v/v)[1].

Induction of AP and dosing of DCQD

First part: Rats were randomly allocated into the following four groups (n = 6 each): a sham-operated group [NG(a)] and three model groups [4hG(a), 12hG(a) and 24hG(a)]. AP models were induced by retrograde perfusion of 3% sodium taurocholate (1 mL/kg.BW) into the biliopancreatic duct[11] at a rate of 6 mL/h with a micro-infusion pump after anesthetization with 10% chloral hydrate (3 mL/kg.BW) injected into the abdominal cavity. The NG(a) group was established by the same procedure, but with use of saline instead of sodium taurocholate. The dosing time of DCQD for the NG(a) and model groups were 4 h, 4 h, 12 h and 24 h after operation.

Second part: Forty-two rats were divided into a sham-operated group [NG(b)], and 4h-, 12h- and 24h-dosing treatment groups [4hG(b), 12hG(b) and 24hG(b), respectively] with three corresponding control groups [MG(b)s]. Only the three treatment groups were administered the same dosage of DCQD at 4 h, 12 h and 24 h, respectively, after AP induction. The three corresponding control groups were given saline instead of DCQD at the same time. Rats were euthanized at 24 h after drug dosing.

Collection and measurement of samples

First part: Plasma samples (0.5 mL) were collected from the tails at 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h after a single dosing of DCQD. Serum samples for amylase (AMS) and pancreatic tissues for the tissue concentrations of the absorbed components of DCQD were collected 24 h after drug dosing. A total of 0.05 mL of internal standard working solution and 0.1 mL of hydrochloric acid buffer solution were added into 0.2 mL of the plasma or tissue homogenate samples, followed by 3.0 mL of ethyl acetate. Then, the mixtures were extracted by vortex mixing for 7 min and centrifuged at 3000 rpm for 7 min at a low temperature. After that, 2.4 mL supernatants were evaporated at 45 ℃, followed by incubation with 0.1 mL of double-solvents (methanol-water: 92:8, v/v). Thereafter, 20 μL of supernatant was injected automatically into the HPLC-MS/MS system for analysis. The 10 major components of DCQD (aloe-emodin, rhein, emodin, chrysophanol, honokiol, rheochrysophanol, magnolol, hesperidin, naringenin and naringin) were detected. The mean contents of the components were detected three times, as in our previous study[12]. The detected DCQD samples were stored in the Public Experiment Platform at West China Hospital (Chengdu, China).

Second part: Serum for AMS and inflammatory cytokines measurement and pancreatic tissues for pathological scoring were collected 24 h after drug dosing. Pancreatic samples were fixed in 10% neutral formalin for paraffin sections and stained with hematoxylin and eosin (HE). All the histopathology specimens were scored in a blinded fashion by two independent pathologists using a scoring system for the extent and severity of tissue injury (0-4, edema, neutrophil infiltration, necrosis and hemorrhage, respectively), as previously described[4,13]. The IL-10 and IL-6 levels were detected by enzyme-linked immunosorbent assay kits. The following formula was used to calculate the value of AMS: AMS (U/dL) = (blankOD - measurementOD)/blankOD × 0.4 × 0.5/10 × 30 min/7.5 min × 100/0.1 × 10

Statistical analysis

The pharmacokinetic parameters were processed by pharmacokinetic statistical software DAS2.0.1 programmed by the Chinese Pharmacological Society. The data were obtained by statistical moment calculation. The following pharmacokinetic parameters were calculated: the maximum plasma concentration (C_{max}), the time to reach maximum concentration (T_{max}), the mean residence time ($MRT_{0\rightarrow t}$), the elimination half-life ($T_{1/2}$) and the area under the plasma concentration-time curve from time 0 to the time of the last measurable concentration ($AUC_{0\rightarrow t}$). The data were processed with statistical software PEMS3.1. All values were expressed as the mean ± SD. A one-way repeated-measure ANOVA, followed by multiple pairwise comparisons using the Student-Newman-Keuls procedure, was used to detect differences among the groups. $P < 0.05$ was considered a statistically significant difference.

RESULTS

Ten major components of DCQD distributed in pancreatic tissues

In this study, all 10 major components of DCQD in pancreatic tissues could be detected by HPLC-MS/MS. The concentrations of naringin, hesperidin, naringenin, aloe-emodin and chrysophanol in pancreatic tissues were relatively high. Compared to the NG(a) group, the concentrations of emodin,

Table 1 Concentrations of the 10 components of DCQD distributed in pancreatic tissues

	NG(a)	4hG(a)	12hG(a)	24hG(a)
Rhein	12.65 ± 3.7	179.15 ± 77.6[a]	199.89 ± 34.8	50.08 ± 17.6[e]
Emodin	18.68 ± 8.7	1.33 ± 0.8[a]	34.46 ± 10.3[c]	39.75 ± 12.4
Naringin	415.30 ± 17.8	19.47 ± 1.4[a]	32.10 ± 2.7[c]	269.16 ± 12.9[e]
Honokiol	2.38 ± 1.2	1.98 ± 0.1	19.79 ± 2.2[c]	4.26 ± 1.0
Magnolol	1.34 ± 0.9	0.97 ± 0.2	0.97 ± 0.2	7.51 ± 0.8[e]
Hesperidin	162.01 ± 34.3	34.94 ± 12.6[a]	24.31 ± 10.3	113.26 ± 13.8[e]
Naringenin	847.98 ± 76.9	858.58 ± 19.6	1077.06 ± 26.8[c]	262.30 ± 100.6[e]
Aloe-emodin	439.05 ± 179.8	89.53 ± 14.8[a]	502.74 ± 70.7[c]	501.45 ± 143.4
Chrysophanol	60.99 ± 16.4	5.16 ± 2.1[a]	197.29 ± 17.9[c]	113.43 ± 23.7[e]
Rheochrysidin	6.79 ± 1.2	0.55 ± 0.2[a]	17.92 ± 1.9[c]	11.67 ± 0.9[e]

Rats were orally administered with DCQD (10 mL/kg.BW). Pancreatic tissues were collected 24 h after a single dosage of DCQD. The concentrations of the 10 components of DCQD were determined by HPLC-MS/MS. The results are presented as the mean ± SD of μg/mg (n = 6). NG(a): the sham-operated group with the dosing time at 4 h after operation. 4hG(a), 12hG(a) and 24hG(a): rats were dosed orally with DCQD at 4 h, 12 h and 24 h, respectively, after AP induction. 4hG(a) *vs* NG(a): [a]P < 0.05, 12hG(a) *vs* 4hG(a): [c]P < 0.05, 24hG(a) *vs* 12hG(a): [e]P < 0.05. AP: Acute pancreatitis; DCQD: Da-Cheng-Qi decoction; HPLC-MS/MS: High-performance liquid chromatography tandem mass spectroscopy.

naringin, hesperidin, aloe-emodin, chrysophanol and rheochrysidin were lower in the 4hG(a) group (P < 0.05), while the rhein concentration was higher (P < 0.05). The concentrations of emodin, naringin, honokiol, naringenin, aloe-emodin, chrysophanol and rheochrysidin in the 12hG(a) group were higher than in the 4hG(a) group (P < 0.05). The concentrations of rhein, naringenin, chrysophanol and rheochrysidin in the 24hG(a) group were lower than in the 12hG(a) group (P < 0.05), while the concentrations of naringin, magnolol and hesperidin in the 24hG(a) group were higher (P < 0.05) (Table 1).

Eight major components of DCQD detected in plasma

Only eight of the ten components were successfully fitted to the concentration-time curves according to the testing data (Figure 1). The T_{max} values were all at 40 min (0.67 h) after a single dosage of DCQD in the NG(a) group. The T_{max} of six components (aloe-emodin, rhein, emodin, chrysophanol, naringenin and naringin) in the 4hG(a) group, all components in the 12hG(a) group and that of five components (aloe-emodin, rhein, rheochrysidin, magnolol and naringin) in the 24hG(a) were delayed (Figure 1).

In the NG(a) group, the C_{max} values of the components were as follows: aloe-emodin, 3218.33 ng/mL; rhein, 8638.42 ng/mL; emodin, 510.97 ng/mL; naringin, 204.56 ng/mL; chrysophanol, 5419.89 ng/mL; rheochrysidin, 146.75 ng/mL; naringenin, 419.94 ng/mL; and magnolol, 19.58 ng/mL. Compared to the NG(a) group, the C_{max} values of aloe-emodin, rhein, emodin, chrysophanol and magnolol were reduced in the 4hG(a) group (Figure 1). Compared to the 4hG(a) or 12hG(a) groups, the C_{max} values of aloe-emodin, rhein, emodin, naringenin and magnolol were higher in the 24hG(a) group, and they were as follows: 5885.13 ng/mL, 8245.18 ng/mL, 88.65 ng/mL, 606.41 ng/mL and 11.018 ng/mL, respectively. Among the three model groups, the C_{max} values of chrysophanol and naringin were highest in the 12hG(a) group, and for rheochrysidin the C_{max} was in the 4hG(a) group; the concentrations were 4054.73 ng/mL, 519.53 ng/mL and 557.06 ng/mL, respectively (Figure 1).

The pharmacokinetic parameters were analyzed by statistical moment calculation, and those in model groups were different from those in the NG(a) group (Table 2). Compared to the NG(a) group, the $AUC_{0\rightarrow t}$ values of aloe-emodin, chrysophanol, emodin and magnolol were smaller in the 4hG(a) group (P < 0.05), while those of rheochrysidin, naringin and naringenin were larger (P < 0.05). The $AUC_{0\rightarrow t}$ of aloe-emodin in the 24hG(a) group was larger than in the 4hG(a) or 12hG(a) groups, and those of rhein, chrysophanol, magnolol and naringin in the 12hG(a) group were larger than in the 4hG(a) or 24hG(a) groups. The $T_{1/2}$ values of rhein, emodin, aloe-emodin, rheochrysidin, naringin and magnolol in the 24hG(a) group were longer than in the 4hG(a) or 12hG(a) groups.

DCQD elevated the IL-10 levels and lowered the IL-6 levels in serum

In serum, the IL-10 and IL-6 levels in the three corresponding control groups were all higher than in the NG(b) group (IL-10: 152.8 ± 18.58/77.46 ± 7.42/99.72 ± 4.7 *vs* 48 ± 12, P < 0.05; IL-6: 147.91 ± 4.36/171.44 ± 13.43/98.48 ± 2.7 *vs* 68 ± 20, P < 0.05). Compared to the corresponding control groups, the IL-10 levels in the 12hG(b) and 24hG(b) groups were increased (96.55 ± 7.84 *vs* 77.46 ± 7.42, 251.22 ± 16.15 *vs* 99.72 ± 4.7, P < 0.05), and the IL-10 level in the 24hG(b) group was higher than in the 4h(b) and 12hG(b) groups (251.22 ± 16.15 *vs* 154.41 ± 12.09/96.55 ± 7.84, P < 0.05) (Figure 2A). The IL-6 levels displayed a decrease in the 4hG(b) and 12hG(b) groups compared to their corresponding control groups (89.99 ± 4.61 *vs* 147.4.36, 90.82 ± 5.34 *vs* 171.44 ±

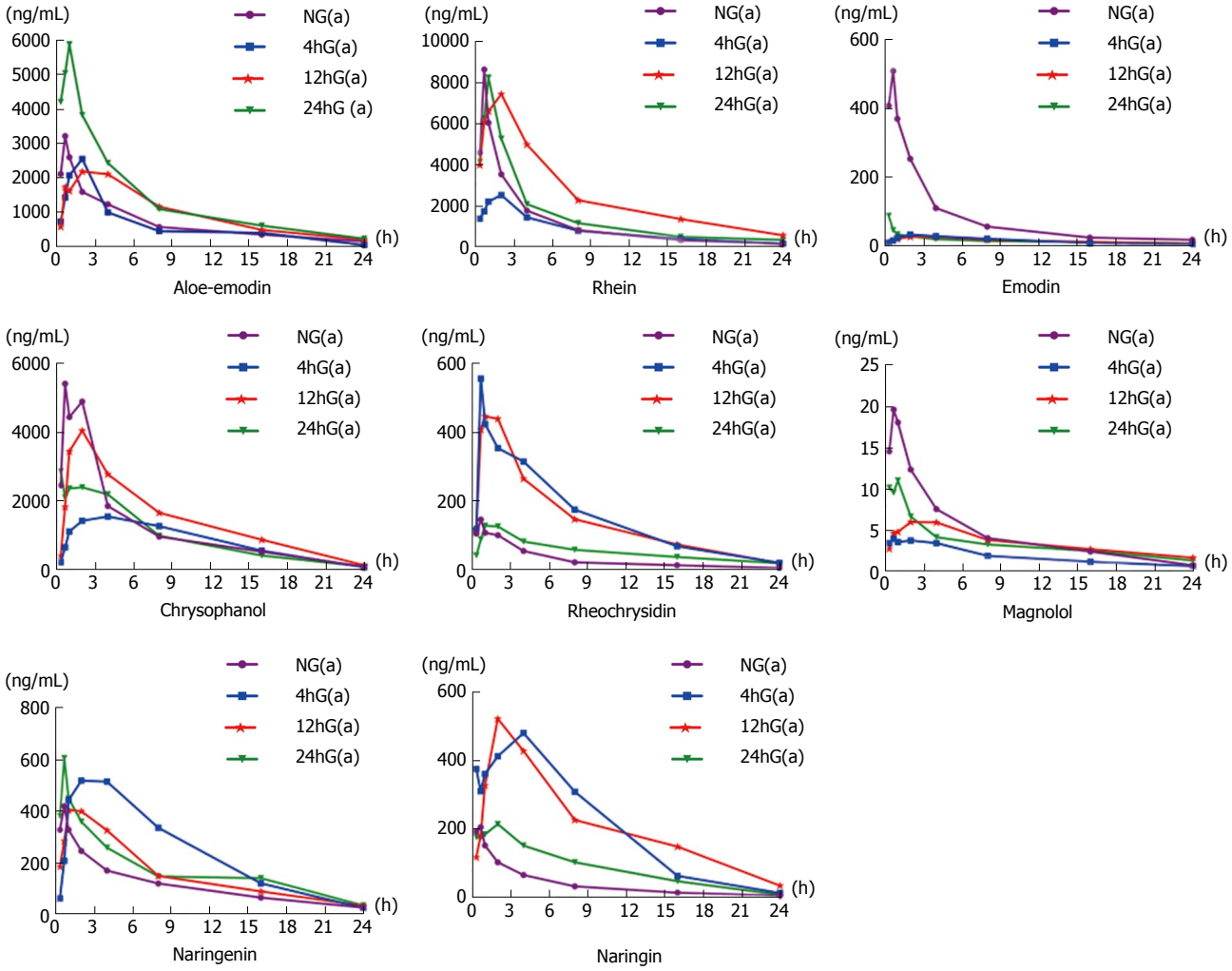

Figure 1 Concentration-time curves of the eight components of Da-Cheng-Qi decoction in plasma of the rats with acute pancreatitis. Rats (n = 6 per group) were orally dosed with DCQD (10 mL/kg.BW). Plasma samples were collected from the tails at 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h after a single dosage of DCQD. Plasma concentrations of the components of DCQD were determined by HPLC-MS/MS. The results are presented as the mean ± SD. NG(a): the sham-operated group with the dosing time 4 h after operation; 4hG(a), 12hG(a) and 24hG(a): the dosing times were 4 h, 12 h and 24 h, respectively, after AP induction. AP: Acute pancreatitis; DCQD: Da-Cheng-Qi decoction; HPLC-MS/MS: High-performance liquid chromatography tandem mass spectroscopy.

13.43, $P < 0.05$) (Figure 2B).

Pathological damages in the pancreatic tissues and serum AMS levels

In the NG(b) group, the pancreatic tissues were edematous with a few neutrophils but without obvious hemorrhage and necrotic acinar tissues. In the MG(b) groups, edema, hemorrhage and neutrophils with some necrotic acinar tissues were obvious. In the 12hG(b) group, the pathological damages were improved, while those in the 4hG(b) or 24hG(b) groups had no obvious improvement (Figure 3A). Compared to the NG(b) group, the pathological scores in the MG(b) groups were increased (1.79 ± 0.3/2.2 ± 0.2/2.67 ± 0.67 *vs* 0.7 ± 0.26, $P < 0.05$). Those in the 12hG(b) group were evidently reduced compared to its control group (1.3 ± 0.4 *vs* 2.2 ± 0.2, $P < 0.05$) or the 4hG(b) and 24hG(b) groups (1.3 ± 0.4 *vs 2.63* ± 0.4/2.5 ± 0.3, $P < 0.05$) (Figure 3B).

The AMS levels in the 4hG(a), 12hG(a) and 24hG(a) groups were all higher than in the NG(a) group (724.17 ± 42.8/673.67 ± 26.64/659.65 ± 41.38 *vs* 273.67 ± 93.23, $P < 0.05$) (Figure 3C). Compared to the NG(b) group, that in the MG(b) groups were higher (718.65 ± 51.04/711.68 ± 55.37/666.4 ± 73.4 *vs* 389 ± 98, $P < 0.05$). The AP model was successful. That in the 12hG(b) group was reduced compared to its control group (649 ± 131.69 *vs* 711.68 ± 55.37, $P < 0.05$) (Figure 3D).

DISCUSSION

According to the present study, AP reduced the concentrations of the major components of DCQD to the target pancreas, and the oral administration time also played an important role; their absorption

Table 2 Pharmacokinetic parameters of the 8 detected components of Da-Cheng-Qi decoction in plasma

Parameter	NG(a)	4hG(a)	12hG(a)	24hG(a)
Aloe-emodin				
T1/2 (h)	7.5 ± 3.4	4.8 ± 1.9	7.3 ± 2.7	7.5 ± 4.3
AUC0→t (μg/mL•h)	16582.3 ± 523.7	15180.5 ± 245.3[a]	23266.6 ± 2848.4[b]	32517.8 ± 3109.6[dc]
MRT0→t (h)	7.0 ± 1.8	6.5 ± 1.6	7.7 ± 0.9	6.2 ± 1.3
Tmax (h)	1.22 ± 1.38	1.45 ± 0.62	2.28 ± 1.44	0.67 ± 0.30[dc]
Cmax (μg/mL)	4080.9 ± 2491.6	2890.5 ± 955.0	2948.2 ± 997.6	7706.6 ± 2366.7[dc]
Rhein				
T1/2 (h)	4.7 ± 1.5	6.3 ± 1.8	6.2 ± 1.2	16.6 ± 2.1[dc]
AUC0→t (μg/mL•h)	27164.9 ± 1686.9	19164.9 ± 1680.3	60705.9 ± 2870.4[b]	35470.0 ± 1910.1[dc]
MRT0→t (h)	4.7 ± 1.0	6.3 ± 1.5[a]	7.4 ± 1.3	5.9 ± 3.2
Tmax (h)	0.7 ± 0.3	1.4 ± 0.9[a]	1.3 ± 0.7	1.3 ± 0.4
Cmax (μg/mL)	11033.4 ± 3248.9	3037.9 ± 1040.5[a]	9439.4 ± 3191.6[b]	9938.6 ± 3349.6[d]
Chrysophanol				
T1/2 (h)	5.2 ± 1.7	6.8 ± 3.6	4.5 ± 0.8	6.2 ± 3.8
AUC0→t (μg/mL•h)	28925.5 ± 7837.2	20214.5 ± 1460.6[a]	34977.3 ± 1927.7[b]	23102.4 ± 1614.8[c]
MRT0→t (h)	5.7 ± 0.8	7.2 ± 1.7[a]	7.3 ± 1.0	6.2 ± 1.2
Tmax (h)	1.2 ± 0.6	3.5 ± 2.5[a]	1.7 ± 0.5	1.3 ± 0.5[d]
Cmax (μg/mL)	7708.1 ± 2234.3	2296.1 ± 7643.8[a]	4521.8 ± 1127.1[b]	4772.6 ± 1078.4[d]
Emodin				
T1/2 (h)	8.2 ± 6.6	14.7 ± 11.7	11.7 ± 6.0	20.8 ± 19.6
AUC0→t (μg/mL•h)	1891.5 ± 1692.8	395.3 ± 159.0[a]	395.4 ± 82.4	391.2 ± 135.8
MRT0→t (h)	5.2 ± 1.9	8.4 ± 1.7[a]	9.4 ± 0.8	8.6 ± 1.9
Tmax (h)	0.6 ± 0.3	3.8 ± 3.3[a]	3.2 ± 2.6	0.7 ± 0.7[dc]
Cmax (μg/mL)	546.2 ± 496.8	37.9 ± 18.4[a]	34.1 ± 9.6	91.8 ± 37.4
Rheochrysidin				
T1/2 (h)	6.4 ± 0.9	4.6 ± 1.9	5.4 ± 2.1	8.3 ± 2.3[dc]
AUC0→t (μg/mL•h)	740.2 ± 623.3	3680.6 ± 1903.0[a]	3489.2 ± 1354.1	1301.8 ± 420.9[dc]
MRT0→t (h)	5.7 ± 1.4	6.4 ± 0.9	6.3 ± 1.5	8.2 ± 1.1[dc]
Tmax (h)	0.9 ± 0.6	2.1 ± 1.6	1.7 ± 1.3	1.5 ± 0.6
Cmax (μg/mL)	184.7 ± 92.4	713.8 ± 201.6[a]	642.3 ± 211.5	155.5 ± 56.4[dc]
Magnolol				
T1/2 (h)	8.5 ± 4.9	9.4 ± 2.8	12.2 ± 3.3	22.8 ± 7.6[dc]
AUC0→t (μg/mL•h)	111.4 ± 37.2	44.4 ± 14.3[a]	83.9 ± 27.2[b]	81.5 ± 25.0[d]
MRT0→t (h)	7.2 ± 0.9	7.9 ± 1.2	9.4 ± 1.1	8.9 ± 1.2
Tmax (h)	0.7 ± 0.3	1.3 ± 0.5Δ	2.8 ± 1.4	0.6 ± 0.3[dc]
Cmax (μg/mL)	24.2 ± 8.6	5.8 ± 2.3Δ	7.7 ± 3.6	15.3 ± 13.1[dc]
Naringin				
T1/2 (h)	5.8 ± 1.2	4.9 ± 1.8	5.3 ± 1.4	5.5 ± 1.4
AUC0→t (μg/mL•h)	918.1 ± 106.8	4920.6 ± 310.7[a]	5054.4 ± 435.1	2044.1 ± 26.9[dc]
MRT0→t (h)	5.9 ± 1.1	6.6 ± 0.8	7.9 ± 0.6	6.9 ± 1.0
Tmax (h)	0.7 ± 0.2	1.4 ± 1.4	2.5 ± 1.2	1.8 ± 1.3
Cmax (μg/mL)	229.3 ± 195.6	724.4 ± 584.7	595.1 ± 338.9	322.9 ± 148.4
Naringenin				
T1/2 (h)	7.2 ± 1.7	4.9 ± 1.1a	9.3 ± 3.6[b]	7.7 ± 1.3[d]
AUC0→t (μg/mL•h)	2705.2 ± 164.6	5795.1 ± 291.4[a]	3758.1 ± 250.1[b]	4099.2 ± 148.8[dc]
MRT0→t (h)	6.4 ± 1.7	7.3 ± 0.4	7.7 ± 1.2	7.9 ± 1.8
Tmax (h)	0.8 ± 0.6	2.3 ± 1.4	1.6 ± 1.3	0.6 ± 0.3[d]
Cmax (μg/mL)	552.9 ± 226.7	720.4 ± 165.9	543.3 ± 110.4	624.9 ± 143.7

Rats were orally dosed with DCQD (10 mL/kg.BW). Plasma samples were collected from the tails at 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h after a single dosage of DCQD. The samples were assessed by HPLC-MS/MS. The pharmacokinetic parameters were processed by pharmacokinetic statistic software DAS2.0.1 and the data were obtained by statistical moment calculation. The results are presented as the mean ± SD. NG(a): the sham-operated group with the dosing time at 4 h after operation. 4hG(a), 12hG(a) and 24hG(a): rats were dosed orally with DCQD at 4 h, 12 h and 24 h, respectively, after AP induction. Compared with NG(a): [a]$P < 0.05$, 12hG(a) *vs* 4hG(a): [b]$P < 0.05$, 24hG(a) *vs* 12hG(a): [c]$P < 0.05$, 24hG(a) *vs* 4hG(a): [d]$P < 0.05$. AP: Acute pancreatitis; DCQD: Da-Cheng-Qi decoction; HPLC-MS/MS: High-performance liquid chromatography tandem mass spectroscopy.

may be better if the oral administration time of DCQD is approximately 12 h after the onset of AP (Table 1). AP delayed the T_{max} and reduced the C_{max} of the components of DCQD in the circulation of rats (Figure 1). The components of DCQD displayed a higher C_{max} and a longer $T_{1/2}$ when the oral administration time of DCQD was approximately 24 h after the onset of AP (Figure 1 and Table 2). The $AUC_{0\rightarrow t}$ was larger when that time was approximately 12 h after the onset of AP (Table 2). DCQD increased the IL-10 levels and lowered the IL-6 levels (Figure 2A and B), and the later administration of the DCQD dose corresponded to higher IL-10 levels (Figure 2A). Therefore, administering the DCQD dose too early may not be

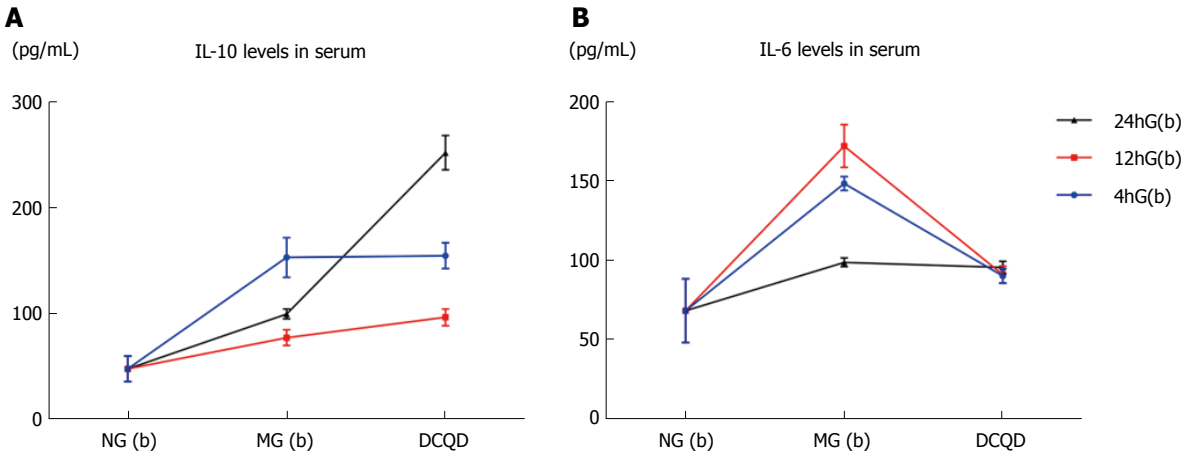

Figure 2 Da-Cheng-Qi decoction elevated IL-10 levels and lowered IL-6 levels in serum of the rats with acute pancreatitis. Rats (*n* = 6 per group) in the three treatment groups were orally dosed with DCQD (10 mL/kg.BW) 4 h, 12 h and 24 h after AP induction. Serum samples were collected at 24 h after a single dosage of DCQD. The results are presented as the mean ± SD. NG(b): the sham-operated group; MG(b): the model group or the control group; 4hG(b), 12hG(b) and 24hG(b): rats were orally dosed with DCQD 4 h, 12 h and 24 h, respectively, after AP induction. AP: Acute pancreatitis; DCQD: Da-Cheng-Qi decoction.

appropriate for AP, and administration should at least be 12 h after the onset of AP.

Pathological circumstances may affect the absorption of the components of DCQD and affect the pharmacokinetics in AP. Pancreatic ischemia, reduced pancreatic blood flow and increased capillary permeability are usually common in AP[14]. The systemic hemodynamic disturbances lead to ischemia of the intestine[15]. In addition, one retrospective analysis (197 patients) showed that 65% of patients with AP had acute gastrointestinal mucosal lesions detected by upper gastrointestinal endoscopy[16]. As well, 59% of patients with AP showed gut barrier dysfunction with increased intestinal permeability in a meta-analysis of 18 studies[17]. Both propulsion and contractility were reduced in necrotizing pancreatitis of rats[18]. It was reported that permeability of the ileum was significantly increased at 6 h, the blood endotoxin level was elevated and bacterial translocation occurred 18 h after induction of SAP-induced by injection of 3% sodium deoxycholate[19].

As we know, oral medicines, including herbs, are generally absorbed by the gastrointestinal mucosa. The process of drug absorption into the blood circulation from the plasma membrane barrier proceeds as follows: drug molecule encounters the gastrointestinal mucous layer, brush border, epithelial cell membrane, intracellular fluid, basal lamina, lamina propria, externa of vessels, cytoplasm of vessels, intima of vessels and then the blood. Therefore, these aforementioned factors may play an important role in the delay of the T_{max} of the components of DCQD in reaching the circulation and establishing their concentrations in pancreatic tissues.

Another factor may be the physicochemical properties of these components. Chrysophanol belongs to the Class Ⅱ poorly water soluble drugs in the Biopharmaceutics Classification System (BCS), with low solubility but high permeability[20,21]. Magnolol, a small-molecule neolignan[22], has an extensive first-pass metabolism and low absorption[23]. Naringin is moderately soluble in water, and it is broken into its aglycon naringenin in the intestine by the gut microflora and then absorbed from the gut[24]. These different characteristics may influence the absorption of these components. Furthermore, the pharmacokinetics of phytochemicals have substantial variation[25] and circulating concentrations of phytochemicals, which could vary widely among individuals, even in the context of controlled feeding studies[26]. In brief, the internal environments of rats and the characteristics of the components of DCQD may be the major factors affecting the absorption of these components.

It is well known that the avoidance of gastric and intestinal secretion has been the cornerstone of management of patients with AP for nearly a century[27]. To espouse the "pancreatic rest" concept, fasting and water deprivation have become the fundamental treating rules. However, the benefits of oral dosing with DCQD in the early stage of AP have been demonstrated, especially in gastrointestinal internal environments. This approach could improve intestinal propulsive function, relieve abdominal distension and abdominal pressure[28], and protect the intestinal immune barrier, with amelioration of the levels of high mobility group box-1 protein (HMGB1) RNA and cyclooxygenase 2 (COX-2) RNA expression[29]. One meta-analysis showed that purgative therapy could shorten the time of first defecation and the hospitalization time[30]. Thus, the advantages of DCQD are obvious. Future studies should be done to determine whether the oral dose of DCQD could increase gastrointestinal and pancreatic secretion.

The finding of DCQD regulating the balance of pro-inflammation and anti-inflammation was consistent with our previous studies[4]. However, the current

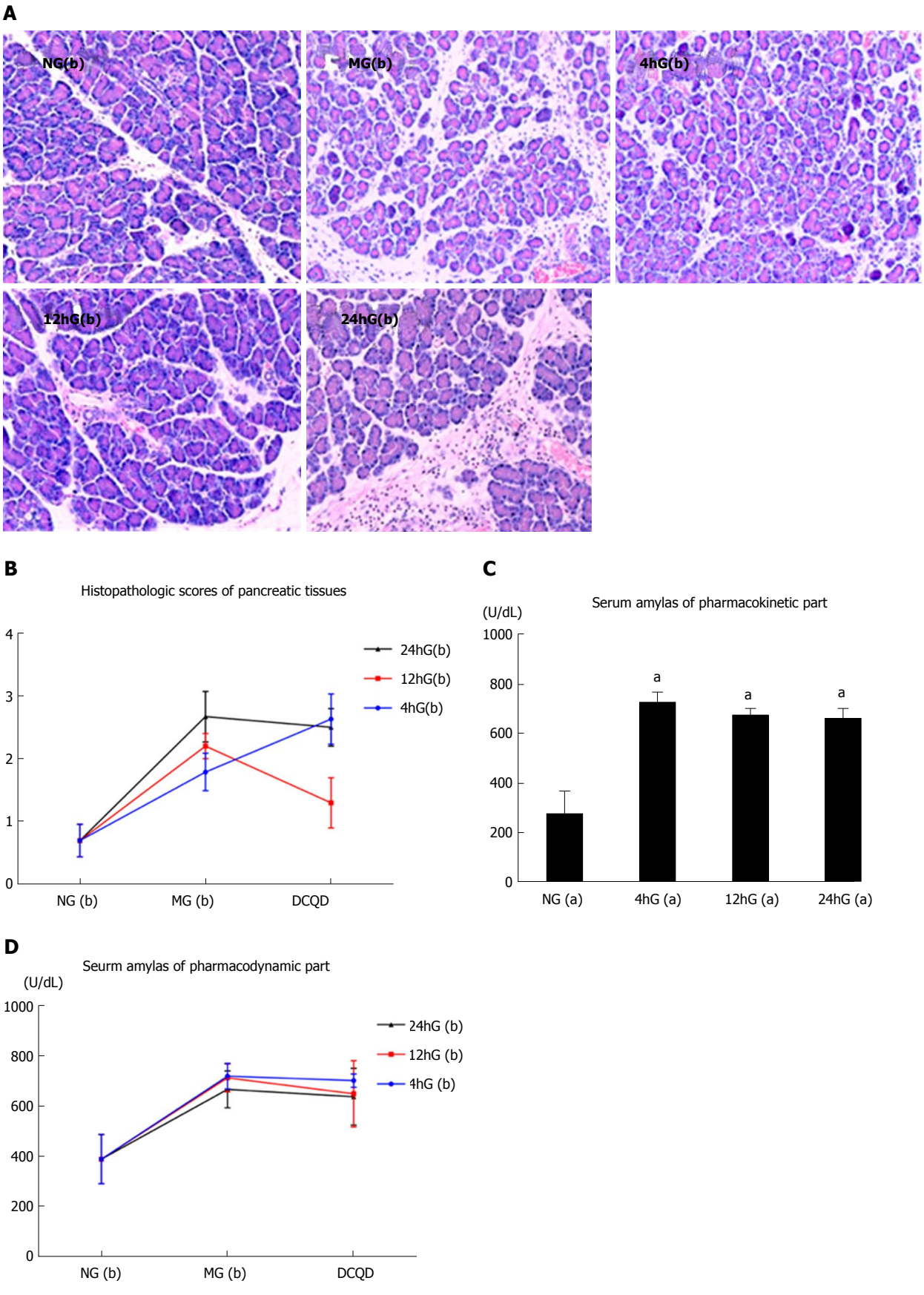

Figure 3 Pathological damages in the pancreatic tissues and changes in the serum amylase concentration. Rats (n = 6 per group) were orally administered DCQD (10 mL/kg.BW) 4 h, 12 h and 24 h after AP induction. Pancreatic tissues were collected for staining with hematoxylin-eosin (A, × 200) 24 h after a single dosage of DCQD; B: Pathological scores of the pancreatic tissues. Serum amylase was detected by enzyme-linked immunosorbent assay (C and D). The results are presented as the mean ± SD. $^{a}P < 0.05$ *vs* NG(a). NG(a/b): the sham-operated group; MG(b): the model group or the control group; 4hG(a/b) 12hG(a/b), and 24hG(a/b): rats were orally dosed with DCQD 4 h, 12 h and 24 h, respectively, after AP induction. AP: Acute pancreatitis; DCQD: Da-Cheng-Qi decoction.

study showed that its oral dosing time might affect the inflammatory cytokines and pharmacokinetics of the effective components of DCQD targeting of pancreatic tissues and plasma. The chronomedicine of TCM, with thousands of years of history and in which the midnight-noon ebb-flow theory is typical, is similar to modern chronobiology. Theoretically, the function of Chinese medicine will be most effective at driving out pathogenic factors when the function of some meridian is at its peak[31]. For example, erythrocyte C3b receptor rosette and erythrocyte immune complex rosette can reach peak value when the kidney meridian has its most active function[32].

Along with the improvement of modern technology, the research on the relationship between the dosing time of Chinese medicine and plasma concentration or curative effect has been performed. Nishioka *et al*[33] demonstrated that the dosing time of Sho-Saiko-To could affect the plasma concentrations of the effective components (glycyrrhizin and baicalein). Additionally, the pharmacokinetic processes of emodin and aloe-emodin of DCQD presented a circadian rhythm phenomenon[34]. According to our study, the oral dosing time also affects the drug tissue concentrations. Therefore, the oral dosing time of DCQD is closely related to its pharmacokinetics and pharmacodynamics.

In this study, the pathological damages of pancreatic tissues had been improved only in the 12hG(b) group, and was not improved in the 4hG(b) or 24hG(b) groups. However, this finding was not consistent with those of a previous study showing that a similar dose of DCQD 2 h after AP induction confers some protection against pancreatic tissue damage. Although the weight of rats showed no difference, this finding may be related to the different experimenters, which may have resulted in large differences among the various groups. We should ensure the consistency of experimenters. In clinical practice, orally dosing or coloclysis of DCQD are performed immediately when patients with AP are admitted to hospital. Whether these approaches could increase secretion of the gastrointestinal tract remains unclear. Recently, little research on the optimal administration time of Chinese herbs has been reported, and there are no definite opinions on this topic in the Chinese guidelines, although the guidelines are generally used in clinical practice in China. Therefore, our conjecture needs further clinical studies to be confirmed.

In conclusion, AP and the oral administration time of DCQD could affect the pharmacokinetics of the absorbed components of DCQD in the pancreatic tissues and plasma of rats. Late-time dosing may result in higher concentrations of the major components of DCQD with better pharmacokinetics and pharmacodynamics of anti-inflammation than seen with early-time dosing, thereby showing the late time to be the optimal dosing time of DCQD for AP.

ARTICLE HIGHLIGHTS

Research background

Oral administration with Da-Cheng-Qi decoction (DCQD) is the conventional therapy at the early phase of acute pancreatitis (AP) patients in China. But oral dosing with DCQD is contrary to the idea of pancreatic rest at the early stage of AP, which may inhibit the absorption of the components of DCQD, influence its pharmacokinetics or pharmacodynamics and even worsen the disease severity.

Research motivation

The necrosis of pancreatic acinar cells would worsen the disease and the induction of apoptosis would relieve the disease severity. In clinical practice, oral dosing or coloclysis of DCQD are performed immediately when patients with AP are admitted to hospital. Whether these approaches could increase secretion of gastrointestinal tract remain unclear. What's more, little research on the optimal administration time of Chinese herbs has been reported, and there are no definite opinions on this topic in the Chinese guidelines. Thus, based on effect of DCQD regulating the apoptosis/necrosis switch of pancreatic acinar cells to ameliorate the pancreatic inflammation and pathological damage, the study aimed to screen the optional oral dosing time of DCQD in rats with AP according to the pharmacokinetics of the absorbed components and the pharmacodynamics of DCQD targeting of pancreas.

Research objectives

This objective was to screen the optional oral dosing time of DCQD in rats with AP based on the pharmacokinetic and pharmacodynamic parameters. We hoped to find an optimal dosing time of DCQD without increasing the severity of AP.

Research methods

This animal experiment was divided into pharmacokinetic and pharmacodynamic parts. AP models were induced by 3% sodium taurocholate. Rats were dosed at three different times. Plasma samples were collected from the tails at nine different times. The main components' concentrations of plasma and pancreatic tissues were detected by high-performance liquid chromatography tandem mass spectroscopy, confirmed as a specific, sensitive, accurate and reproducible method. The pharmacokinetic parameters [the maximum plasma concentration (C_{max}), the time to reach maximum concentration (T_{max}), the mean residence time ($MRT_{0\rightarrow t}$), the elimination half-life ($T_{1/2}$) and the area under the plasma concentration-time curve from time 0 to the time of the last measurable concentration ($AUC_{0\rightarrow t}$)] were processed by pharmacokinetic statistical software DAS2.0.1 programmed by the Chinese Pharmacological Society. The IL-10, IL-6 and amylase concentrations in serum and pathological scores of pancreatic tissues were calculated.

Research results

According to the present study, AP reduced the concentrations of the major components of DCQD to the target pancreas, and the oral administration time also played an important role. AP delayed the T_{max} and reduced the C_{max} of the components of DCQD in the circulation of rats. The $AUC_{0\rightarrow t}$ was larger when that time was approximately 12 h after the onset of AP. DCQD increased the IL-10 levels and lowered the IL-6 levels, and the later administration of the DCQD dose corresponded to higher IL-10 levels. Therefore, administering the DCQD dose too early may not be appropriate for AP. However, our results need further clinical studies to be confirmed.

Research conclusions

Late-time dosing may result in higher concentrations of the major components of DCQD with better pharmacokinetics and pharmacodynamics of anti-inflammation than seen with early-time dosing, thereby showing the late time to

be the optimal dosing time of DCQD for AP.

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ORIGINAL ARTICLE

Retrospective Study

Short- and long-term results of endoscopic ultrasound-guided transmural drainage for pancreatic pseudocysts and walled-off necrosis

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Abstract

AIM

To evaluate the short- and long-term results of endoscopic ultrasound-guided transmural drainage (EUS-GTD) for pancreatic fluid collection (PFC) and identify the predictive factors of treatment outcome for walled-off necrosis (WON) managed by EUS-GTD alone.

METHODS

We investigated 103 consecutive patients with PFC who

underwent EUS-GTD between September 1999 and August 2015. Patients were divided into four groups as follows: WON (n = 40), pancreatic pseudocyst (PPC; n = 11), chronic pseudocyst (n = 33), and others (n = 19). We evaluated the short- and long-term outcomes of the treatment. In cases of WON, multiple logistic regression analyses were performed to identify the predictor variables associated with the treatment success. In addition, PFC recurrence was examined in patients followed up for more than 6 mo and internal stent removal after successful EUS-GTD was confirmed.

RESULTS

In this study, the total technical success rate was 96.1%. The treatment success rate of WON, PPC, chronic pseudocyst, and others was 57.5%, 90.9%, 91.0%, and 89.5%, respectively. Contrast-enhanced computed tomography using the multivariate logistic regression analysis revealed that the treatment success rate of WON was significantly lower in patients with more than 50% pancreatic parenchymal necrosis (OR = 17.0; 95%CI: 1.9-150.7; P = 0.011) and in patients with more than 150 mm of PFC (OR = 27.9; 95%CI: 3.4-227.7; P = 0.002).The recurrence of PFC in the long term was 13.3% (median observation time, 38.8 mo). Mean amylase level in the cavity was significantly higher in the recurrence group than in the no recurrence group (P = 0.02).

CONCLUSION

The reduction of WON by EUS-GTD alone was associated with the proportion of necrotic tissue and extent of the cavity. The amylase level in the cavity may be a predictive factor for recurrence of PFC.

Key words: Endoscopic ultrasound-guided transmural drainage; Pancreatic fluid collection; Revised Atlanta Classification; Walled-off necrosis

Core tip: It remains unclear that which patients with walled-off necrosis (WON) can be resolved by endoscopic ultrasound-guided transmural drainage (EUS-GTD) alone and which ones should be treated by endoscopic necrosectomy or other additional treatment. In addition, some pancreatic fluid collections (PFCs) develop recurrent fluid collection, and it is also unclear which types of PFCs show recurrence. In this study, we demonstrated that PFC size and proportion of pancreatic parenchymal necrosis were related to the resolution of WON treated by EUS-GTD alone. Regarding long-term follow-up patients, mean amylase level in the cavity was associated with PFC recurrence, suggesting a prolonged stent placement in patients with predicted recurrence.

Watanabe Y, Mikata R, Yasui S, Ohyama H, Sugiyama H, Sakai Y, Tsuyuguchi T, Kato N. Short- and long-term results of endoscopic ultrasound-guided transmural drainage for pancreatic pseudocysts and walled-off necrosis. *World J Gastroenterol* 2017; 23(39): 7110-7118 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7110.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7110

INTRODUCTION

Pancreatic fluid collection (PFC) is a local complication after pancreatitis. Although most PFCs spontaneously improve, some PFCs remain and become infectious, thereby needing therapeutic intervention. In 2012, the revision of the Atlanta classification categorized PFC into the following four types: acute peripancreatic fluid collection, acute necrotic collection, pancreatic pseudocyst (PPC), and walled-off necrosis (WON). According to this revised classification, the development of PPC is considered to be extremely rare in acute pancreatitis, and most PFCs over 4 wk are classified as WON[1]. This suggests that many PFCs after acute pancreatitis that were treated as PPC because of slight debris in the cavity should be addressed as WON, according to the revised classification.

Endoscopic ultrasound-guided transmural drainage (EUS-GTD) is now widely accepted as a minimally invasive method for managing PFC with minimal complications[2]. According to previous reports, most patients with PPC achieved treatment success by EUS-GTD alone[3-6]. Conversely, the treatment success rate of WON by EUS-GTD alone was relatively lower than that of PPC[4,7]. Endoscopic necrosectomy (EN) was performed in patients with no clinical improvement by EUS-GTD. In a recent review of 10 series of EN, the overall treatment success rate was 76%, with 5% procedure-related mortality and 27% morbidity[8]. Although EN is less invasive than surgical necrosectomy, serious complications associated with EN have been reported lately. More recently, novel, fully covered biflanged metal stents have been reported to be effective and feasible for the treatment of WON[9-12]. However, the criteria for WON that should be treated by EN or/and with these metal stents remain unclear. Therefore, it is essential to clarify the cases that can be resolved by EUS-GTD alone and those that should be treated with EN and/or metallic stents in addition to EUS-GTD. To the best of our knowledge, to date, no report has investigated the predictive factors of treatment outcome managed by EUS-GTD alone using the definition of WON in the 2012 Atlanta classification.

Despite initial treatment success, some PFCs develop recurrent fluid collection owing to disconnected pancreatic duct syndrome (DPDS)[13,14]. Although long-term PFC recurrence is unknown, recommendations for permanent stent placement have been reported; however, stent migration or obstruction should be considered[13].

In this study, we classified PFC into the following four groups according to the 2012 Atlanta classification: WON; PPC; chronic pseudocyst; and others, including trauma, pancreatic cancer, and pancreatic fistula (after pancreatic surgery). We evaluated patients characteristics, technical success, treatment success, and complications in these four groups. In particular, we compared the clinical features between the treatment success and failure to identify the factors that affect the treatment outcome in patients with WON managed by EUS-GTD alone. The long-term follow-up results of patients who underwent EUS-GTD were assessed and predictive factors for recurrence of PFC were identified.

MATERIALS AND METHODS

Patients

We retrospectively investigated 103 consecutive patients with PFC who underwent EUS-GTD between September 1999 and August 2015 at Chiba University Hospital (Chiba, Japan). Mean age of patients was 54.7 years, and a majority of patients were males. PFC caused by acute pancreatitis was classified according to the 2012 Atlanta classification and definition. We distinguished between WON and PPC by contrast-enhanced computed tomography (CT) 1-4 wk after the onset of pancreatitis and evaluated the existence and extent of pancreatic parenchymal necrosis in all patients with PFC. A chronic pseudocyst is defined as a well-demarcated fluid collection without solid debris occurring in the setting of known chronic pancreatitis and the absence of recent severe acute pancreatitis[14]. Indications for EUS-GTD were as follows: (1) infected cases (fever and leukocytosis) despite the administration of intravenous antibiotics; and (2) symptomatic cases, such as abdominal pain or obstruction of the gastric outlet, intestinal system, or biliary system. Informed procedural consents were obtained from all patients.

Procedures

Before EUS-GTD, a CT scan was obtained from all patients. The standard technique for EUS-GTD involved the following steps. A curved linear array EUS was used to visualize the extent of PFC and to determine the puncture site. Before puncturing, color Doppler was used to identify the regional vessels that needed to be avoided. The cavity was punctured with a 19-gage needle, and then PFC was performed for conducting blood biochemical tests and cultures. A guidewire was inserted through the puncture needle and coiled in PFC under fluoroscopic guidance. The punctured site was dilated by a dilator and a balloon dilator (sometimes an electric dilator was also used). Finally, a 7Fr double pigtail stent and a 7Fr nasocystic drainage catheter were emplaced. The nasocystic drainage catheter was removed after reduction of PFC, and the internal stent was removed within 6 mo after the treatment success.

We assessed the efficacy of EUS-GTD using CT. If the size of the cavity was not reduced after 1-2 wk, we performed additional procedures, such as the multiple transmural gateway technique, percutaneous drainage, or EN.

Outcomes

In this study, we defined technical success as achieving stent and/or nasocystic drainage catheter placement. We defined the treatment success as any reduction in the cavity size to less than 20 mm within 8 wk, as determined by a follow-up CT using EUS-GTD alone, without an additional treatment such as multiple transmural gateway technique, percutaneous drainage, or EN. The treatment success also included the improvement of symptoms. Recurrence was considered to have occurred if the size of the cavity increased to more than 20 mm, regardless of symptoms over 6 mo after EUS-GTD.

Statistical analysis

The factors associated with the clinical success and recurrence were determined using statistical comparisons. Continuous variables were presented as means (with standard deviations) and medians (with range) and compared using Mann-Whitney *U* test. Categorical variables were expressed as frequencies and proportions and compared using χ^2 tests with Yates' correction or Fisher's exact test. In patients with WON, multiple logistic regression analyses were performed to identify the predictor variables associated with the treatment success. The optimal cut-off value of the variables that differentiated between recurrence and no recurrence was determined by the receiver-operating characteristic analysis. In addition, the area under the curve was calculated. The statistical significance was determined as $P < 0.05$, and datasets were compiled using Microsoft Excel (Microsoft Corporation, Redmond, WA, United States). In addition, the IBM SPSS Statistics software version 20.0 (IBM Corporation, Chicago, IL, United States) was used to perform all the statistical analyses. The statistical methods of this study were reviewed by Kengo Nagashima, PhD from Department of Global Clinical Research, Graduate School of Medicine, Chiba University.

RESULTS

Patients

According to the 2012 Atlanta classification and definition for acute pancreatitis, each PFC was classified as WON (n = 40), PPC (n = 11), chronic pseudocyst (n = 33), and others (n = 19) for 103 patients who underwent EUS-GTD. More than 50% of PFCs were located mainly in the pancreatic body or tail. Mean

Table 1 Demographic and clinical characteristics of patients who underwent endoscopic ultrasound-guided transmural drainage *n* (%)

characteristics	Value
Age, yr	
mean (SD)	54.7 (15.5)
Range	15-89
Median	56
Gender	
Male	73 (70.9)
Female	30 (29.1)
Type of pancreatitis	
Acute pancreatitis	46(44.6)
Chronic pancreatitis	38 (36.9)
Other	19 (18.4)
Etiology of PFC	
Alcohol	51 (49.5)
Idiopathic	17 (16.5)
Gallstones	15 (14.5)
Trauma	6 (5.9)
Post-surgery (pancreatic fistula)	7 (6.8)
Post-ERCP	3 (2.9)
Pancreatic cancer	4 (3.9)
Category of PFC	
WON	40 (38.8)
Pancreatic pseudocyst	11 (10.7)
Chronic pseudocyst	33 (32.0)
Others (cancer/trauma/fistula)	19 (18.5)
Main location of cavity	
Head	19 (18.4)
Body or tail	84 (81.6)
Size of cavity, mm (long axis)	
mean (SD)	104.4 (49.0)
Range	30-246
Median	100

EUS-GTD: Endoscopic ultrasound-guided transmural drainage; PFC: Pancreatic fluid collection; SD: Standard deviation; ERCP: Endoscopic retrograde cholangio-pancreatogram; WON: Walled-off pancreatic necrosis.

cavity size was 104.9 mm (Table 1).

Technical success

Of 103 patients, 4 technically failed, resulting in the technical success rate of 96.1% (95%CI: 90.3%-98.9%). In the technically failed cases, each patient required one of the following additional treatments: surgery, transpapillary drainage, percutaneous drainage, extracorporeal shock wave lithotripsy (ESWL), and observation.

Treatment success

In the WON group (n = 40), a technical failure was reported in 1 patient and treatment success in 23 patients (57.5%; 95%CI: 40.9%-73.0%). The treatment success group comprised 2 patients who underwent percutaneous drainage for PFC distant from the main lesion treated by EUS-GTD. We could not achieve the treatment success by EUS-GTD alone in 16 patients, of whom 2 needed surgical treatment, 11 needed no surgical treatment, and 1 died. In the group treated without surgery, we performed EN in 5 patients, multiple transluminal gateway technique in 2 patients, percutaneous drainage in 5 patients, and continuing conservative treatment until reduction over 8 wk in 3 patients. After additional treatment, 2 patients died. All 3 patients who died after EUS-GTD or EN had a respiratory or renal failure.

In the PPC group (n = 11), there were no technical failures, and we achieved the treatment success in 10 patients (90.9%; 95%CI: 58.4%-99.8%). In the chronic pseudocyst group (n = 33), there were two technical failures, and of 31 patients, we achieved the treatment success in 30 patients (96.8%; 95%CI: 83.8%-99.9%). In the treatment success group of chronic pancreatitis, 3 patients underwent EUS-GTD for PFC distant from the main lesion treated by EUS-GTD. In the others group (n = 19), there was one technical failure, and we achieved the treatment success in 17 patients (94.4%; 95%CI: 73.2%-99.9%).

Complications

The procedural complications were encountered in 15 of 103 patients (14.6%), with cases of bleeding (n = 2), stent migration (n = 3), infection (n = 3), pneumoperitoneum (n = 2), localized peritonitis (n = 3), puncture into another organ (n = 1), and mediastinal emphysema (n = 1). All patients were managed conservatively without surgery.

Predictors of treatment success for WON

We compared the treatment success group with the treatment failure group. Patients with more than 50% pancreatic parenchymal necrosis (P = 0.004) and a PFC of more than 150 mm (P < 0.001) on CT were significantly associated with treatment failure based on the univariate analysis. However, PFC with infection was not significant (Table 2). Patients with more than 50% pancreatic parenchymal necrosis (OR = 17.0; 95%CI: 1.9-150.7; P = 0.011) and with a PFC of more than 150 mm (OR = 27.9; 95%CI: 3.4-227.7; P = 0.002) on CT were also significantly associated with treatment failure based on the multiple logistic regression analysis (Table 3).

The treatment success by EUS-GTD alone was not achieved in any patient with WON with more than 50% parenchymal necrosis and a with PFC of more than 150 mm diameter on CT but was achieved in 90% of patients with under 50% pancreatic parenchymal necrosis and with a PFC of less than 150 mm (Table 4). There were two cases of failure with fewer than 50% necrosis and with a PFC of less than 150 mm. In one case with a multilocular type of WON, the necrotic collection remained in the posterior pararenal extraperitoneal space, and we performed percutaneous drainage. In another case, extrapancreatic necrosis without pancreatic parenchymal necrosis extended widely, and EN was conducted.

Table 2 Comparison of patient demographics and clinical characteristics in patients underwent endoscopic ultrasound-guided transmural drainage of walled-off necrosis *n* (%)

		Treatment success		*P* value
		Yes (*n* = 23)	No (*n* = 16)	
Age, yr	Mean (SD)	57.8 (18.1)	55.8 (13.1)	
	Range	15-85	30-83	0.484
Gender	Male	16 (69.6)	14 (87.5)	0.359
Etiology of pancreatitis	Alcohol	8 (34.8)	6 (37.5)	0.862
Body mass index	Mean (SD)	23.3 (5.5)	24.2 (3.6)	0.203
ASA classification ⩾ 3	Yes	12 (52.2)	13 (81.2)	0.09
Pancreatic parenchymal necrosis ⩾ 50%	Yes	2 (8.7)	9 (56.3)	0.004
Duration from onset of pancreatitis to drainage, wk	Mean (SD)	11.1 (7.8)	9.4 (10.9)	
	Range	3.1-25.3	2.0-47.7	0.219
Size of cavity, mm (long axis)	Mean (SD)	109.9 (35.7)	156.9 (35.7)	
	Range	70-246	66-207	< 0.001
Size of cavity ⩾ 150 mm	Yes	2 (8.7)	11 (68.8)	< 0.001
PFC with infection	Yes	13 (56.5)	13 (81.3)	0.203
Follow-up durations, mo	Mean (SD)	26.9 (30.8)	28.1 (33.9)	0.808
	Range	0.7-133.5	1.3-128.3	

EUS-GTD: Endoscopic ultrasound-guided transmural drainage; WON: Walled-off pancreatic necrosis; PFC: Pancreatic fluid collection; SD: Standard deviation.

Table 3 Multiple logistic regression analysis examining factors associated with treatment success for walled-off necrosis

Multiple logistic regression	OR	95%CI	*P* value
Pancreatic parenchymal necrosis (< 50% *vs* ⩾ 50%)	17.0	1.9-150.7	0.011
Size of cavity (< 150 mm *vs* ⩾ 150 mm)	27.9	3.4-227.7	0.002

Table 4 Treatment success rate of walled-off necrosis according to two parameters; pancreatic parenchymal necrosis and size of cavity

	Size of cavity < 150 mm	Size of cavity ⩾ 150 mm
Pancreatic parenchymal necrosis < 50%	90.5% (19/21)	28.6% (2/7)
Pancreatic parenchymal necrosis ⩾ 50%	40.0% (2/5)	0% (0/6)

Recurrence at long-term follow-up

Overall, 75 patients were followed up for more than 6 mo, and the internal stent removal was confirmed. The median observation time was 38.8 mo. Of 75 patients, 10 patients suffered a recurrence, and the overall recurrence rate was 13.3%. The additional treatment for recurrence cases was additional EUS-GTD for 5 patients, transpapillary drainage for one patient, ESWL for one patient, surgery for 2 patients, and observation for one patient. In the recurrence group, the amylase level in the cavity was significantly higher than that in the no recurrence group (P = 0.01; Table 5) based on the univariate analysis. The amylase level at 63,100 in the cavity represented the most sensitive (83.3%) and specific (78.2%) point on the receiver-operating characteristic curve and corresponded to the largest area under the curve (0.820). No recurrence rate of the amylase level in the cavity ⩾ 63100 was significantly lower than that of the amylase level in the cavity < 63100 (P = 0.02; Figure 1).

DISCUSSION

In this study, we revealed the usefulness and feasibility of EUS-GTD for PFC; however, it was less effective in achieving the treatment success for WON as compared with other etiologies of PFC. The PFC size and the proportion of pancreatic parenchymal necrosis were related to the short-term outcomes of WON. In addition, mean amylase level in the cavity was associated with PFC recurrence, suggesting a prolonged stent placement for patients with predicted recurrence.

The treatment success rate of WON by EUS-GTD alone was 61.1%. Some retrospective studies have demonstrated a 45%-63% treatment success rate of EUS-GTD for WON[4,7]. A comparison of these results with our study is difficult because the definition of treatment success was different in each study, which in turn differed from our study, which was based on the 2012 Atlanta classification. A recent report, based on the 2012 Atlanta classification, revealed that the standard EUS-GTD using plastic stents or self-expandable metal stents resolved 70% of sterile WON and 40% of infected WON; however, the rest of WON required EN[15].

After treatment failure by EUS-GTD for WON, EN or other intervention, including surgical treatment, should be considered. A recent randomized controlled trial suggested that the IL-6 level following EN was significantly lower than that following surgical necrosectomy and that major complications or death occurred less frequently after EN compared to those after surgical necrosectomy[16]. Although EN should be

Table 5 Pancreatic fluid collection recurrence in patients followed-up over 6 mo and confirmed internal stent removal after successful endoscopic ultrasound-guided transmural drainage (*n* = 75) *n* (%)

		Recurrence		*P* value
		Yes (*n* = 10)	No (*n* = 65)	
Age, yr	Mean (SD)	54.4 (12.6)	55.1 (15.9)	
	Range	37-79	15-89	0.719
Gender	Male	9 (90)	45 (69.2)	0.266
Type of pancreatitis	Chronic	6 (60)	22 (33.8)	0.162
Main location of cavity	Head	1 (10)	16 (24.6)	0.677
External drainage only	Yes	2 (20)	9 (13.8)	0.634
Duration of internal stent, days (*n* = 64)	Mean (SD)	194.9 (106.1)	243.2 (217.3)	
	Range	86-399	21-1387	0.659
Spontaneous dislodgement of stent (*n* = 64)	Yes	4 (50)	17 (30.4)	0.421
Size of cavity, mm (long axis)	Mean (SD)	91.6 (38.0)	102.6 (49.3)	
	Range	44-167	30-230	0.612
Amylase in cavity, IU/L (*n* = 57)	Mean (SD)	96930 (55599)	44719 (53790)	
	Range	31380-188000	30-273700	0.011
	Median	83075	31200	
PFC with infection	Yes	2 (20)	28 (43.1)	0.298

PFC: Pancreatic fluid collection; SD: Standard deviation; EUS-GTD: Endoscopic ultrasound-guided transmural drainage.

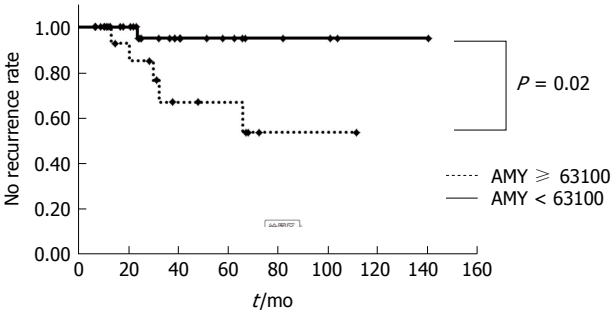

Figure 1 Kaplan-Meier curve comparing no recurrence rate of amylase in cavity ⩾ 63100 with that of amylase in cavity < 63100.

considered for WON that cannot be resolved by EUS-GTD, serious complications of EN have been reported. Recently, while three multiple-center trials revealed 75%-91% success rate of EN, the associated mortality and morbidity rated were 5.8%-11% and 26%-33%, respectively[17-19]. In our study, we performed EN on 4 patients, and one patient died due to multiple organ failure. Therefore, if possible, it is better to accomplish the resolution of WON by EUS-GTD alone.

Some factors associated with the failed resolution of WON by EN or standard drainage and EN have been reported, including body mass index > 32[18], American Society of Anesthesiologists physical status classification ⩾ 3[19], and multilocular morphology[15]. In this study, we elucidated that the treatment success rate of EUS-GTD alone for WON was significantly lower in patients with more than 50% pancreatic parenchymal necrosis or with a PFC of more than 150 mm on CT. All patients with WON with more than 50% parenchymal necrosis and with a PFC of more than 150 mm needed an additional treatment such as EN, whereas the treatment success was achieved in 90% patients with under 50% pancreatic parenchymal necrosis and within a PFC of 150 mm by EUS-GTD alone (Figure 2). If we can predict the treatment outcome by EUS-GTD with these parameters, we may be able to avoid unnecessary invasive therapy or make an earlier decision to perform additional treatment in a few days.

Instead of EUS-GTD, multiple transmural gateway technique for WON has been reported, and the treatment success rate of multiple transmural gateway technique was significantly better than that of EUS-GTD (94.4% *vs* 62.1%)[20,21]. More recently, novel, fully covered biflanged metal stents have been reported to be effective for the treatment of WON[9-12]. Regarding the use of these stents, high cost, stent migration, and other potential adverse events have been concerning[22]; therefore, optimal selection for using these stents is needed. According to our study, patients with PFC more than 50% pancreatic parenchymal necrosis or patients with more than 150 mm may be appropriate candidates for these treatments.

The proportion of pancreatic parenchymal necrosis could be associated with the amount of solid debris in the cavity of WON. Reportedly, the morphological findings of WON on EUS have therapeutic implications owing to the large size and more solid debris needing a more aggressive therapeutic method[23]. However, it is often challenging to estimate the necrotic component with the whole observation of PFC on EUS. In addition, even if rich debris seemed to exist in the cavity by EUS, some cases of WON could be resolved by EUS-GTD alone, as shown in Figure 2.

In this study, PPC was relatively rare (10.7%) based on the values in the 2012 Atlanta classification. Notably, 8 of 11 patients categorized as PPC were caused by acute pancreatitis occurring in the setting of known chronic pancreatitis and 3 were atypical etiology of pancreatitis (drug-induced in 2 patients and idiopathic in 1 patient). The treatment success rate of 90.9% for PPC in this study was higher than that of WON.

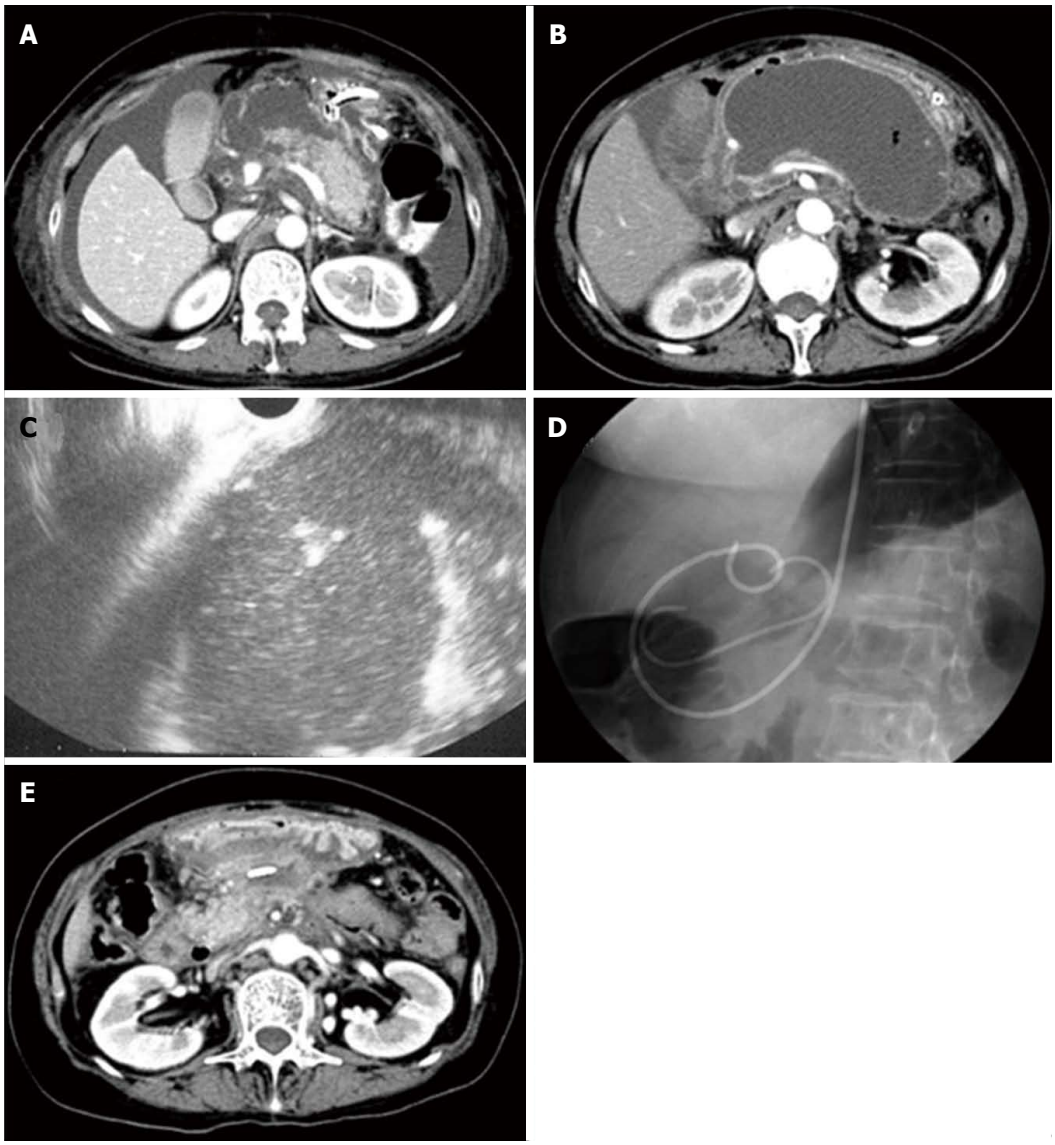

Figure 2 Representative case of walled-off necrosis resolved by endoscopic ultrasound-guided transmural drainage alone. A: Computed tomography of the abdomen (axial image) showing less than 50% pancreatic parenchymal necrosis; B: Pancreatic fluid collection was 135 mm in diameter; C: Rich debris seemed to exist in the cavity as shown by endoscopic ultrasound; D: Endoscopic ultrasound guided transmural drainage was performed and pancreatic fluid collection culture was positive; E: Cavity was reduced at 6 wk after intervention.

EUS-GTD for chronic pseudocysts was successful. In chronic pancreatitis, PFC often communicates with the main pancreatic duct and stricture of this duct exists. To manage chronic pseudocysts, a combination of EUS-GTD with transpapillary drainage or ESWL for pancreatic stones might be effective.

After successful initial treatment following EUS-GTD for PFC, recurrence of PFC in the long term was higher in patients with higher amylase level in the cavity, indicating the communication of PFC with the pancreatic duct such as chronic pseudocysts or DPDS. DPDS is characterized by the main pancreatic duct cut-off, with an inability to access the upstream pancreatic duct during an ERCP, and CT evidence of viable pancreatic tissue upstream (toward the spleen), in association with a persistent non-healing pancreatic fistula or PFC[24,25]. We routinely removed internal stents following the resolution of PFC almost 6 mo after EUS-GTD. Although a permanent stent placement significantly reduced PFC recurrence in comparison with scheduled stent removal, migrated stents caused bowel obstruction that required surgery[21]. In cases with higher amylase levels in the cavity, transpapillary treatment or prolonged stent placement for more than 6 mo should be considered. However, the timing of stent removal and permanent stent placement remains controversial, and further study is required.

There were some limitations in this study. First, this was a retrospective study conducted at a single tertiary center and the number of patients with WON was relatively less. Second, the study period was long (16 years) and a learning curve might have influenced the results, although the standard technique for EUS-GTD has not changed. Third, there may have

been a selection bias because we could not examine all patients with the amylase level in the cavity consecutively.

In conclusion, EUS-GTD is a successful and safe therapeutic technique in a majority of patients with PFC. The cavity size and proportion of pancreatic parenchymal necrosis are predictors for a successful treatment of WON. Higher amylase levels in the cavity might lead to PFC recurrence after stent removal. A prolonged stent placement should be considered in such cases.

ARTICLE HIGHLIGHTS

Research background

The 2012 Atlanta classification categorized PFC into four types. The revised classification suggests that many PFCs after acute pancreatitis that were treated as pancreatic pseudocyst (PPC) because of slight debris in the cavity should be addressed as walled-off necrosis (WON). Most patients with PPC achieved treatment success by EUS-GTD alone. Although endoscopic necrosectomy (EN) was performed in patients with no clinical improvement by EUS-GTD, serious complications of EN have been reported. However, the criteria for WON that should be treated by EN or other additional treatment remained unclear. Therefore, it is crucial to clarify the cases that can be resolved by EUS-GTD alone and those that should be treated with EN or other treatment in addition to EUS-GTD. Some PFCs develop the recurrent fluid collection in long term. Recommendations of permanent stent placement have been reported to reduce recurrence; however, stent migration or obstruction is concerned. A predictive factor of PFC recurrence should also be clarified.

Research motivation

The treatment success rate of WON by EUS-GTD alone was relatively lower than that of PPC. It is unclear that which patients with WON can be treated by EUS-GTD alone and which ones by EN or other additional treatment including metallic stents. Despite initial treatment success, some PFCs develop the recurrent fluid collection in the long term. It is also unclear which types of PFCs show recurrence.

Research objectives

We aimed to evaluate the short- and long-term results of EUS-GTD for PFC following a revision of the PFC framework by the 2012 Atlanta classification and identify the predictive factors of treatment outcome for WON managed by EUS-GTD alone and predictive factors for recurrence of PFC.

Research methods

The authors retrospectively investigated 103 consecutive patients with PFC who underwent EUS-GTD between September 1999 and August 2015 at Chiba University Hospital. The factors associated with clinical success and recurrence were determined using statistical comparisons. In patients with WON, multiple logistic regression analyses were performed to identify the predictor variables associated with the treatment success. In addition, PFC recurrence was examined in patients followed up over 6 mo and confirmed internal stent removal after successful EUS-GTD. The optimal cut-off value of the variables that differentiated between recurrence and no recurrence was determined by the receiver-operating characteristic analysis. In addition, area under the curve was calculated. The statistical significance was determined as $P < 0.05$.

Research results

The treatment success rate of WON, PPC, chronic pseudocyst, and others was 57.5%, 90.9%, 91.0%, and 89.5%, respectively. The treatment success rate of WON was significantly lower in patients with more than 50% pancreatic parenchymal necrosis (OR = 17.0: 95%CI: 1.9-150.7; $P = 0.011$) and in patients with more than 150 mm of PFC (OR = 27.9; 95%CI: 3.4-227.7; $P = 0.002$) on contrast-enhanced computed tomography using the multivariate logistic regression analysis. The recurrence of PFC in the long term was 13.3% (median observation time, 38.8 mo). Mean amylase level in the cavity was significantly higher in the recurrence group than in the no recurrence group ($P = 0.02$). In cases with higher amylase levels in the cavity, transpapillary treatment or prolonged stent placement for more than 6 mo should be considered. However, the timing of stent removal and permanent stent placement remains controversial, and further study is required.

Research conclusions

Reduction of WON by EUS-GTD alone was associated with the proportion of necrotic tissue and the extent of the cavity. Amylase level in the cavity may be a predictive factor for recurrence of PFC.

According to our study, additional treatments, such as EN, should be considered after EUS-GTD in patients with WON with more than 50% parenchymal necrosis and a PFC of more than 150 mm, whereas it may not be needed with under 50% pancreatic parenchymal necrosis and within a PFC of 150 mm.

After successful initial treatment following EUS-GTD for PFC, recurrence of PFC in the long term was higher in patients with higher amylase level in the cavity. This finding indicates the communication of PFC with the pancreatic duct such as chronic pseudocyst or disconnected pancreatic duct syndrome. Therefore, in cases with higher amylase levels in the cavity, transpapillary treatment or prolonged stent placement should be considered.

Research perspectives

In this study, we confirmed that the PFC size and the proportion of pancreatic parenchymal necrosis were related to the resolution of WON treated by EUS-GTD alone. If we can predict treatment outcome of WON by EUS-GTD alone, we might be able to avoid unnecessary invasive therapy or make an earlier decision to perform an additional treatment. Moreover, if we can predict recurrence of PFCs by the amylase level in the cavity, we might be able to reduce the recurrence of PFCs with prolonged stent placement or transpapillary treatment. Prospective studies with larger numbers of patients will be needed to confirm the reliability of these predictive factors.

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ORIGINAL ARTICLE

Retrospective Study

Laparoscopic finding of a hepatic subcapsular spider-like telangiectasis sign in biliary atresia

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Informed consent statement: Informed consent was obtained from all the patients.

Conflict-of-interest statement: The authors declare that they have no competing interests.

Data sharing statement: The technical appendix, statistical code, and dataset are available from the corresponding author at tshaotao83@hust.edu.cn. All the participants gave informed consent for data sharing when they enrolled in this study. No additional data are available.

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Abstract

AIM

To assess the diagnostic value of a laparoscopic finding of a hepatic subcapsular spider-like telangiectasis (HSST) sign in biliary atresia.

METHODS

A retrospective study was conducted first and then a validation set was used to investigate the value of an HSST sign in predicting biliary atresia (BA). In the

retrospective study, laparoscopic images of the liver surface were reviewed in 126 patients with infantile cholestasis (72 BA patients and 54 non-BA cholestasis patients) and a control group of 38 patients with non-hepatic conditions. Analysis was first made by two observers separately and finally, a consensus conclusion was achieved. Then, the diagnostic value of the HSST sign was validated in an independent cohort including 45 BA and 45 non-BA patients.

RESULTS

In the retrospective investigation, an amplified HSST sign was found in all BA patients, while we were unable to detect the HSST sign in 98.1% of the 54 non-BA patients. There was no HSST sign in any of the control subjects. In the first review, the sensitivity and specificity from one reviewer were 100% and 98.1%, respectively, and the results from the other reviewer were both 100%. The consensus sensitivity and specificity were 100% and 98.1%, respectively. The HSST sign was defined as being composed of several enlarged tortuous spider-like vascular plexuses with two to eight branches distributed on all over the liver surface, which presented as either a concentrated type or a dispersed type. In the independent validation group, the sensitivity, specificity, positive predictive value and negative predictive value of the HSST sign were 100%, 97.8%, 97.8% and 100%, respectively.

CONCLUSION

The HSST sign is characteristic in BA, and laparoscopic exploration for the HSST sign is valuable in the diagnosis of BA.

Key words: Laparoscopic hepatic subcapsular spider-like telangiectasis sign; Infantile cholestasis; Biliary atresia; Infantile hepatitis; Laparoscopy; Diagnosis; Pediatric surgery

Core tip: Color Doppler ultrasound finding of hepatic subcapsular flow has shown much potential for discriminating biliary atresia (BA). While in clinical practice, we have noticed a similar but more intuitive phenomenon - a laparoscopic finding of hepatic subcapsular spider-like telangiectasis (HSST) sign, which may be a specific marker for BA. Based on the current data, we found that the sensitivity and specificity of the HSST sign were each generally close to 100%.

Zhou Y, Jiang M, Tang ST, Yang L, Zhang X, Yang DH, Xiong M, Li S, Cao GQ, Wang Y. Laparoscopic finding of a hepatic subcapsular spider-like telangiectasis sign in biliary atresia. *World J Gastroenterol* 2017; 23(39): 7119-7128 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7119.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7119

INTRODUCTION

Biliary atresia (BA), as a common neonatal cholangiopathy that leads to cirrhosis[1], is the most common indication for liver transplantation in infants[2,3]. The incidence of BA ranges from 1 in 5000 (Asian countries) to 1 in 19000 (European countries) live births, and it presents with typical symptoms and signs such as persistent jaundice, acholic stools and hepatomegaly within the first months of life[2]. This disease is characterized by a progressive fibro-obliterative obstruction of extrahepatic bile ducts. A younger age (in the first 2 mo of life) at the time of Kasai portoenterostomy has been found to be life-saving in restoring bile flow and preventing the worsening of liver function[4,5]. Thus, early discrimination is vital for the prognosis. Nevertheless, it is still challenging to differentiate BA from other cholestatic diseases as there are no characteristic manifestations found in the early stage of BA.

Clinic features, such as prolonged jaundice, pale stools and high conjugated hyperbilirubinemia, are remarkable, but they are not specific[6]. The ultrasonographic findings of an abnormal gall bladder and triangular cord sign have been widely used in early discrimination of BA[6,7], but the accuracy of these measurements varies[8-15]. A recent study showed that the absence of a gallbladder and the triangular cord sign could both have the highest specificity, up to 99%, but a sensitivity of 28% and 80%, respectively[14]. Other examinations are also compelling, but the value is limited. A duodenal fluid test is difficult to perform. Definite biliary excretion shown by scintigraphy can exclude BA, however, the absence of excretion is not specific in BA because of it can be attributed to any form of severe cholestasis[2,16]. Due to the small diameter of and inadequate fluid in biliary ducts, magnetic resonance cholangiopancreatography (MRCP) plays a limited role in showing the biliary tree in normal infants younger than 3 mo[17]. Therefore, the high false positive rate in predicting BA is inevitable. Moreover, the pathological features of BA are bile ductular proliferation, canalicular and cellular bile stasis, bile plugs, and portal or perilobular fibrosis with preservation of the basic hepatic lobular architecture[5,18,19], which overlap with α-1-antitrypsin deficiency, and occasionally Alagille syndrome, cystic fibrosis, and total parenteral nutrition (TPN)-related cholestasis[5].

Cholangiography is the gold standard, and a definitive diagnosis of BA can be made when cholangiography fails to show the biliary tree[1]. Laparotomy cholangiography was previously used and was refused by some parents because of the abdominal trauma and incision complications, which finally led to the delay of diagnosis. Recently, with the application

of laparoscopy in pediatric surgery, laparoscopy-assisted cholangiography (LAC) has become possible. In our center, we performed laparoscopy-assisted cholangiography on infants who were highly suspected of having BA before the Kasai portoenterostomy was developed[20]. During the procedure, we found that a prominent pathological change on the liver surface - a hepatic subcapsular spider-like telangiectasis (HSST) sign - had a strong correlation with BA.

The current report includes a retrospective study and a prospective study, which aimed to investigate the significance of the laparoscopic finding of an HSST sign in differentiating BA from other causes of neonatal cholestasis.

The study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, and all parents of the patients signed the inform consent. Before the parents signed the consent form for surgery, they were informed that the procedure was minimally invasive and that the procedure would be carried out *via* a small incision at the umbilicus ring and one stab incision in the left abdominal wall. Parents were told that there was a chance of needing additional trocars or conversion to an open technique. Advantages and disadvantages were reviewed to ensure that the parents were fully educated about the procedure.

MATERIALS AND METHODS

Retrospective and clinical investigation

An HSST sign in laparoscopic images was evaluated in cholestasis cases and a control group.

Patients: Laparoscopic images of the liver surface were reviewed in 126 patients with infantile cholestasis who underwent laparoscopic cholecystocholangiography or laparoscopic exploration at our center between February 2011 and February 2015. Among the 126 patients, 72 (31 boys, 41 girls; mean age, 58 d ± 29 d) were diagnosed with BA on the basis of laparoscopic cholangiography or exploration (when cholangiography failed due to the presence of atrophic gallbladder), and the other 54 (24 boys, 30 girls; mean age, 59 d ± 21 d) were diagnosed with non-BA conditions, which included infantile hepatitis (n = 46), biliary hypoplasia (n = 5) and total parenteral nutrition (TPN) induced cholestasis (n = 3). Their final diagnoses were confirmed by LAC, pathological examination or clinical follow-up. Another 38 patients (19 boys, 19 girls; mean age, 63 d ± 22 d) without jaundice or liver disease who underwent laparoscopic surgery served as controls. Their clinical diagnoses were Hirschsprung's disease (n = 34) and intussusception (n = 4).

Laparoscopic findings: Laparoscopic images (5 mm or 10 mm optical laparoscopy, pediatric HOPKINS Ⅱ 26003BA, STORZ, Germany) of the liver surface were evaluated by two observers separately and blindly (Zhou Y and Jiang M) to screen for the presence or absence of the HSST sign. The HSST sign was preliminarily defined as enlarged tortuous spider-like vascular plexuses distributed on the liver surface.

Statistical analysis: The results are expressed as the mean ± SD or number (percentage). For quantitative data, statistical significance between groups was tested using a two-sample t test. For qualitative data (sex), significance between groups was tested by a χ^2 test. Sensitivity and specificity were calculated for the HSST sign with the method of Newcombe[21]. Individual readings were calculated first; consensus readings were calculated thereafter (by professor Tang ST). Meanwhile, we applied Cohen's κ coefficient to assess the agreement between the analyses conducted by the two observers. Logistic regression analysis was applied to determine whether the presence of the HSST sign was useful in discriminating BA. A P-value of < 0.05 was considered statistically significant. SPSS 13.0 software (SPSS, IL, United States) was used for data analyses.

Validation of the HSST sign

The diagnostic value of the HSST sign was validated in an independent cohort composed of 45 BA and 45 non-BA patients.

Patients: Between February 2015 and April 2017, we enrolled a consecutive series of patients who were highly suspected of having BA. The enrollment criteria in our series included the following: (1) icterus was gradually aggravated after the first 3-4 wk; (2) consistent depigmented stool; and (3) traditional diagnostic examinations (*i.e.*, physical examination, blood biochemical examination, ultrasonography, hepatobiliary scintigraphy, MRCP, *etc*.) failed to achieve a definitive diagnosis[20]. The children's general information, including gender, age and biochemical parameters [the level of total and direct serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gammaglutamyl transpeptidase (GGT)] were carefully documented.

Validation protocol: All enrolled patients underwent laparoscopic exploration for the HSST sign before LAC. The patient was considered to be BA when the HSST sign was detected. The final diagnosis was confirmed by LAC.

Pathologic evaluation: After surgery, patients underwent pathologic examination. The hepatic subcapsular spider-like vessels were confirmed by gross inspection, well preserved on the prepared tissue, and evaluated with a microscope.

Statistical analysis: The results are expressed as the mean ± SD or number (percentage). A two-sample *t* test was used to compare quantitative parameters between patients with BA and non-BA patients. A χ^2 test was used to compare the male-to-female ratio between patients with BA and non-BA patients. The cut-off values for optimal clinical performance (best sensitivity and best specificity simultaneously) of individual parameters were determined from the receiver-operating characteristic (ROC) curve. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the HSST sign using the method of Newcombe[21]. A *P*-value of < 0.05 was considered statistically significant. SPSS 13.0 software (SPSS, IL, United States) was used for data analysis.

RESULTS

Retrospective and clinical investigation

The two groups were age- and sex- matched (P > 0.05 for both). The mean levels of total bilirubin, direct bilirubin, AST and ALT in the BA group showed no significant difference compared to the non-BA group. The mean level of GGT was significantly higher in the BA group than in the non-BA group (P = 0.02). Total bilirubin, direct bilirubin, ALT, AST and GGT levels were normal in the controls (Table 1).

An amplified HSST sign was identified in all patients with BA (Figure 1A) and was not found in any of the control subjects by both observers (Figure 1 B). One observer (Jiang M) determined that there was no HSST sign in 53 (98%) non-BA patients (Figure 1C-E), except for one patient with cytomegalovirus (CMV) hepatitis (Figure 1F). The other observer (Zhou Y) had the opinion that there was no HSST sign in all patients with non-BA. After discussion, the discrepancy was resolved. The sensitivity and specificity of the HSST sign were 100% and 98.1%, respectively (Table 2).

The HSST sign was widely distributed on the surface of both left and right hepatic lobes, including on the visceral and diaphragmatic facies (Figure 2). Thus, we redefined the HSST sign as follows: it is composed of several enlarged tortuous spider-like vascular plexuses with four to eight branches distributed all over the liver surface, which presents as a dispersed type (radiating branches originate from more than one central point, but close to each other to form a spider-like sign) (Figure 3A) or a concentrated type (radiating branches originate from one central point) (Figure 3B).

Logistic regression analysis showed that the presence of the HSST sign was a significant predictor of BA (P < 0.01). In addition, the concordance between the two observers was also robust (κ = 0.94, 95%CI: 0.87–1.0).

Validation of the HSST sign

Characteristics of patients: The mean age of the BA group was 60 d ± 29 d. The mean age of the non-BA group was 61 d ± 23 d. In the non-BA group, the final diagnoses were infantile hepatitis (n = 43) and biliary hypoplasia (n = 2). As shown in Table 3, there was no significant difference between the BA and non-BA groups in age or male-to-female ratio (P > 0.05 for both). The mean levels of total bilirubin, direct bilirubin, AST and ALT in BA patients had no significant difference compared to those in the non-BA group. The mean level of GGT was significantly higher in the BA group than in the non-BA group (P < 0.001).

Diagnostic performance of the HSST sign: On high-definition laparoscopic images (5 mm or 10 mm optical laparoscopic, pediatric HOPKINS Ⅱ 26003BA, STORZ, Germany), the HSST sign in all BA patients was easily observed (Figure 4A). There were no HSST sign observed in non-BA patients, except for one patient (Figure 4B). The patient was considered to have BA due to the presence of the HSST sign, whose final diagnosis was biliary hypoplasia. The sensitivity, specificity, PPV and NPV of the HSST sign were 100%, 97.8%, 97.8% and 100%, respectively.

Pathologic evaluation: The hepatic subcapsular spider-like vessels were clearly identified in the stained tissue. The vessels in the BA group were revealed as dilated branches of hepatic arteries in the hepatic subcapsular area (Figure 4C) and the vessels in hypoplasia were revealed as hyperplastic capillaries in the hepatic subcapsular area (Figure 4D).

DISCUSSION

A large sample study focused on GGT showed that it was higher in the BA group than in other cholestatic disorders for all age groups[22], which was coincident with our results. In our validation set, we found that the area under the receiver operating characteristic curve was 0.745 and GGT at a cut-off value of > 404 U/L had a 64.4% sensitivity and 84.4% specificity in discriminating BA. A level of 300 U/L was also previously reported by Liu *et al*[16] with 85% accuracy. However high it may be, we cannot confirm BA directly by GGT alone.

In the entire study, there were 14 BA patients (6 in the retrospective study and 8 in the validation set) below the age of 40 d (with the minimum age of 20 d), in whom the HSST sign was observed but there was no liver cirrhosis at gross inspection (Figure 5A). The HSST sign was also found in 10 patients with BA (8 in the retrospective study and 4 in the validation set) who were older than 90 d (91-163 d) (Figure 5B). Based on the above data, the presence of the HSST

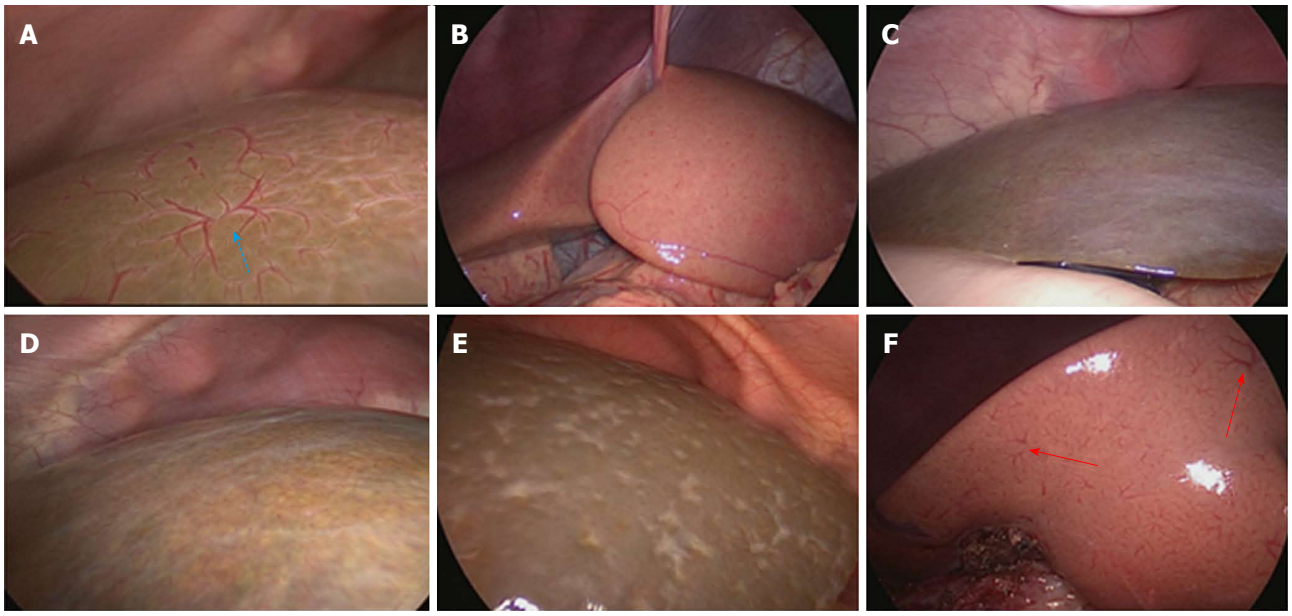

Figure 1 Laparoscopic images of the liver surface in the retrospective study. A: HSST sign in a 70-day-old boy with biliary atresia (blue arrow); B: Image of a 64-day-old boy with Hirschsprung's disease; C: Image of an 82-day-old boy with infantile hepatitis; D: Image of a 72-day-old girl with biliary hypoplasia; E: Image of a 70-day-old boy with total parenteral nutrition-induced cholestatic cirrhosis; F: Small vessel plexuses (red arrows) observed in a 55-day-old boy with cytomegalovirus hepatitis. The HSST sign does not exist in the images B-E. HSST: Hepatic subcapsular spider-like telangiectasis.

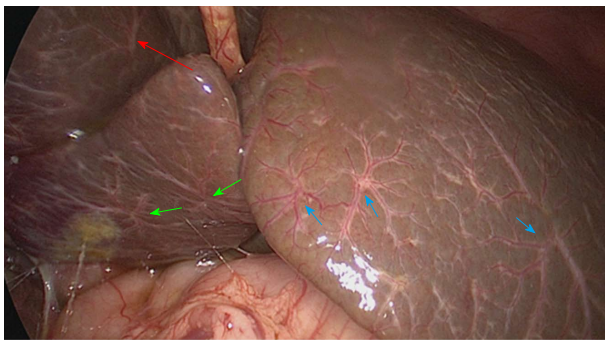

Figure 2 Hepatic subcapsular spider-like telangiectasis sign in a 68-day-old boy with biliary atresia. A hepatic subcapsular spider-like telangiectasis sign was widely distributed on the liver surface, including on the visceral facies of the right liver lobe (red arrow), on diaphragmatic facies of the left liver lobe (blue arrows) and on the surface of the caudate lobe (green arrows).

sign may not have a close relationship with hepatic fibrosis or cirrhosis. It is known that CMV infection was one causative factor of BA and may result in a delayed diagnosis and surgical treatment[23,24]. Mohanty *et al*[25] reported two cases of CMV infection, initially presenting with intrahepatic cholestasis, who subsequently developed BA. Whether the pathological change in the perivascular arterial tufts is the causative factor or results from the developmental of BA is still unknown and needs further investigation.

Ultrasonography (US) has been widely used in differentiating BA from other causes of jaundice. Hepatic subcapsular flow (HSF) in color Doppler US was documented as being useful to predict BA[8,15,18,26] and had the highest performance in discriminating BA from non-BA conditions among several parameters[18]. However, most of the neonates or infants were uncooperative during color Doppler US examination and other factors, such as the doctor's experience, machines, and patients' respiration intensities, also played an important role. Additionally, Takamizawa *et al*[9] debated that US alone could not rule out BA and laparotomy remained mandatory if BA was suspected. Therefore, we conducted this study and finally determined that the HSST sign was useful for predicting BA with a 100% sensitivity and 97.8% specificity. Here, we had a 53-day-old patient with a pre-operative icteric index of 149.9 but unobvious yellowing of the skin. As shown in the picture, the laparoscopic HSST sign (Figure 6A) and the result of LAC (Figure 6B) were easier to identify than distinguishing HSF in a color Doppler US image (Figure 6C).

A similar phenomenon was also observed in Lee's[8] and Ramesh's[27] studies. These authors reported that patients with BA who had hepatic subcapsular flow on color Doppler US images also had subcapsular telangiectatic vessels on the liver surface at the time of the Kasai procedure. In these reports, four patients with TPN-induced cholestasis and two with CMV hepatitis were considered as having HSF[8,15,18]. In our retrospective study, three patients with TPN-induced cholestasis cirrhosis were encountered, but no HSST sign was detected (Figure 2E). In our validation set, there were also two cases of infantile hepatitis who were considered as having HSF, while the HSST sign did not exist. The laparoscopic amplification effect on an intuitive view of the liver surface and ultrasonic

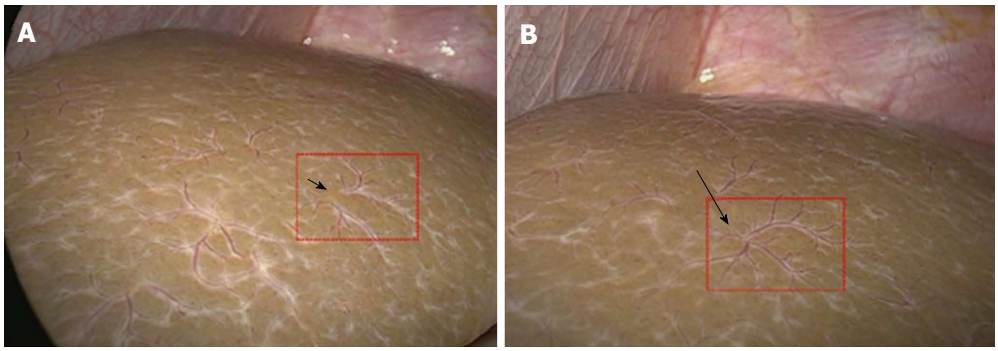

Figure 3 Different types of hepatic subcapsular spider-like telangiectasis signs. A: Dispersed type (short arrow) of the HSST sign in a 75-day-old boy with BA; B: Concentrated type (long arrow) of the HSST sign in a 77-day-old boy with BA. HSST: Hepatic subcapsular spider-like telangiectasis; BA: Biliary atresia.

Table 1 Clinical characteristics of patients with biliary atresia, non-biliary atresia patients and control subjects in the retrospective study

Characteristic	BA (n = 72)	Non-BA (n = 54)	P value[1]	Control (n = 38)	P value[2]	P value[3]
Age (d)	58 ± 29 (20-180)	59 ± 21 (35-125)	0.89	63 ± 22 (36-109)	0.35	0.34
Male-to-female ratio	31:41	24:30	0.88	19:19	0.49	0.60
Total bilirubin (μmol/L)	197.6 ± 72.8	163.6 ± 62.1	0.062	19.3 ± 18.3	<0.0001	<0.0001
Direct bilirubin (μmol/L)	132.8 ± 143.8	99.3 ± 50.8	0.29	5.7 ± 5.6	0.003	<0.0001
ALT (U/L)	162.9 ± 122.6	170.2 ± 362.6	0.91	21.3 ± 7.4	<0.0001	0.15
AST (U/L)	239.7 ± 156.7	183.5 ± 253.1	0.27	30.0 ± 11.1	<0.0001	0.037
GGT(U/L)	684.7 ± 450.6	454.2 ± 450.9	0.052	46.5 ± 22.4	<0.0001	0.003

[1]BA *vs* non-BA; [2]BA *vs* controls; [3]Non-BA *vs* controls. The results are expressed as the mean ± SD or number (percentage). HSST: Hepatic subcapsular spider-like telangiectasis; BA: Biliary atresia; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase.

Table 2 Diagnostic value of the hepatic subcapsular spider-like telangiectasis sign in first and consensus reviews by two reviewers in the retrospective study n (%)

	First review					
	Observer 1[1]		Observer 2[2]		Consistent result	
Imaging feature	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
HSST	72 (100)	54 (100)	72 (100)	53 (98.1)	72 (100)	53 (98.1)

[1]Observer 1 = Zhou Ying; [2]Observer 2 = Jiang Meng.

Table 3 Clinical characteristics of biliary atresia and non-biliary atresia patients in the validation group

Characteristic	BA (n = 45)	Non-BA (n = 45)	P value
Age (d)	60 ± 29	61 ± 23	0.90
Male-to-female ratio	21:24	25:20	0.40
Total bilirubin (μmol/L)	192.3 ± 63.5	173.6 ± 77.0	0.21
Direct bilirubin (μmol/L)	131.6 ± 140.8	104.5 ± 52.3	0.23
ALT (U/L)	172.8 ± 120.9	124.3 ± 264.1	0.27
AST (U/L)	255.2 ± 162.1	188.7 ± 193.9	0.08
GGT (U/L)	661.8 ± 466.3	320.7 ± 384.1	<0.001

The results are expressed as the mean ± SD. BA: Biliary atresia; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase.

artifacts may be the cause of this difference. Moreover, better concordance (κ = 0.937) was achieved through the amplified intuitive view.

Although the signal of hepatic subcapsular vessels was found with US, these vessels on laparoscopic images had their own characteristics: (1) intuitive and stable view of liver surface that was not influenced by interference (machines, doctors' experiences, thickness of the abdominal wall, *etc.*); (2) the HSST sign could be observed not only on the diaphragmatic surface but also on the visceral surface; and (3) images were magnified 4-8 times, which made the view of liver surface clear and vessels easily identified.

Microscopic examinations revealed dilated small arteries in the hepatic subcapsular area in patients with BA, which is consistent with Lee's[8] and El-Guindi's[15] studies. These vessels were branches of hepatic arteries, instead of capillaries in the hepatic capsule. Until now, this phenomenon was not mentioned for other liver diseases. The findings of arterial hyperplasia or hypertrophy in liver specimens and the enlargement of the hepatic artery on color Doppler US imaging in BA patients were also documented[15]. It has also been

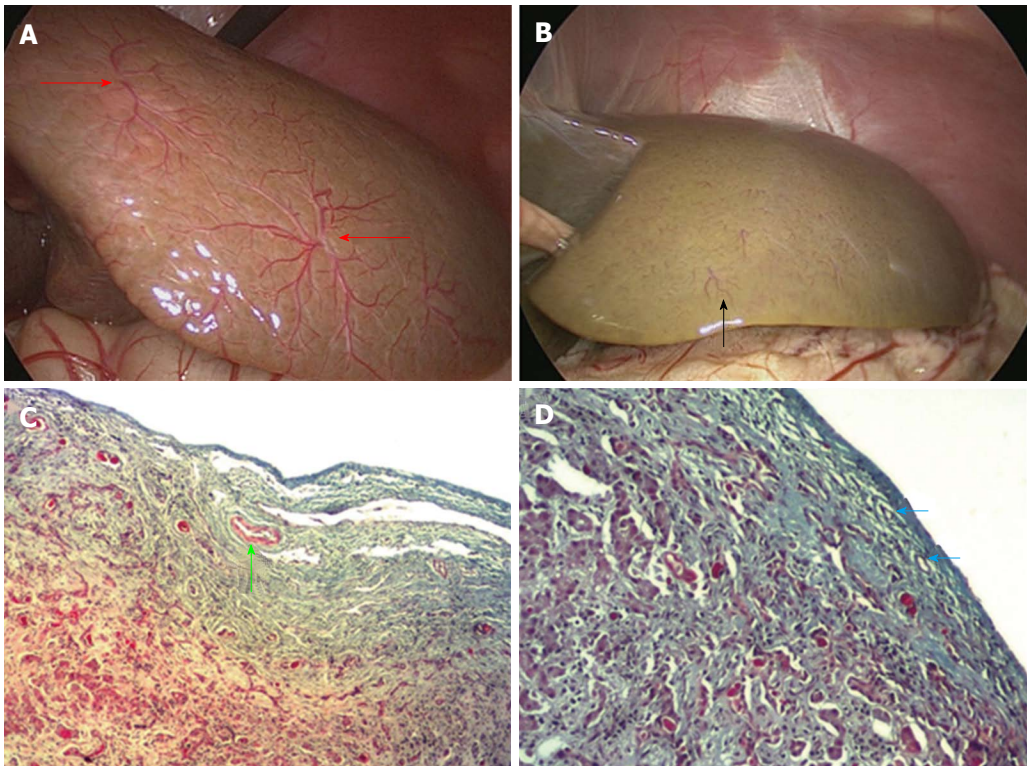

Figure 4 Hepatic subcapsular spider-like telangiectasis sign and pathologic evaluation in the validation set. A: Concentrated type of the HSST sign (red arrow) in a 68-day-old boy with BA; B: Vessels (black arrow) in biliary hypoplasia; C: Vessels in (A) was revealed as dilated small arteries (green arrow) in the hepatic subcapsular area; D: Vessels in biliary hypoplasia (B) was revealed as dilated capillaries (blue arrow) in the hepatic subcapsular area. (Trichrome staining; ×100). HSST: Hepatic subcapsular spider-like telangiectasis; BA: Biliary atresia.

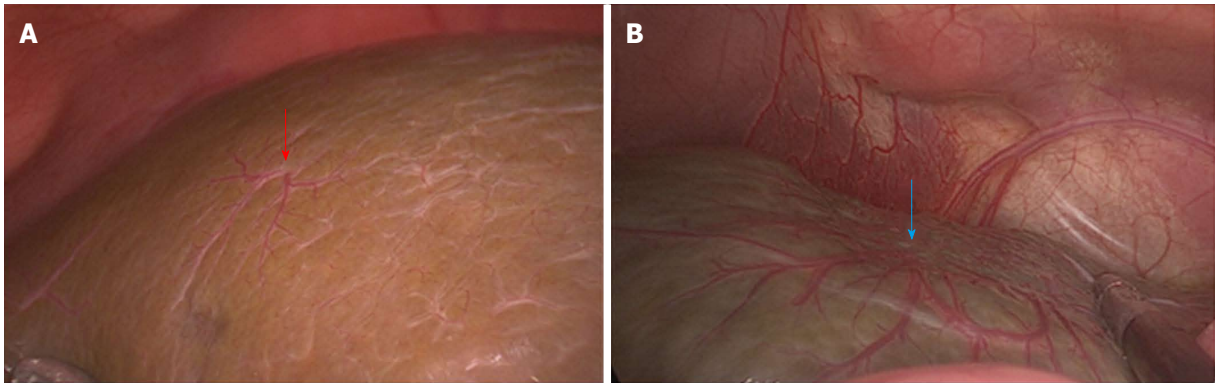

Figure 5 Laparoscopic images of hepatic subcapsular spider-like telangiectasis sign in biliary atresia patients at different ages. A: HSST sign (red arrow) in a 40-day-old boy with BA. There was no cirrhosis at gross inspection; B: HSST sign (blue arrow) in a 156-day-old boy with BA, who had hepatic cirrhosis with ascites and underwent liver transplantation. The HSST sign is obvious in both patients. HSST: Hepatic subcapsular spider-like telangiectasis; BA: Biliary atresia.

reported that the arterial changes might be driven by primary growth signals, or represent a response to a diseased micro-environment that might be rich in growth promoting signals that favor angiogenesis[28-32]. Similarly, the HSST sign is one of the arterial changes in an intuitive view. As with the HSST sign in images 1-5, they were not quite the same and this difference might help in predicting prognoses in the future. Whether it is a primary change of BA or secondary to the bile duct inflammation is unknown and will be our next focus of study.

At present, the procedure for addressing BA patients is a routine examination followed by laparotomy or laparoscopic cholangiography and a laparotomy Kasai operation. For the laparoscopic cholangiography, two or three 5-mm trocars and 30-45 min are needed with one or two instances of radiation exposure. However, with the application of the HSST sign, the

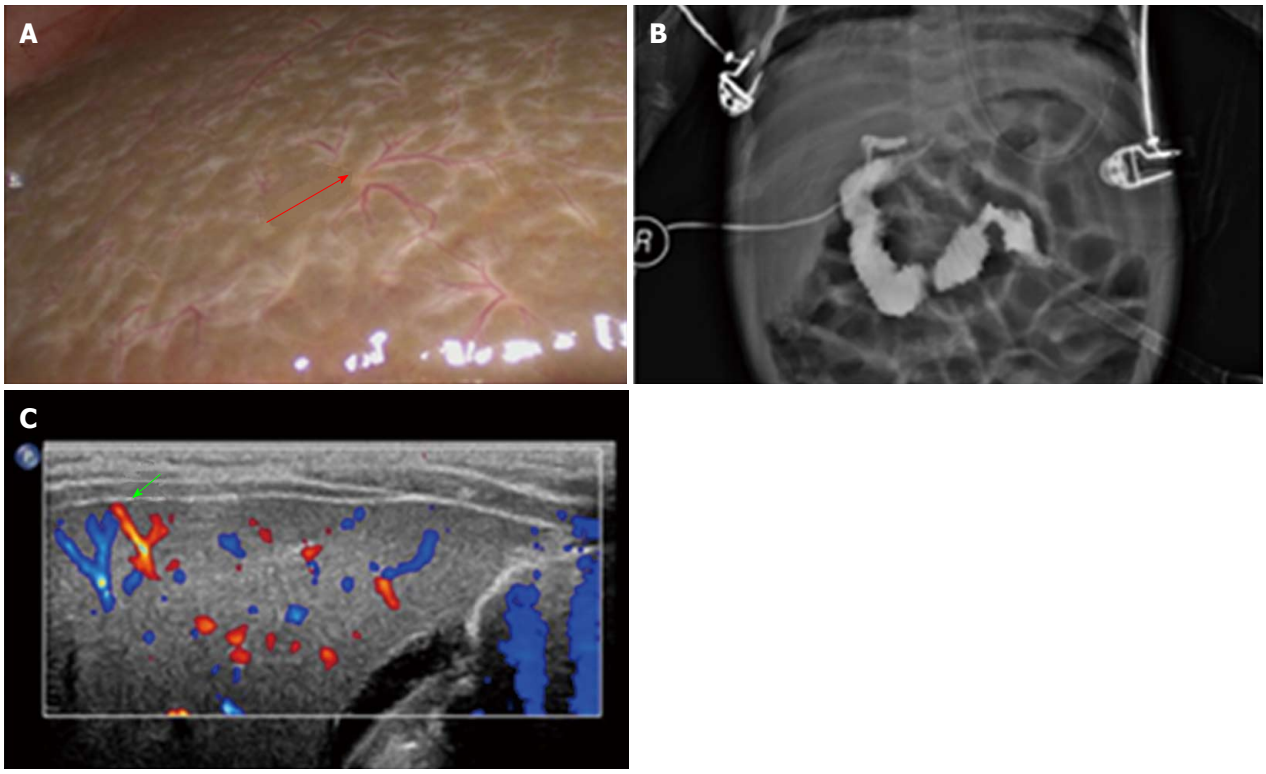

Figure 6 Images in a 53-day-old patient with biliary atresia. A: Dispersed type of the HSST sign (red arrow); B: Image of laparoscopic cholangiography indicates biliary atresia; C: Hepatic arterial flow extending to the hepatic surface on color Doppler US image (green arrow) indicates positive HSF. Laparoscopic HSST sign and the result of laparoscopic cholangiography were easier to identify than distinguishing HSF in a color Doppler US image. HSST: Hepatic subcapsular spider-like telangiectasis; US: Ultrasonography; HSF: Hepatic subcapsular flow.

procedure for addressing BA may become a routine examination followed by laparoscopic exploration for the HSST sign and a laparotomy Kasai operation in the future, which would shorten the operative time and radiation exposure and only needs one 5-mm trocar. The advantage is obvious especially for non-BA patients. In addition, it reflects the diagnostic function of laparoscopy in BA, similar to a laparoscopic diagnosis of hermaphroditism and inguinal hernias.

In conclusion, the HSST sign was preliminarily found to have a good performance in diagnosing or excluding BA with a 100% sensitivity and 97.8% specificity. According to an early laparoscopic exploration of the HSST sign, we can rapidly differentiate BA from non-BA cases and potentially further prevent the delay of a diagnosis of BA. However, given the limited sample size included in the analysis, the value of the HSST sign should be further confirmed in the future. Along with the further research, the HSST sign might be widely applied in clinical practice through a multicenter and large sample study in the future.

COMMENTS

Background

As a common neonatal cholangiopathy that leads to cirrhosis, biliary atresia (BA) is the most common indication for liver transplantation in infants. This disease is characterized by a progressive fibro-obliterative obstruction of extrahepatic bile ducts. A younger age (in the first 2 mo of life) at the time of Kasai portoenterostomy has been found to be life-saving in restoring bile flow and preventing the worsening of liver function. Nevertheless, it is still challenging to differentiate BA from other cholestatic diseases as there are no characteristic manifestations found in the early stage of BA. The demand for a specific and reliable test to diagnose BA has increased.

Research frontiers

Color Doppler ultrasound finding of hepatic subcapsular flow has shown much potential for discriminating BA. While in clinical practice, we have noticed a similar but more intuitive phenomenon - a laparoscopic finding of hepatic subcapsular spider-like telangiectasis (HSST) sign, which may be a specific marker for BA. This has not been published in previous literature.

Innovations and breakthroughs

According to these preliminary investigation, the HSST sign showed promising diagnostic performance for differentiating BA from any other cholestasis diseases, such as infantile cholestasis and cholestatic syndrome. The authors found that the sensitivity and specificity of the HSST sign were each generally close to 100%.

Applications

With the application of the HSST sign, the procedure for addressing BA may become a routine examination followed by laparoscopic exploration for the HSST sign and a laparotomy Kasai operation in the future, which would shorten the operative time and radiation exposure.

Terminology

The HSST sign is composed of several enlarged tortuous spider-like vascular

plexuses with four to eight branches distributed all over the liver surface, which presents as a dispersed type (radiating branches originate from more than one central point, but close to each other to form a spider-like sign) or a concentrated type (radiating branches originate from one central point).

Peer-review

Very interesting findings. It is worth to be published because of new findings of laparoscopic view for the surface of liver in biliary atresia

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ORIGINAL ARTICLE

Retrospective Study

Digestive tract reconstruction using isoperistaltic jejunum-later-cut overlap method after totally laparoscopic total gastrectomy for gastric cancer: Short-term outcomes and impact on quality of life

Ze-Ning Huang, Chang-Ming Huang, Chao-Hui Zheng, Ping Li, Jian-Wei Xie, Jia-Bin Wang, Jian-Xian Lin, Jun Lu, Qi-Yue Chen, Long-Long Cao, Mi Lin, Ru-Hong Tu, Ju-Li Lin

Ze-Ning Huang, Chang-Ming Huang, Chao-Hui Zheng, Ping Li, Jian-Wei Xie, Jia-Bin Wang, Jian-Xian Lin, Jun Lu, Qi-Yue Chen, Long-Long Cao, Mi Lin, Ru-Hong Tu, Ju-Li Lin, Department of Gastric Surgery, Fujian Medical University Union Hospital, Fuzhou 350001, Fujian Province, China

Author contributions: Huang ZN and Huang CM conceived the study, analyzed the data, drafted the manuscript, and made the video; Zheng CH, Li P, Xie JW and Wang JB helped revise the manuscript critically for important intellectual content; Lin JX, Lu J, Chen QY, Cao LL, Lin M, Tu RH and Lin JL helped collect the data and design the study; all authors read and approved the final manuscript.

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Abstract

AIM

To evaluate the short-term outcomes and quality of life (QoL) in gastric cancer patients undergoing digestive tract construction using the isoperistaltic jejunum-later-cut overlap method (IJOM) after totally laparoscopic total gastrectomy (TLTG).

METHODS

A total of 507 patients who underwent laparoscopic gastrectomy (D2) from January 2014 to March 2016 were originally included in the study. The patients were divided into two groups to undergo digestive tract construction using either IJOM after TLTG (group T, *n* = 51) or Roux-en-Y anastomosis after laparoscopic-

assisted total gastrectomy (LATG) (group A, n = 456). The short-term outcomes and QoL were compared between the two groups after 1:2 propensity-score matching (PSM). We used a questionnaire to assess QoL.

RESULTS

Before matching, age, sex, tumor size, tumor location, preoperative albumin and blood loss were significantly different between the two groups (P < 0.05). After PSM, the patients were well balanced in terms of their clinicopathological characteristics, although both blood loss and in-hospital postoperative days in group T were significantly lower than those in group A (P < 0.05). After matching, group T reported better QoL in the domains of pain and dysphagia. Among the items evaluating pain and dysphagia, group T tended to report better QoL ("Have you felt pain" and "Have you had difficulty eating solid food") (P < 0.05).

CONCLUSION

The IJOM for digestive tract reconstruction after TLTG is associated with reduced blood loss and less pain and dysphagia, thus improving QoL after laparoscopic gastrectomy.

Key words: Esophagojejunostomy; Overlap; Later-cut; Totally laparoscopic total gastrectomy; Quality of life

Core tip: This paper used propensity score-matched analysis and questionnaire survey to evaluate the short-term outcomes and quality of life (QoL) in patients who underwent digestive tract reconstruction using the isoperistaltic jejunum-later-cut overlap method (IJOM) after totally laparoscopic total gastrectomy (TLTG) and in patients who underwent Roux-en-Y anastomosis after laparoscopic-assisted total gastrectomy. We found the IJOM for digestive reconstruction after TLTG is associated with reduced blood loss and less pain and dysphagia, thus improving the QoL after laparoscopic gastrectomy.

Huang ZN, Huang CM, Zheng CH, Li P, Xie JW, Wang JB, Lin JX, Lu J, Chen QY, Cao LL, Lin M, Tu RH, Lin JL. Digestive tract reconstruction using isoperistaltic jejunum-later-cut overlap method after totally laparoscopic total gastrectomy for gastric cancer: Short-term outcomes and impact on quality of life. *World J Gastroenterol* 2017; 23(39): 7129-7138 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7129.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7129

INTRODUCTION

Since Kitano *et al*[1] reported laparoscopic-assisted distal gastrectomy in 1991, laparoscopic techniques and instruments have improved substantially. Consequently, totally laparoscopic distal gastrectomy is increasingly employed and has a proven history of safety and feasibility[2-8]. However, although scholars have reported a variety of totally laparoscopic total gastrectomy (TLTG) methods[3,9-13], this technique has not been widely adopted because of the technological difficulty inherent in digestive reconstruction. Interest in improving the postoperative appearance and quality of life (QoL) in patients with gastric cancer has been increased. This goal, combined with the reduced trauma associated with TLTG, has heightened interest in developing ways to improve TLTG. Surgeons have primarily adopted the overlap or functional end-to-end method for digestive tract reconstruction after TLTG. However, these methods have drawbacks, such as jejunal freeness, which is seen particularly frequently with large anastomoses. Therefore, we devised the isoperistaltic jejunum-later-cut overlap method (IJOM), which involves esophagojejunostomy anastomosis after TLTG. Using this technique, we believe that the jejunum can be positioned with greater ease, thereby reducing the difficulties encountered with the anastomosis. However, little is known about the short-term outcomes and QoL of patients following the implementation of this IJOM for digestive reconstruction after TLTG. Thus, this study aimed to assess the short-term outcomes and QoL in gastric cancer patients undergoing digestive tract reconstruction with IJOM after TLTG and with Roux-en-Y anastomosis after LATG using propensity-score matching (PSM)[14,15] and a QoL assessment scale.

MATERIALS AND METHODS

Study population and inclusion/exclusion criteria

Between January 2014 to March 2016, data were collected from 703 patients who underwent laparoscopic gastrectomy at Fujian Medical University Union Hospital. The including criteria were: (1) pathologically proved gastric cancer by endoscopic biopsy specimen analysis; (2) the aforementioned examination indicated no evidence of distant metastasis; and (3) postoperative pathological diagnosis was curative R0. The exclusion criteria were: (1) intraoperatively proved distant metastasis; (2) T4b stage; (3) missing pathological data; (4) neoadjuvant therapy; and (5) comorbidities that could influence QoL (*e.g.*, previous or combined malignancies; cardiovascular disease; cerebrovascular disease; neurological conditions, such as dementia and seizure; and severe chronic obstructive pulmonary disease requiring persistent medical aid). A number of 507 patients were eligible. Group T consisted of 51 patients who underwent the IJOM after TLTG, and group A comprised 456 patients who received a Roux-en-Y anastomosis after LATG. The 1:2 PSM was performed. Ultimately, group T included 51 patients and group A included 102 patients (Figure 1).

Table 1 Characteristics, merit and demerit of different esophagojejunostomy anastomosis techniques

Anastomosis surgeon	Characteristics	Merit	Demerit
Uyama *et al*[12]	The anastomosis line is vertical with esophageal long axis Jejunum is located in the right side of the esophagus.	Anastomotic is large enough	The number of anastomosis linear staplers is much
Matsui *et al*[37]	Complete the anastomosis before severed esophagus Close the stoma and resect specimens at the same time Jejunum is located in the right side of the esophagus	The number of anastomosis linear staplers is reduced	Probably develop dysphagia 6 mo after operation
Lee *et al*[13]	Suture esophagus, jejunum and right angle of diaphragm after anastomosis Jejunum is located in the right side of the esophagus	Reduce the incidence of esophageal hiatal hernia and anastomotic fistula	Increase the operation time
Okabe *et al*[38]	Before anastomosis, the specimens was removed Jejunum is located in the left side of the esophagus	The size of anastomotic stoma is bigger	The technique is difficult
Inaba *et al*[29]	Overlap anastomosis Divide the jejunum before anastomosis	Isoperistaltic anastomosis meets the physiological needs	The jejunum is free and difficult for anastomosis
Matsui *et al*[39]	Overlap anastomosis Divide the esophagus after anastomosis	Isoperistaltic anastomosis meets the physiological needs	The jejunum is free and difficult for anastomosis

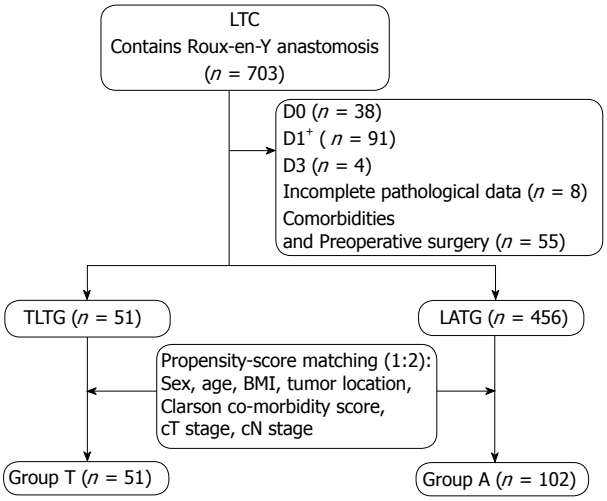

Figure 1 The flow chart of patient selection. LTG: Laparoscopic total gastrectomy; TLTG: Totally laparoscopic total gastrectomy; LATG: Laparoscopic assisted total gastrectomy.

Anastomosis step

In group T, the IJOM was performed after TLTG. After dissecting the lymph nodes laparoscopically and mobilizing the esophagus (Figure 2A) and the duodenum (Figure 2B), an endoscopic linear stapler was used to transect them sequentially in predetermined locations. Two small incisions were made on the left side of the resection margin of the esophagus (Figure 2C) and the antimesenteric border of the jejunum (Figures 2D) approximately 20 cm away from the ligament of Treitz, respectively.

Then, the two limbs of the stapler were inserted into each incision, respectively, and the forks of the stapler were closed and fired, achieving a side-to-side esophagojejunostomy (Figure 2E). After confirming that there was no bleeding *via* common stab incision (Figure 2F), the common stab was manually sutured (Figure 2G). Then, the jejunum was transected after baring the mesenteric border approximately 1 cm into the jejunum wall and approximately 3 cm away from the esophagojejunostomy (Figure 2H). After a small incision was made each on the antimesenteric border of the margin of the proximal jejunum and the distal jejunum roughly about 45 cm from esophagojejunostomy, the two limbs of the stapler were inserted into each incision, and the forks of the stapler were closed and fired to achieve a side-to-side jejunojejunostomy (Figure 2I). After confirming that there was no bleeding or damage to jejunal mucosa by common stab incision, the common stab incision was sutured laparoscopically (Figure 2J). Finally, we removed the specimen through a 3.5-cm incision on the lower abdomen. The differences between this method and other esophagojejunostomy anastomosis techniques are summarized in Table 1.

For patients in group T, the lymph nodes were dissected laparoscopically, and the esophagus and duodenum were mobilized. Then, the traditional open Roux-en-Y anastomosis was performed using a circular stapler[16].

Definition

All patients signed the informed consent form before operation. Preoperative computed tomography (CT) scanning, ultrasonography of the abdomen and endoscopic ultrasonography were routinely performed. When distant metastasis was suspected, positron emission tomography was performed. Preoperative morbidities were scored according to the Charlson score system[17]. Tumor staging was performed according to the 7th edition of the International Union against Cancer (UICC) classification[18]. Postoperative anastomosis-related complications were diagnosed by gastrografin esophagram or clinical manifestations and stratified using the Clavien-Dindo classification[19]. Perioperative death was defined as death that occurred during hospitalization. The Institutional Review Board

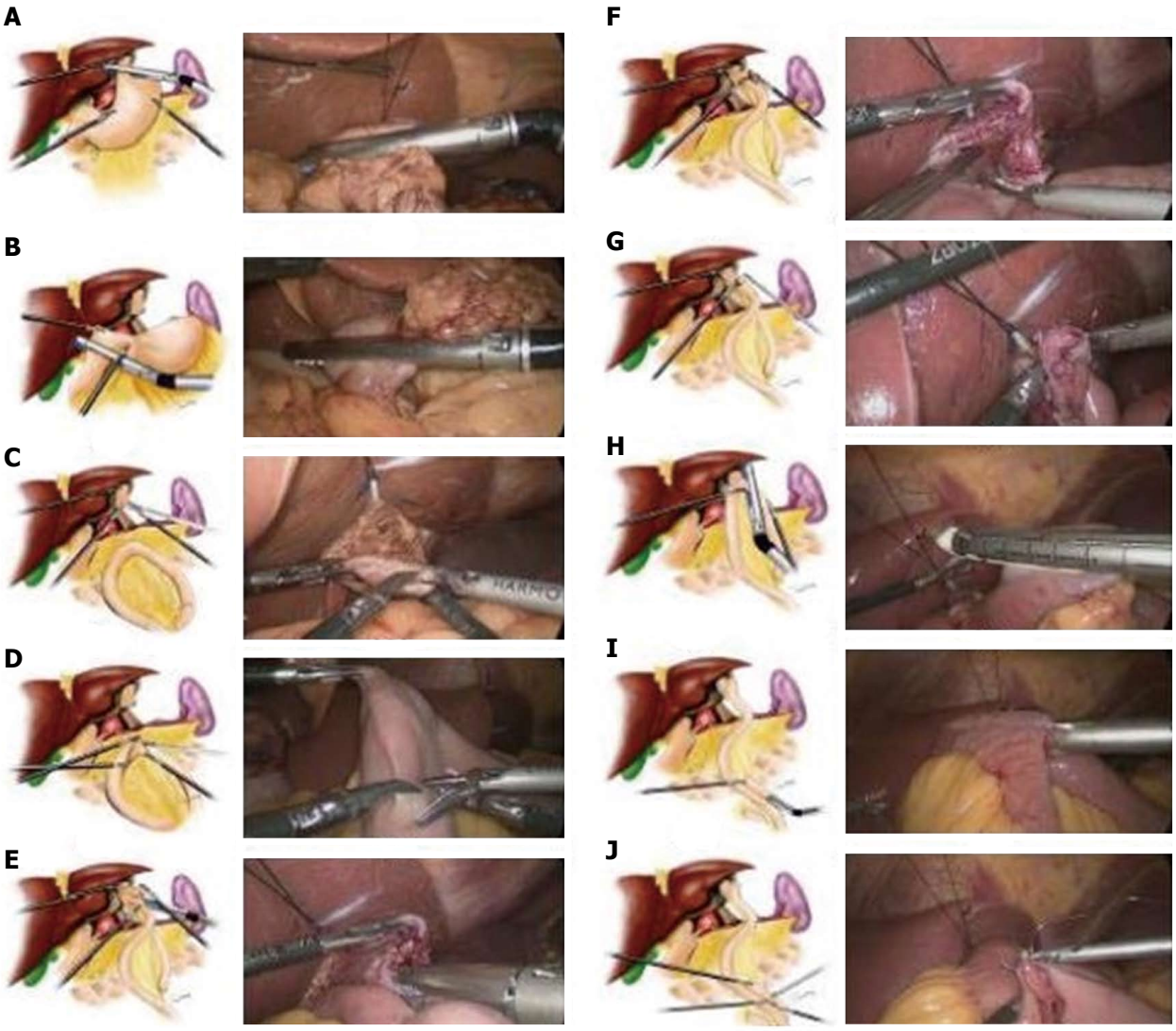

Figure 2 The schematic diagram of anastomosis.

of Fujian Medical University Union Hospital approved this study.

QoL questionnaire

We used the validated Chinese version of the European Organization for Research and Treatment of Cancer 30-item core QoL questionnaire (EORTC-QLQ-C30)[20] to assess QoL. The questionnaire includes a global health status, five functional items, three symptom dimensions, and six individual symptom items (Table 2). Furthermore, we used the validated Chinese version of the 22-item EORTC-QLQ gastric cancer module (EORTC-QLQ-STO22)[21] to assess tumor-specific QoL. This questionnaire includes a functional scale, five symptom dimensions, and three individual symptom scales. QoL is represented by a score ranging from 0 to 100 for every scale. Better QoL is indicated by the higher scores in the functional scales of the EORTC-QLQ-C30 and lower scores in the symptom scores of EORTC-QLQ-C30 and STO22 (Table 2).

Follow-up

The follow-ups were performed *via* mailing, telephone call, or outpatient service. We explained the content of each item of the questionnaire to the patients 6 mo postoperatively, and the patients chose their own responses. Most patients underwent physical examinations, laboratory tests, chest radiography, abdominal ultrasonography or CT, and annual endoscopic examinations.

Statistical analysis

The Statistical Package for Social Science version 18.0 (SPSS, Chicago, IL, United States) was used to perform statistical analyses. The *t* tests or paired *t* tests were performed to compare continuous variables. χ^2 tests were performed to compare categorical variables. $P < 0.05$ was considered statistically significant.

RESULTS

Demographics and clinical characteristics

Before matching, age, sex, tumor location, tumor size, and preoperative albumin level differed significantly between the two groups ($P < 0.05$). After 1:2

Table 2 Structure of EORTC QLC-C30 and EORTC QLQ-STO22

Scale	Number of constituting items
EORTC QLQ-C30	
Global health status/QoL scale[1]	2
Functional scales[1]	
Physical functioning	5
Role functioning	2
Emotional functioning	4
Cognitive functioning	2
Social functioning	2
Symptom scales[2]	
Fatigue	3
Nausea and vomiting	2
Pain	2
Dyspnoea	1
Insomnia	1
Appetite loss	1
Constipation	1
Diarrhoea	1
Financial difficulties	1
EORTC-QLQ-STO22[2]	
Dysphagia	3
Chest and abdominal pain	4
Reflux	3
Eating restrictions	4
Anxieties	3
Dry mouth	1
Taste problem	1
Body image	1
Hair loss	2

[1]Higher scores represent better QoL; [2]Higher scores represent worse QoL. EORTC QLQ indicates European Organization for Research and Treatment of Cancer Quality-of-life Questionnaire; QoL: Quality-of-life.

matching, 51 and 102 patients were included in groups T and A, respectively, and well balanced in their clinicopathological characteristics (Table 3).

Perioperative results

After matching, blood loss and postoperative days of hospital stay were significantly less in group T than in group A ($P < 0.05$). The number of harvested lymph nodes, operative time, time to first flatus, time to fluid diet, and hospitalization costs were similar in the two groups (Table 4).

Postoperative complications

Before matching, one patient had an anastomotic fistula in group T. In contrast, Group A included 22 patients with anastomotic fistula, one with anastomotic hemorrhage and four with anastomotic obstruction. The incidences of anastomosis-related complications were 2.0% and 5.9% in groups T and A, respectively; the two rates were similar. After matching, there was one patient with anastomotic fistula in group T, whereas there were four patients with anastomotic fistula, one with anastomotic hemorrhage, and two with an anastomotic obstruction in group A. The incidence of anastomosis-related complications had no statistical difference in the two groups, and no perioperative deaths occurred (Table 5).

Functional scales

After matching, six patients died and one was lost to follow-up 6 mo postoperatively in group T. Eventually, 44 patients joined the questionnaire survey. In group A, ten patients died, and three were lost to follow-up. A total of 89 patients from group A participated in the questionnaire survey. The functional scales of EORTC-QLQ-C30 were all similar in both groups (Figure 2).

Symptom scales

After matching, the symptom scales of EORTC-QLQ-C30 and STO22 were compared. Based on the pain scales of EORTC-QLQ-C30 and dysphagia scales of EORTC QLQ-STO22, group T reported better QoL (Figure 3). Subgroup analyses of two items in the pain scale ("Have you felt pain?" and "Has your life been affected by pain?"), group T tended to report better QoL in "Have you felt pain?"($P = 0.018$). In the dysphagia scale, subgroup analyses of three items ("Have you had difficulty eating solid food?", "Have you had difficulty swallowing liquid or eating soft food?", and "Have you had difficulty drinking water?") revealed that group T tended to report better QoL in response to the question "Have you had difficulty eating solid food?" than group A ($P = 0.039$) (Table 6).

DISCUSSION

Laparoscopy offers several advantages over traditional laparotomy, such as reduced trauma, faster recovery, fewer postoperative complications, and greater aesthetic appeal. These benefits are attributable to the minimal invasiveness of laparoscopy and the good clinical outcomes that have been reported[22-25]. Currently, the methods of digestive tract reconstruction employed after LG include LATG and TLTG. In LATG, the digestive tract reconstruction is performed *via* a small incision after lymphadenectomy, although this decreases the advantages of laparoscopic minimally invasive surgery. Since Uyama *et al*[12] reported totally laparoscopic digestive tract reconstruction, substantial research in Japan and Korea has revealed that TLTG has desirable short-term outcomes and is also safe and effective[11,26-28].

Currently, the digestive tract reconstruction methods used after TLTG include overlap[12] and functional end-to-end[29] techniques. However, jejunal freeness is one of several drawbacks associated with these methods and makes the anastomosis more difficult. Therefore, we devised the IJOM, which involves esophagojejunostomy anastomosis after TLTG. We believe that, using this technique, the jejunum can be more easily positioned, thereby reducing the difficulty in creating the anastomosis. Moreover, because the proximal jejunum is divided after the anastomosis, the length of the blind loops can be easily grasped. We found that blood loss was reduced in group T, and this may be attributed to the clearer,

Table 3 Demographic and clinical characteristics of patients in the two groups

	All patients			Propensity-matched patients		
	Group T (n = 51)	Group A (n = 456)	P value	Group T (n = 51)	Group A (n = 102)	P value
Age (mean ± SD, yr)	55.5 ± 12.1	61.6 ± 11.2	< 0.001	55.5 ± 12.1	55.9 ± 11.0	0.916
Gender			< 0.001			1.000
Male	34	345		34	68	
Female	17	111		17	34	
Charlson comorbidity index			0.281			0.608
0	48	418		48	92	
1-2	3	38		3	10	
BMI (mean ± SD, kg/m^2)	22.5 ± 13.1	22.3 ± 13.5	0.919	22.5 ± 13.1	22.6 ± 12.8	0.965
Tumor size (mean ± SD, cm)	4.5 ± 1.5	4.9 ± 1.3	0.041	4.5 ± 1.5	4.7 ± 1.7	0.142
Tumor location			< 0.001			0.177
Upper third	4	188		4	12	
Middle third	34	169		34	76	
Whole	13	99		13	14	
Histology type			0.453			0.482
Differentiation	47	416		47	97	
Undifferentiation	4	40		4	5	
Preoperative albumin (mean ± SD, g/L)	40.8 ± 4.3	39.1 ± 5.2	0.025	40.8 ± 4.3	40.6 ± 4.6	0.796
Depth of infiltration (T)			0.174			0.643
T1	15	82		15	23	
T2	8	83		8	18	
T3	10	135		10	16	
T4a	18	166		18	45	
Nodal status (N)			0.729			0.534
N0	21	190		21	34	
N1	11	77		11	18	
N2	5	66		5	10	
N3	14	123		14	40	
UICC stage			0.319			0.502
Ⅰ	13	78		13	18	
Ⅱ	17	159		17	40	
Ⅲ	21	219		21	44	

BMI: Body mass index; UICC stage: 7th edition of the International Union against Cancer.

Table 4 Operative variables of the patients

	All patients			Propensity-matched patients		
	Group T (n = 51)	Group A (n = 456)	P value	Group T (n = 51)	Group A (n = 102)	P value
Operative time, min (mean ± SD)	209.3 ± 41.0	203.6 ± 49.3	0.427	209.3 ± 41.0	200.5 ± 55.6	0.318
Blood loss, mL (mean ± SD)	48.3 ± 38.5	98.4 ± 149.1	0.017	48.3 ± 38.5	105.4 ± 147.9	0.008
Harvested LNs (mean ± SD)	44.5 ± 15.0	41.2 ± 14.2	0.237	44.5 ± 15.0	42.6 ± 15.2	0.465
Time to first flatus, days (mean ± SD)	3.8 ± 1.2	3.5 ± 1.7	0.220	3.8 ± 1.2	3.6 ± 1.2	0.332
Time to fluid diet, days (mean ± SD)	5.6 ± 1.4	5.6 ± 1.6	1	5.6 ± 1.4	5.5 ± 1.9	0.739
Postoperative days (mean ± SD)	12.6 ± 4.3	14.7 ± 8.9	0.097	12.6 ± 4.3	15.4 ± 8.9	0.035
hospitalization costs, yuan	75450 ± 20038	73308 ± 21932	0.505	75450 ± 20038	70407 ± 13254	0.065
Chemotherapy	33	310	0.635	33	78	0.123

LN: Lymph node.

amplified field of vision achieved during laparoscopic digestive tract reconstruction. As a result, blood vessels in the muscles and mesentery can be more readily identified and are less likely to be transected during the procedure. Consistent with our findings, previous studies have shown that reduced blood loss is associated with better postoperative recovery[30]. We found that the length of the postoperative stay in group T was significantly shorter than that in group A, confirming that less trauma and blood loss during TLTG can promote faster recovery.

Lee *et al*[31] showed that distal gastrectomy decreases the QoL because of problems with eating restrictions and body image. However, compared with open surgery, laparoscopic-assisted distal gastrectomy improves QoL, specifically by reducing the incidence of postoperative intestinal obstruction[32]. Fujii *et al*[33] suggested that this reduction in postoperative intestinal obstruction may be attributable to the less abdominal manipulation required for laparoscopic surgery, which may blunt the systemic cytokine and inflammatory responses[34,35]. Similarly, totally laparoscopic technology reduces the amount of intestinal manipulation required during digestive tract reconstruction and may also

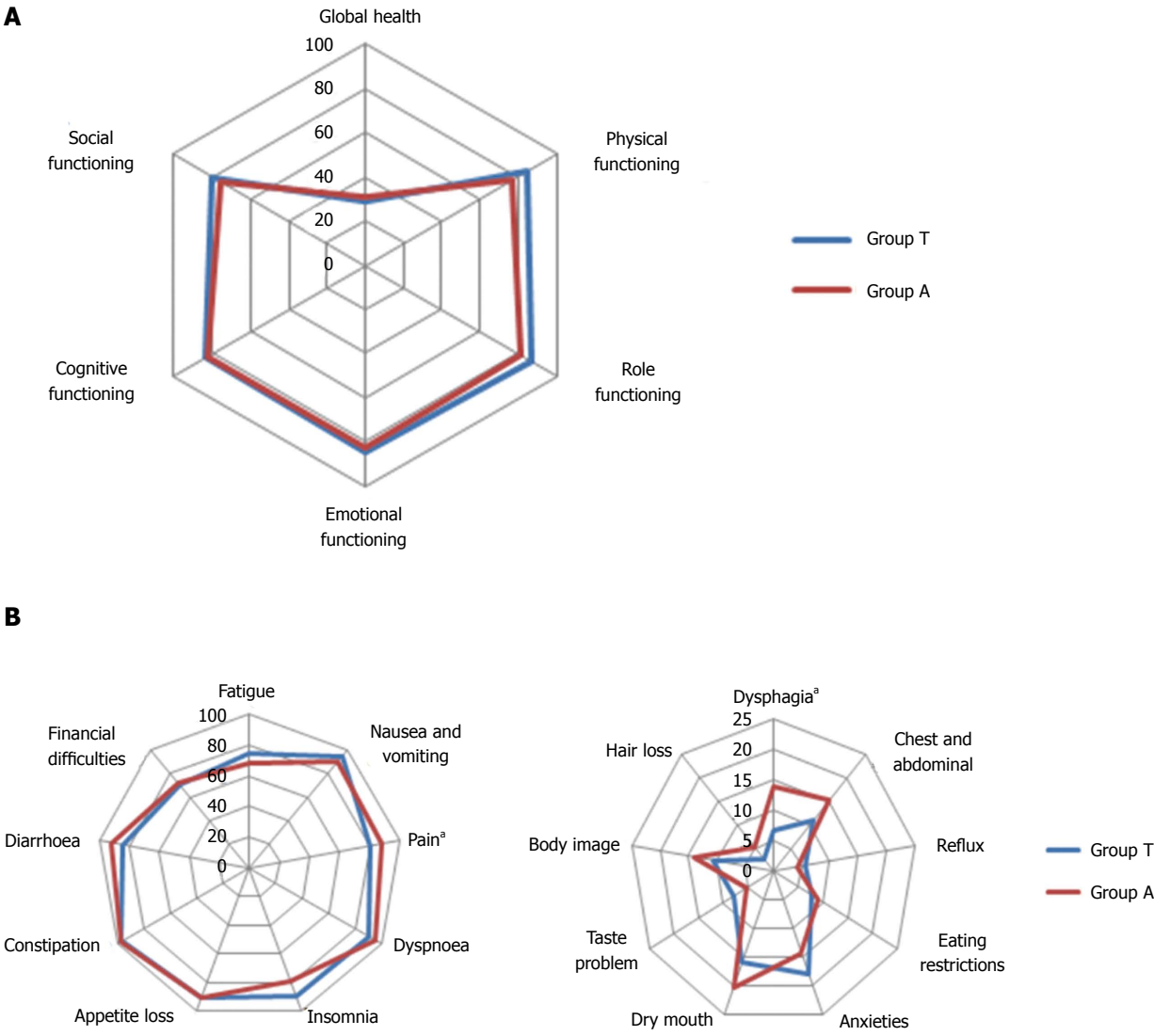

Figure 3 The functional scales. A: EORTC-QLQ-C30; B: EORTC-QLQ-C30 and EORTC-QLQ-STO22. [a]*P* < 0.05, EORTC-QLQ-C30 *vs* EORTC-QLQ-STO22.

Table 5 Morbidity and mortality associate with anastomosis

	All patients			Propensity-matched patients		
	Group T, % (*n* = 456)	Group A, % (*n* = 51)	*P* value	Group T, % (*n* = 102)	Group A, % (*n* = 51)	*P* value
Morbidity	1 (2.0)	27 (5.9)	0.893	1 (1.9)	6 (11.8)	0.552
Anastomotic fistula	1	22		1	4	
Anastomotic hemorrhage	0	1		0	0	
Anastomotic obstruction	0	4		0	2	
Mortality	0	0	/	0	0	/

reduce the incidence of postoperative intestinal obstruction. However, no studies have evaluated QoL following totally laparoscopic surgery. Schneider *et al*[36] reported that the diameter of the anastomotic stoma obtained using the linear stapler was significantly larger than that achieved with the circular stapler, which benefits the passage of food. At 6 mo post-surgery, we found that symptoms of dysphagia were better in group T, especially in terms of eating solid food. This finding indicates that using a linear stapler can expand the diameter of the anastomotic stoma and that the decreased intestinal manipulation involved in TLTG can reduce the incidence of intestinal obstruction.

Patients who underwent TLTG reported significantly less pain than those undergoing LATG. We believe that this is because the incision in the abdominal wall involved in TLTG is shorter, leading to less pain from inflammation and scar formation. In addition, less intra-abdominal manipulation likely contributes to decreasing the formation of intra-abdominal adhesions

Table 6 Constituent items of pain of EORTC-QLQ-C30 and dysphagia of EORTC-QLQ-STO22 as compared between group T and group A

	Propensity-matched patients		
	Group T (n = 44)	Group A (n = 89)	P value
Constituent items of pain of EORTC-QLQ-C30			
Have you felt pain?			0.018
Not at all	28	66	
A little	12	7	
Quite a lot	3	14	
Very much	1	2	
Have your life affected by pain?			0.271
Not at all	39	73	
A little	4	7	
Quite a lot	1	9	
Very much	0	0	
Constituent items of dysphagia of EORTC-QLQ-STO22			
Have you felt difficult to eat solid food?			0.039
Not at all	26	31	
A little	11	32	
Quite a lot	7	21	
Very much	0	5	
Have you felt difficult to eat liquid of soft food?			0.275
Not at all	38	67	
A little	5	15	
Quite a lot	1	7	
Very much	0	0	
Have you felt difficult to drink water?			0.194
Not at all	39	80	
A little	5	5	
Quite a lot	0	4	
Very much	0	0	

and, thus, the associated discomfort.

This is the first study investigating the differences in short-term outcomes and QoL between the IJOM after TLTG and Roux-en-Y anastomosis after LATG using PSM and a QoL assessment scale. The results show that utilizing the IJOM after TLTG can reduce intra-operative blood loss and relieve symptoms of pain and dysphagia. However, this study has several limitations. First, the follow-up period was short. Second, a retrospective, single-center design was used. Therefore, a prospective, multiple-center study with a longer follow-up period is needed.

COMMENTS

Background

Surgeons have primarily adopted several methods for digestive tract reconstruction after totally laparoscopic total gastrectomy (TLTG). However, these methods have drawbacks, such as jejunal freeness and difficult to perform. Therefore, we devised the isoperistaltic jejunum-later-cut overlap method (IJOM), but little is known about the short-term outcome and quality-of-life (QoL) in patients following the implementation of this digestive reconstruction.

Research frontiers

The QoL after distal gastrectomy was reported to be affected by eating restrictions and body image. For TLTG, scholars have reported a variety of digestive reconstruction methods which are safe and effective, but the QoL is uncertain.

Innovations and breakthroughs

The authors used propensity score-matched analysis and questionnaire survey to perform this research. The authors found that IJOM for digestive reconstruction can reduce blood loss compared with Roux-en-Y anastomosis and was associated with less pain and dysphagia, thus improving QoL after laparoscopic gastrectomy.

Applications

Through this study, we can focus the symptoms which probably happen after surgery and accordingly improve the QoL of patients. However, a prospective, multiple-center study with a longer follow-up period is needed.

Terminology

EORTC-QLQ-C30: Chinese version of the European Organization for Research and Treatment of Cancer 30-item core QoL questionnaire. EORTC-QLQ-STO22: the validated Chinese version of the 22-item EORTC-QLQ gastric cancer module. PSM: A statistical matching method which can reduce the bias of variables.

Peer-review

This study is a pioneer study about esophagojejunostomy anastomosis after TLTG.

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ORIGINAL ARTICLE

Observational Study

Adalimumab efficacy in enteropathic spondyloarthritis: A 12-mo observational multidisciplinary study

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Abstract

AIM

To report adalimumab (Ada) efficacy on articular-gastrointestinal disease and health-related quality of life (HRQoL) in patients with enteropathic spondyloarthritis (ES).

METHODS

A cohort of 52 patients with ES was evaluated in the departments of gastroenterology and internal medicine. At baseline, all patients underwent assessment by an integrated gastro-rheumatologic evaluation of articular and gastrointestinal activity, as well patient reported outcomes (PROs) of the HRQoL questionnaires. After this integrated evaluation and following a specific working flowchart, the Ada anti-tumor necrosis factor (TNF)-inhibitor was assigned to a cohort of 30 patients and its clinical efficacy was evaluated at baseline and

after 6-mo and 12-mo treatment by the following tests: (1) Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein (ASDAS-CRP); Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Metrology Index (BASMI) for articular activity; (2) Inflammatory Bowel Disease Questionnaire (IBDQ), Crohn's Disease Activity Index (CDAI) and partial Mayo (pMayo) score for gastrointestinal symptoms and activity; and (3) Health Assessment Questionnaire (HAQ), Patient Global Assessment (PGA) and Short Form-36 health survey (SF-36) questionnaires for PROs of the HRQoL.

RESULTS

Integrated evaluation and management of the patients affected by ES, carried out simultaneously by a gastroenterologist and a rheumatologist, allowed clinicians to choose the optimal therapeutic strategy. In a cohort of 30 ES patients affected by active articular and gastrointestinal disease, or axial active articular inflammation, Ada led to fast and sustained improvement of both articular and gastrointestinal disease activities. In fact, all the clinimetric evaluation tests exploring articular or gastrointestinal activity, as well as all the HRQoL scores, showed a significant improvement having been achieved at the earliest (6-mo) assessment. This important clinical improvement was maintained at the 12-mo follow-up. Importantly, global and gastrointestinal quality of life significantly correlated with articular disease activity, providing evidence to support that the integrated evaluation is the best option to manage patients with ES.

CONCLUSION

Ada treatment, upon multidisciplinary (gastro-rheumatologic) evaluation, significantly improves both articular and gastrointestinal inflammation, thereby improving the HRQoL in patients affected by ES.

Key words: Clinimetric assessment; Patient reported outcomes; Inflammatory bowel diseases; Enteropathic spondyloarthritis; Tumor necrosis factor-inhibitors; Multidisciplinary evaluation

Core tip: Enteropathic spondyloarthritis (ES) is characterized by articular inflammation in patients with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis. Correct management, especially covering both of the two clinical manifestations (gastro-rheumatologic), remains a challenge. In this study, we demonstrated that the integrated gastroenterological and rheumatologic evaluation of ES patients achieved the best therapeutic approach. In particular, we demonstrated that in a real-life cohort of ES patients, the tumor necrosis factor-inhibitor, adalimumab, led to fast and sustained improvement of articular and gastrointestinal inflammation, with a consequent improvement in the global and gastrointestinal quality of life.

Luchetti MM, Benfaremo D, Ciccia F, Bolognini L, Ciferri M, Farinelli A, Rossini M, Mosca P, Triolo G, Gabrielli A. Adalimumab efficacy in enteropathic spondyloarthritis: A 12-mo observational multidisciplinary study. *World J Gastroenterol* 2017; 23(39): 7139-7149 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7139.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7139

INTRODUCTION

Enteropathic spondyloarthritis (ES) is a seronegative spondyloarthropathy (SpA) characterized by the presence of articular inflammation in patients affected by inflammatory bowel diseases (IBDs), such as Crohn's disease (CD) or ulcerative colitis (UC)[1,2]. Arthritis is the most frequent extra-intestinal manifestation in patients with IBDs[1] and it may primarily involve the axial joints, presenting with definite ankylosing spondylitis (AS) and/or isolated sacroiliitis, or peripheral joints and/or peri-articular structures, such as tendons and entheses[3]. The articular manifestations significantly affect health-related quality of life (HRQoL) of ES patients[4].

Although a link between inflammation of the joints and gut has been demonstrated[5,6], only half of ES patients are actually evaluated by a rheumatologist for proper diagnosis and, thus, for an integrated therapeutic approach through a coordinated action with the treating gastroenterologist[7]. Thus, an integrated clinical evaluation and therapeutic approach encompassing both the intestinal and articular features in ES patients, will likely be beneficial, particularly for the clinical outcomes. It has been demonstrated by recent studies that tumor necrosis factor (TNF)-alpha inhibitors could be effective therapeutic agents against ES[8], but few to date have reported on their real-life efficacy in this disease.

Herein, we have investigated the role of a gastro-rheumatologic multidisciplinary management and therapeutic approach in ES patients through evaluation of the efficacy of the TNF-alpha inhibitor adalimumab (Ada), assessing the efficacy on both gastrointestinal and rheumatologic disease activities and on the patient-reported HRQoL.

MATERIALS AND METHODS

Patients and study design

This study was carried out in a cohort of 52 patients with ES, including 31 affected by CD (59.6%) and 21 by UC (40.3%), collectively representing 23.6% of the 220 overall patient population with IBD in the

Table 1 Enteropathic spondyloarthritis patient features

	ES-AN, *n* = 52	ES-AN/Ada, *n* = 30
Crohn's disease:Ulcerative colitis	31 (60):21 (40)	19 (63):11 (37)
Males:Females	22 (42):30 (58)	17 (57):13 (43)
Age in years	47.2 ± 14.2	46.2 ± 14.4
Disease duration of IBD in years	11.3 ± 10.1	8.8 ± 7.9
Smokers:Ex-smokers	9 (17):20 (38)	
HLA-B27 positivity	5 (10)	4 (13)
Prior surgical intervention for IBD	13 (25)	5 (17)
Previous extra-intestinal disease	6 (11)	5 (17)
Eritema nodosum	2	1
Uveitis	3	3
Pioderma gangrenosum	1	1
Crohn's disease activity by CDAI		
Inactive	14 (45)	7 (37)
Moderate	10 (32)	8 (42)
Moderate-to-Severe	7 (23)	4 (21)
Ulcerative colitis activity by partial Mayo		
Mild	18 (86)	8 (73)
Moderate	3 (14)	3 (27)
Severe	0	0
Current medication at baseline		
Non-steroids anti-inflammatory drugs	3	0
Sulfasalazine	3	2
Mesalazine	25	12
Cyclosporine	1	1
Azathioprine	9	5
Oral steroids	12	7
Topical steroids	3	2
Metotrexate	2	1
Infliximab	6	4
Adalimumab	2	0
Spondyloarthritis features		
Ankylosing spondylitis according to Modified New York Criteria	16 (31)	10 (33)
Non-radiographic Axial-Spondyloarthritis by ASAS Criteria	13 (25)	10 (33)
Peripheral- Spondyloarthritis	23 (44)	10 (33)
Type of axial involvement	*n* = 29	*n* = 20
Syndesmophytosis	8 (28)	6 (30)
Bamboo spine	2 (7)	2 (10)
Sacroiliitis by MRI and/or X-ray	29 (100)	20 (100)
Type of articular involvement in Crohn's disease	*n* = 31	*n* = 19
Axial	16 (52)	11 (58)
Axial and peripheral	4	3
Peripheral only	15 (48)	8 (42)
Enthesitis	9 (29)	5 (26)
Type of articular involvement in ulcerative colitis	*n* = 21	*n* = 11
Axial	13 (62)	9 (82)
Axial and peripheral	9	5
Peripheral only	8 (38)	2 (18)
Enthesitis	4 (19)	2 (18)

Data are presented as *n*, *n* (%) or mean ± SD. CDAI: Crohn's Disease Activity Index; ES-AN: Patient cohort with enteropathic spondyloarthritis from the SPIB Program, Ancona, Italy; ES-AN/Ada: Patients of the ES-AN cohort treated with adalimumab; IBD: Inflammatory bowel disease; NSAID: Non-steroidal anti-inflammatory drug.

Spondyloarthritis in IBD Project (commonly referred to as SPIB), described elsewhere[9]. The patients' clinical and laboratory data are shown in Table 1, and the patient group is henceforth defined as the "ES-AN" cohort (Enteropathic spondyloarthritis from Ancona, Italy).

At each clinical observation, both the rheumatologist and the gastroenterologist collaborated in a shared session to develop the therapeutic strategy by applying a specifically designed algorithm (Figure 1) that was mainly based upon gastrointestinal-articular disease activity and the site of articular involvement at diagnosis (axial or peripheral arthritis). Briefly, the ES-AN patients were primarily separated into the following two groups for comparative analysis:

Group I (biological drugs-naïve group): This group encompassed three treatment subgroups. In the axial-ES-AN (Ax-ES-AN) subgroup, patients were administered Ada as first-line therapy, due to the absolute contraindication for a long-course treatment with nonsteroidal anti-inflammatory drugs (NSAIDs)

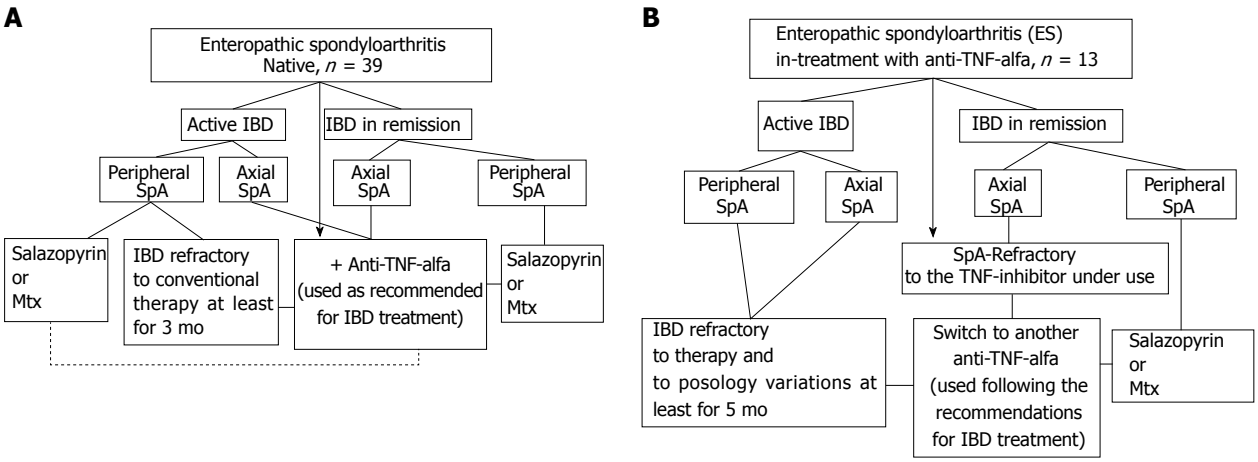

Figure 1 Therapeutic algorithm of the ES-AN patient cohort at the time of entry into the study. A: ES patients biological drugs-naïve were treated depending on IBD activity and site of the articular involvement, as follows: a) Ax-ES-AN with Ada as the TNF-inhibitor in first-line therapy, due to the absolute contraindication for a long-course treatment with NSAIDs in cases of IBDs; b) Per-ES-AN in cases of active IBD and in those patients who were non-responders to short-course corticosteroid treatment (not > 3 mo) or NSAIDs (not > 2 wk), with DMARD (methotrexate or sulfasalazine), or in cases of ESR > 30 mm/h and/or CRP > 0.5 mg/dL, and polyarticular inflammatory involvement with Ada; c) Per-ES-AN in inactive IBD, with steroids or DMARDs, depending on the number of inflamed joints and systemic inflammation (evaluated by ESR and/or CRP); B: ES patients already in treatment with infliximab for the IBD. In the ES-AN patients already treated with IFX: a) Per-ES-AN with active IBD were switched to Ada; b) Per-ES-AN with IBD in remission received a DMARD in addition to the IFX already in use; c) Ax-ES-AS were switched to Ada, regardless of IBD activity. Dashed line: Patients refractory to therapy. Therapeutic doses: DMARDs were prescribed at the standard dose regimens (salazopyrine 2 gr bid; methotrexate 10-20 mg once a week); Ada was used following the therapeutic dosage and indications for IBDs (160 mg at d 1 and 80 mg after 2 wk, followed by 40 mg every 2 wk). Ada: Adalimumab; AX-ES-AN: Patients with axial spondyloarthritis in the ES-AN cohort; CRP: C-reactive protein; ES: Enteropathic spondyloarthritis; ES-AN: Patients affected by ES in the Ancona's cohort; ESR: Erythrocyte sedimentation rate: DMARD: Disease modifying anti-rheumatic drug; IBD: Inflammatory bowel disease; NSAID: Non-steroidal anti-inflammatory drug; Per-ES-AN: Patients with peripheral spondyloarthritis in the ES-AN cohort.

in case of IBD. In the peripheral-ES-AN (Per-ES-AN) subgroup, patients with active IBD or who were non-responders to a short course of corticosteroids (not more than 3 mo) or NSAIDs (not more than 2 wk) were administered either a disease-modifying anti-rheumatic drug (DMARD), such as either methotrexate (MTX) or sulfasalazine (SSZ), or Ada if erythrocyte sedimentation rate (ESR) was > 30 mm/h and/or C-reactive protein (CRP) concentration was > 0.5 mg/dL and/or in the presence of polyarticular inflammatory involvement. In the Per-ES-AN in inactive IBDs subgroup, patients were administered steroids or DMARDs, depending on count number of inflamed joints and systemic inflammation (evaluated by ESR and/or CRP).

Group II (TNF inhibitor-treated group): This group also encompassed three treatment subgroups. For the first, the Per-ES-AN consisting of patients with still active IBD were switched to another TNF-inhibitor (Ada). For the second, the Per-ES-AN consisting of patients with IBD in remission were administered a DMARD in addition to the TNF-inhibitor already in use. For the third, the Ax-ES-AN patients were switched to Ada, regardless of IBD activity.

In all patients, the TNF-inhibitor Ada was used as recommended for IBD treatment: 160 mg in the first week, 80 mg for the next 2 wk, and thereafter 40 mg once every 2 wk.

Study measures and evaluation

All patients of the ES-AN cohort were assessed for clinical disease activity and HRQoL (Table 2). Briefly, articular (SpA) disease activity was assessed by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[10] and the Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein (ASDAS-CRP) calculation[11]; gastrointestinal (IBD) disease activity was assessed with the Crohn's Disease Activity Index (CDAI) for CD[12] and the partial Mayo (pMAYO) score for UC[13].

Patient-reported outcomes (PROs) were assessed with tests specific for articular-related symptoms [*i.e.* the Bath Ankylosing Spondylitis Functional Index (BASFI)][14] and gastrointestinal-related symptoms [*i.e.* the Inflammatory Bowel Disease Questionnaire (IBDQ)][15]. Global wellness was assessed by use of the Health Assessment Questionnaire (HAQ), Patient Global Assessment (PtGA), and the Short Form-36 health survey (SF-36)[16].

Statistical analysis

Endpoints of this study were the disease activity indexes at baseline and at 6-mo and 12-mo follow-ups. Variables are presented as mean ± standard deviation. Comparisons between groups at baseline

Table 2 Clinimetric test for articular-gastrointestinal activity and patient reported outcomes of health-related quality of life

Test	Items and interpretation
Bath Ankylosing Spondylitis Disease Index[10]	6 items: (1) fatigue, (2) back pain, (3) peripheral pain/swelling, (4) discomfort at pressure, (5) morning discomfort, and (6) duration of morning stiffness; Range from 0 to 10, with lower number representing less severe disease activity; Score > 4 = active disease.
Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein[11]	5 items: (1) back pain, (2) morning stiffness, (3) patient global, (4) peripheral pain/swelling, and (5) C-reactive protein; Score < 1.3 = inactive disease; Score 1.3 to < 2.1 = moderate activity; Score 2.1 to ⩽ 3.5 = high activity; Score > 3.5 = very high activit; Change ⩾ 1.1 = clinically important improvement; Change ⩾ 2.0 = major improvement.
Crohn's Disease Activity Index[12]	8 items: (1) liquid stools, (2) abdominal pain, (3) general well-being, (4) extra-intestinal manifestations (including arthralgia), (5) use of anti-diarrheals, (6) abdominal masses, and (7) hematocrit, 8) weight; Final score is the sum of items, weighted by different factors; Score < 150 = non-active disease; Score > 150 = active disease; Score > 450 = extremely severe disease.
Partial MAYO score[13]	3 items: (1) stool frequency, (2) rectal bleeding, and (3) physician global assessment; Range from 0 to 9; Score < 2 = disease remission; Score 2-4 = mild disease activity; Score 5-7 = moderate disease activity; Score > 7 = severe disease activity.
Bath Ankylosing Spondylitis Functional Index[14]	10 questions designed to determine the degree of functional limitation; Final score ranges from 0 to 10, with lower score indicating less functional limitation.
Inflammatory Bowel Disease Questionnaire[15]	32 questions divided into 4 subscales: (1) bowel symptoms (10 questions); (2) systemic symptoms, including sleep disorders and fatigue (5 questions); (3) emotional function, such as depression, aggression and irritation (12 questions); and (4) social function, meaning the ability to participate in social activities and to work (5 questions); The patient is invited to choose from 1 to 7 for every question; Total score ranges from 32 to 224 points, with lower scores reflecting worse quality of life.
Patient Global Assessment (PtGA)	Collected on a numeric rating scale ranging from 0 to 10 for the question asking the patient: "Considering all the ways your disease affects you, how much do you think is active today?"
Short Form-36 health survey[16]	Generic health status instrument with 8 domains: (1) physical function, (2) body pain, (3) role limitations–physical, (4) general health, (5) vitality, (6) social function, (7) role limitations–emotional, and (8) mental health; Greater scores reflect better health status; Summarized in two summary scores defined as the (1) physical component score (Sf-36/PCS) and (2) mental component score (Sf-36/MCS).

and between baseline and subsequent assessments were performed, respectively, with unpaired and paired Student's *t*-tests. Correlations between variables were assessed using Pearson's correlation coefficient. Data were analyzed using the SPSS software (v22.0; IBM SPSS Statistics for Windows, Armonk, NY, United States).

RESULTS

Baseline assessment of the patients

In the ES-AN cohort, 29 (57.6%) patients were affected by predominant axial SpA (Ax-ES-AN) and 23 (42.3%) by peripheral SpA (Per-ES-AN) (Table 1). Only 5 patients showed positivity for human leukocyte antigen-B27 (9.6%), including 4 affected by Ax-ES-AN and 1 by Per-ES-AN. Sacroiliitis was found in all the Ax-ES-AN patients, but 17 (55%) patients fulfilled the Modified New York Criteria for AS, whereas 13 (45%) were affected by non-radiographic axial-SpA[2]. Syndesmophytosis was found in 8 (27.6%) of the Ax-ES-AN patients at different levels, but only 2 (6.9%) presented a bamboo spine radiologic feature at baseline. Concurrent peripheral arthritis and enthesitis were present in about half of the Ax-ES-AN patients (44.8%).

According to the established criteria, IBD was active in 63.2% of the CD patients and in 98% of the UC patients, although the degrees of activity varied. At baseline, no differences were observed between the axial and peripheral spondyloarthritis patients and between the CD and UC patients for the articular disease activity (BASDAI, ASDAS-CRP) and the PROs [IBDQ, BASFI, PtGA, HAQ, SF-36 Physical Component Score (PCS) and Mental Component Score (MCS)] (data not shown). Following findings from the integrated evaluation and according to the algorithm presented in Figure 1, the criteria employed in our study to guide the therapeutic choice were (1) the presence and/or absence of active IBD (evaluated by both the gastroenterologist and by the results of the gastrointestinal tests and PROs); and (2) the site of articular involvement (axial or peripheral joints). Thus, Ada was assigned to a cohort of 30 patients, henceforth defined as the ES-AN/Ada cohort. Among this cohort, 4 were switched from infliximab for inefficacy, and the total was comprised of 20 patients with Ax-ES-AN and 10 patients with Per-ES-AN (Table 1).

Evaluation of articular and gastrointestinal disease activity

In most of the ES-AN/Ada patients, articular disease activity, as assessed by ASDAS-CRP and BASDAI, was significantly improved at 6-mo compared to baseline

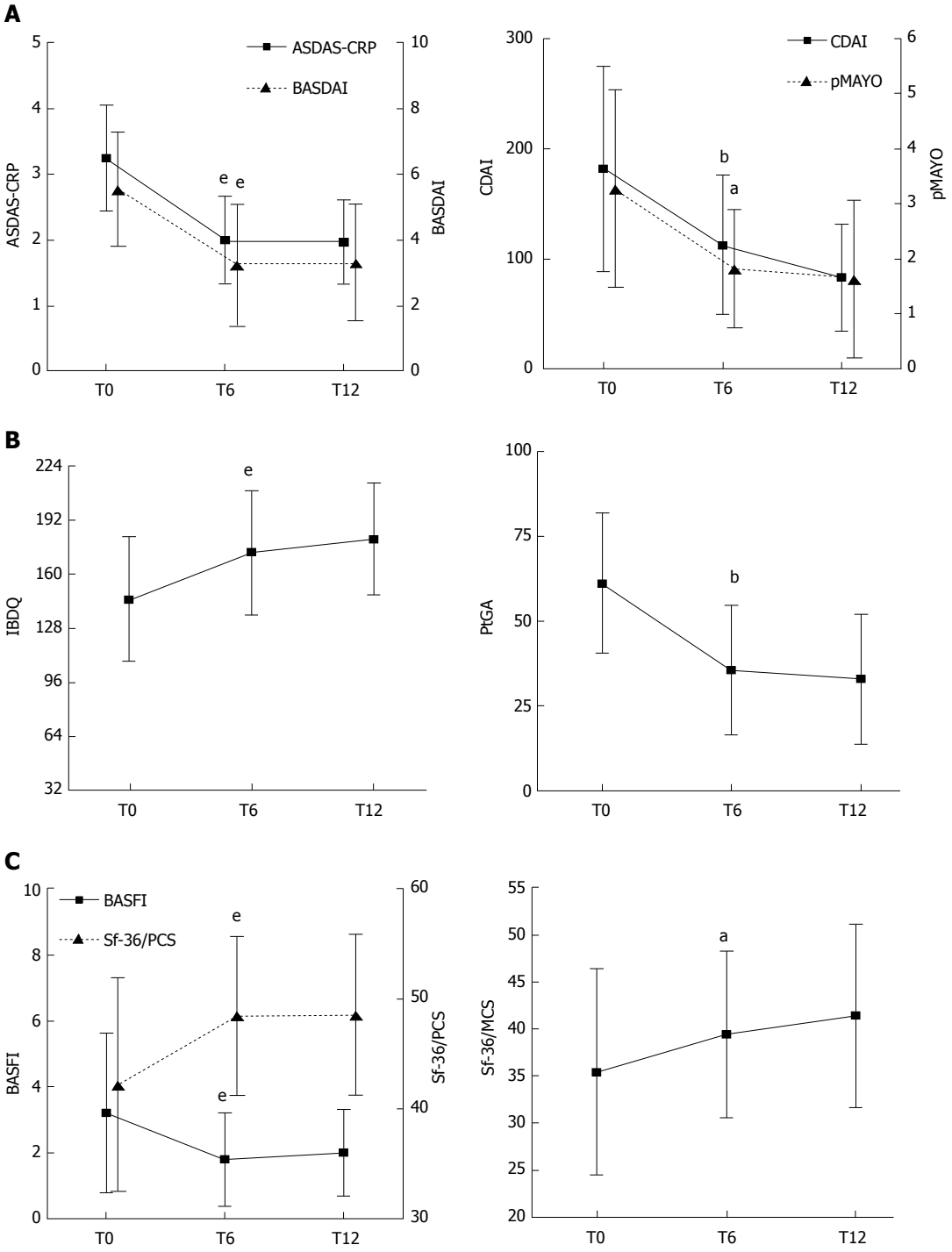

Figure 2 Evaluation of articular-gastrointestinal disease activity and HRQoL in the ES-AN patient cohort at baseline and after adalimumab therapy. All data of the ES-AN patients were collected at baseline (T0), and after 6 mo (T6) and 12 mo (T12) of therapy. A: Evaluation of the articular disease activity with BASDAI or ASDAS-CRP (Left), and of the gastrointestinal disease activity by CDAI for CD and pMAYO for UC (Right); B: Evaluation of PROs of gastrointestinal disease activity by IBDQ (Left), and of global wellness by the PtGA (Right). $^{a}P < 0.05$, $^{b}P < 0.01$, $^{e}P < 0.001$. ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score/C-Reactive Protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CDAI: Crohn's Disease Activity Index; ES: Enteropathic spondyloarthritis; ES-AN: Patients affected by ES in the Ancona's cohort; HRQoL: Health-Related Quality of Life; IBDQ: Inflammatory Bowel Disease Questionnaire; pMAYO: Partial Mayo score; PROs: Patient reported outcomes.

(ASDAS-CRP: 3.2 ± 0.8 *vs* 1.9 ± 0.6, $P < 0.001$; BASDAI: 5.5 ± 1.7 *vs* 3.2 ± 1.8, $P < 0.001$). A major clinical improvement (ASDAS change of ⩾ 2) occurred in 21% and an important clinical improvement (ASDAS change of ⩾ 1.1) occurred in 52% (Figure 2A). The improvement of articular disease was significant in both the axial and the peripheral subgroups of patients ($P < 0.001$ for both comparisons). The clinical improvement at the articular level was maintained at the 12-mo examination (Figure 2A).

Regarding the gastrointestinal disease activity, in the CD patients the treatment led to a fast, consistent

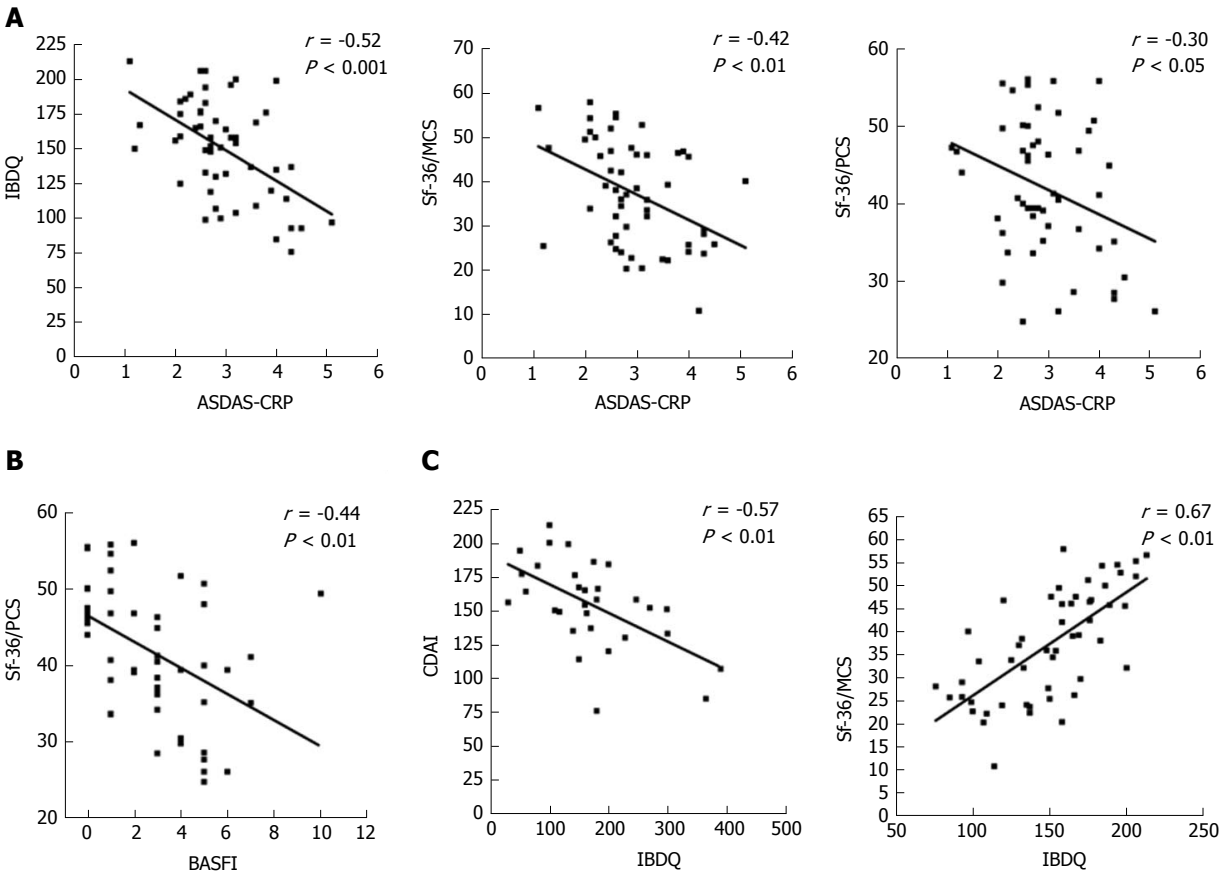

Figure 3 Correlations between articular and gastrointestinal disease activity and HRQoL scores in the ES-AN patient cohort at baseline. A: Correlation between the PROs of gastrointestinal disease activity (assessed by IBQD) scores and SF-36 summary scores (assessed by PCS and MCS) with articular activity scores (assessed by ASDAS-CRP); B: Correlation of the articular function (assessed by BASFI with SF-36/PCS); C: Correlation of the IBDQ with crohn's disease gastrointestinal disease activity (assessed by CDAI) and the SF-36/MCS. ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein; CDAI: Crohn's Disease Activity Index; ES: Enteropathic spondyloarthritis; ES-AN: Patients affected by ES in the ancona's cohort; HRQoL: Health-Related Quality of Life; IBDQ: Inflammatory Bowel Disease Questionnaire; PROs: Patient reported outcomes; SF-36/MCS: Summary of "Mental Component Score" of the Short Form-36 health survey; SF-36/PCS: Summary of "Physical Component Score" of the Short Form-36 health survey.

and significant improvement of the gastrointestinal symptoms at 6 mo, as assessed by the CDAI score (181.3 ± 93.2 *vs* 112.3 ± 63.0, $P < 0.01$; Figure 2A), and maintained at 12 mo, when the clinical remission was observed in almost all patients (Figure 2A). In parallel, a significant clinical improvement was observed also in the UC patients, as shown by the decrease of the pMAYO score at the 6-mo and 12-mo examinations (Figure 2A). All the values and comparisons are detailed in Table 3.

Evaluation of the HRQoL

ES-AN/Ada patients reported significant improvement, from baseline to 6 mo, in the IBDQ (145.3 ± 36.8 *vs* 172.8 ± 36.7, $P < 0.01$), PtGA (61.1 ± 20.6 *vs* 35.6 ± 19.1, $P < 0.01$; Figure 2B) and HAQ (4.8 ± 7.9 *vs* 2.0 ± 4.2, $P < 0.05$) scores, and this improvement was maintained at 12 mo. Moreover, regarding the PROs impacting articular function and global health wellness, significant improvements were observed from baseline to 6 mo in the BASFI (3.2 ± 2.4 *vs* 1.8 ± 1.4, $P < 0.01$), Sf-36/PCS (42.2 ± 9.7 *vs* 48.4 ± 7.3, $P < 0.01$) and Sf-36/MCS scores (35.4 ± 10.9 *vs* 39.4± 8.8, $P < 0.05$; Figure 2C); again, the improvements were maintained at 12 mo. The improvement of all the scores for PROs was similar in both the Ax-ES-AN/Ada and Per-ES-AN/Ada subgroups at each follow-up observation. All the values and comparisons are detailed in Table 3.

Correlations between variables

At baseline, it is noteworthy that a consistent significant correlation was observed between the IBDQ test (specific for the evaluation of the PROs related to the gastrointestinal disease activity) and the clinimetric scores of articular activity, as assessed by ASDAS-CRP (Figure 3A), BASDAI, and with most of the PROs of the HRQoL, as assessed by Sf-36/MCS and CDAI (Table 4). Moreover, articular disease activity at baseline was significantly correlated with worse results in the HRQoL tests, as shown by the Sf-36/PCS and Sf-36/MCS scores (Table 4). After 12 mo of Ada treatment, improvement of the articular activity (ASDAS-CRP) correlated significantly with decrease in the gastrointestinal disease activity scores, as assessed by CDAI in the CD patients (Figure 4A) and IBDQ in all of the patients, and with most of the PROs of the HRQoL, as

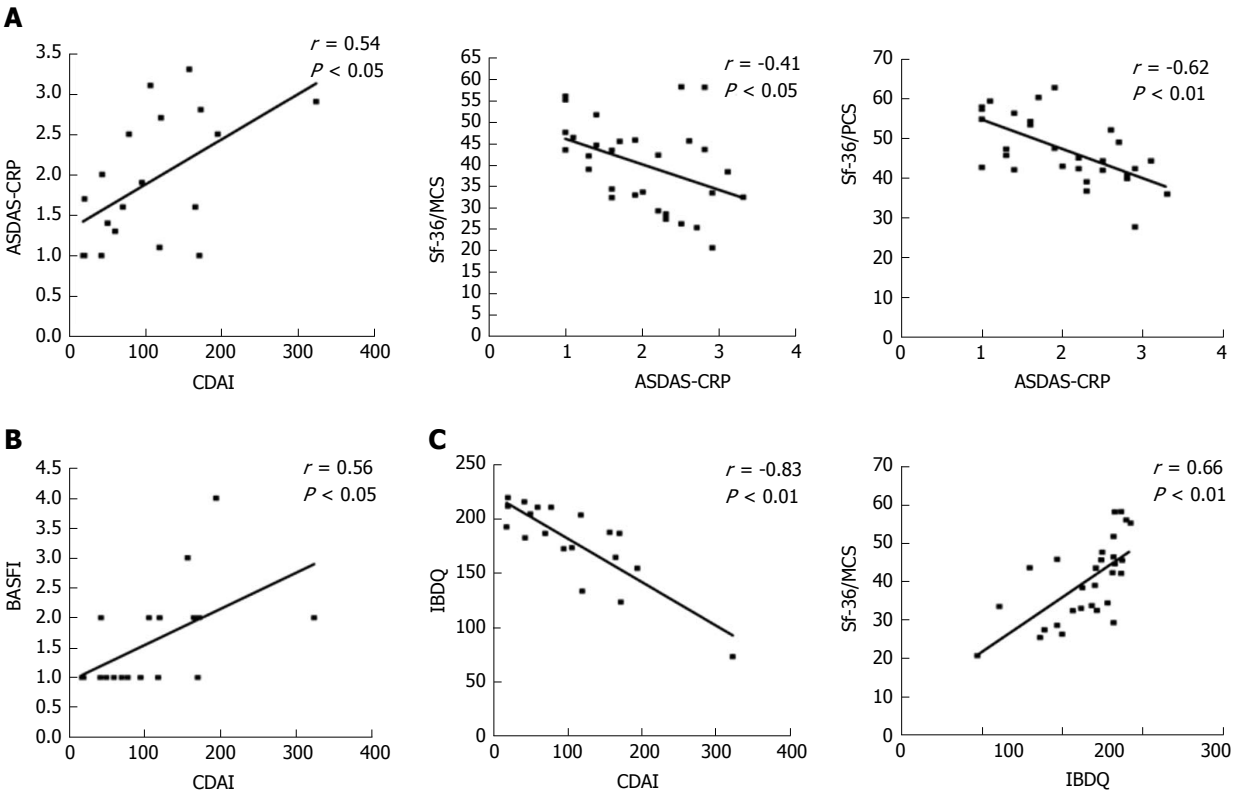

Figure 4 Correlations between articular and gastrointestinal disease activity and HRQoL scores in the ES-AN patient cohort after adalimumab therapy. A: Evaluation data collected for the 30 ES-AN/Ada patients after 12 mo of therapy, showing correlation between gastrointestinal and articular disease activity in Crohn's disease patients (assessed by CDAI) and ASDAS-CRP respectively, and between ASDAS-CRP and SF-36 summary scores (PCS and MCS); B: Correlation between CDAI and articular function (assessed by BASFI); C: Correlation between the gastrointestinal quality of life (assessed by IBDQ) and CDAI and SF-36/MCS. ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein; CDAI: Crohn's Disease Activity Index; ES-AN: Patients affected by ES in the Ancona's cohort; ES-AN/Ada: Patients of the ES-AN cohort treated with adalimumab; HRQoL: Health-Related Quality of Life; IBDQ: Inflammatory Bowel Disease Questionnaire; SF-36/MCS: Summary of "Mental Component Score" of the Short Form-36 health survey; SF-36/PCS: Summary of "Physical Component Score" of the Short Form-36 health survey; Sf-36/PCS: Summary of "Physical Component Score" of the short form-36 health survey; PROs: Patient reported outcomes.

Table 3 Scores of the clinimetric test for articular-gastrointestinal activity and patient reported outcomes of health-related quality of life

	Baseline	T6	T12
CDAI, *n* = 19	181.3 ± 93.2	112.3 ± 63.0[b]	82.7 ± 48.7[b]
pMAYO, *n* = 11	3.27 ± 1.79	1.81 ± 1.07[a]	1.63 ± 1.43[a]
IBDQ	145.3 ± 36.8	172.8 ± 36.7[e]	180.7 ± 33.0[e]
BASDAI	5.5 ± 1.7	3.2 ± 1.8[e]	3.3 ± 1.7[e]
BASFI	3.2 ± 2.4	1.8 ± 1.4[e]	2.0 ± 1.3[a]
ASDAS-CRP	3.2 ± 0.8	1.9 ± 0.6[e]	1.9 ± 0.6[e]
PtGA	61.1 ± 20.6	35.6 ± 19.1[b]	33.0 ± 19.1[b]
HAQ	4.7 ± 8.5	2.0 ± 4.2[a]	1.6 ± 3.5[a]
Sf-36/PCS	42.2 ± 9.7	48.4 ± 7.2[e]	48.5 ± 7.3[b]
Sf-36/MCS	35.4 ± 10.9	39.4 ± 8.8[a]	41.4 ± 9.7[a]
CRP in mg/dL	2.7 ± 3.7	0.8 ± 1.2[a]	0.5 ± 0.6[a]

[a]$P < 0.05$, [b]$P < 0.01$, [e]$P < 0.001$. ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; CDAI: Crohn's Disease Activity Index; CRP: C-Reactive Protein; pMAYO: partial Mayo score; HAQ: Health Assessment Questionnaire; IBDQ: Inflammatory Bowel Disease Questionnaire; PtGA: Patient Global Assessment; SF-36/MCS: Summary of "Mental Component Score" of the Short Form-36 health survey; SF-36/PCS: Summary of "Physical Component Score" of the Short Form-36 health survey; T6 and T12: Results of the evaluation after 6 mo and 12 mo of treatment with adalimumab respectively.

shown by the Sf-36/PCS (r = -0.30, $P < 0.05$) and Sf-36/MCS (r = -0.41, $P < 0.01$) scores (Figure 2C), as well as the BASFI (r = 0.57, $P < 0.001$), PtGA (r = 0.37, $P < 0.01$) and HAQ (r = 0.28, $P < 0.05$) scores. All the correlations for the values at 12-mo examination are detailed in Table 4.

Tolerability

Ada was well tolerated throughout the follow-up period. Side effects reported included recurrent upper respiratory tract infections (n = 2), bothersome hair loss (n = 1), and widespread itch (n = 2); but none necessitated suspension of the treatment. One patient suffered from a serious infection (axillary suppurative hidradenitis) that required prolonged antibiotic treatment. The other minor side effects reported were headache and fatigue.

DISCUSSION

The association between SpAs and IBDs has been known since the beginning of the last century[17]. However, gastroenterologists and rheumatologists continue to carry out their clinical evaluations and

Table 4 Correlations between the scores of clinimetric tests for articular-gastrointestinal activity and patient reported outcomes of health-related quality of life scores

	CDAI	pMAYO	IBDQ	BASDAI	BASFI	ASDAS-CRP	PtGA	HAQ	Sf-36/ PCS	Sf-36/ MCS	CRP
At baseline											
CDAI	1	1	-0.57[b]	0.19	0.29	0.21	0.14	0.12	-0.24	-0.33	0.35
pMAYO	1	1	-0.48[a]	0.34	0.38	0.37	0.15	0.18	-0.14	-0.11	0.28
IBDQ	-0.57[b]	-0.48[a]	1	-0.38[b]	-0.34[a]	-0.52[b]	-0.26	-0.19	0.27	0.67[b]	-0.26
BASDAI	0.19	0.34	-0.38[b]	1	0.64[b]	0.69[b]	0.24	0.26	-0.22	-0.31[a]	0.00
BASFI	0.29	0.38	-0.34[a]	0.64[b]	1	0.57[b]	0.37[b]	0.36[b]	-0.44[b]	-0.15	0.37[b]
ASDAS-CRP	0.21	0.37	-0.52[b]	0.69[b]	0.57[b]	1	0.37[b]	0.36[b]	-0.30[a]	-0.41[b]	0.32[a]
PtGA	0.14	0.15	-0.26	0.24	0.37[b]	0.37[b]	1	0.22	-0.14	-0.22	0.14
HAQ	0.12	0.18	-0.19	0.26	0.36[b]	0.36[b]	0.22	1	-0.46[b]	-0.03	0.07
Sf-36/PCS	-0.24	-0.14	0.27	-0.22	-0.44[e]	-0.30[a]	-0.14	-0.46[b]	1	0.05	-0.13
Sf-36/MCS	-0.33	-0.11	0.67[b]	-0.31[a]	-0.15	-0.41[b]	-0.22	-0.03	0.05	1	-0.08
After 12 mo of therapy with adalimumab (ES-AN/Ada cohort)											
CDAI	1	1	-0.83[b]	0.16	0.56[a]	0.54[a]	0.53[a]	0.53[a]	-0.71[b]	-0.67[b]	0.75[b]
pMAYO	1	1	-0.51	0.18	0.49	0.17	0.56	-0.29	-0.14	-0.25	0.6
IBDQ	-0.83[b]	-0.51	1	-0.23	-0.56[b]	-0.55[b]	-0.61[b]	-0.36[a]	0.53[b]	0.66[b]	-0.52[b]
BASDAI	0.16	0.18	-0.23	1	0.58[b]	0.68[b]	0.51[b]	0.39[a]	-0.52[b]	-0.11	-0.14
BASFI	0.56[a]	0.49	-0.56[b]	0.58[b]	1	0.56[b]	0.64[b]	0.54[b]	-0.52[b]	-0.45[a]	0.16
ASDAS-CRP	0.54[a]	0.17	-0.55[b]	0.68[b]	0.56[b]	1	0.67[b]	0.38[a]	-0.62[b]	-0.41[a]	0.31
PtGA	0.53[a]	0.56	-0.61[b]	0.51[b]	0.64[b]	0.67[b]	1	0.51[b]	-0.37[a]	-0.59[b]	0.26
HAQ	0.53[a]	-0.29	-0.36[a]	0.39[a]	0.54[b]	0.38[a]	0.51[b]	1	-0.47[b]	-0.40[a]	0.10
Sf-36/PCS	1	1	-0.83[b]	0.16	0.56[a]	0.54[a]	0.53[a]	0.53[a]	-0.71[b]	-0.67[b]	0.75[b]
Sf-36/MCS	1	1	-0.51	0.18	0.49	0.17	0.56	-0.29	-0.14	-0.25	0.60
CRP	-0.83[b]	-0.51	1	-0.23	-0.56[b]	-0.55[b]	-0.61[b]	-0.36[a]	0.53[b]	0.66[b]	-0.52[b]

Correlations between variables were assessed in the 30 patients with enteropathic spondyloarthritis from Ancona, Italy, at baseline and after 6 mo (data not shown) and 12 mo of therapy with adalimumab (ES-AN/Ada), using Pearson's correlation coefficient; [a]$P < 0.05$, [b]$P < 0.01$. ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; CDAI: Crohn's Disease Activity Index; CRP: C-Reactive Protein; HAQ: Health Assessment Questionnaire; IBDQ: Inflammatory Bowel Disease Questionnaire; pMAYO: partial Mayo score; PtGA: Patient Global Assessment; SF-36/MCS: Summary of "Mental Component Score" of the Short Form-36 health survey; SF-36/PCS: Summary of "Physical Component Score" of the Short Form-36 health survey.

the therapeutic management of these diseases, now known collectively as ES, independently. In this regard, the different clinical guidelines employed by the two medical specialists may lead to different therapeutic decisions for the same clinical scenario, ultimately harboring potential for different clinical outcomes of the disease. A critical issue in the clinical management of ES is the correct therapeutic choice, and an important role has emerged recently for the TNF-alpha inhibitors in this regard. Infliximab was the first TNF-alpha inhibitor used in ES[18], but Ada has recently been applied to ES patients affected by CD and has achieved rates of clinical remission up to 50%[19].

To our knowledge, this is the first study demonstrating the efficacy of the integrated gastrorheumatologic approach for evaluation and therapeutic management of ES patients, with efficacy evidenced through assessments of disease activity and HRQoL over a 12-mo period. As dictated by our operative algorithm, both the gastroenterologist and the rheumatologist evaluated each of the patients in the study cohort, with both of the specialists collaborating to choose the optimal therapy. This integrated multidisciplinary approach, therefore, considered the gastrointestinal and articular disease activities simultaneously and, most importantly in the latter case, the site of articular inflammation (axial or peripheral). Thus, for example, in the case of axial spondyloarthritis, the therapy with anti-TNF-alpha inhibitors was mandatory, following the latest rheumatologic indications[20].

Since only few studies in the literature have so far evaluated the efficacy and safety of anti-TNF-alpha in ES, we employed the up and coming Ada treatment in our study. The results indicated that in ES patients, regardless of IBD type and/or site of articular involvement, Ada significantly improves the inflammation states of both the joints (assessed by ASDAS-CRP and BASDAI) and gut (assessed by CDAI or pMAYO score), as well as the HRQoL. Moreover, the benefits were already observable at 6 mo and the improvements were maintained at 12 mo. Ada also consistently improved not only the physical function in almost all ES patients (assessed by PtGA, BASFI, HAQ and Sf-36/PCS) but also their psychological function (assessed by Sf-36/MCS and IBDQ), from baseline to 6 mo.

It is noteworthy that in our study the strong linear relationship between articular-gastrointestinal disease activity and the PROs confirms the strong link between gut and joint inflammations. In fact, the IBDQ (specific for the gastrointestinal-related quality of life) strongly correlated with results of the clinimetric tests of articular activity (ASDAS-CRP, BASDAI and BASFI); additionally, the articular disease activity (assessed by ASDAS-CRP) strongly correlated with all of the PROs studied, even those specific for IBDs. To

the contrary, however, at baseline the gastrointestinal disease activity scores (except for those of the IBDQ) did not correlate well with the other variables, but at 12 mo of therapy the CDAI correlated with most of the articular and PROs of HRQoL. This latter finding may be consequent to the different degrees of disease activities reported by the patients at baseline, which, after the treatment, tend to be globally ameliorated both at the gastrointestinal and articular levels.

Thus, since only the ASDAS-CRP test proved to be the most reliable in all patients in our study, we think that it should be necessary to develop composite scores and HRQoL questionnaires specific and suitable for ES patients at diagnosis, similar to those developed for other multidisciplinary diseases, such as psoriatic arthritis[21]. In conclusion, in our work that was carried out in a real-life cohort of a significant number of ES patients, we demonstrated that Ada produced a fast and significant improvement of both the gastrointestinal and articular scores of disease activity and, moreover, of the HRQoL. This clinical result was achieved by employing an integrated outpatient clinic specific for ES patients, as recently endorsed, particularly with regard to early diagnosis[22,23]. The integrated approach provided the optimal management of both the multidisciplinary clinical evaluation and the therapy of these patients. Further studies are certainly warranted to assess the long-term outcomes, and tolerability, of Ada and other TNF-alpha inhibitors in patients affected by ES.

COMMENTS

Background

Enteropathic spondyloarthritis (ES) is characterized by articular inflammation in patients with inflammatory bowel diseases (IBDs), such as Crohn's disease or ulcerative colitis. Arthritis is the most frequent extra-intestinal manifestation found in IBD patients, yet only half of the ES patients are actually evaluated by a rheumatologist for a proper diagnosis and, thereafter, for receipt of an integrated therapeutic approach through a coordinated action between the two specialists.

Research frontiers

Considering the multidisciplinary intrinsic "face" of IBDs and the novel therapeutic opportunities that comprise the biological drugs, as in the case of anti-tumor necrosis factor (TNF)-alpha inhibitors, an integrated approach and evaluation of all ES patients should be routinely employed and strongly encouraged to obtain the best therapeutic efficacy on all the clinical manifestations of this disease.

Innovations and breakthroughs

In this work, the authors have demonstrated that the integrated (simultaneous) evaluation of patients affected by ES, through the coordinated efforts of a gastroenterologist and rheumatologist, led to the best therapeutic approach, thereby allowing the patients to achieve a consistent clinical remission of both the articular and gastrointestinal inflammations.

Applications

Through this study authors have been able to generate a simple working flowchart of the multidisciplinary clinical care process applied during the patients' integrated assessment. They suggest that in patients with ES and in consideration of the therapeutic choice, particular attention should be paid to the presence of active intestinal disease, presence of active articular (arthritis) and/or periarticular (enthesitis) disease and localization of the joints' inflammation (peripheral or axial, as in the case of sacroiliitis).

Terminology

ES belongs to the group of seronegative spondyloarthritis (SpA) and, as such, is characterized by the presence of arthritis in patients affected by IBDs; it is also known as SpA-IBD.

Peer-review

This is an interesting and well-conducted work.

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ORIGINAL ARTICLE

Observational Study

Presence of columnar-lined esophagus is negatively associated with the presence of esophageal varices in Japanese alcoholic men

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Abstract

AIM

To determine whether the presence of columnar-lined esophagus (CLE) is associated with the presence of

esophageal varices (EVs) in male Japanese alcoholics.

METHODS

The subjects were 1614 Japanese alcohol-dependent men (≥ 40 years of age) who had undergone upper gastrointestinal endoscopic screening. Digitalized records of high-quality endoscopic images that included the squamocolumnar junction and esophagogastric junction were retrospectively jointly reviewed by four expert endoscopists for the purpose of diagnosing CLE. The authors investigated whether and to what extent there were associations between the presence of CLE and the presence of EVs, especially in the group with liver cirrhosis (LC).

RESULTS

CLE ≥ 5 mm in length was found in 355 subjects (≥ 30 mm in 6 of them), LC without EVs in 152 subjects, LC with EVs in 174 subjects, and EVs without LC in 6 subjects. Advanced EVs, *i.e.*, nodular, large or coiled forms, red color sign, or post-treatment, were found in 88 subjects. The incidence of CLE ≥ 5 mm decreased in the following order ($P < 0.0001$): 23.3% in the group without EVs, 17.4% in the group with small and straight EVs, and 5.7% in the group with advanced EVs. The multivariate ORs (95%CI) for EVs and advanced EVs in the group with LC were lower when CLE ≥ 5mm was present [0.46 (0.23-0.93) and 0.24 (0.08-0.74), respectively, *vs* 0-4 mm CLE].

CONCLUSION

The presence of CLE in male Japanese alcoholics was negatively associated with the presence of EVs.

Key words: Alcohol; Columnar-lined esophagus; Hiatal hernia; Liver cirrhosis; Portal hypertension; Esophageal varices

Core tip: A positive association between excessive drinking and the presence of short-segmental columnar-lined esophagus (CLE) has been reported in Asians. Endoscopic screening of 1614 Japanese alcohol-dependent men revealed the presence of CLE ≥ 5 mm in length in 355 subjects and esophageal varices (EVs) in 180 subjects. The presence of CLE was negatively associated with the presence of EVs, and even more negatively associated with the presence of advanced forms of EVs. Since the first resistance vessels to EVs are the mucosal palisade vessels and submucosal veins at the lower end of the esophagus, the development of CLE may impede the development of EVs.

Yokoyama A, Hirata K, Nakamura R, Omori T, Mizukami T, Aida J, Maruyama K, Yokoyama T. Presence of columnar-lined esophagus is negatively associated with the presence of esophageal varices in Japanese alcoholic men. *World J Gastroenterol* 2017; 23(39): 7150-7159 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7150.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7150

INTRODUCTION

Esophageal varices (EVs) develop as a result of portal hypertension, which is mainly due to liver cirrhosis (LC) in alcoholics, and excessive alcohol drinking increases the risk of variceal bleeding and mortality[1-3]. Excessive alcohol consumption has been reported to be associated with the presence of gastroesophageal reflux disease (GERD) and a short-segmental columnar-lined esophagus (CLE) in East-Asian studies[4-8]. Long-segmental CLE is rare in Asians[8], and a large pooled analysis of the cases in Western studies did not show a positive association between heavy drinking and GERD or long-segmental CLE[9]. The differences between the results in East Asia and the West may be attributable to the differences between East Asia and Western countries in abdominal obesity, incidences of *Helicobacter pylori* infection, and gene polymorphisms of alcohol-metabolizing enzymes.

Our empirical impression based on the results of endoscopic screening examinations of Japanese alcoholic men is that EVs are less common among men with short-segmental CLE. The development of CLE has been shown to be accompanied by several histological changes around the palisade vessels, including the development of a double muscularis mucosae[10,11]. Since dilatation of the palisade vessels at the lower end of the esophagus has been suspected of being one of the major initial events in the development of EVs secondary to portal hypertension[12,13], some of the histological changes accompanying the development of CLE may protect against the development of EVs.

It is widely accepted that genetic polymorphisms of alcohol dehydrogenase-1B (ADH1B, rs1229984) and aldehyde dehydrogenase-2 (ALDH2, rs671) affect the susceptibility of East-Asians to alcoholism[14-16], and the presence of fast-metabolizing ADH1B encoded by the *ADH1B*2* allele in Japanese alcoholics has been reported to be positively associated with the presence of advanced liver disease[17-19].

The aim of the present study was to determine whether and to what extent associations exist between the presence of EVs and the presence of CLE in Japanese alcoholic men based on the results of endoscopic screening examinations and their ADH1B/ALDH2 genotypes.

MATERIALS AND METHODS

Subjects

The reference population of this study consisted of 1902 Japanese alcoholic men 40 years of age and over who: (1) Came to the Kurihama Medical and Addiction Center for treatment of alcohol dependence for the first

time between May 2004 and December 2011; (2) Were evaluated for the presence of physical comorbidities; (3) Underwent routine upper gastrointestinal endoscopic screening; (4) And underwent ADH1B and ALDH2 genotyping[19]. After excluding the 194 subjects with a history of either gastrectomy or treatment for esophageal cancer and the 94 subjects without a digitalized record of high-quality endoscopic images that included the squamocolumnar junction and esophagogastric junction, 1614 patients were selected as subjects.

All of the alcoholics who participated in this study met the DSM-Ⅳ criteria for alcohol dependence[20]. Just before the endoscopic screening examination we asked each participant when he was in a sober state about his drinking and smoking habits. Usual alcohol consumption during the preceding year was expressed in grams of ethanol per day calculated by using standard conversion factors for alcoholic beverages. Beer and low-malt beer were assumed to be 5% ethanol (v/v); wine, 12%; sake, 16%; shochu, 25%; and whiskey, 40%.

The clinical diagnoses of comorbidities were made after alcohol detoxification. Patients received a routine examination that included a physical examination, blood tests, chest X-ray and abdominal X-ray, upper gastrointestinal endoscopy, abdominal ultrasound examination, and abdominal computed tomography. The clinical diagnosis of LC was made on the basis of the results of the physical examination, blood tests, and imaging studies or detection of esophagogastric varices during the endoscopic examination. The severity of LC was graded according to the Child-Pugh scoring system based on the findings at the first visit[21]. Hepatitis B surface (HBs) antigen and second generation anti-hepatitis C (HCV) antibody were measured with Abbott enzyme immunosorbent assays (Abbott Japan Inc., Tokyo).

Endoscopic procedure

Endoscopy was performed with an Olympus XQ230, Q240, Q240Z, or Q260 panendoscope (Olympus Optical Co. Ltd., Tokyo, Japan). Esophagogastric varices were diagnosed according to the grading system for esophagogastric varices adopted by the Japanese Society for Portal Hypertension[22]; *e.g.*, based on their form [small and straight (F1), nodular (F2), and large or coiled (F3)] and red color (RC) sign (Figure 1). The severity of portal hypertensive gastropathy (PHG) was evaluated according to Toyonaga's grading system, which has been adopted by the Japanese Society for Portal Hypertension (Figure 2)[23]: All PHG lesions exhibit a snake-skin (mosaic) pattern in their background mucosa; Grade 1, erythematous flecks or maculae; Grade 2, red spots or diffuse redness; and Grade 3, intramucosal or luminal hemorrhage. Three patients could not be evaluated for PHG because of food residue in the stomach.

Assessment for columnar-lined esophagus and hiatal hernia

The digitalized images that included the squamocolumnar junction and esophagogastric junction acquired before advancing the endoscope into the stomach were stored by the medical imaging communication system. The endoscopic digitalized images were retrospectively assessed during a joint review by four expert endoscopists (Yokoyama A, Hirata K, Nakamura R, Omori T) to diagnose CLE and hiatal hernia according to the classification system adopted by the Japan Esophageal Society[24]. The endoscopic esophagogastric junction was defined as the lower limit of the palisade longitudinal vessels (Figure 3)[10,11]. It was defined as the upper limit of the gastric mucosal folds when the lower limit of the palisade vessels was unclear. The greatest axial lengths of CLE were classified into four categories: 0-4 mm, 5-9 mm, 10-29 mm, and $\geqslant$ 30 mm. The axial length of a hiatus hernia was defined as the distance between the esophagogastric junction and the hiatus represented by the diaphragmatic pinch. The images were examined for the presence or absence of a hiatal hernia whose axial length was $\geqslant$ 10 mm.

Alcohol dehydrogenase-1B and aldehyde dehydrogenase-2 genotyping

ADH1B and ALDH2 genotyping of every subject was performed by PCR-RFLP methods on a lymphocyte DNA sample[19].

Statistical analysis

The data have been summarized as means and standard errors or as percentage values. Student's *t*-test was used to compare normally distributed continuous variables between groups; the Mann-Whitney *U*-test was used to compare non-normally distributed continuous variables between groups; and Fisher's exact test or Cochran-Mantel-Haenszel test for trend was used to compare proportions between groups. Multiple logistic regression models were used to calculate adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). A *P* value less than 0.05 was considered statistically significant. All analyses were performed by a biomedical statistician (Yokoyama T) using the SAS statistical package (version 9.4; SAS Institute, Cary, NC United States).

RESULTS

LC without EVs was diagnosed in 152 subjects, LC with EVs in 174 subjects, and EVs without LC in 6 subjects. The EVs were classified as F1 in 92 subjects, F2 or F3 in 41 subjects, RC-sign-positive in 21 subjects, and post-treatment in 26 subjects, 17 of whom were reported to have been treated for variceal rupture. All of the EVs in the subjects without LC were classified as F1. Table 1 shows the background factors of the 1614

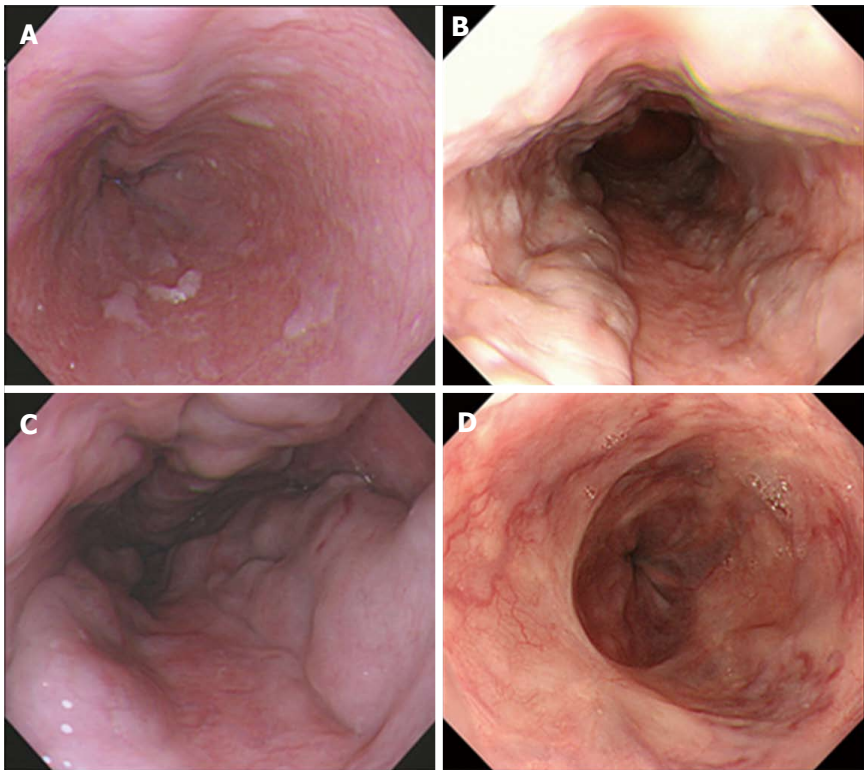

Figure 1 Classification of esophageal varices. A: F1 varices, small and straight; B: F2 varices, nodular; C: F3 varices, large or coiled; D: post-treatment varices.

Table 1 Background of the Japanese alcoholic men with and without esophageal varices *n* (%)

	Esophageal varices		
	Absent	Present	*P* value
n	1434	180	
Age (yr)			
mean ± SE	55.8 ± 0.3	54.4 ± 0.7	0.079
Alcohol consumption (g ethanol/d)			
mean ± SE	121 ± 2	133 ± 7	0.066
Ever smoker	1310 (91.4)	159 (88.3)	0.21
Liver cirrhosis			
Absent	1282 (89.4)	6 (3.3)	
Present	152 (10.6)	174 (96.7)	< 0.0001
Gastric Varices			
Absent	1418 (98.9)	147 (81.7)	
Cardia, cardia/fornix	2 (0.1)	23 (12.8)	
Fornix	14 (1.0)	10 (5.6)	< 0.0001
Portal hypertensive gastropathy	1432	179	
Absence	1172 (81.8)	59 (33.0)	
Grade 1	191 (13.3)	80 (44.7)	
Grade 2	53 (3.7)	25 (14.0)	
Grade 3	16 (1.1)	15 (8.4)	< 0.0001
Anti-HCV antibody positive	69 (4.8)	13 (7.2)	0.20
HBs antigen positive	17 (1.2)	4 (2.2)	0.28
ALDH2 genotype			
*1/*1	1216 (84.8)	161 (89.4)	
*1/*2	218 (15.2)	19 (10.6)	
*2/*2	0 (0.0)	0 (0.0)	0.12
ADH1B genotype			
*1/*1	406 (28.3)	41 (22.8)	
*1/*2	470 (32.8)	69 (38.3)	
*2/*2	558 (38.9)	70 (38.9)	0.20

ADH1B: Alcohol dehydrogenase-1B; ALDH2: Aldehyde dehydrogenase-2; *P* values were calculated by Student's *t*-test for mean values, and Fisher's exact test for percent data.

subjects according to whether they had EVs. There were no significant differences between the group with EVs and the group without EVs in age, usual alcohol consumption, smoking, hepatitis C and B infection status, or ALDH2 and ADH1B genotypes. LC, gastric varices, and higher grade PHG were more common in the group with EVs than in the group without EVs ($P < 0.0001$). Most of the gastric varices in the group without EVs were found in the fornix alone, and gastric varices located in the cardia or cardia plus fornix predominanted in the group with EVs.

CLE $\geqslant$ 5 mm was found in 355 (22.0%) of the subjects (5-9 mm, 13.6%; 10-29 mm, 8.1%; and $\geqslant$ 30 mm, 0.4%; Table 2). When advanced forms of EVs were defined as F2 - F3 varices, RC-sign-positive varices, and post-treatment varices, the proportion of subjects with CLE $\geqslant$ 5 mm decreased in the following order ($P < 0.0001$): 23.3% in the group without EVs, 17.4% in the group with F1 varices, and 5.7% in the group with advanced varices, and the findings were similar in the subgroup of subjects with LC ($P = 0.0004$). The incidence of CLE $\geqslant$ 5 mm in the group of subjects with advanced EVs did not differ significantly according to whether they had or had not received endoscopic treatment (7.7% and 4.8%, respectively, $P = 0.63$). The proportions with a hiatal hernia $\geqslant$ 10 mm (24.4%, 20.7%, and 13.6%, respectively) decreased in the same order as the order in which the proportions of subjects with CLE $\geqslant$ 5 mm decreased ($P = 0.017$), but the trend was not significant in the subgroup of subjects with LC.

Table 2 Columnar-lined esophagus and hiatal hernia and the degree of esophageal varices *n* (%)

Esophageal varices	Absent	F1 varices	Advanced varices	*P* value
n	1434	92	88	
Columnar-lined esophagus				
0-4 mm	1100 (76.7)	76 (82.6)	83 (94.3)	
⩾ 5 mm	334 (23.3)	16 (17.4)	5 (5.7)	< 0.0001
5-9 mm	208 (14.5)	8 (8.7)	3 (3.4)	
10-29 mm	120 (8.4)	8 (8.7)	2 (2.3)	
⩾ 30 mm	6 (0.4)	0 (0.0)	0 (0.0)	
Hiatal hernia ⩾ 10 mm				
Absent	1084 (75.6)	73 (79.3)	76 (86.4)	
Present	350 (24.4)	19 (20.7)	12 (13.6)	0.017
Number of patients with liver cirrhosis	152	86	88	
Columnar-lined esophagus				
0-4 mm	117 (77.0)	73 (84.9)	83 (94.3)	
⩾ 5 mm	35 (23.0)	13 (15.1)	5 (5.7)	0.0004
5-9 mm	22 (14.5)	8 (9.3)	3 (3.4)	
10-29 mm	12 (7.9)	5 (5.8)	2 (2.3)	
⩾ 30 mm	1 (0.7)	0 (0.0)	0 (0.0)	
Hiatal hernia ⩾ 10 mm				
Absent	124 (81.6)	71 (82.6)	76 (86.4)	
Present	28 (18.4)	15 (17.4)	12 (13.6)	0.36

Advanced varices: F2-F3 varices, red-color-sign positive varices, or post-treatment varices. *P* values are for trend calculated by the Cochran-Mantel-Haenszel test.

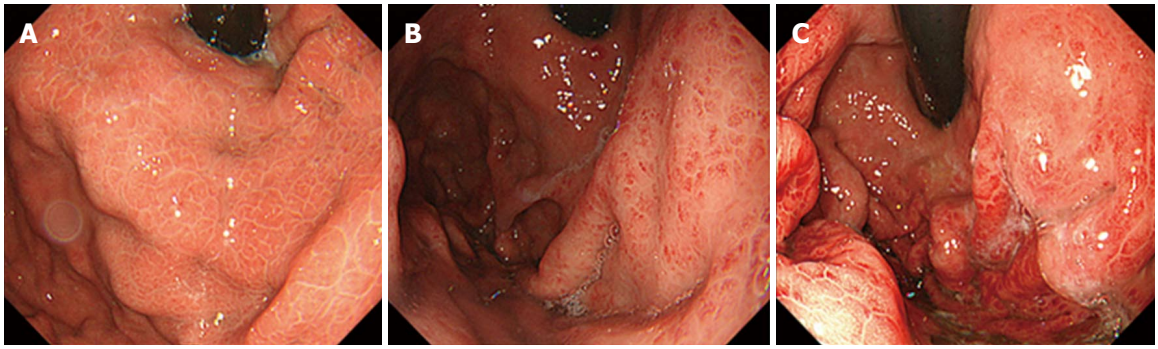

Figure 2 Portal hypertensive gastropathy lesions exhibiting a snake-skin (mosaic) pattern in their background mucosa. A: Grade 1, erythematous flecks or maculae; B: Grade 2, red spots or diffuse redness; C: Grade 3, intramucosal or luminal hemorrhage.

Table 3 shows the background factors of the 1614 subjects according to whether they had CLE ⩾ 5 mm, and there were no significant differences between the two groups in age, usual alcohol consumption, smoking, or ALDH2 and ADH1B genotypes. Hiatal hernia was more common in the group with CLE ⩾ 5 mm than in the other group (45.9% *vs* 17.3%, *P* < 0.0001). There was a significant difference between the group with CLE ⩾ 5 mm and the group without CLE ⩾ 5 mm in the proportions of 'no LC and no EVs' subjects, 'LC and no EVs' subjects, and the 'EVs' subjects they contained (*P* = 0.001). There were no significant difference between the group with CLE ⩾ 5 mm and group without CLE ⩾ 5 mm in the presence of gastric varices or the grade of PHG.

A multiple logistic regression analysis to predict the presence of CLE ⩾ 5 mm showed that the ORs (95%CI) for CLE ⩾ 5 mm increased with age per +10 years [1.15 (1.00-1.32)] and with the presence of hiatal hernia ⩾ 10 mm [4.33 (3.31-5.67)] and decreased with the presence of EVs [0.47 (0.26-0.87); Table 4]. The presence of LC, presence of gastric varices, and grade of PHG were not associated with the presence of CLE ⩾ 5 mm.

Table 5 shows the background factors of the 326 subjects with LC according to whether they had EVs, and there were no significant differences between the two groups in age, usual alcohol consumption, smoking, hepatitis C and B infection status, or ALDH2 and ADH1B genotypes. The absence of CLE ⩾ 5 mm (*P* = 0.003), presence of gastric varices (*P* < 0.0001), and higher grade PHG (*P* < 0.0001) were all more common in the LC group with EVs than in the LC group without EVs. Advanced Child-Pugh class (*P* = 0.011) was more common in the LC group with advanced EVs.

A multiple logistic regression analysis showed

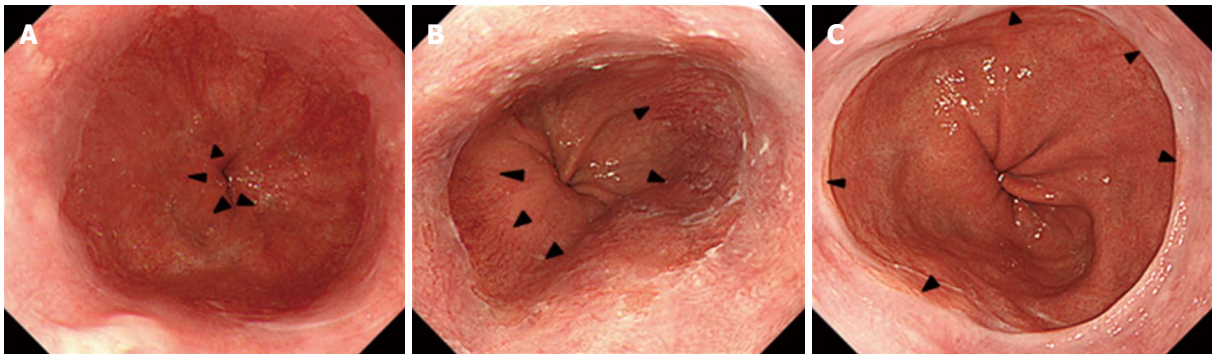

Figure 3 Columnar-lined esophagus and hiatal hernia. A: CLE 10-29 mm; B: CLE 5-9 mm; C: CLE 0-4 mm and hiatal hernia ⩾ 10 mm. The endoscopic esophagogastric junction was defined as the lower limit of the palisade longitudinal vessels (arrows). The axial length of a hiatal hernia was defined as the distance between the esophagogastric junction and the hiatus represented by the diaphragmatic pinch. CLE: Columnar-lined esophagus.

Table 3 Background of the Japanese alcoholic men with and without a columnar-lined esophagus *n* (%)

	Columnar-lined esophagus ⩾ 5 mm		
	Absent	Present	*P* value
n	1259	355	
Age (yr), mean ± SE	55.6 ± 0.3	55.8 ± 0.5	0.75
Usual alcohol consumption (g ethanol), mean ± SE	123 ± 2	121 ± 4	0.69
Ever smoker	1151 (91.4)	318 (89.6)	0.29
Hiatal hernia ⩾ 10 mm	218 (17.3)	163 (45.9)	< 0.0001
No liver cirrhosis and no EVs	983 (78.1)	299 (84.2)	
Liver cirrhosis and no EVs	117 (9.3)	35 (9.9)	
EVs	159 (12.6)	21 (5.9)	0.001
Gastric varices			
Absent	1215 (96.5)	350 (98.6)	
Cardia, cardia and fornix	23 (1.8)	2 (0.6)	
Fornix	21 (1.7)	3 (0.8)	0.13
Portal hypertensive gastropathy	1256	355	
Absent	947 (75.4)	284 (80.0)	
Grade 1	218 (17.4)	53 (14.9)	
Grade 2,3	91 (7.2)	18 (5.1)	0.17
ALDH2 genotype,			
*1/*1	1077 (85.5)	300 (84.5)	
*1/*2	182 (14.5)	55 (15.5)	
*2/*2	0 (0.0)	0 (0.0)	0.61
ADH1B genotype,			
*1/*1	348 (27.6)	99 (27.9)	
*1/*2	407 (32.3)	132 (37.2)	
*2/*2	504 (40.0)	124 (34.9)	0.15

ADH1B: Alcohol dehydrogenase-1B; ALDH2: Aldehyde dehydrogenase-2; EVs: Esophageal varices; *P* values are according to the results of Student's *t*-test for age; the Mann-Whitney *U*-test for alcohol consumption; and Fisher's exact test for the other variables.

that the ORs (95%CI) for EVs and advanced EVs in the subgroup of subjects with LC decreased with the presence of CLE ⩾ 5 mm [0.46 (0.23-0.93) and 0.24 (0.08-0.74), respectively, *vs* CLE 0-4 mm; Table 6]. Child-Pugh class was not associated with EV status. The ORs for EVs and advanced EVs increased with progression of the grade of PHG, and the OR for advanced EVs increased with the presence of gastric varices.

DISCUSSION

The analysis of the results of endoscopic screening of 1614 Japanese alcoholic men in this study demonstrated that the presence of CLE was negatively associated with the presence of EVs, and even more negatively associated with the presence of advanced forms of EVs. The prevalence of CLE ⩾ 30 mm has been reported to be very low (0.0%-0.2%) in Asians[8,25-27]. The prevalence of CLE ⩾ 30 mm in the present study was 0.4%, and most of the CLE was short-segmental. The presence of a hiatal hernia was a strong determinant of the presence of CLE in this study, a finding that was consistent with the results of previous studies[5,7,8,26,28], suggesting that GERD in patients with a hiatal hernia contributes to the development of CLE. Asian studies have demonstrated positive associations between excessive alcohol consumption and both GERD and short-segmental CLE[4-8,26]. The prevalence of CLE ⩾ 10 mm has been reported to be 1.8% in unselected Taiwanese, 4.0% in symptomatic Koreans[27], and 5.3% in a group of Japanese who underwent an endoscopic examination in a university hospital[8]. Thus, the high prevalence (8.1%) of CLE ⩾ 10 mm in the population of alcoholics in our study was at least in part attributable to chronic heavy drinking.

As expected, the presence of EVs was positively associated with the presence of LC, gastric varices, and PHG. However, these factors were not associated with the presence of CLE, and adjustment for these factors revealed a negative association between the presence of CLE and the presence and severity of EVs. These findings taken together suggested a special relationship between CLE and EVs in the presence of LC and portal hypertension.

In the majority of LC patients the left gastric vein is the afferent vein to the EVs[29], and the first resistance vessels to the EVs are the mucosal palisade vessels and submucosal veins at the lower end of the

Table 4 Multivariate analyses to predict the presence of columnar-lined esophagus in Japanese alcoholic men

	CLE, ⩾ 5 mm *vs* 0-4 mm		
Independent variables	**OR**	**95%CI**	***P* value**
Age, per +10 yr	1.15	(1.00-1.32)	0.043
Usual alcohol consumption, per +22 g ethanol	0.99	(0.96-1.03)	0.75
Ever smoker *vs* never smoker	0.75	(0.49-1.14)	0.17
Hiatal hernia ⩾ 10 mm, presence *vs* absence	4.33	(3.31-5.67)	< 0.0001
Liver cirrhosis, presence *vs* absence	1.04	(0.68-1.60)	0.86
Esophageal varices, presence *vs* absence	0.47	(0.26-0.87)	0.015
Gastric varices, presence *vs* absence	0.59	(0.21-1.64)	0.31
Portal hypertensive gastropathy			
Absent	1.00	Referent	
Grade 1	1.23	(0.86-1.78)	0.26
Grade 2,3	0.91	(0.52-1.60)	0.74
Anti-HCV antibody, positive *vs*, negative	0.93	(0.53-1.66)	0.81
HBs antigen, positive *vs*, negative	0.56	(0.13-2.47)	0.44
ALDH2 genotype, *1/*1 *vs* *1/*2	0.94	(0.67-1.33)	0.73
ADH1B genotype, *2 carrier *vs* *1/*1	0.97	(0.73-1.28)	0.81

ADH1B: Alcohol dehydrogenase-1B; ALDH2: Aldehyde dehydrogenase-2; OR: Multivariate odds ratio; CI: Confidence interval; CLE: Columnar-lined esophagus. ORs and CIs were calculated by using a multiple logistic regression model.

Table 5 Background of the Japanese alcoholic men with liver cirrhosis according to whether they had esophageal varices *n* (%)

	Esophageal varices				
	Absent	**All forms**	***P* value**	**Advanced forms**	***P*[1] value**
n	152	174		88	
Age (yr)					
mean ± SE	55.9 ± 0.7	54.5 ± 0.7	0.16	54.3 ± 1.0	0.21
Alcohol consumption (g ethanol/d)					
mean ± SE	124 ± 7	133 ± 7	0.37	142 ± 11	0.18
Ever smoker	132 (86.8)	155 (89.1)	0.61	83 (94.3)	0.081
Columnar-lined esophagus ⩾ 5 mm	35 (23.0)	18 (10.3)	0.003	5 (5.7)	0.0005
Hiatal hernia ⩾ 10 mm	28 (18.4)	27 (15.5)	0.55	12 (13.6)	0.37
Child-Pugh class					
A	93 (61.2)	88 (50.6)		41 (46.6)	
B	50 (32.9)	64 (36.8)		32 (36.4)	
C	9 (5.9)	22 (12.6)	0.053	15 (17.0)	0.011
Gastric varices					
Absent	137 (90.1)	141 (81.0)		65 (73.9)	
Cardia, cardia and fornix	2 (1.3)	23 (13.2)		17 (19.3)	
Fornix	13 (8.6)	10 (5.7)	< 0.0001	6 (6.8%)	< 0.0001
Portal hypertensive gastropathy	151	173			
Absent	88 (58.3)	55 (31.8)		27 (30.7)	
Grade 1	50 (33.1)	78 (45.1)		39 (44.3)	
Grade 2	10 (6.6)	25 (14.5)		12 (11.4)	
Grade 3	3 (2.0)	15 (8.7)	< 0.0001	15 (8.4)	< 0.0001
Anti-HCV antibody positive	8 (5.3)	13 (7.5)	0.50	8 (9.1)	0.29
HBs antigen positive	4 (2.6)	4 (2.3)	1.00	1 (1.1)	0.65
ALDH2 genotype					
*1/*1	134 (88.2)	156 (89.7)		77 (87.5)	
*1/*2	18 (11.8)	18 (10.3)		11 (12.5)	
*2/*2	0 (0.0)	0 (0.0)	0.72	0 (0.0)	1.00
ADH1B genotype					
*1/*1	32 (21.1)	36 (20.7)		15 (17.0)	
*1/*2	51 (33.6)	68 (39.1)		37 (42.0)	
*2/*2	69 (45.4)	70 (40.2)	0.55	36 (40.9)	0.41

[1]Advanced forms of EVs *vs* no EVs. ADH1B: Alcohol dehydrogenase-1B; ALDH2: Aldehyde dehydrogenase-2; EVs: Esophageal varices; *P* values were calculated by Student's *t*-test for mean values, and Fisher's exact test for percent data.

esophagus, where there are many palisade vessels and few submucosal veins[12,13]. The palisade vessels penetrate the muscularis mucosae and connect to the submucosal veins 3-5 cm above the esophagogastric junction, the critical area for EV rupture[12,30,31]. Greatly enlarged palisade vessels disrupt the muscularis mucosae and form EVs across the proper mucosae and the submucosa[13], and they cause advanced EVs by

Table 6 Multivariate analyses to predict the presence of esophageal varices in Japanese alcoholic men with liver cirrhosis

	Cirrhosis with EVs *vs* cirrhosis without EVs			
	All forms of EVs		Advanced forms of EVs	
Independent variables	OR (95%CI)	*P* value	OR (95%CI)	*P*[1] value
Age, per +10 yr	0.88 (0.67-1.16)	0.35	0.90 (0.64-1.26)	0.55
Usual alcohol consumption, per +22 g ethanol	1.00 (0.94-1.06)	0.97	1.02 (0.95-1.10)	0.52
Ever smoker *vs* never smoker	1.04 (0.50-2.16)	0.93	2.13 (0.69-6.57)	0.19
Columnar-lined esophagus, ⩾ 5 mm *vs* 0-4 mm	0.46 (0.23-0.93)	0.030	0.24 (0.08-0.74)	0.013
Hiatal hernia ⩾ 10 mm, presence *vs* absence	1.10 (0.54-2.21)	0.80	0.97 (0.38-2.49)	0.95
Child-Pugh class				
A	1.00 Referent		1.00 Referent	
B	1.18 (0.70-1.98)	0.53	1.06 (0.55-2.05)	0.87
C	1.56 (0.64-3.82)	0.33	1.85 (0.67-5.16)	0.24
Gastric varices, presence *vs* absence	1.94 (0.96-3.91)	0.065	2.89 (1.29-6.47)	0.010
Portal hypertensive gastropathy				
Absent	1.00 Referent		1.00 Referent	
Grade 1	2.24 (1.32-3.80)	0.003	1.92 (0.97-3.77)	0.060
Grade 2,3	4.40 (2.11-9.15)	< 0.0001	5.29 (2.18-12.81)	0.0002
Anti-HCV antibody, positive *vs* negative	1.09 (0.41-2.93)	0.87	1.54 (0.49-4.88)	0.46
HBs antigen, positive *vs*, negative	1.16 (0.26-5.16)	0.85	0.61 (0.06-6.36)	0.68
ALDH2 genotype, *1/*1 *vs* *1/*2	1.02 (0.47-2.20)	0.96	1.00 (0.38-2.60)	1.00
ADH1B genotype, *2 carrier *vs* *1/*1	1.10 (0.61-1.99)	0.76	1.59 (0.72-3.51)	0.25

[1]Advanced forms of EVs *vs* no EVs, EVs: Esophageal varices; ADH1B: Alcohol dehydrogenase-1B; ALDH2: Aldehyde dehydrogenase-2; OR: Multivariate odds ratio; ORs and CIs were calculated by using a multiple logistic regression model; Advanced forms of EVs were F2-F3 varices, red-color-sign positive varices, or post-treatment varices.

connecting with submucosal veins or sub- and intra-epithelial channels accompanied by 'varices on varices' and positive RC signs[12,13,32].

Although there is no difference between the maximal size of the palisade vessels in the CLE and normal lower esophagus[11], it is conceivable that the development of CLE may change the microenvironment of the interstitium around the palisade vessels, *e.g.*, increased interstitial fibrosis and the formation of the shallow muscularis mucosae of a double muscularis mucosae. A double muscularis mucosae was seen in 71% of the specimens obtained by endoscopic resection of a CLE[11]. The double muscularis mucosae in CLE divides the proper mucosae into two restricted compartments, and may increase the resistance to enlargement of palisade vessels and prevent the vessels from communicating with the submucosal vessels or sub- and intra-epithelial channels[13,32], thereby inhibiting the development of enlarged EVs and RC signs. Scars secondary to endoscopic ligation or sclerotherapy were present in some of the LC subjects with post-treatment varices and their presence may have influenced the development of CLE. However, the incidence of CLE in the LC group did not differ significantly according to whether there was a history of endoscopic treatment.

The presence of the *ADH1B*2* allele in Japanese alcoholics has been demonstrated to be positively associated with the presence of advanced liver disease and the progression of Child-Pugh class[17-19], but the results of the present study showed no significant associations between the genetic polymorphism and the presence of EVs. The effect of the *ADH1B*2* allele on EVs was probably eclipsed, because 46.6% of the LC subjects lacked EVs.

Our study had several potential limitations. The first potential limitation was that it was a cross-sectional study based on the results of the endoscopic screening, and the progression of CLE and EVs was not evaluated directly. Identification of a causal relationship between these endoscopic findings would require longitudinal follow-up examinations. The second potential limitation was that we evaluated the degree of CLE by retrospectively reviewing the endoscopic images, however, the review was performed jointly by four expert endoscopists. Pathological studies of autopsied cirrhotic subjects are warranted to clarify the pathological background underlying the negative association between the presence of CEL and the presence of EVs. We did not observe any effects of alcohol consumption during the preceding year on the presence of CLE or EVs, but that may have been because of the homogeneity of the study population in terms of their extremely high alcohol consumption. Generalization of the results obtained in our study based on investigations of alcoholic men treated in the Center requires confirmation in various drinking populations, including in a population with mild alcoholism.

Helicobacter pylori infection and chronic atrophic gastritis protect against the development of GERD and CLE[5,6,33]. If the observed associations between CLE and EVs and advanced EVs reflect causal relationships, the current trend toward lower *Helicobacter pylori* infection rates in Japan may result in lower EV rates and advanced EV rates in the future, and examination for CLE may benefit alcoholics with advanced liver disease by identifying their risk for the development or

progression of EVs.

In conclusion, this cross-sectional observational study revealed a negative association between the presence of CLE and the presence of EVs in Japanese alcoholic men. Further studies should be conducted prospectively in a longitudinal fashion to confirm this finding.

ARTICLE HIGHLIGHTS

Research background

Esophageal varices (EVs) develop as a result of portal hypertension, which is mainly due to liver cirrhosis (LC) in alcoholics. Excessive alcohol consumption has been reported to be associated with the presence of a short-segmental columnar-lined esophagus (CLE) in East-Asian studies. It has not been evaluated whether associations exist between the presence of EVs and the presence of CLE in alcoholics.

Research motivation

Our empirical impression based on the results of endoscopic screening examinations of Japanese alcoholic men is that EVs are less common among men with short-segmental CLE. The development of CLE is accompanied by several histological changes around the palisade vessels at the lower end of the esophagus. Some of the histological changes accompanying the development of CLE may protect against the development of EVs.

Research objectives

To determine whether and to what extent associations exist between the presence of EVs and the presence of CLE in Japanese alcoholic men based on the results of endoscopic screening examinations.

Research methods

The subjects were 1614 Japanese alcohol-dependent men (≥ 40 years of age) who had undergone upper gastrointestinal endoscopic screening. Digitalized records of high-quality endoscopic images that included the squamocolumnar junction and esophagogastric junction were retrospectively jointly reviewed by four expert endoscopists for the purpose of diagnosing CLE. The authors investigated whether and to what extent there were associations between the presence of CLE and the presence of EVs, especially in the group with LC.

Research results

CLE ≥ 5 mm in length was found in 355 subjects, LC without EVs in 152 subjects, LC with EVs in 174 subjects, and EVs without LC in 6 subjects. Advanced EVs, *i.e.*, nodular, large or coiled forms, red color sign, or post-treatment, were found in 88 subjects. The incidence of CLE ≥ 5 mm decreased in the following order ($P < 0.0001$): 23.3% in the group without EVs, 17.4% in the group with small and straight EVs, and 5.7% in the group with advanced EVs. The multivariate ORs (95%CI) for EVs and advanced EVs in the group with LC were lower when CLE ≥ 5 mm was present [0.46 (0.23-0.93) and 0.24 (0.08-0.74), respectively, *vs* 0-4 mm CLE]. To clarify the pathological backgrounds of the negative association between the presence of CEL and the presence of EVs, the further pathological studies of autopsied cirrhotic subjects may be warranted.

Research conclusions

The presence of CLE was negatively associated with the presence of EVs, and even more negatively associated with the presence of advanced forms of EVs. Since the first resistance vessels to EVs are the mucosal palisade vessels and submucosal veins at the lower end of the esophagus, the development of CLE may impede the development of EVs. *Helicobacter pylori* infection and chronic atrophic gastritis protect against the development of GERD and CLE. If the observed associations between CLE and EVs and advanced EVs reflected causal relationships, the current trend toward to lower *Helicobacter pylori* infection rates in Japan may influence EV rates and advanced EV rates in the future, and examination for CLE may benefit alcoholics with advanced liver disease by identifying their risk for the development or progression of EVs. The further studies should be conducted prospectively in the longitudinal fashion to test this finding.

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ORIGINAL ARTICLE

Observational Study

Characteristics and outcomes of cholangiocarcinoma by region in Thailand: A nationwide study

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Abstract

AIM

To identify the potential risk factors of cholangiocarcinoma, we determined the characteristics of cholangiocarcinoma patients among 5 different regions of Thailand.

METHODS

All patients diagnosed with cholangiocarcinoma between 2008 and 2013 were identified using the Nationwide Hospital Admission Data registry (n = 39421). Baseline characteristics, comorbidities and survival were abstracted.

RESULTS

The annual incidence during the study period was stable in all regions. Most patients lived in the Northeast (62.8%), followed by the North (16.9%), Central (12.3%), Bangkok (5.4%), and South (n = 2.6%) regions (P < 0.0001). Significantly more cholangiocarcinoma patients had diabetes, cirrhosis, and chronic viral hepatitis B/C infection than non-cholangiocarcinoma participants (diabetes: 11.42% *vs* 5.28%; cirrhosis: 4.81% *vs* 0.92%; hepatitis B: 0.74% *vs* 0.12%; and hepatitis C: 0.50% *vs* 0.10%, P < 0.0001 for all, respectively). The overall 1-year mortality rate was 81.7%, with a stable trend over time.

CONCLUSION

Diabetes and chronic liver diseases may be associated with cholangiocarcinoma in the Thai population.

Key words: Bile duct cancer; Population-based study; Epidemiology; Liver fluke infection

Core tip: Cholangiocarcinoma is highly prevalent in Thailand, particularly in the Northeast region. The high cholangiocarcinoma incidence in this region is known to be associated with a high prevalence of liver fluke infection. Cirrhosis, diabetes, and chronic viral hepatitis B and C infections have been recently identified as risk factors for cholangiocarcinoma in Western countries. In this study, we found that diabetes and chronic liver diseases may be associated with cholangiocarcinoma in the Thai population. Further study to determine the magnitude of the impact of these factors on cholangiocarcinoma development in the Thai population is necessary.

Chaiteerakij R, Pan-ngum W, Poovorawan K, Soonthornworasiri N, Treeprasertsuk S, Phaosawasdi K. Characteristics and outcomes of cholangiocarcinoma by region in Thailand: A nationwide study. *World J Gastroenterol* 2017; 23(39): 7160-7167 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7160.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7160

INTRODUCTION

Cholangiocarcinoma (CCA), a malignancy of the biliary tract epithelium, is of increasing importance due to its continually increasing incidence worldwide[1-7]. The incidence of CCA varies substantially from region to region, with a high incidence in Asia [*e.g.*, 22.9, 7.5, and 5.6 cases per 100000 persons in Thailand[2], China[7] and South Korea[5], respectively] and a low incidence in the United States and Europe [*e.g.*, 3.4, 1.6, 1.3, and 1.2 cases per 100000 persons in Italy[3], the United States[6], France[8], and the United Kingdom[4], respectively]. Variation in the global incidence of CCA is due to variations in risk factors, particularly host and environmental risk factors. Within Thailand, the incidence of CCA also varies geographically[9]. The Northeast region of Thailand has the most CCA cases, with an incidence rate of 85 cases per 100000 persons per year, whereas the South region has the lowest number of cases at 5.7 cases per 100000 persons per year. The high incidence of CCA in Northeast Thailand is related to the high prevalence of liver fluke or *Opisthorchis viverrini* (OV) infection[10].

In addition to liver fluke infection, primary sclerosing cholangitis (PSC) is another major risk factor for CCA[11]. PSC is a major cause of CCA in Western populations and is associated with an 80- to 160-fold increase in CCA risk[12,13]. Although PSC markedly increases CCA risk, PSC is present in only 20% of all CCA patients[14]. Most CCA patients do not have identifiable risk factors.

Cirrhosis, chronic viral hepatitis B and C infection, diabetes, obesity, and smoking have recently been shown to be associated with increased CCA risk[12-14]. These factors were believed to contribute to the increasing incidence of CCA in the United States[15]. The question regarding whether or not these pre-existing conditions contribute to CCA development in the Thai population, particularly in people living in areas that are non-endemic for liver fluke infection, has not yet been studied. Accordingly, the objectives of this study were to compare the characteristics of CCA patients among the 5 different regions of Thailand. Secondarily, survival outcomes were determined. This population-based study was conducted using data from the National Health Security Office, Thailand.

MATERIALS AND METHODS

Ethical considerations

The protocol of this study was approved by the

Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB No. 113/58).

Data source

The data were obtained from the Nationwide Hospital Admission Data (NHAD) registry. The data were already de-identified before the investigators accessed them. Briefly, the NHAD registry is a database that contains hospitalization data of Thai citizens who are covered by the national Medical Welfare Scheme (MWS). The MWS was established in Thailand in 2000 and is currently one of 3 major health insurance systems. During the study period, the MWS covered approximately 47 million of the 64 million total Thai people in all age groups across Thailand. Those who have health insurance coverage by the MWS can access health services in hospitals that are registered with the National Health Security Office. Patients have access to the hospitals that are assigned to each individual by the household registration system. At the time of data collection, there were 39421 hospitals in the registry, of which 14402 (36.5%) were primary or community hospitals that provide primary healthcare, 11277 (28.6%) were secondary hospitals that provide general healthcare, and 13742 (34.9%) were tertiary referral hospitals that provide specialized care for complex cases.

Study population

CCA cases: All patients diagnosed with CCA who were admitted to affiliated hospitals during the January 2008 to December 2013 study period were identified in the NHAD registry using the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) codes of C22.1 (intrahepatic cholangiocarcinoma), C24.0 (extrahepatic cholangiocarcinoma), C24.1 (cancer of the ampulla of Vater), C24.8 (cholangiocarcinoma whose subtype cannot be classified), and C24.9 (cholangiocarcinoma, unspecified subtype). Cancer of the ampulla of Vater was included in this study because it occurs within 2 cm of the distal end of the common bile duct. Demographic data, including age, gender, and residential area, were collected and recorded. Potential risk factors for CCA, including cirrhosis, diabetes, and chronic viral hepatitis B and C infection, were collected using the corresponding ICD-10 codes, as follows: HBV infection: B18.0 and B18.1; HCV infection: B18.2; cirrhosis: K74, K74.0, K74.1, K74.2, K74.6, K70.2, K70.3, and K70.9; and diabetes mellitus type 2: E11. Date of death was obtained from the death certificate.

Controls: There were 2 control cohorts in this study. First, to compare demographic differences between CCA patients and non-CCA individuals, all Thai citizens were used as controls (n = 64076033). Demographic data of the Thai citizens were obtained in the year 2011 (the midpoint of the study period) from the Department of Provincial Administration, Ministry of the Interior, Thailand (Table 1). Second, to explore the potential associations between cirrhosis, diabetes and chronic viral hepatitis B and C infections and CCA development in the Thai population, patients without CCA who were admitted to hospitals during the study period were used as controls (n = 18508448). The data of non-CCA controls were obtained from discharge summary notes in the same manner as CCA cases.

Statistical analysis

All data analyses were performed using PASW® Statistics version 18 (SPSS, Inc., Chicago, IL, United States) and STATA version 12.1 (StataCorp LP, College Station, TX, United States). The patients were divided into 1 of the following 5 geographic region groups: Northeast, North, Central, Bangkok, or South. Bangkok is the capital of Thailand and is located in the Central geographic region. However, we designated Bangkok as a separate region because it has several major university hospitals that may be able to provide more technologically advanced treatment options than most hospitals located in other parts of the country. The annual incidence rate of CCA was calculated by dividing the number of new patients who were admitted with a diagnosis of CCA annually by the general population in the same year. Underlying diseases and one-year mortality rates were compared among regions using Student's t-test for continuous variables and chi-square test for categorical variables.

Demographic data of CCA patients and all Thai citizens were compared using chi-square and t-tests. The proportions of individuals with cirrhosis, diabetes and chronic viral hepatitis B and C infections between CCA and non-CCA patients who were admitted to the hospital were compared during the study period using a χ^2 test. Data are presented as a number, number (%), or mean ± SD.

One-year mortality was calculated using the per-patient information in which the survival time was calculated from the subtraction between the date of death and the date of the first admission. One-year mortality was then defined as 1 if the survival time was less than a year and 0 otherwise. An analysis of the trend in CCA incidence and mortality was performed using "nptrend", the nonparametric test for trend across ordered groups developed by Cuzick (1985), which is an extension of the Wilcoxon rank-sum test. A P-value of < 0.05 indicated statistical significance.

RESULTS

Baseline characteristics

There were 39421 patients diagnosed with CCA who

Table 1 Baseline characteristics of cholangiocarcinoma patients diagnosed between 2009 and 2013 *n* (%)

Variable	CCA cases (*n* = 39421)	Thai population (*n* = 64076033)	*P* value
Gender			
Male	24120 (61.2)	31529148 (49.2)	< 0.001
Age (mean ± SD, yr)	64.1 ± 11.7	34.9[1]	
Geographic region			< 0.0001
Northeast	24239 (61.5)	21585883 (33.7)	< 0.001
North	6699 (17.0)	11783311 (18.4)	< 0.001
Central	5295 (13.4)	16060141 (25.1)	< 0.001
Bangkok	1114 (2.8)	5674843 (8.8)	< 0.001
South	1056 (2.7)	8971855 (14.0)	< 0.001

[1]Weighted average age calculated using the midpoint; Standard deviation was undefined; thus, a *t*-test was not performed. CCA: Cholangiocarcinoma.

Table 2 Incidence (per 100000 persons) of cholangiocarcinoma in Thailand during the 2009 to 2013 study period

Region	Year					P_{trend} value
	2009	2010	2011	2012	2013	
All	15.80	15.63	16.13	16.07	16.43	0.13
Northeast	28.71	28.33	28.96	29.30	28.83	0.25
North	19.59	18.80	19.46	18.80	20.84	0.70
Central	7.23	7.42	7.98	8.40	9.20	0.06
Bangkok	5.87	6.17	6.27	5.52	6.01	0.85
South	2.76	2.74	2.98	2.81	2.98	0.17

Table 3 Proportions of individuals with potential predisposing conditions to cholangiocarcinoma development *n* (%)

Conditions	CCA patients (*n* = 39421)	Non-CCA patients (*n* = 18508448)	*P* value
Cirrhosis	1896 (4.81)	170255 (0.92)	< 0.001
Chronic viral hepatitis B infection	291 (0.74)	21797 (0.12)	< 0.001
Chronic viral hepatitis C infection	196 (0.50)	18339 (0.10)	< 0.001
Diabetes	4502 (11.42)	977973 (5.28)[1]	< 0.001

[1]The number was estimated from the proportion of individuals with diabetes among 1900000 patients on whom data on diabetes were available, assuming the proportion of individuals with diabetes was similar between those with and without data on diabetes. CCA: Cholangiocarcinoma.

were admitted to the affiliated hospitals during the study period. The mean ± SD age was 64.0 ± 11.7 years, and most patients (61.2%) were male. When compared by geographic area, the number of CCA patients varied significantly. The Northeast region had the greatest number of CCA cases (24239; 61.5%), followed by the North (6699; 17.0%) and Central regions (5295; 13.4%) ($P < 0.0001$) (Figure 1). Bangkok had a relatively low number of CCA cases (1114; 2.8%), and the South region had the lowest number of CCA patients (1056; 2.7%) (Table 1). Differences in the demographic characteristics between CCA cases and the general population are shown in Table 1. During the study period, the annual incidence rate was stable over time, *i.e.*, 15.8, 15.6, 16.3, 16.1, and 16.4 cases per 100000 persons-year in the years 2009, 2010, 2011, 2012, and 2013, respectively, $P_{trend} = 0.13$. When classified by region, the annual incidence rate in each region remained stable over the course of the study period (Table 2).

Potential factors contributing to CCA in Thailand

Among the comorbid diseases potentially associated with CCA that were studied, diabetes was the most frequently detected, affecting 11.42% ($n = 4502$) of the entire CCA cohort. Cirrhosis and chronic viral hepatitis B and C infection were present in 1896 (4.81%), 291 (0.74%), and 196 (0.50%) patients, respectively (Table 3). CCA patients with cirrhosis ($n = 1896$) were significantly more likely to have hepatitis B infection, hepatitis C infection and alcoholic liver disease than CCA patients without cirrhosis ($n = 37525$), *i.e.*, 124 (6.5%) *vs* 132 (0.3%), 100 (5.3%) *vs* 84 (0.2%), and 197 (10.4%) *vs* 0 (0%) for hepatitis B infection, hepatitis C infection and alcoholic liver disease, respectively; $P < 0.0001$ for all.

In a comparison of the proportion of individuals with underlying diabetes and chronic liver diseases between CCA *vs* non-CCA patients, we found that a greater proportion of CCA patients had diabetes than non-CCA patients, *i.e.*, 4502/39421 (11.42%) *vs* 977973/18508448 (5.28%), $P < 0.001$, respectively. Similarly, a significantly greater proportion of CCA patients had viral hepatitis B and C infection and cirrhosis, *i.e.*, 291/39421 (0.74%) *vs* 21797/18508448 (0.12%), 196/39421 (0.50%) *vs* 18339/18508448 (0.10%), and 1896/39421 (4.81%) *vs* 170255/18508448 (0.92%) for viral hepatitis B, viral hepatitis C and cirrhosis, respectively; $P < 0.001$ for all comparisons (Table 3).

Compared to the Northeast region, all regions had a higher proportion of CCA cases with cirrhosis (5.39%, 6.84%, 7.46%, and 5.97% *vs* 3.97% for North, Central, Bangkok, and South *vs* Northeast, respectively; $P < 0.0001$ for all comparisons). Similarly, all regions except for Bangkok had a significantly greater proportion of CCA patients with chronic viral hepatitis B infection than the Northeast region (11.36%, 1.52%, and 1.11% in the North, South, and Central regions *vs* 0.44% in the Northeast, respectively; $P < 0.001$ for all comparisons) (Table 4). Bangkok had a borderline significantly greater proportion of individuals with chronic hepatitis B than the Northeast ($P = 0.05$) (Table 4). Taken together, these findings suggest that cirrhosis and chronic viral hepatitis B infection may be partly associated with the

Table 4 Proportion of cholangiocarcinoma patients who had underlying diabetes and chronic liver diseases *n* (%)

	Total (n = 39421)	Northeast (n = 24237)	North (n = 6696)	$P_{\text{North vs Northeast}}$	Central (n = 5294)	$P_{\text{central vs Northeast}}$	Bangkok (n = 1113)	$P_{\text{Bangkok vs Northeast}}$	South (n = 1055)	$P_{\text{South vs Northeast}}$	$P_{\text{for all regions}}$
Diabetes	4502 (11.42)	2803 (11.56)	572 (8.54)	< 0.0001	689 (13.01)	0.11	389 (18.36)	< 0.0001	133 (12.60)	0.25	< 0.0001
Cirrhosis	1896 (4.81)	963 (3.97)	440 (5.39)	< 0.0001	362 (6.84)	< 0.0001	83 (7.46)	< 0.0001	63 (5.97)	< 0.0001	< 0.0001
Chronic hepatitis B	291 (0.74)	106 (0.44)	91 (1.36)	< 0.0001	59 (1.11)	< 0.0001	18 (1.62)	0.05	12 (1.52)	0.002	< 0.0001
Chronic hepatitis C	196 (0.50)	92 (0.38)	57 (0.85)	< 0.0001	20 (0.38)	0.33	17 (1.53)	0.12	3 (0.28)	< 0.001	< 0.0001

P-value < 0.05 indicates statistical significance.

Table 5 One-year mortality rate in cholangiocarcinoma patients by region during the 2009 to 2013 study period *n* (%)

Year	Region					Total
	Northeast	North	Central	Bangkok	South	
2009	3825 (79.6)	1153 (85.5)	754 (80.6)	170 (78.7)	149 (74.5)	6051 (80.6)
2010	3928 (82.4)	1114 (86.1)	782 (81.0)	181 (80.4)	148 (74.0)	6153 (82.6)
2011	3988 (82.1)	1097 (83.0)	830 (80.0)	176 (78.2)	172 (78.5)	6263 (81.8)
2012	4087 (82.5)	1068 (82.3)	893 (79.7)	174 (81.3)	176 (83.4)	6398 (82.0)
2013	3973 (81.9)	1208 (84.2)	986 (79.9)	169 (72.5)	165 (73.3)	6501 (81.5)
P_{trend}	0.57	0.25	0.13	0.57	0.85	0.85

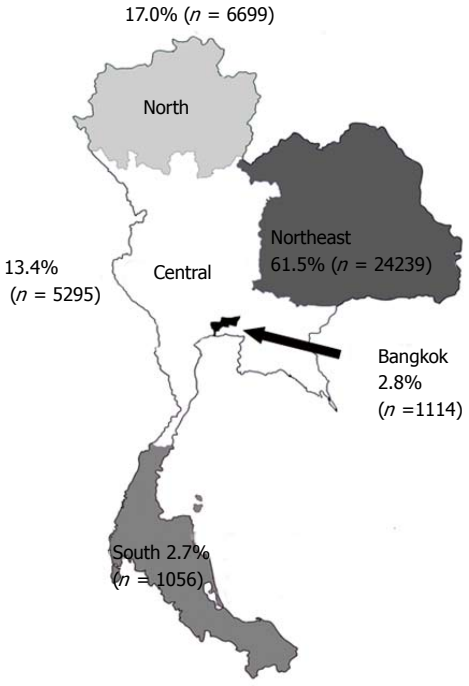

Figure 1 Northeast region had the greatest number of cholangiocarcinoma cases, followed by the North and Central regions.

development of CCA in our population.

Outcome of CCA patients

At the end of the study period, 35190 (89.27%) patients had died, of whom 31366 had died within 1 year after CCA diagnosis, corresponding to a 1-year mortality rate of 81.68%. The study period mean one-year mortality rates were stable (80.6%, 82.6%, 81.8%, 82.0%, and 81.5% for the years 2009 to 2013; P_{trend} = 0.85) (Table 5). A stable one-year mortality rate was also observed when evaluated by region (Table 5).

DISCUSSION

In this population-based study of 39421 Thai CCA patients, we observed substantial heterogeneity in the CCA incidence across various regions of Thailand. To a lesser degree, we also observed variation in mortality across the country. Our findings suggest that diabetes, liver cirrhosis, and chronic viral hepatitis B and C infection may contribute to CCA risk in the Thai population.

As expected, we found that approximately 62% of patients resided in Northeast Thailand, whereas only 3% of patients lived in the South region. The frequency of CCA cases in each region was correlated with the reported prevalence of OV infection in each area (as high as 67% in the Northeast and only 0.1% in the South)[16,17]. OV infection was shown to be associated with a 10-fold increased risk of CCA[10]. Accordingly, the eradication of OV infection could potentially reduce the incidence of CCA in Thailand. Indeed, a national program to control OV infection has been rigorously applied since 2000[17]. After the implementation of a comprehensive control program in a certain area in the Northeast in which the prevalence of OV infection was as high as 67%, the OV infection rate declined to 16%[17]. With the continuation of this control program, we may see a decline in CCA incidence in this particular study area over the next decade.

There is a body of evidence that suggests that chronic liver diseases and diabetes are potential risk factors for CCA[12-14,18,19]. Cirrhosis was shown to be associated with at least a 5-fold increase in CCA

risk[12-14,20]. Previous studies have shown that CCA patients had a higher proportion of individuals with cirrhosis than controls, with proportions ranging from 3% to 10% depending on the enrolled population, study design, and subtype of CCA studied[12-14,20]. In this study, a significantly greater proportion of CCA patients had cirrhosis than non-CCA patients. Additionally, the proportions of CCA cases with cirrhosis in non-endemic areas of OV infection (Central, Bangkok, and South regions) ranged from 5.6% to 7.5%, which were comparable to the rate of 7.8% reported in a Korean study[20]. Interestingly, the proportions of CCA patients with cirrhosis in the non-endemic areas of OV infection were significantly higher than the proportion in the Northeast region. Taken together, these findings suggest that cirrhosis may be a potential factor that contributes to CCA in Thai individuals, particularly in those who are not infected with OV.

Chronic viral hepatitis B and C infections have recently been recognized as potential risk factors for CCA[12-14,20-22]. In line with previous reports, we found that significantly more Thai patients with CCA had chronic viral hepatitis B and C infections than non-CCA patients. Two recent meta-analyses reported that hepatitis B and C infection confer risks of CCA with odds ratios of 2.0 and 5.4, respectively[21,22]. The magnitude of the effect of hepatitis B infection on CCA risk was more pronounced in Asian than in non-Asian populations. For example, the odds ratios for hepatitis B infection were 4.1 and 8.9 in Korean and Chinese cohorts[18,23], but only 2.8 in an American cohort[13]. Data related to the effect of hepatitis B and C infection on CCA risk in the Thai population are scarce[24]. The only report we were able to identify in the literature did not identify a significant association between hepatitis B/C infection and CCA; however, the study was conducted in an area endemic to OV infection, and the effect of hepatitis B and C on CCA risk may have been masked by the higher effect magnitude of OV infection[24].

We observed that an approximately 2-fold greater proportion of CCA patients had diabetes than non-CCA patients (11.4% *vs* 5.3%). A recent large case-control study of 2395 CCA cases and 4769 controls conducted in the United States reported that the proportion of subjects with diabetes among CCA cases and controls was 18.2% and 10.0%, respectively[13]. Accordingly, diabetes increased the risk of developing CCA by 2.7-fold, and this magnitude of risk was consistent with a reported odds ratio of 2.6 for developing CCA among Korean individuals with diabetes[13,18].

Taken together, our findings suggest that all of the studied underlying comorbid diseases potentially contribute to the risk of developing CCA in a Thai population. Future studies to confirm these findings and to determine the magnitude of the effect of these factors in a Thai population are warranted.

The major strength of this study is that our data were obtained from the Nationwide Hospital Admission Data (NHAD) registry, which is a database that includes the hospitalization data of 73% of all Thai citizens. As such, these findings most likely reflect an overall characterization of this cancer in Thailand. This study also has some mentionable limitations. First, the annual incidence estimated in this study might be underestimated due to the unique features of this database. The database solely includes information during hospitalization. Because data of patients seen at outpatient departments were not available, this could raise a concern that some patients, particularly those with advanced stage CCA who were only seen at outpatient departments and had never been admitted to a hospital, might be missed. We admit that this is possible; however, based on the current practice in our country, most CCA patients require at least one hospitalization, either for performing an investigation for a definite diagnosis or for receiving treatment by surgery, biliary drainage with palliative chemotherapy or even the best supportive care. Thus, we believe that the number of CCA patients who did not have any hospitalization was not large, and this number did not significantly affect the overall findings observed in this study. Second, the diagnosis of CCA was made by ICD codes that were obtained from hospital discharge summary notes and without histopathological confirmation. Consequently, the actual number of CCA patients during the study period could be higher than the number presented here. However, the proportions of CCA patients by region were consistent with the rates reported in a previous study[2]. As a result, it is likely that the different incidence rates among the 5 different regions are a valid finding. Another concern was that the proportion of CCA patients in each region shown in this study might not reflect the actual region of residence of patients because some local people might migrate to other regions; in particular, a portion of Northeastern people migrate to work in Bangkok. Thai people are covered by the national MWS only if they visit their primary hospital, which is designated by household registration. Patients who require hospitalization are usually referred back to their primary hospital. If necessary, the primary hospital will further refer patients to the nearby secondary or tertiary hospitals within the same region. Thus, the effect of migration across regions had a minimal impact on the proportion of patients in each region. Data on hepatitis B/C infection, diabetes, smoking, alcohol drinking, obesity, and metabolic syndrome were not available in the discharge summary notes of all participants, thus precluding us from estimating the effect of these conditions on CCA risk. Similarly, the prevalence of OV infection and the relationship between OV infection and CCA incidence cannot be

determined because the diagnosis of OV infection was not available in the medical records. The diagnosis of CCA was obtained from ICD codes, which group hilar and distal CCA together as extrahepatic CCA; thus, an analysis by CCA subtypes could not be performed. Choledocholiathiasis, hepatolithiasis, choledochal cysts, Caroli's syndrome and bile duct adenoma have been shown to be associated with CCA[1,14]. These conditions were not included in the analysis because this study aimed to explore the conditions that have recently been proposed to be related to CCA and may in part contribute to the increasing incidence of CCA worldwide.

In summary, CCA-related incidence and mortality among Thai patients vary by geographical region. Our findings yield indirect evidence that supports current epidemiological data that suggests that diabetes, cirrhosis, and chronic viral hepatitis B and C infection are associated with an increased risk of developing CCA. These conditions may be important factors contributing to CCA in Thai individuals.

COMMENTS

Background

The incidence of cholangiocarcinoma (CCA) has been increasing for a few decades for unclear reasons. Primary sclerosing cholangitis, liver fluke infection, and conditions with chronic biliary tract inflammation are established predisposing factors to CCA development. Accumulating evidence suggests that cirrhosis, chronic viral hepatitis B/C infection and diabetes are associated with CCA.

Research frontiers

The results of this study provide indirect evidence supporting the current epidemiological knowledge about risk factors of CCA.

Innovations and breakthroughs

This study used the Nationwide Hospital Admission Data (NHAD) registry to identify potential risk factors for CCA among 5 different regions of Thailand. The two key findings were that the incidence of CCA varied geographically in Thailand and that significantly more CCA patients had diabetes, cirrhosis, and chronic viral hepatitis B/C infection than non-CCA participants.

Applications

This study suggests that diabetes, cirrhosis, and chronic viral hepatitis B and C infection may contribute to CCA development in the Thai population. Further study to confirm these associations and determine the magnitude of the impact of these factors on cholangiocarcinoma development in the Thai population is warranted.

Terminology

Cholangiocarcinoma is a cancer of the bile duct epithelium. CCA can be categorized into 3 subtypes: intrahepatic, perihilar and distal cholangiocarcinoma. Although the three subtypes share some common features, each has its own characteristics, including genetic alterations, presentation, treatment and prognosis. Obtaining a diagnosis of cholangiocarcinoma using ICD codes precludes the analysis by subtypes because the ICD codes group perihilar and distal cholangiocarcinoma together as extrahepatic cholangiocarcinomas.

Peer-review

This study addresses an important healthcare problem in Thailand. This is an informative article about the risk factors of cholangiocarcinoma in Thailand using a nationwide database. This study aimed to identify potential risk factors of cholangiocarcinoma among 5 different regions of Thailand. The fact that even in Thailand, diabetes, viral hepatitis B/C infection and cirrhosis are risk factors of cholangiocarcinoma in a region where fluke infection is not endemic, is interesting.

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ORIGINAL ARTICLE

Observational Study

Expression of annexin A5 in serum and tumor tissue of patients with colon cancer and its clinical significance

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Abstract

AIM

To investigate the expression of annexin A5 in serum and tumor tissue of patients with colon cancer and to analyze its clinical significance.

METHODS

Ninety-three patients with colon cancer treated at our hospital between February 2013 and March 2016 were included in an observation group, and 40 healthy individuals were included in a control group. Enzyme-linked immunosorbent assay was performed to determine the serum level of annexin A5, while immunohistochemistry was performed to determine the expression of annexin A5 in cancer tissues.

RESULTS

The serum level of annexin A5 was 0.184 ± 0.043 ng/mL in the observation group, which was significantly higher than that in the control group ($P < 0.05$). Annexin A5 expression was detected in 79.31% of the patients with lymph node metastasis, which was significantly higher than that in patients without lymph node metastasis ($P < 0.05$). Moreover, annexin A5 expression was detected in 86.96% of the patients with stage Ⅲ to Ⅳ disease, which was significantly higher than that in patients with stage Ⅰ to Ⅱ disease ($P < 0.05$). The serum level of annexin A5 was 0.215 ± 0.044 ng/mL in patients whose tumors were positive for annexin A5 expression, which was significantly higher than that in patients whose tumors were negative for annexin A5 expression ($P < 0.05$). The serum level of annexin A5 was correlated with annexin A5 expression in colon cancer tissues (*r*

= 0.312, $P < 0.05$). When a cutoff value of > 0.148 ng/mL for serum level of annexin A5 was used in the diagnosis of colon cancer, the sensitivity was 83.90%, and the specificity was 57.50%.

CONCLUSION

For patients with colon cancer, annexin A5 expression in cancer tissues is related to lymph node metastasis and tumor grade. Serum level of annexin A5 is related to annexin A5 expression in cancer tissues and is of diagnostic relevance.

Key words: Immunohistochemistry; Annexin A5; Colon cancer; Serum

Core tip: For patients with colon cancer, annexin A5 expression in cancer tissues is related to lymph node metastasis and tumor grade. Serum level of annexin A5 is related to annexin A5 expression in cancer tissues and is of diagnostic relevance.

Sun CB, Zhao AY, Ji S, Han XQ, Sun ZC, Wang MC, Zheng FC. Expression of annexin A5 in serum and tumor tissue of patients with colon cancer and its clinical significance. *World J Gastroenterol* 2017; 23(39): 7168-7173 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7168.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7168

INTRODUCTION

Colon cancer is a common malignancy of the digestive tract. Studies have shown that the incidence of colon cancer is ⩾ 0.005%[1] and that the incidence has continued to trend upwards in recent years because of risk factors such as diet and smoking[2,3] .

Basic cancer research has shown that changes in the levels of certain molecules affect tumor cell proliferation and differentiation, which in turn affect the development and progression of malignant tumors[4]. Annexin A5, first isolated from human placenta, was found to bind to phosphatidylserine in a calcium-dependent manner[5,6]. In the present study, 93 patients with colon cancer who were treated at our hospital between February 2013 and March 2016 were included to investigate the expression of annexin A5 in serum and in cancer tissues, with an aim to investigate its clinical significance.

MATERIALS AND METHODS

General information

Ninety-three patients with colon cancer (observation group) who were treated at our hospital between February 2013 and March 2016 were included in this study. The inclusion criteria were as follows: (1) pathologically confirmed colon cancer; (2) complete clinical and pathological data; and (3) being willing to provide informed consent. The exclusion criterion was incomplete clinical or pathological data. Forty healthy individuals who underwent a routine health checkup at our hospital were included as controls. No significant difference was observed with respect to age or gender between the two groups (Table 1).

Detection of serum level of annexin A5

A fasting venous blood sample was collected from each subject in the morning and centrifuged at 10000 *r*/min to separate the serum, which was then stored at -20 ℃ and tested within one week to determine the annexin A5 level. The Roche automated biochemical analyzer E170 module was used for testing, and the assay kit was purchased from Shanghai Taikang Biotechnology Co., Ltd. The assay was performed according to the instructions given in the package insert. Control serum or standard was included with the kit.

Immunohistochemistry

Paraffin sections were deparaffinized, rehydrated, and cut into 3 mm sections. The sections were incubated in 3% H_2O_2 at room temperature for 5 min, rinsed with deionized water (3 min × 3 times), blocked with 10% milk protein (1 g protein in 100 mL of purified water), and incubated at room temperature for 5 min. Next, the sections were incubated with a mouse anti-annexin A5 antibody (Nanjing Biyuntian Biotechnology Co., Ltd.) for 2 h at 37 ℃, followed by a PBS wash (5 min × 3 times). Then, the slides were incubated with a horseradish peroxidase-labeled rabbit secondary antibody (Roche) for 30 min at 37 ℃, followed by a PBS wash (5 min × 3 times). After that, the slides were incubated with NBT/BCIP reagent, which was used to develop the reaction, for 5 min. Finally, the sections were counterstained, dehydrated, cleared, mounted, and observed under an OLIPICS microscope (Shanghai Precision Instrument Co., Ltd). All the required reagents were purchased from Nanjing Taikang Biotechnology Co., Ltd.

Evaluation criteria for immunohistochemical staining

Immunohistochemical staining was considered positive if yellow granules were present in the cytoplasm of tumor cells or stromal cells. The staining intensity was graded as follows: 0, no staining; 1, light yellow; 2, yellow; and 3, brown. The percentage of positive cells was scored as follows: 0, < 5%; 1, 5% to 24%; 2, 25% to 50%; 3, 51% to 74%; and 4, and ⩾ 75%. The product of the staining intensity and the percentage of positive cells was either < 2 (negative) or ⩾ 2 (positive).

Table 1 General information (mean ± SD)

Group	*n*	M/F	Age (yr)
Observation group	93	54/39	53.29 ± 9.49
Control group	40	27/13	52.17 ± 8.14
t/χ^2		1.046	0.65
P value		> 0.05	> 0.05

Table 2 Relationship between Annexin A5 and clinicopathological features of patients with colon cancer *n* (%)

Clinicopathological feature	*n*	Positive	χ^2	*P* value
Gender				
M	54	31 (57.41)	0.023	> 0.05
F	39	23 (58.97)		
Age, yr				
≥ 55	47	29 (61.70)	0.516	> 0.05
< 50	46	25 (54.35)		
Lymph node metastasis				
Yes	29	23 (79.31)	7.812	< 0.05
No	64	31 (48.44)		
Tumor diameter (cm)				
≥ 5	51	29 (56.86)	0.067	> 0.05
< 5	42	25 (59.52)		
Tumor stage				
Ⅰ to Ⅱ	47	14 (29.79)	31.204	< 0.05
Ⅲ to Ⅳ	46	40 (86.96)		

Table 3 Serum levels of Annexin A5 in the two groups (mean ± SD, ng/mL)

Group	*n*	Annexin A5	*t*	*P* value
Observation group	93	0.184 ± 0.043	2.904	< 0.05
Control group	40	0.159 ± 0.051		

Statistical analysis

SPSS v19.0 was used for statistical analyses. Measurement data are expressed as mean ± SD and were analyzed by the *t*-test. Count data were analyzed by the χ^2 test. Spearman rank correlation analysis was performed to analyze potential correlations between variables. A receiver operating characteristic curve was used to analyze the diagnostic value of serum annexin A5 level. $P < 0.05$ was considered statistically significant.

RESULTS

Annexin A5 expression in cancer tissue

No significant difference was observed in the positive expression rates of annexin A5 among patients of different ages or genders, or those with different tumor diameters. Moreover, 79.31% of the patients with lymph node metastasis expressed annexin A5, which was significantly higher than the percentage of patients without lymph node metastasis ($P < 0.05$); 86.96% of the patients with stage Ⅲ to Ⅳ disease expressed annexin A5, which was significantly higher than the percentage of patients with stage Ⅰ to Ⅱ disease ($P < 0.05$) (Table 2).

Serum levels of annexin A5 in the two groups

The serum level of annexin A5 was significantly higher in the observation group than in the control group ($P < 0.05$) (Table 3).

Correlation between serum level of annexin A5 and expression of annexin A5 in tumor tissue

The serum level of annexin A5 was 0.215 ± 0.044 ng/mL in patients whose colon tumors were positive for annexin A5 expression, which was significantly higher than the corresponding value in patients whose colon tumors were negative for annexin A5 (0.180 ± 0.021 ng/mL) ($t = 4.599$, $P < 0.05$). A Spearman rank correlation analysis showed that the serum level of annexin A5 was related to the expression of annexin A5 in tumor tissues ($r = 0.312$, $P < 0.05$).

Diagnostic value of serum level of annexin A5

The ROC curve for the serum level of annexin A5 in the diagnosis of colon cancer showed an area under the curve of 0.732 ($P < 0.05$). At a cutoff value of 0.148 ng/mL, the sensitivity was 83.90%, and the specificity was 57.50% (Figure 1).

DISCUSSION

Changes in diet, excessive alcohol consumption, and genetic susceptibility factors promote the development and progression of colon cancer. In particular, among elderly male smokers aged 45 or older, the incidence of colon cancer is 0.005% or higher and has continued to trend upwards in recent years[7,8]. For colon cancer, the incidence of early metastasis is high, which results in poor patient outcomes: the five years survival rate is < 35%, and the median survival time is < 32 mo[9-11]. Studies on the genetic and biological mechanisms of the development and progression of colon cancer may provide new targets for immune therapy or strategies of comprehensive biological therapy for colon cancer[12,13].

Molecular changes play an important regulatory role in the development of malignant tumors. Cell surface Connexins or membrane proteins can induce the transcription initiation activity of downstream oncogenes, which promotes aberrant activation of the cell cycle in colonic epithelial cells and leads to excessive proliferation of cancer cells[14]. Accumulating experimental data indicate that phosphatidylserine exposition is associated with apoptosis and other cell death programs[15-17], which renders it an attractive target in imaging overall cell death. Annexin A5 is identified in blood vessel as a blood anticoagulation factor and it builds voltage-dependent calcium channel in phosphatidylserine bilayers[18,19]. Corsten *et al*[20] showed that through binding with strong affinity to phosphatidylserine, annexin A5 offers an interesting opportunity for visualization of aggregate

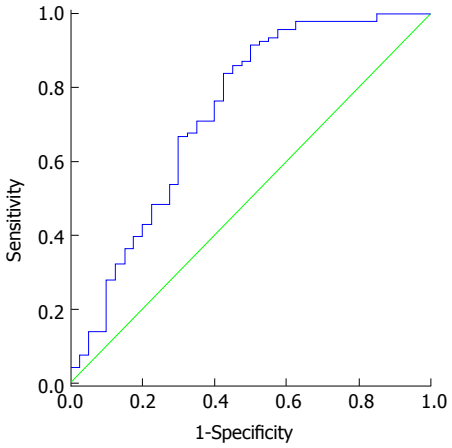

Figure 1 Receiver operating characteristic curve for the value of serum level of annexin A5 in the diagnosis of colon cancer.

cell death[21,22], thus providing a fit benchmark for *in vivo* monitoring of anticancer treatment[23-26]. Recently, annexin A5 has been reported as a new mediator of cisplatin-induced apoptosis by inducing voltage-dependent anion channel oligomerization in human kidney epithelial cells[27,28]. Annexin A5 forms N6-acetyllysine at specific positions of the amino-terminal region of the membrane protein, and as a result, it affects the formation of a transcriptional co-inhibitory complex and participates in transcriptional repression and silencing of tumor suppressor genes via H1 phosphorylation[29,30]. Previous studies have investigated the relationship between annexin A5 and liver cancer or esophageal cancer and showed that uH2B-related monotone generalization increased the risk of malignant digestive tumors and promoted clinical progression[31-34]. This study explored not only the expression of uH2B in colon cancer tissue but also the diagnostic value of its serum level in the diagnosis of colon cancer.

In this study, immunohistochemical staining showed significantly high expression of annexin A5 in colon cancer tissue and demonstrated that the positive expression rate of annexin A5 was significantly higher in patients with lymph node metastasis than in those without. This suggests that annexin A5 may play a role in the promotion of the invasion of lymph nodes by colon cancer cells. Furthermore, approximately 80% of the patients with late-stage (Ⅲ and Ⅳ) colon cancer expressed annexin A5, which was significantly higher than the percentage of patients with stage Ⅰ or Ⅱ disease, which suggests that annexin A5 significantly promotes the clinical progression and worsening of colon cancer. Annexin A5 induces the activation of second messengers in cancer cells, which promotes the production of cancer cell differentiation antigens, the proliferation and differentiation of colon cancer cells, and clinical progression.

In conclusion, annexin A5 is highly expressed in serum and tumor tissues of patients with colon cancer, and its expression is closely related to the clinical stage and presence of lymph node metastasis in patients with colon cancer. Nevertheless, this study has certain limitations. For instance, we did not investigate the relationship between the expression of annexin A5 and the long-term survival of patients with colon cancer.

COMMENTS

Background

Colon cancer is a common malignancy of the digestive tract. Studies have shown that the incidence of colon cancer is ⩾ 0.005% and that the incidence has continued to trend upwards in recent years because of risk factors such as diet and smoking.

Research frontiers

Basic cancer research has shown that changes in the levels of certain molecules affect tumor cell proliferation and differentiation, which in turn affect the development and progression of malignant tumors. Annexin A5 is a glycoprotein that contains a multiplex carboxyl terminus binding domain, which influences the differentiation of surface antigens on cancer cells and promotes tumor proliferation and invasion.

Innovations and breakthroughs

The objective was to investigate the clinical significance of annexin A5 expression in colon cancer.

Applications

For patients with colon cancer, annexin A5 expression in cancer tissues is related to lymph node metastasis and tumor grade. Serum level of annexin A5 is related to annexin A5 expression in cancer tissues and is of diagnostic relevance.

Peer-review

In this study, the authors investigated the expression of annexin A5 in serum and tumor tissues of patients with colon cancer and its clinical significance.

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CASE REPORT

Faecal microbiota transplantation in patients with *Clostridium difficile* and significant comorbidities as well as in patients with new indications: A case series

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Abstract

Fecal microbiota transplantation (FMT) is effective in recurrent *Clostridium difficile* infection (rCDI). Knowledge of the safety and efficacy of FMT treatment in immune deficient patients is scarce. FMT has been

suggested as a potential method for an increasing number of new indications besides rCDI. Among our FMT-treated rCDI patients, we reviewed those with major comorbidities: two human immunodeficiency virus patients, six haemodialysis patients, two kidney transplant patients, two liver transplant patients and a patient with chronic lymphatic leukaemia. We also reviewed those treated with FMT for indications other than rCDI: *Salmonella* carriage (two patients), trimethylaminuria (two patients), small intestinal bacterial overgrowth (SIBO; one patient), and lymphocytic colitis (one patient), as well as a common variable immunodeficiency patient with chronic norovirus infection and ESBL-producing *Escherichia coli* (*E. coli*) carriage. Of the thirteen rCDI patients treated with FMT, eleven cleared the CDI. The observed adverse events were not directly attributable to FMT. Concerning the special indications, both *Salmonellas* and ESBL-producing *E. coli* were eradicated. One trimethylaminuria patient and one SIBO-patient reported a reduction of symptoms. Three patients did not experience a benefit from FMT: chronic norovirus, lymphocytic colitis and the other fish malodour syndrome. There were no reported side effects in this group. FMT appeared to be safe and effective for immunocompromised patients with rCDI. FMT showed promise for the eradication of antibiotic-resistant bacteria, but further research is warranted.

Key words: Faecal microbiota transplantation; Antibiotic resistance; *Clostridium difficile* infection; Microbiota; Immunodeficiency; *Salmonella* infection

Core tip: Knowledge of faecal microbiota transplantation (FMT) in immunocompromised patients and patients with conditions other than recurrent *Clostridium difficile* infection (rCDI) is scarce. We reviewed 13 FMT-treated patients with rCDI and major comorbidities as well as 8 patients with new indications. In our cohort, FMT appeared to be safe and effective for immunocompromised patients: dialysis patients, human immunodeficiency virus patients, solid organ transplant patients and a patient with chronic lymphatic leukaemia. Of the patients treated for indications other than rCDI, the most promising results were successful eradication of antibiotic-resistant bacteria. Eradication of chronic *Salmonella* carriage in two patients with FMT represents the first cases reported to date.

Lahtinen P, Mattila E, Anttila VJ, Tillonen J, Teittinen M, Nevalainen P, Salminen S, Satokari R, Arkkila P. Faecal microbiota transplantation in patients with *Clostridium difficile* and significant comorbidities as well as in patients with new indications: A case series. *World J Gastroenterol* 2017; 23(39): 7174-7184 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7174.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7174

INTRODUCTION

The intestinal microbiota is an area of active research. Knowledge of the human microbiota has been accumulating rapidly in recent years. The gut bacteria was previously regarded as passive or harmful waste, but the intestinal microbiota is currently respected as a well-orchestrated organism with an active role in the development of immunity and maintenance of health[1-4].

Microbial imbalance, dysbiosis, is suggested to play a role in many different diseases. The microbiota holds many expectations as a new treatment target. Faecal microbiota transplantation (FMT) is a straightforward way to change the microbial composition of the intestine[1-4]. FMT has been shown to induce profound and long-lasting changes in the microbiota, offering a means to modify the gut microbiota relatively permanently for the treatment of microbiota-associated diseases[5].

FMT has become a widely accepted treatment for recurrent *Clostridium difficile* infection (rCDI)[6-9]. We, among others, have shown that FMT through colonoscopy is an effective treatment for rCDI[5]. Experience in many centres has shown that FMT is also safe when performed to a high standard[10-13].

The risk of adverse events of FMT is low in well performed studies[6]. Rigorous screening of the donor is mandatory. Without proper screening, there is a possibility of transmitting infectious diseases and a possibility of transmitting conditions that FMT is suspected to ameliorate, such as obesity[14].

All FMT should be performed at a health care unit by professionals who are familiar with the procedure[15]. FMT is currently indicated only for rCDI[15]. If FMT is used for other indications, then patients should be carefully evaluated, and the results, whether positive or negative, should be reported. Our group has treated a few patients with conditions other than rCDI and outside of on-going study programmes. Such cases have been evaluated carefully on an individual basis by a gastroenterologist, an infectious disease specialist and a microbiologist.

Although FMT has been effective and generally safe in published studies, data concerning FMT in certain special groups are scarce[16]. More information is needed regarding the safety of FMT in immunosuppressed and other special groups such as patients undergoing haemodialysis[12]. In our FMT-treated patients, we gathered information on patients with significant comorbidities, such as immune-deficient patients, patients with an organ transplant and hae-

modialysis patients.

CASE REPORT

Study design

Of the patients treated with FMT in Helsinki University Hospital, we searched for those treated for rCDI and having major comorbidities and those treated for indications other than rCDI. We included dialysis patients and patients with known immune deficiencies in the rCDI group; human immunodeficiency virus (HIV), organ transplant or hematologic disease. We found 13 patients with rCDI and major comorbidities and 8 patients with conditions other than rCDI. We review the patient histories, the outcomes and adverse events of each patient.

Ethical aspects

Since FMT policy and legislation differ from country to country[17,18], we consulted the Finnish Medicines Agency (Fimea), which is the national competent authority for regulating pharmaceuticals and blood and tissue products. Fimea noted that stool does not fall under the category of pharmaceuticals and did not consider it relevant to the establishment of new regulations specifically for FMT. In Finland, FMT studies do not require approval of the drug authority Fimea.

In Finland, FMT may be used by doctors based on their own judgement. We recommend that clinicians using FMT follow international guidelines[15,19]. FMT is indicated for rCDI treatment; for other indications, it should be used only in a clinical trial setting.

FMT protocol

Some of the patients described in this article have been treated with stool from a donor familiar to them. Most of the described patients have been treated with stool from a universal donor - frozen and thawed stool from our local faecal bank. The faecal banking protocol is described in detail in our previous article[10]. Briefly, faecal material from a healthy donor is mixed with saline and glycerol and frozen at -80 ℃. In some cases, we used the faecal suspension stored at -20 ℃, which did not seem to affect the results or safety of FMT. The frozen suspension is thawed a few hours before FMT and collected into two 100-mL syringes. When necessary, the suspension is passed through a pre-sterilized, stainless steel tea strainer to remove larger particles.

Donor screening was performed as described in detail by Mattila *et al*[6] and Satokari *et al*[10]. Briefly, the donors were screened for hepatitis C and B, HIV, *Treponema pallidum* and common enteric pathogens. The donor was required to lack antibiotics for the previous six months and to present no gastrointestinal symptoms.

The preferred route of administration of FMT in our practice is colonoscopy. The suspension is administered into the cecum and ascending colon. Preceding the colonoscopy, the patients undergo bowel lavage by polyethylene glycol solution (between 3-5 L). The patients with rCDI underwent pre-treatment with vancomycin or metronidazole, and the treatment was stopped 36 h before the FMT. In some special cases, we administered FMT during gastroscopy, injecting the suspension into the duodenum as distally as possible.

Although FMT has become the routine treatment after two relapses for CDI in our hospital, the decision to assess FMT in immunosuppressed patients and in special indications is based on a very thorough consideration, especially of safety concerns, by gastroenterologist, specialist of infectious diseases and microbiologist with FMT experience.

Thirteen patients treated with FMT for rCDI and having significant comorbidities

In this article, we report the results of thirteen patients with major comorbidities who underwent rCDI and were treated with FMT (Table 1). Two of the patients had HIV, six were haemodialysis patients, two had a kidney transplant, two had a liver transplant and one had chronic lymphatic leukaemia (CLL).

Eleven of the thirteen rCDI patients (85%) treated with FMT successfully cleared the CDI. Six of the patients had major adverse events, of which two died at 2 and 5 mo post-FMT; however, these events were not directly attributable to FMT. A detailed description of each patient is published online as a supplement.

Eight patients treated with FMT and not having rCDI

Eight patients received FMT as an experimental form of treatment for various special indications. The patients and outcomes are compiled in Table 2 and described in detail below.

A carrier of salmonella #1

A 17-year-old male was found to be a carrier of *Salmonella* in a routine check-up on the 1st of September 2015. He had not had previous GI symptoms. According to Finnish health authority instructions, a *Salmonella*-positive person may not work in food processing. The patient was about to start his studies to become a cook and *Salmonella* was delaying his plans.

The *Salmonella* strain was resistant to doxycycline and ciprofloxacin. He had a mild knee symptom that was thought to be reactive arthritis. Ciprofloxacin was administered briefly and stopped as the sensitivity results of the *Salmonella* became apparent. He was receiving tetracycline for acne since the 18th of August, which was stopped on the 26th of October. In October, faecal salmonella was negative twice, but it was positive again on the 16th of November.

A 2-wk course of i.v. ceftriaxone was considered an

Table 1 The results of fecal microbiota transplantation treatments of thirteen patients with different comorbidities and *Clostridium difficile* infection

	Patient characteristics	Medical history	Post FMT situation	Adverse events in 1 mo
1	A patient with HIV, ulcerative colitis and rCDI	28-year-old male with HIV, antiviral medication and virus undetectable, previous suspicion of ulcerative colitis. Recurrent diarrhoea with *C. difficile* positivity, slow response to vancomycin.	No further relapses Two months after diarrhoea recurred at the same time with mild alcohol associated pancreatitis. In colonoscopy final diagnosis of ulcerative colitis was made. *C. difficile* remained negative.	No
2	A patient with HIV, alcoholism and rCDI	59-year-old female, depression, continuous heavy smoking and consumption of alcohol. HIV and antiviral therapy. rCDI after antibiotic treatment for respiratory infection.	No further relapses Diarrheal continued due to exocrine pancreatic insufficiency and excessive alcohol consumption 5 mo after FMT *C. difficile* reinfection treated with vancomycin and fidaxomicin	No
3	A Haemodialysis patient with rCDI #1	60-year-old female, rheumatoid arthritis and in haemodialysis due to amyloidosis. Chronic atrial fibrillation, polypectomies of rectum adenomas. Had *Enterococcus* sepsis 2012.	No further relapses. Half a year after FMT *Enterococcus faecalis* sepsis and an epidural abscess.	No
4	A Haemodialysis patient with rCDI #2	19-year-old female, haemodialysis due to Goodpasture syndrome complicated with pulmonary haemorrhage. Immunosuppressive therapy.	No further relapses.	No
5	A Haemodialysis patient with rCDI #3	77-year-old male, haemodialysis after renal carcinoma operation, diabetes Ⅱ and COPD. *Pseudomonas* septicaemia followed by rCDI.	No further relapses. One week after FMT hospitalized due to generalized enema and possible cystitis. Two months after FMT hospitalized due to gastroenteritis, faecal clostridium was negative.	One week after hospitalized due to enema and cystitis
6	A Haemodialysis patient with rCDI #4	80-year-old male. Haemodialysis because of chronic glomerulonephritis, type II diabetes, hypertension, epilepsy, AV-block and a pace maker. *Staphylococcus aureus* septicaemia followed by rCDI.	No further relapses. *Staphylococcus aureus* sepsis 5 mo after the FMT.	No
7	A Haemodialysis patient with rCDI #5	66-year-old male, haemodialysis due to microscopic polyangiitis. Chronic atrial fibrillation.	No further relapses	No
8	A Haemodialysis patient with rCDI #6	79-year-old female. Hypertension, dyslipidaemia, atrophic gastritis. TIA 2004 and 2005, a pace maker due to bifascicular block. Coronary disease. Haemodialysis due to an episode of rhabdomyolysis.	2 wk after FMT reinfection after an antibiotic treatment of cystitis. No further FMT's due to poor general condition. Patient died 2 mo after FMT to underlying diseases	2 wk after *C. difficile* reinfection
9	A Kidney transplant patient with rCDI #1	78-year-old female. Kidney transplant due to polycystic renal disease. Polycystic liver, type Ⅱ diabetes, hypertension and asthma. Operated for cholecystectomy and hysterectomy. *E. coli* sepsis and one month after another infectious episode treated with meropenem followed by severe rCDI.	No further relapses 3 d after FMT gastroenteritis, *Clostridium* was not tested. Restarted vancomycin for 2 d. 12 d after FMT the patient was hospitalized due to infection, CT scan did not reveal the aetiology.	Gastroenteritis 3 d after FMT Hospitalized 12 d after FMT
10	A Kidney transplant patient with rCDI #2	61-year-old female. A kidney transplant due to polycystic renal disease. rCDI after clindamycin for dental infection.	No further relapses	No
11	A Liver transplant patient with rCDI	56-year-old female. Liver transplant due to mushroom intoxication, a moderate renal failure.	No further relapses	No
12	A Patient with a liver transplant, renal insufficiency, haemodialysis and rCDI	69-year-old male. Liver transplantation due to alcohol cirrhosis, followed by renal insufficiency and haemodialysis.	No further relapses	No
13	A Patient with chronic lymphatic leukaemia, chronic norovirus infection and rCDI	65-year-old female. Chronic lymphatic leukaemia since 1996. Autologous stem cell transplantation in 2003. Cytostatic interventions from 2009-2011, after which she had prolonged pancytopenia, infections and hypogammaglobinaemia. In summer 2011, she had chronic norovirus infection and recurrent CDI, several vancomycin courses and gammaglobulin infusions. March 2012 FMT	No primary complications Hospitalized 2 wk after FMT due to diarrhoea. Both norovirus and *Clostridium difficile* stayed positive in stool samples. Patient died in August 2012, 5 mo after FMT for complications of her haematological disease.	CDI and norovirus related diarrhoea continued.

FMT: Fecal microbiota transplantation; HIV: Human immunodeficiency virus; rCDI: Recurrent *Clostridium difficile* infection; *C. difficile: Clostridium difficile*; *E. coli: Escherichia coli*.

Table 2 The results of fecal microbiota transplantation treatments of eight patients with different new indications

	Patient and diagnosis	Age at 1st FMT and gender	Route of administration	Outcome	FMT related complications
1	A carrier of *Salmonella* #1	17-year-old male	Colonoscopy	Successful eradication of Salmonella	No
2	A carrier of *Salmonella* #2	52-year-old female	Colonoscopy	Successful eradication of Salmonella	No
3	A patient with TMAU #1	24-year-old male	Gastroscopy	Moderate self-reported benefit up to 6 mo, at 12 mo symptoms had recurred to former severity	No
4	A patient with TMAU #2	49-year-old female	Gastroscopy	No change in self-reported symptom severity	No
5	A patient with SIBO	66-year-old male	Gastroscopy (treated 3 times using 2 donors)	Self-reported decrease in symptom severity	No
6	A patient with lymphocytic colitis	21-year-old female	Colonoscopy	Two week decrease in self-reported symptoms, then recurrence of symptoms to former severity	No
7	A carrier of norovirus	32-year-old female	Colonoscopy	No change in self-reported symptom severity, no success in virus eradication	No
8	A carrier of ESBL-producing	31-year-old female	Colonoscopy	Successful eradication of ESBL-producing *E. coli*	No

FMT: Fecal microbiota transplantation; TMAU: Trimethylaminuria; SIBO: Small intestinal bacterial overgrowth; *E. coli: Escherichia coli*.

option, but the patient did not accept this treatment due to his needle phobia. A two-week course of trimethoprim -sulfadiazine 160 mg/500 mg 1 × 2 per orally was started on the 26th of November since the *Salmonella* strain was found to be sensitive. Unfortunately, the faecal *Salmonella* test was still positive after this treatment.

On the 29th of January 2016, the patient was given FMT as described in detail in the FMT protocol section. Preceding the FMT, he received a 5-d course of ceftriaxone 2 g × 1 i.m. The colonoscopy findings were normal, as well as the histology of routine biopsies. There were no complications during the procedure, but the patient fainted soon afterward, which might have been caused in part by the pain and anxiety-relieving medications used during the colonoscopy. He recovered rapidly.

An upper abdomen ultrasound was performed and did not reveal any gallstones. Gallstones are a known risk factor for resistant *Salmonella*.

On the 9th of February - less than two weeks after FMT - the stool test was salmonella-positive and the treatment was considered a failure at first. However, the subsequent three tests (the 2nd, the 8th and the 11th of May) were all negative. According to the instructions of Finnish health authorities, the patient is considered free from *Salmonella* after having three negative samples in a row, and our patient could continue his studies.

There were no reported side effects of the FMT treatment. We consider it likely that the transplanted new gut microbiota played a role in the eradication of *Salmonella*, although we cannot exclude the possibility that the *Salmonella* would have been eradicated spontaneously.

A carrier of salmonella #2

A 52-year-old woman had *Salmonella* enteritis in March 2016. Her symptoms ceased, but she remained a chronic carrier. She had been treated with courses of trimethoprim-sulphadiatzine and amoxicillin. She had also undergone a two-week course of intravenous ceftriaxone, but the *Salmonella* culture remained positive. The bacterial strain was resistant to ciprofloxacin. She was on sick leave during this time because of her work in food production. FMT treatment was administered through colonoscopy on the 17th of November 2016. Prior to FMT, a course of ceftriaxone 2 g 1 × 1 i.v. was administered for six days. The three subsequent faecal tests after FMT (the 2nd, 12th and 19th of December 2016) for *Salmonella* were all negative, and she could return to work. No side effects were observed.

The rationale for treating salmonella carriage with FMT

The prevalence of chronic *Salmonella* carriage is estimated to be 2%-5% in endemic areas. Symptomless carriage of *Salmonella,* especially of individuals working in food production, is considered to be the main route of distribution of the disease among people. Furthermore, persistent carriage of *Salmonella* is associated with gallstones. Fluoroquinolones are the drug of choice for the treatment of chronic carriage of *Salmonella*[20], but strains that are resistant to ciprofloxacin pose a special challenge.

In animal models, *Salmonella* carriage is associated with changes in the intestinal microbiota[21]. It is not known whether people with *Salmonella* carriage possess alterations of the gut microbiota. To the best of our knowledge, there are no reported cases of eradication of *Salmonella* with FMT. FMT has been shown to reduce antibiotic resistance genes in the gut microbiota[22,23]. FMT has shown potential in eradicating faecal carriage of different multidrug-resistant bacteria in case reports[24]. Therefore, changing or diversifying the intestinal microbiota through FMT is a promising

new option to eradicate chronic *Salmonella* in cases where antibiotics have failed.

A patient with trimethylaminuria (fish malodour syndrome, TMAU) #1

A 24-year-old male had been diagnosed with fish malodour syndrome (trimethylaminuria, TMAU) two years earlier, but the symptoms had started at the age of 16 years. Choline loading resulted in a TMA/TMA-n-oxide-ratio of 0.43 mg/mmol creatinine (reference range 0.05-0.21). He had a severe odour problem, especially when sweating. He had been treated with riboflavin and activated charcoal without effect. A choline restricted diet and occasional two-week courses of metronidazole followed by lactobacilli treatment had a slight positive effect. Copper chlorophyllin was prescribed, but he did not initiate the treatment. After metronidazole pre-treatment, he was given experimental FMT through gastroscopy on the first of December 2015. Six weeks after FMT, he reported a slight reduction of the odour. Six months after the treatment, he reported fewer odour problems, but after one year, the malodour had returned to its former severity. He did not report any side effects.

A patient with TMAU #2

A 49-year-old female with TMAU. Odour problems started at the age of 12 when menstruation began. The odour problem was at its worst 7-10 d post-ovulation. The diagnosis was confirmed based on the urine TMA-oxide and TMA ratio. TMA-oxide was 59.1 mg/mmol creatinine (reference 17-147), and TMA was 16.5 mg/mmol creatinine (reference 2.5-10.8) ratio 0.28 (reference 0.05-0.21).

She was in the perimenopausal phase with hot flashes and excess paroxysmal sweating, causing the odour problem to worsen, but it was partly in control with hormonal treatment. Two-week metronidazole courses only helped temporarily. She had used a strict choline-restricted diet, vitamin B2 and high doses of lactobacilli. Copper chlorophyllin and activated charcoal had been ineffective. She had previously subjectively felt less of an odour problem for a few weeks after bowel cleansing for colonoscopy. FMT was given as an experimental therapy though colonoscopy. As a pre-treatment, the patient was prescribed metronidazole 400 mg three times per day for 7 d to facilitate engraftment of the donor's microbiota. Metronidazole was stopped 36 h prior to FMT. No relief of the malodour was achieved after FMT.

The rationale for treating fish malodour syndrome with FMT

Trimethylaminuria (TMAU) is a condition in which body odour resembles that of a dead fish. In TMAU, trimethylamine (TMA) accumulates in the body. Primary trimethylaminuria is genetic and caused by an inability to convert the fish smelling TMA into non-odorous trimethylamine-N-oxide (TMAO) in the liver due to a deficiency of the hepatic microsomal flavin-containing monooxygenase (FMO3). Secondary TMAU is defined as an accumulation of TMA without inherited FMO3 deficiency. The aetiology of secondary TMAU is not fully known. One causal factor may be the gut microbiota, which can produce TMA through the metabolism of certain food compounds such as TMAO and choline[25]. Thus, altering TMA metabolism may be possible through manipulating the intestinal microbiota. To our knowledge, there are no previous reported cases of TMAU treated with FMT.

A choline-restricted diet and copper chlorophyllin are the recommended treatments for TMAU[26]. Some patients experience partial relief for symptoms by using antibiotics followed by high doses of lactobacilli, riboflavin or charcoal tablets. Our hypothesis is that TMAU can be ameliorated by manipulating the gut microbiota through FMT. Some short-term positive effects were achieved in one patient, but the bacterial spectrum of the present single FMT did not seem to be effective for the treatment of TMAU. More data concerning the effect of FMT on TMAU are needed.

A patient with small intestinal bacterial overgrowth

The patient was 66 years old in January 2015 when he received his first FMT. He had colectomy and ileal pouch-anal anastomosis (IPAA) surgery in 2008 for ulcerative colitis and adenocarcinoma of the caecum. He had experienced bloating and flatulence during his adult life, but it had become worse since IPAA. He had bowel movements on average 6 times per day.

Endoscopic examination of the pouch showed no inflammation. On the small bowel passage X-ray, there was a small bowel dilatation of 10 cm on the left side of the abdomen. Small bowel MRI did not show an indication for surgery.

Before FMT, the patient underwent several treatments with inadequate results. He was treated with dietary changes and dimethicone to decrease bloating and flatulence. A probiotic - *Escherichia coli* (*E. coli*) *Nissle* up to 2 × 2 capsules (2.5×10^9-25×10^9 CFU/capsule) was administered to remediate dysbiosis. Antibiotics were given to decrease small intestinal bacterial overgrowth (SIBO). He received a course of metronidazole and two courses of rifaximin 200 mg 1 × 4, with one course lasting two weeks and the other four weeks with a tapering dosage. He reported a slight benefit from all of these treatments, but continued to suffer from flatulence and bloating.

On the 20th of January 2015, the patient received FMT *via* gastroscopy as an experimental treatment. For this treatment, 200 mL of the frozen and thawed faecal material was infused through a gastroscope deep into the descending duodenum. Biopsies were obtained *via* gastroscopy and revealed *Helicobacter*-negative atrophic gastritis.

Six weeks after FMT, the patient reported that his

symptoms and bowel movements decreased 50% and that the scent of his flatus was milder. The patient was considered to have reached a partial response and was scheduled for a new FMT.

The second FMT *via* gastroscopy was performed on the 2nd of October using the stool of the same donor as in the first FMT. This time, macrogol bowel preparation was used. The patient reported some benefit from the second treatment, but since disturbing flatulence continued, a third FMT was scheduled with a one-week course of per oral penicillin pre-treatment because the patient had previously experienced relief of his symptoms when using penicillin for a dental infection.

The third FMT *via* gastroscopy was performed on the 29th of January 2016. The transplant was from another donor who was an unfamiliar, tested and generally healthy person, whose stool was frozen and thawed on the day of the transplantation. The last contact with the patient was on the 21th of June 2016, *i.e.*, five months after the third FMT. He reported having fewer symptoms, but some flatulence persisted, though with a milder scent than previously.

The patient was considered to have gained a partial response to his SIBO symptoms from these three FMT treatments.

The rationale for treating SIBO with FMT

A SIBO is defined as an increase in the number or alterations of the type of bacteria in the small bowel. It may be associated with several features, such as alterations of the small bowel anatomy, motility, and immunity, among others. Alterations of the gut microbiota are associated with SIBO by definition. SIBO causes bloating and diarrhoea. Malabsorption, malnutrition and weight loss may also be present. SIBO can most accurately be diagnosed with jejunal aspirate, but this is not widely used due to the invasiveness of the procedure[27]. Hydrogen or methane breath tests are used more widely, but in many centres, including our own, these tests are not in clinical use. We diagnose SIBO clinically based on symptoms and signs. Our SIBO patient had an altered GI anatomy due to a J-pouch and altered immunity due to ulcerative colitis. He received three FMTs through gastroscopy and reported reduced symptoms. For a more objective evaluation of the FMT effect on SIBO, hydrogen breath tests before and after the treatments would have been valuable. More research on the effect of FMT on SIBO is warranted.

A patient with lymphocytic colitis

A female patient who was diagnosed with microscopic colitis in 2013 at the age of 18 had diarrhoea up to 20 times per day. Faecal calprotectin was constantly negative. She had an inadequate response to medications - mesalamine 2.4 mg/d, budesonide 9 mg/d for two months, loperamide or fibres. She had tried various diets to relieve the symptoms. The patient wished to be treated with FMT. For her case, there were no on-going scientific study protocols to follow.

After repeated requests from the patient and with no other rational treatment options available, FMT through colonoscopy was administered as an experimental treatment on the 21st of June 2016.

In the follow-up telephone conversation on the 7th of July, the patient reported to have gained a benefit from the procedure for two weeks, after which the diarrhoea recurred as before. The outcome was considered negative and no further transplants were given.

The rationale for treating lymphocytic colitis with FMT

Lymphocytic colitis is a subtype of microscopic colitis. It is a cause of diarrhoea that is more common in elderly people, but it may even affect children. Microscopic colitis may be associated with an altered gut microbiota. In a small study, patients with microscopic colitis had a decrease in *Akkermansia* species compared with the healthy controls. *Akkermansia* is considered to have a protective effect on the intestinal epithelium[28,29]. Our patient with lymphocytic colitis was treated once with FMT through colonoscopy. She experienced short-term (two weeks) relief of her symptoms, after which the symptoms recurred. The outcome was considered negative. In possible future studies, it might be worth considering a recurrent treatment-protocol with FMT, which has shown some promising results in IBD patients[30,31] and in a single case of collagenous colitis[32], as well as pre-treatment with antibiotics prior to FMT, which may facilitate engraftment of the donor´s microbiota[5].

A carrier of norovirus

A 32-year-old woman was treated with FMT for being a chronic carrier of norovirus. As a long-term diagnosis, she had common variable immunodeficiency (CVI), coeliac disease and osteoporosis. She had chronic diarrhoea since 2009, malabsorption since 2012 and partial parenteral nutrition since March 2015. Her norovirus infection was diagnosed in September 2013. Previously, she did not have acute gastroenteritis. Several medications for her norovirus infection had been attempted without success: interferon alfa, interferon with ribavirin and nitazoxsanide.

FMT was administered in March 2016 through gastroscopy. She was susceptible to bacterial infections due to CVI and bronchiectasis. She had received trimethoprim -sulfamethoxazole as a long-term prophylactic treatment, which was ceased 36 h before FMT. Bowel lavage was not administered prior to FMT. Routine biopsies of the gastroscopy revealed partial villus atrophy.

After FMT, the symptoms of the patient remained unchanged, with four to six bowel movements per day.

Faecal norovirus remained positive. Thus, the patient did not benefit from the experimental FMT treatment. No side effects related to the FMT were observed.

Rationale for treating chronic norovirus infection with FMT

Approximately 5% of CVI patients have enteropathy. In some case reports, chronic norovirus infection has been the cause of CVI-associated enteropathy, and eradication of the virus has cured the symptoms. It has even been hypothesized that chronic norovirus could be a key player in most of these cases[33].

It is suspected that the gut microbiota plays a role in regulating norovirus infection and its pathogenesis[34], and the relationship between the gut bacteria and norovirus infection is undergoing active analysis using murine models. We did not find any published cases of chronic norovirus or CVI enteropathy treated with FMT, and to our knowledge, the patient presented herein is the first reported case.

A carrier of ESBL-producing E. coli

A 31-year-old female patient with asthma was a carrier of the multidrug-resistant *E. coli*-extended spectrum beta-lactamase producing strain (ESBL). She had received pyelophritis caused by ESBL-producing *E. coli* two times, in October 2015 and June 2016. Both episodes of pyelonephritis had been treated with intravenous ertapenem. The duration of the second ertapenem treatment was ten days.

She had studied scientific literature concerning ESBL and *E. coli* virulence factors. After her second pyelonephritis, a consultant infectious disease specialist recommended ESBL eradication with FMT. Prior to FMT, faecal cultures for ESBL-producing *E. coli* were collected five times between August 2016 and February 2017. All the cultures were positive with ESBL-producing *E. coli*.

The *E. coli* strain was resistant to amoxicillin-clavulanic acid, ampicillin, cephalexin, ceftriaxone, cefuroxime, levofloxacin and trimethoprim-sulfamethoxazole, susceptible to ertapenem, meropenem, tobramycin, fosfomycin and nitrofurantoin and showed intermediate susceptibility to ceftazidime and piperacillin-tazobactam.

The patient was hospitalized for meningitis in November 2016. She was treated with ceftriaxone and acyclovir. The meningitis was shown to be caused by an enterovirus. She recovered fully, but the episode delayed her FMT treatment.

FMT was performed *via* colonoscopy on the 31st of January 2017. The endoscopic finding was normal, as were the routine biopsies. Six weeks later, on the 20th of March, the faecal culture of ESBL-producing *E. coli* was negative. The patient had symptoms of cystitis, and the urine test showed elevated leukocytes and *E. coli*, but this time they were susceptible to all the tested antibiotics. She was treated with a two and a half day course of nitrofurantoin 75 mg twice a day.

The rationale for treating ESBL-producing E. coli carriage with FMT

Antibiotic resistance is an emerging global health problem. One of the most common and clinically relevant types of antibiotic-resistant bacteria are the ESBL-producing enterobacteria, especially *E. coli*, which occurs worldwide[35]. Antibiotic resistance is largely caused by excessive use of wide spectrum antibiotics. We, among other authors, have reported the reduction of antibiotic resistance genes in the intestinal microbiome of patients with rCDI after FMT[22,23].

FMT has been successfully used for the eradication of ESBL-producing *E. coli* and other multidrug-resistant bacteria in a small number of published case reports[36-38]. Clinical use of FMT for eradicating resistant bacteria requires further study in larger groups of patients.

DISCUSSION

We report the results obtained for 21 FMT-treated patients; thirteen of the patients had rCDI with a significant underlying comorbidity, and eight of the patients had a condition other than rCDI. The 21 reviewed patients consisted of a heterogeneous group with many comorbidities. This establishes a limitation to our study; definitive conclusions cannot be drawn for the patients as a group. The strength of our study is that we review real life patients who are often excluded from studies due to their comorbidities.

There remains a paucity of data about FMT treatments of patients with different comorbidities. In particular, immunocompromised patients have been excluded from many studies due to the suspected risk of infectious complications. Published data from case series to date suggest that FMT is acceptably safe and effective, even for immunocompromised patients[11,12,39].

Of our thirteen patients with rCDI, *Clostridium difficile* (*C. difficile*) was successfully eradicated from eleven patients. Of those eleven, a patient with HIV and alcoholism experienced reinfection four months after FMT. One patient had gastroenteritis symptoms three days after the FMT and took vancomycin for two days without consulting a doctor. Faecal *C. difficile* was not tested. Her CDI relapses before FMT had been severe. There were no relapses of CDI documented over 8 mo of follow-up, and thus the outcome was considered positive. Two patients experienced a relapse, of which one had received antibiotics less than a week after FMT.

At one month of follow-up after FMT, two of the thirteen rCDI patients had relapsed. Two of the patients were hospitalized due to infections that were

not related to FMT. Two dialysis patients had sepsis in the months following FMT. One dialysis patient died two months after FMT. A patient with CLL and chronic norovirus did not clear the CDI or norovirus; she died due to complications of CLL five months after FMT. The HIV patient resolved the *C. difficile* infection through FMT but experienced an activation of underlying ulcerative colitis two months after FMT. The patient group had many comorbidities, and all the adverse events were considered likely to be unrelated to the FMT.

We also report eight cases of patients treated with FMT for a reason other than rCDI. These patients had prolonged *Salmonella* infection (two cases), ESBL-producing *E. coli* carriage, fish malodour syndrome (two cases), chronic norovirus infection, small bowel bacterial overgrowth and lymphocytic colitis. The acknowledged indication for FMT is recurrent *C. difficile* infection. When performed outside of this indication, FMT should preferably be conducted in a clinical trial setting[19]. However, experimental treatment in carefully considered cases is justified when other treatment options are limited. Such cases also provide preliminary results regarding the use of a specific treatment for new indications.

In the past few years, an increasing number of diseases have been shown to be associated with alterations of the gut microbiota, yet the causality is in most cases undefined. FMT has been suggested to be investigated in many of these diseases[1-4]. Although promising data about FMT in new indications such as autism[40], constipation[41] and epilepsy[42] have been reported, careful consideration of the associated risks is necessary.

The eight patients treated for causes other than rCDI all had a condition in which disruption of the gut microbiota was a possible etiological factor. All eight patients expressed a strong wish to try FMT for their condition, for which other treatments had previously failed. The patients were informed of the experimental nature of the procedure. The justification of each treatment was considered by at least three specialists, including the performing gastroenterologist, the referring physician and the head of the Gastroenterology Department.

The carriers of *Salmonella* and ESBL-producing *E. coli*, and the SIBO patient seemed to have benefitted from FMT. One of the fish malodour syndrome patients received only short-term relief for malodour, and the other did not gain any benefit. The patient suffering from lymphocytic colitis and the CVI patient with chronic norovirus infection did not gain a benefit from FMT. The positive outcome of the carriers of *Salmonella* and ESBL-producing *E. coli* was objectively defined with a laboratory test, as was the negative outcome for the norovirus patient. The outcomes of the other three patients was based on self-reported symptoms and thus were less objective. None of these patients reported any side effects.

The eradication of antibiotic resistant bacteria with FMT has been studied by many research groups, and the results to date are promising. Successful eradications have been described with several multidrug-resistant bacteria, such as ESBL-producing and carbapenemase-producing *Enterobacteriaceae*, vancomycin-resistant *Enterococci*, or methicillin-resistant *Staphylococcus aureus*[36-38]. To the best of our knowledge, the two eradications of *Salmonella* carriage are the first reported cases. The efficacy of FMT for chronic norovirus infection and fish malodour syndrome has also not been reported previously. The treatment options for multidrug-resistant organisms are scarce - eradication and increasing colonization resistance by FMT may offer a new means to counter the problem.

We think it necessary to further study the effect of FMT in conditions where other treatment options are limited. Placebo-controlled trials should be preferred due to the high risk of a placebo effect in conditions in which the diagnosis relies mostly on symptoms, although randomized controlled trials may not be an option for infrequent conditions due to the small number of patients. Thus, case series provide valuable guidance for clinical practice and future clinical trials.

In conclusion, in our cohort, FMT appeared to be a safe and effective treatment for rCDI for patients with significant comorbidities, although further conclusions cannot be drawn due to the small sample size. FMT also shows promise for the eradication of antibiotic-resistant bacteria, for which further research is warranted. FMT is only indicated for rCDI; for other indications, FMT should still be performed only in a clinical trial setting.

COMMENTS

Case characteristics

The authors reviewed 21 fecal microbiota transplantation (FMT)-treated patients, of which 13 had recurrent *Clostridium difficile* infection (rCDI) and major comorbidities: two human immunodeficiency virus patients, six haemodialysis patients, two kidney transplant patients, two liver transplant patients and a patient with chronic lymphatic leukaemia. In addition, the authors reviewed 8 patients treated with FMT for new indications: *Salmonella* carriage (two patients), trimethylaminuria (two patients), small intestinal bacterial overgrowth, lymphocytic colitis, ESBL-producing *Escherichia coli* carriage and a common variable immunodeficiency-patient with chronic norovirus infection.

Treatment

The patients were treated with FMT. Most of the patients received FMT *via* colonoscopy, and stool from a universal donor was mainly used. In a minority of cases, FMT was administered through gastroscopy.

Related reports

Immunocompromised patients have been excluded from the majority of FMT studies, but case reports and series have started to emerge. The number of case reports of patients treated with FMT for indications other than *Clostridium*

difficile is growing. To our knowledge, eradication of *Salmonella* carriage with FMT has not been reported previously.

Experiences and lessons

FMT is acceptably safe for the treatment of rCDI in immunocompromised patients. FMT is promising as a treatment for the eradication of antibiotic-resistant bacteria. There is a great demand for further research on FMT for many new indications.

Peer-review

This article gives a clear description of 21 cases and reasonable discussion, and it can provide a good reference in the daily performance of FMT. The case description in the article is very detailed, the analysis is also very thorough. The article has a good clinical significance.

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CASE REPORT

Oval mucosal opening bloc biopsy after incision and widening by ring thread traction for submucosal tumor

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Author contributions: Mori H was responsible for devising the research and writing the manuscript; Kobara H, Guan Y, Goda Y, Kobayashi N and Nishiyama N participated equally in the work; Masaki T provided a critical revision of the manuscript for intellectual content and was responsible for final approval of the manuscript.

Institutional review board statement: This study was approved by the ethics committees of Kagawa University Hospital (approval No. 51), and it is in accordance with the Declaration of Helsinki.

Informed consent statement: Patients were provided verbal and written informed consent.

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Abstract

Gastric submucosal tumors (SMTs) less than 2 cm are generally considered benign neoplasms, and endoscopic observation is recommended, but SMTs over 2 cm, 40% of which are gastrointestinal stromal tumors (GISTs), have malignant potential. Although the Japanese Guidelines for GIST recommend partial surgical resection for GIST over 2 cm with malignant potential as well as en bloc large tissue sample to obtain appropriate and large specimens of SMTs, several reports have been published on tissue sampling of SMTs, such as with endoscopic ultrasound sound fine needle aspiration, submucosal tunneling bloc biopsy, and the combination of bite biopsy and endoscopic mucosal resection. Because a simpler, more accurate method is needed for appropriate treatment, we developed oval mucosal opening bloc biopsy after incision and widening by ring thread traction for submucosal tumor (OMOB) approach. OMOB was

simple and enabled us to obtain large samples under direct procedure view as well as allowed us to restore to original mucosa.

Key words: Gastric submucosal tumors; Gastrointestinal stromal tumor; Reversible opening biopsy; Endoscopic ultrasonography; Large sample

Core tip: Gastric submucosal tumors (SMTs) less than 2 cm are generally considered benign neoplasms, and endoscopic observation is recommended, but SMTs over 2 cm, 40% of which are gastrointestinal stromal tumors (GISTs), have malignant potential. Although partial surgical resection for GIST over 2 cm with malignant potential as well as en bloc large tissue sample to obtain appropriate and large specimen of SMTs is recommended, several reports have been published on tissue sampling of SMTs. Because a simpler, more accurate method is needed for appropriate treatment, we developed oval mucosal opening bloc biopsy after incision and widening by ring thread traction approach.

Mori H, Kobara H, Guan Y, Goda Y, Kobayashi N, Nishiyama N, Masaki T. Oval mucosal opening bloc biopsy after incision and widening by ring thread traction for submucosal tumor. *World J Gastroenterol* 2017; 23(39): 7185-7190 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7185.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7185

INTRODUCTION

Gastric submucosal tumors (SMTs) less than 2 cm are generally considered benign neoplasms, and endoscopic observation is recommended[1]; however, SMTs over 2 cm, 40% of which are gastrointestinal stromal tumors (GISTs), have malignant potential[2]. The Japanese Guidelines for GIST over 2 cm with malignant potential recommend removal by partial surgical resection as well as en bloc large tissue sample collection to obtain an accurate diagnosis before surgery[3]. To obtain appropriate and large specimens of SMTs and diagnose them accurately, there have been several reports related to tissue sampling of SMTs, such as endoscopic ultrasound sound fine needle aspiration (EUS-FNA)[4,5], submucosal tunneling bloc biopsy (STB)[6], and the combination of bite biopsy and endoscopic mucosal resection (CB-EMR) by which the crown of SMTs was partially resected by EMR[7]. Because a simpler, more accurate method is needed for appropriate treatment, we developed oval mucosal opening bloc biopsy after incision and widening by ring thread traction for submucosal tumor (OMOB) approach.

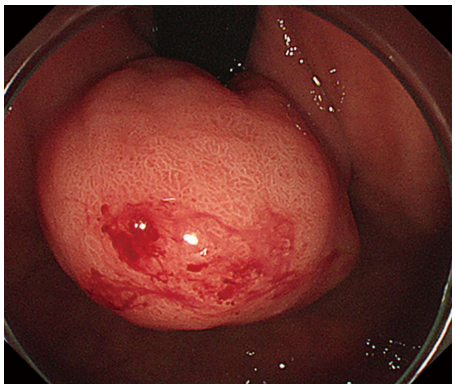

Figure 1 Endoscopic findings of gastric submucosal tumor. A gastric submucosal tumor (30 mm in diameter) is shown in the fornix of the stomach.

CASE REPORT

A forty-seven-year-old woman was diagnosed with a gastric SMT that was 30 mm in diameter in the fornix (Figure 1). As the tumor located in the fornix where EUS-FNA was unable to puncture its needle due to maximum bended endoscope position and STB was also difficult to create submucosal tunnel under maximum bended endoscope position, it was difficult to obtain sufficient tissue sample of this tumor (Figures 1 and 2A). A 5-10 mm straight incision was made on the top of the SMT by Dual knife (KD-650L, OLYMPUS Co., Tokyo, Japan) (Figures 2B and 3). After a 5-mm ring-shaped thread was delivered by grasping forceps and clipped on the left side mucosa of the incision edge (Figure 2C), second clip was hooked the ring-shaped thread (Figure 2D) and moved to be tied up the left gastric wall.

The same procedures were performed on the right side of the incision mucosa (Figure 4) making a straight incision like an oval-shaped incision (Figure 5). With more insufflation, both ring threads expanded the oval incision to a round-shaped incision from which the tumor capsule was clearly recognized (Figure 6). An approximately 5 mm incision of the tumor capsule by Dual knife made it possible to confirm the tumor itself which had abundant tumor vessels (Figures 2E and 6). A 5-mm piece of tumor tissue was obtained by cutting the tumor surface with a Dual knife. After both sides of the ring threads were detached, the opened mucosa was closed by hemoclips to restore it back to the original mucosa (Figures 2F and 7). The total procedure time was only 10 min, and there were no complications, such as bleeding or perforation. The histological result was gastrointestinal stromal tumor. Three weeks after this new bloc biopsy, the incised mucosa was completely recovered with a linear scar. Laparoscopy and endoscopy cooperative

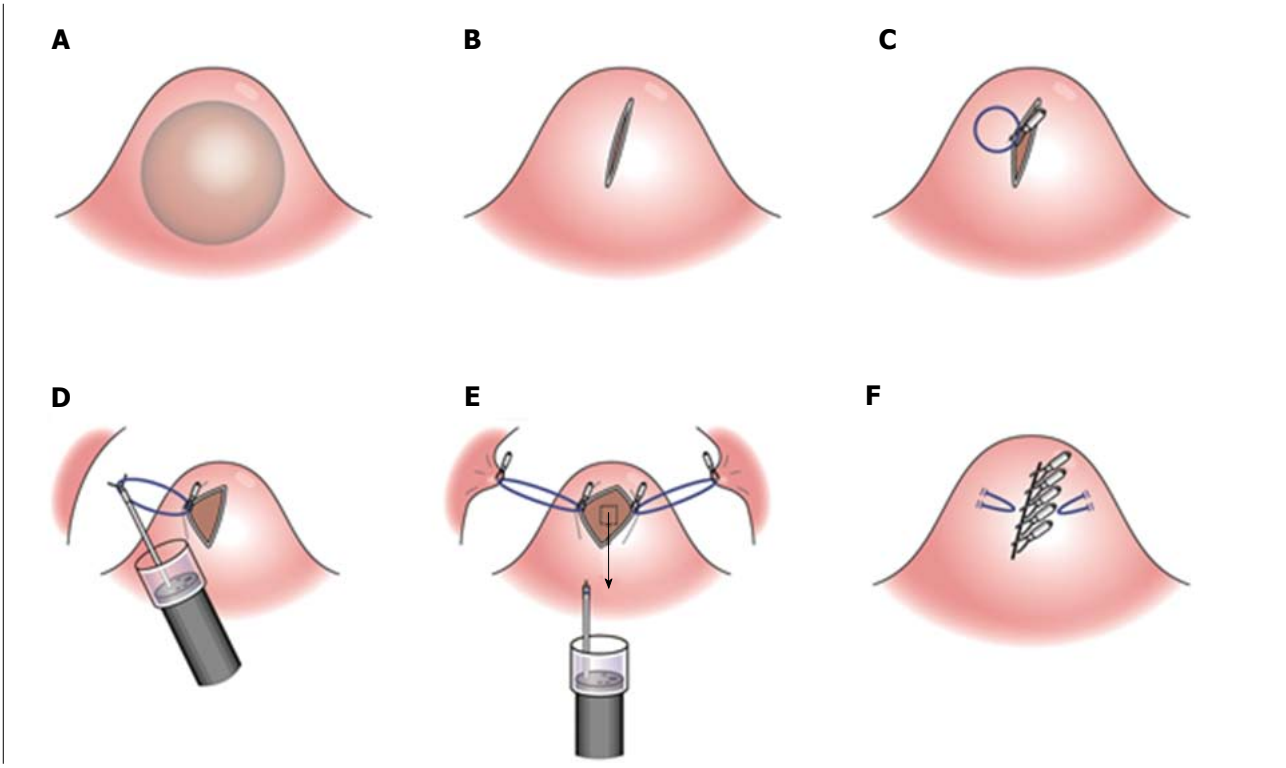

Figure 2 Oval mucosal opening bloc biopsy after incision and widening by ring thread traction. A: A gastric submucosal tumor (SMT) (30 mm in diameter) is shown in the fornix of the stomach; B: A 5-10 mm incision on the top of SMT was made; C: After a 5-mm ring-shaped thread was delivered by grasping forceps; D: Second clip was hooked the ring-shaped thread and moved to be tied up the left gastric wall; E: The same procedures were performed on the right side of the incision mucosa and made a straight incision like an oval-shaped incision; F: After both sides of the ring threads were detached, the opened mucosa was closed by hemoclips to restore it back to the original mucosa.

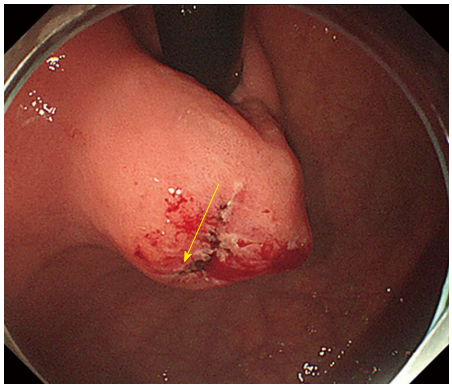

Figure 3 Incision at the top of the submucosal tumor. As endoscopic ultrasound sound fine needle aspiration and submucosal tunneling bloc biopsy were impossible due to the tumor's location, a 5-10 mm incision on the top of submucosal tumor was made (yellow arrow).

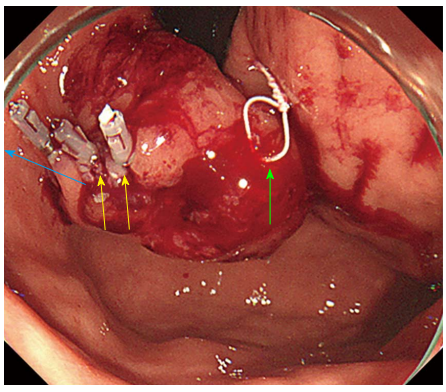

Figure 4 Ring- shaped thread counter traction. After clipping the 5-mm ring-shaped thread on the left side mucosa of the incision edge (yellow arrows), the other side of this ring thread was hooked and pulled to the posterior wall of the stomach (blue arrow). A 2nd white ring thread was placed on the other side of the incision edge (green arrow).

surgery (LECS) was successfully performed, and the histological finding of the GIST was low risk in accordance with Fletcher's classification. An endoscopic image revealed that straight incision on the top of the SMT was completely scarred and closed (yellow ring) (Figure 8) when laparoscopy and endoscopy cooperative surgery (LECS) was performed six week after oval mucosal opening bloc biopsy.

DISCUSSION

The natural history of 2-5 cm GISTs is unknown. In the Japanese Guidelines of GIST, accurate diagnosis, including the histological grade based on a sufficient

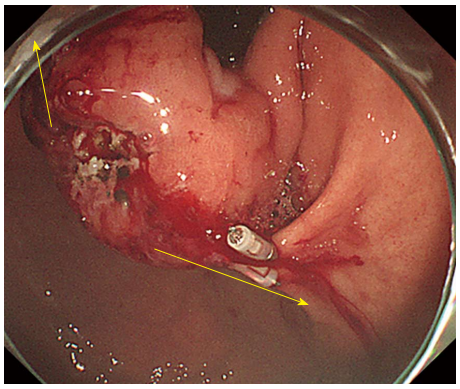

Figure 5 Oval mucosal opening after incision and widening by ring thread traction. The same procedures were performed on both sides of the incision mucosa with a straight incision to an oval shaped incision (yellow arrows).

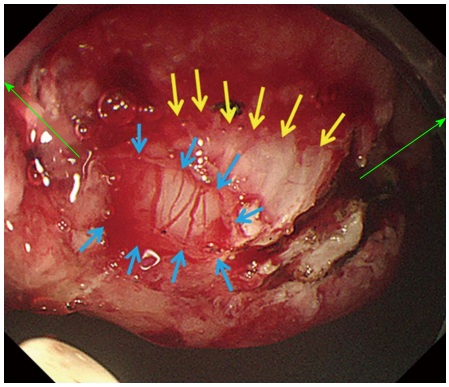

Figure 6 Direct view of capsule and abundant vessels of gastrointestinal stromal tumors. With more insufflation, both ring threads expanded the oval incision to a round shaped incision (green arrows) from which the tumor capsule was clearly recognized. An approximately 7-mm cut of the tumor capsule (yellow arrows) by Dual knife made it possible to confirm the tumor (blue arrows) with abundant tumor vessels.

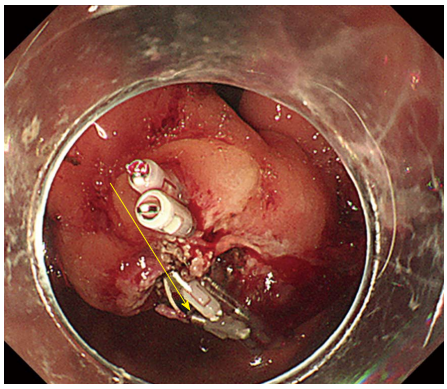

Figure 7 Reversible mucosa closure by hemoclips. After both sides of the ring threads were detached, the opened mucosa was closed by hemoclips to restore it back to the original mucosa (yellow arrow).

tissue sample, is recommended for GIST less than 2 cm, which is growing rapidly, or 2-5 cm GIST rather than endoscopic observation alone[8].

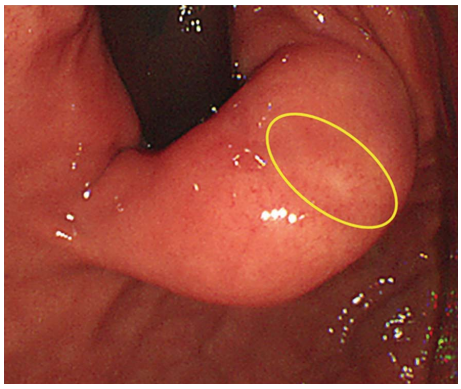

Figure 8 A mucosal incision six week after oval mucosal opening bloc biopsy. An endoscopic image revealed that straight incision on the top of the submucosal tumor was completely scarred and closed (yellow ring) when laparoscopy and endoscopy cooperative surgery was performed six week after oval mucosal opening bloc biopsy.

EUS-FNA is very useful for accurate diagnosis for SMTs since it was reported in 1992[9]. Its diagnostic sensitivity for GIST is very high at approximately 70% and the specificity is approximately 85%[10]. On the other hand, EUS-FNA does not always obtain sufficient tissue by needle sample for one of the grading factors of malignancy, such as the mitotic count under a 50 high power microscope field. The diagnostic rate for EUS-FNA was approximately 60% as the obtained samples were too small to pathologically diagnose the mitotic counts[11]. The combination of bite biopsy and endoscopic mucosal resection (CB-EMR) using a snare to cut the top of SMTs enabled us to obtain a large bloc specimen. However, the bleeding rate was very high at approximately 50%-60% from the snare resection site[12]. Bleeding after snare resection occurred due to a large mucosal defect at approximately 15-20 mm in diameter. Compared to CB-EMR, OMOB enable us to perform en bloc large tissue sampling without complications, such as bleeding, for GIST with rich vessels. OMOB consists of a 1-cm linear incision to round shaped excision using ring threads that expand with insufflation. After obtaining large bloc tissue, coagulation of bleeding vessels is performed followed by closure of the opening mucosa. Closure and recovery of mucosal incision is an important point of OMOB. STB using the ESD technique is another way to obtain a large tissue sample of GIST. As STB was safely performed using flexible endoscopic knives, only ESD experts could perform STB. It is difficult for ordinary endoscopists to perform STB[13], because making appropriate size and location of mucosal incision suitable for creating submucosal tunnel was very difficult for ESD beginner. And creating submucosal tunnel to correct direction and adjusting correct depth of submucosal dissection within the submucosal tunnel were more difficult than conventional gastric

ESD. Another disadvantage of STB is the creation of a submucosal tunnel that leaves an extra 1-cm tunnel scar outside of the GIST. This extra linear scar makes the surgical margin of LECS larger than that of OMOB.

In conclusion, OMOB was simple and enabled us to obtain a large sample under the direct procedure view; it also allowed us to restore to the original mucosa.

COMMENTS

Case characteristics

A forty-seven-year-old woman was diagnosed with a gastric submucosal tumor (SMT) that was 30 mm in diameter in the fornix.

Clinical diagnosis

The tumor located in the fornix was considered as gastric submucosal tumor.

Differential diagnosis

Gastrointestinal stromal tumor (GIST), leiomyoma, schwannoma, leiomyosarcoma, malignant lymphoma, ectopic pancreas and lipoma.

Laboratory diagnosis

All labs were within normal limits.

Imaging diagnosis

Esophagogastroduodenoscopy showed gastric SMT 30 mm in diameter in the fornix .

Pathological diagnosis

The histopathological finding of the SMT was low risk GIST in accordance with Fletcher's classification.

Treatment

Complete surgical excision of lesion.

Related reports

Several reports have been published on tissue sampling of SMTs, such as with endoscopic ultrasound sound fine needle (EUS-FNA) aspiration, submucosal tunneling bloc biopsy, and the combination of bite biopsy and endoscopic mucosal resection.

Term explanation

Oval mucosal opening bloc biopsy by ring thread traction for submucosal tumor is new method for diagnosis of gastric SMT.

Experiences and lessons

Development of oval mucosal opening bloc biopsy after incision and widening by ring thread traction for submucosal tumor (OMOB) approach was useful for simpler, more accurate method for appropriate treatment of gastric SMT.

Peer-review

This case report presented a new biopsy method for GIST of the stomach. The authors demonstrate clearly that "reversible hinged double doors method" is useful to obtain large tissue sample. This method may certainly be of use for tough case even if we use EUS-FNA. This manuscript is well-written in terms of language and seems to be informative to the readers.

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CASE REPORT

Evidence from a familial case suggests maternal inheritance of primary biliary cholangitis

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Abstract

Primary biliary cholangitis (PBC) is an idiopathic autoimmune liver disease characterized by chronic cholestasis and destruction of the intrahepatic bile ducts. Similar to other autoimmune diseases, the pathogenesis of PBC is considered to be a complex etiologic phenomenon involving the interaction of genetic and environmental factors. Although a number of common variants associated with PBC have been reported from genome-wide association studies, a precise genetic mechanism underlying PBC has yet to be identified. Here, we describe a family with four sisters who were

diagnosed with PBC. After the diagnosis of the index patient who was in an advanced stage of PBC, one sister presented with acute hepatitis, and two sisters were subsequently diagnosed with PBC. Notably, one half-sister with a different mother exhibited no evidence of PBC following clinical investigation. Our report suggests the possibility of a maternal inheritance of PBC susceptibility. Moreover, judging from the high-penetrance of the disease observed in this family, we inferred that a pathogenic genetic variant might be the cause of PBC development. We describe a family that exhibited diverse clinical presentations of PBC that included asymptomatic stages with mildly increased liver enzyme levels and symptomatic stages with acute hepatitis or advanced liver fibrosis. Additional studies are needed to investigate the role of genetic factors in the pathogenesis of this rare autoimmune disease.

Key words: Primary biliary cholangitis; Family history; Genetic susceptibility

Core tip: The precise genetic mechanism underlying primary biliary cholangitis (PBC) has yet to be identified. Here, we describe a family with four siblings who were diagnosed with primary biliary cholangitis. This is the first case report to provide evidence of a maternal inheritance mechanism for PBC based on the identification of a non-PBC half-sibling. This report also highlights the occurrence of all clinical presentations of PBC in one family. Identification of a causal variant is important for a better understanding of the mechanism underlying PBC pathogenesis.

Shin S, Moh IH, Woo YS, Jung SW, Kim JB, Park JW, Suk KT, Kim HS, Hong M, Park SH, Lee MS. Evidence from a familial case suggests maternal inheritance of primary biliary cholangitis. *World J Gastroenterol* 2017; 23(39): 7191-7197 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i39/7191.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i39.7191

INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic autoimmune cholestatic liver disease that predominantly affects middle-aged women. PBC is characterized by immune-mediated destruction of the intrahepatic bile ducts that gradually leads to fibrosis, cirrhosis, and eventually liver failure. Most patients are diagnosed when asymptomatic with an elevation of alkaline phosphatase (ALP) or with pruritus and mild elevations on liver biochemical tests. PBC can be diagnosed if two of the three following criteria are met: the presence of anti-mitochondrial antibody (AMA); cholestatic biochemical test results, such as an elevated ALP level; and histologic evidence of non-suppurative cholangitis with destruction of the interlobular bile ducts[1,2].

The current hypothesis regarding the etiology of PBC is that it is a multifactorial disease that occurs due to a combination of genetic, immunologic, and environmental factors[3]. The influence of genetic factors is evidenced by familial clusters and twin studies[4]. Epidemiological studies have demonstrated that family members of patients with PBC are at a higher risk of developing PBC[5-7]. Associations between a number of genetic loci and PBC have been reported in genome-wide association studies (GWAS), which is similar to the cases of many other autoimmune diseases[8-10]. However, no precise genetic mechanism underlying PBC is known.

Herein, we report a family with four sisters who were diagnosed with PBC. The possible implications of a monogenic etiology of PBC are discussed.

CASE REPORT

A 56-year-old woman (Table 1, patient 1) was referred to Hallym University Kangnam Sacred Heart Hospital with abnormal liver function test results. Her past history was unremarkable, and she had no history of alcohol or drug abuse. Here, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP, gamma-glutamyltranspeptidase (GGT), and immunoglobulin (Ig) M were elevated, and she was positive for AMA. A liver biopsy revealed non-suppurative destructive cholangitis and granulomatous inflammation with fibrosis (Figure 1A). The patient was diagnosed with PBC, and her family members, including three sisters, one brother, and one half-sister were evaluated (Figure 2).

Patient 2 had no symptoms but exhibited a mild elevation of ALP. She also exhibited positivity for AMA and non-suppurative cholangitis on a liver biopsy (Figure 1B) and was diagnosed with early-stage PBC.

Patient 3 was admitted to an outside university hospital with acute hepatitis of undetermined etiology. Here, liver enzymes were markedly elevated at the time of admission, *i.e.*, her AST was 744 IU/L, and her ALT was 1273 IU/L. Viral markers of acute hepatitis pathogens, including hepatitis A, hepatitis E, herpes simplex virus, Epstein-Barr virus, and cytomegalovirus, were negative. She then presented to our hospital, where laboratory tests revealed AMA positivity, an elevated IgM level, and liver histologic findings consistent with PBC (Figure 1C).

Patient 4 had no symptoms but exhibited mild elevations of the serum levels of AST, ALT, and GGT. She was AMA-positive, and her IgG and IgM levels were elevated. A subsequent liver biopsy revealed advanced histologic findings consistent with PBC, including portal inflammation, non-suppurative chol-

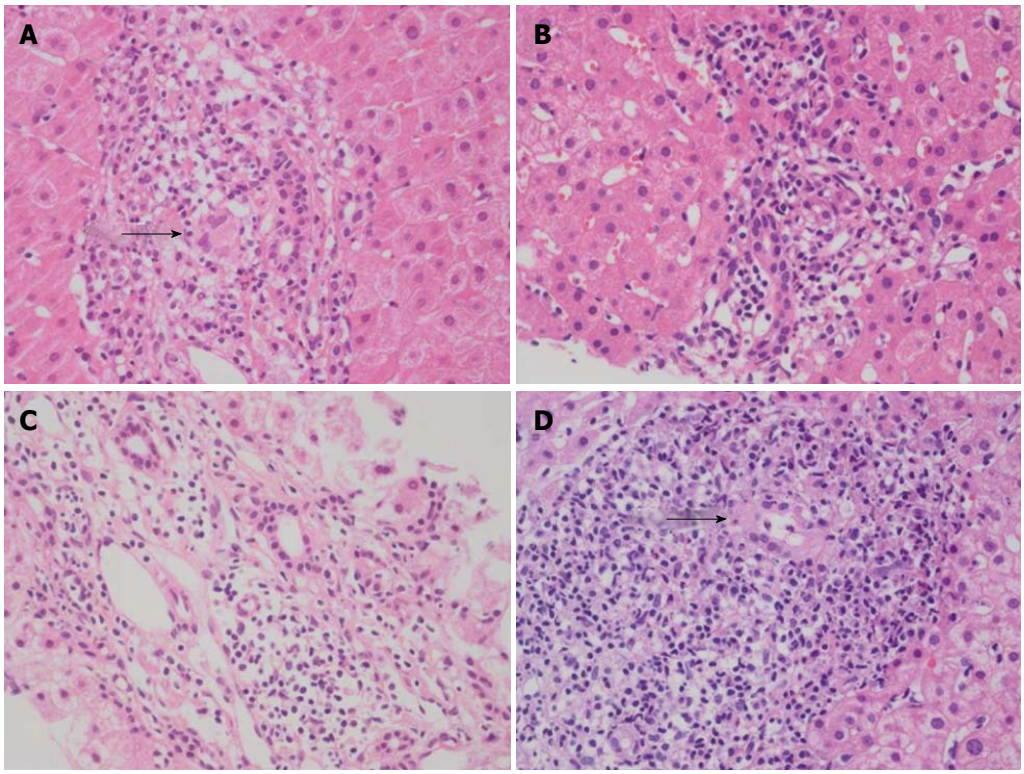

Figure 1 Microscopic features of the patients' liver biopsies (H&E stain, × 400). A: Patient 1: A non-necrotizing granuloma is surrounded by lymphoid infiltrates (arrow); B: Patient 2: Aggregates of lymphocytes indicate the early phase of the disease; C: Patient 3: Mild portal inflammation without bile duct damage or granuloma is present; D: Patient 4: A damaged bile duct is identified in a background of lymphocytes and plasma cells (arrow).

Table 1 Characteristics and initial laboratory findings from the family members

Characteristics (reference ranges)	Patient 1	Patient 2	Patient 3	Patient 4	Brother	Half-sister
Sex, age	F, 56	F, 54	F, 44	F, 38	M, 52	F, 48
Symptom	Fatigue, pruritus	(-)	Fatigue, nausea	(-)	(-)	(-)
Underlying disease	(-)	Thyroid cancer	(-)	(-)	(-)	(-)
AST, IU/L (9-39)	127	29	311	63	16	17
ALT, IU/L (6-45)	160	24	509	90	11	13
Total/direct bilirubin, mg/dL (0.4-1.3/0.1-0.4)	1.0 / 0.3	0.8 /0.2	1.3 / 0.7	0.6 / 0.2	0.5 / 0.2	0.9 / 0.3
ALP, IU/L (35-104)	132	122	122	101	83	58
GGT, IU/L (8-35)	169	63	188	112	14	15
HBs antigen/antibody	(-/+)	(-/+)	(-/-)	(-/+)	(-/+)	Not done
Anti-HCV antibody	(-)	(-)	(-)	(-)	(-)	Not done
Anti-mitochondrial antibody, titer	(+, > 1:1280)	(+, 1:640)	(+, > 1:1280)	(+, 1:640)	(-)	(-)
Anti-smooth muscle antibody	(-)	(-)	(-)	(-)	(-)	(-)
Anti-liver-kidney microsome antibody	(-)	(-)	(-)	(-)	Not done	Not done
Antinuclear antibody	(-)	(-)	(-)	(-)	(-)	(-)
IgG, mg/dL (700-1600)	1630	1150	1790	2070	1130	1210
IgA, mg/dL (70-400)	316	226	362	268	287	177
IgM, mg/dL (40-230)	421	289	542	542	247	94.4

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyltranspeptidase; HBs: Hepatitis B surface; HCV: Hepatitis C virus; Ig: Immunoglobulin.

angitis, granuloma, lymphoplasmacytic infiltrates, and peri-portal fibrosis (Figure 1D).

One brother (52 years old) with the same mother and one half-sister (48 years old) with a different mother were clinically evaluated for PBC; however, they both exhibited normal liver biochemistry and negative AMA results (Table 1).

The four patients were treated with ursodeoxycholic acid (UDCA; 15 mg/kg·d), and liver biochemistry results indicated good responses (Figure 3). The patients have been followed and maintained on UDCA therapy without complications.

DISCUSSION

Here, we report a family that displayed evidence of maternal inheritance and exhibited all possible clinical presentations of PBC. The index patient (patient 1) was

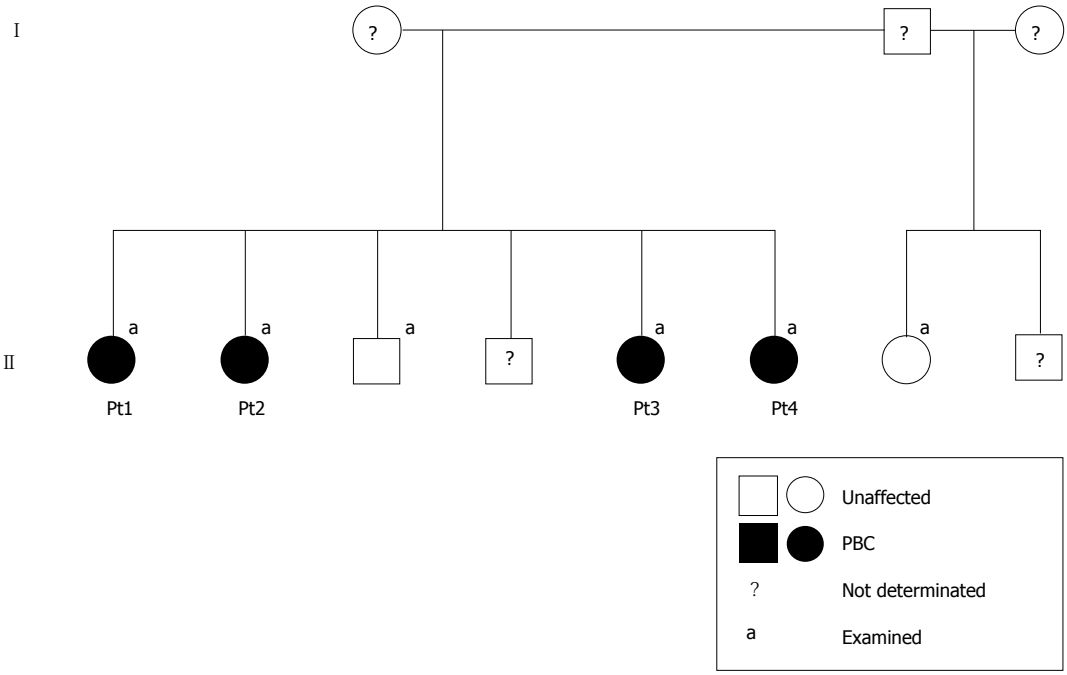

Figure 2 Pedigree of a family with four sisters with diagnoses of primary biliary cholangitis. The black symbols indicate clinically affected family members, and the white symbols indicate non-affected members. No phenotypically relevant information was available for the members with question marks. Pt: Patient; PBC: Primary biliary cholangitis.

diagnosed in a fairly advanced stage of the disease and with early cirrhosis. In contrast, two patients were diagnosed in asymptomatic stages with mildly increased liver biochemistry results (patients 2 and 4). Interestingly, one patient (patient 3) presented with an acute hepatitis-like condition with markedly elevated serum aminotransferases. In PBC, acute presentations have very rarely been reported[11]. Because acute presentations of an overlapping syndrome, *i.e.*, autoimmune hepatitis, are common, it has previously been suggested that autoimmune hepatitis might exhibit overlap with the early stage of PBC in PBC patients with acute presentations[12]. However, we were unable to find any relevant evidence of autoimmune hepatitis in patient 3, including in examinations of liver histology, auto-antibody studies, the serum IgG level, and the treatment response to UDCA[13,14].

There are some factors that are known to trigger PBC symptoms or signs that include adverse drug reactions, pregnancy, and delivery[12]. The mechanisms by which these factors affect disease state are thought to be related to an immunological influence[12]. However, our patient with the acute presentation was not pregnant and had no history of medication use. Our findings suggest that PBC should be considered in the differential diagnosis of acute hepatitis of unknown etiology. Further study is needed to identify possible undiagnosed cases and to investigate the mechanisms that trigger the acute phase of PBC.

Previous GWA studies have contributed to our understanding of the genetic architecture of PBC[8-10]. A number of susceptibility loci are located in genes with known immunologic functions, such as human leukocyte antigen (*HLA*), interleukin 12 receptor subunit beta 2 (*IL12RB2*), interleukin 12A (*IL12A*), C-X-C motif chemokine receptor 2 (*CXCR2*), and the CD80 molecule (*CD80*)[8-10]. However, the identified polymorphisms are also found in the general population at high frequencies and could partially explain the disease heritability. Based on the existence of familial cases with many affected members, as we report here[5,15-17], we infer the contribution of a rare, disease-causing variant to the development of PBC. Although the previous literature also reports a high prevalence of PBC in siblings of patients (four or more cases in 6-10 siblings)[5,15,16], the unique and interesting feature in our case is that PBC was diagnosed in all four sisters but not in one half-sister with a different mother. This finding suggests the possibility of maternal inheritance mechanism and a genetic pattern, such as X chromosome-linked or mitochondrial inheritance.

With rapid progression of next-generation sequencing (NGS) technology, the cost and time required to sequence data have substantially declined[18]. Using high-throughput NGS technology, it is technically feasible to quickly and efficiently investigate rare variants that cannot be identified by GWAS[18]. Therefore, we plan to detect the causal genetic variant in this family using whole-exome sequencing. The identification of a rare pathogenic variant is important for a better understanding of the mechanism underlying the pathogenesis of PBC and for identifying novel

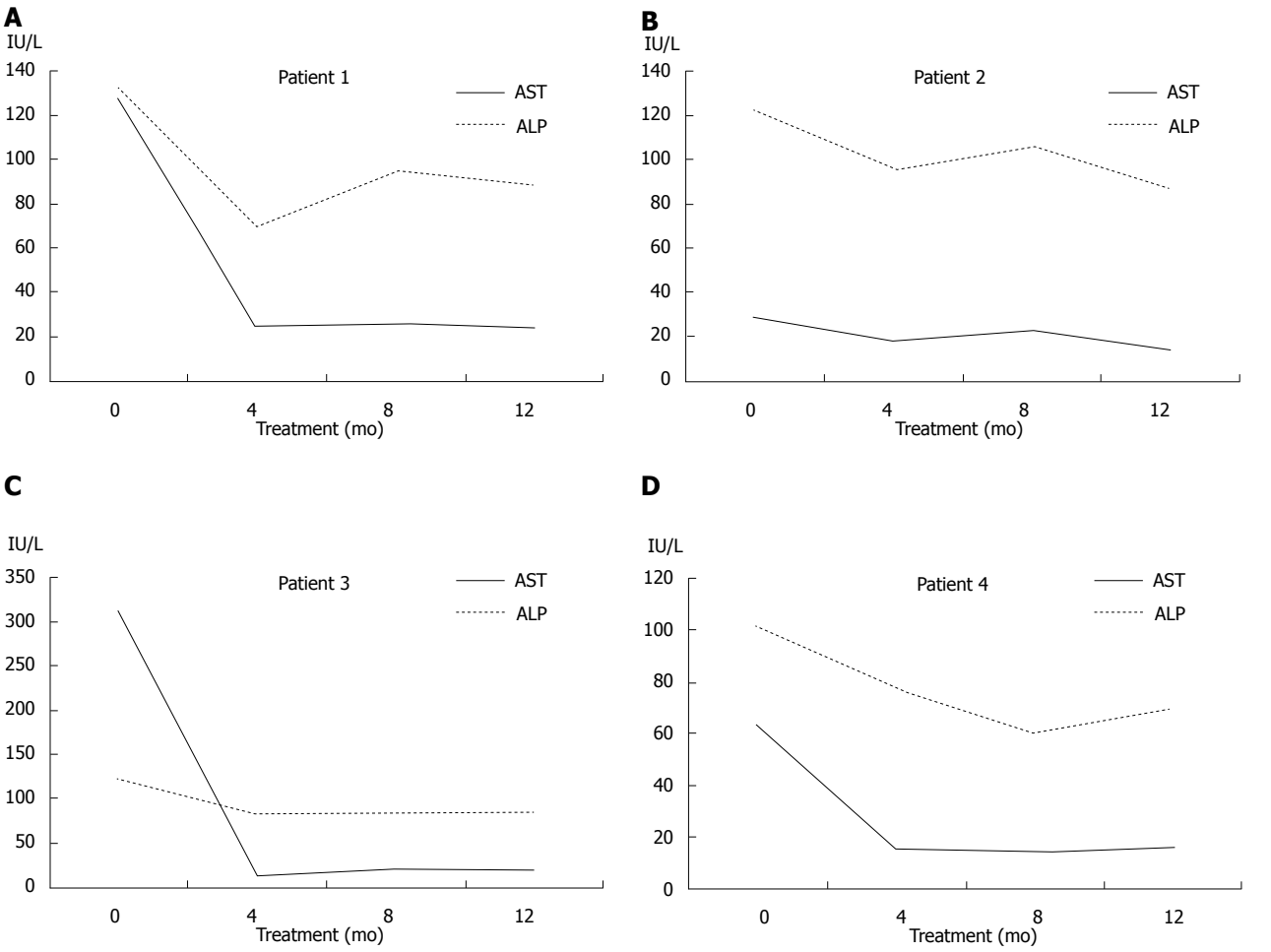

Figure 3 Biochemical responses to ursodeoxycholic acid treatment. A-D: Serum activities of aspartate aminotransferase (AST; solid lines) and alkaline phosphatase (ALP; dotted lines) showing good responses to ursodeoxycholic acid in patients 1-4.

therapeutic targets.

The early detection of PBC is important because UDCA treatment before the development of late-stage disease may normalize the life expectancy[19-21]. Long-term observational studies have demonstrated the benefits of UDCA on serum liver tests, histologic features, and improved survival[22,23]. The efficacy of a novel bile acid analogue, obeticholic acid, has also recently been demonstrated in patients who exhibit inadequate responses to UDCA[24,25]. Luckily, all of our patients responded well to UDCA therapy.

From our case and prior evidence indicating familial clustering of PBC, if one patient is diagnosed with PBC, screening with AMA and liver function tests should be recommended to other family members for the early detection and management of this condition, especially for female relatives.

The genetic etiology of PBC remains elusive despite much effort. To the best of our knowledge, this is the first case to provide evidence of a maternal inheritance mechanism for PBC based on the identification of a non-PBC half-sibling. This report also highlights the occurrence of all clinical presentations of PBC in one family. Additional studies are needed to identify a causal genetic variant in this family and the exact genetic mechanism that leads to the development of PBC.

COMMENTS

Case characteristics

Two patients presented with fatigue and nausea, and the other two patients exhibited no symptoms.

Clinical diagnosis

Four sisters in a family were diagnosed with primary biliary cholangitis (PBC), although one brother with the same mother and one half-sister with a different mother showed no evidence of PBC.

Differential diagnosis

Drug-induced cholestasis (history for medication), bile duct obstruction (ultrasound for gallstones or malignancy), autoimmune hepatitis [liver histology, auto-antibodies studies, serum immunoglobulin G level, and treatment response to ursodeoxycholic acid (UDCA)].

Laboratory diagnosis

All four patients showed the presence of anti-mitochondrial antibodies at high

titers (≥ 1:640) and elevated serum liver biochemistry results including those for alkaline phosphatase and aspartate aminotransferase.

Imaging diagnosis

For all patients, ultrasounds revealed no evidence of biliary obstruction due to gallstones or malignancy.

Pathological diagnosis

For all patients, microscopic observations of liver biopsy tissue revealed histologic findings consistent with PBC.

Treatment

The four patients were treated with UDCA.

Related reports

To date, many PBC candidate loci have been reported in genome-wide association studies. However, these loci are very heterogeneous, and the exact genetic cause of PBC remains elusive.

Explanations of terms

Whole-exome sequencing: also called WES or exome sequencing, is a technique for the sequencing of all human protein-coding exons.

Experiences and lessons

From the observations of the presentations of the four siblings diagnosed with PBC, we recommend that PBC occurrence should be considered in family members of any identified patients.

Peer-review

This is an interesting clinical observation that suggests the possibility of a maternal inheritance pattern of PBC. The presented data may provide an incentive for further research.

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LETTERS TO THE EDITOR

Duplicate publication bias weakens the validity of meta-analysis of immunosuppression after transplantation

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Author contributions: Fairfield CJ wrote this letter; Harrison EM and Wigmore SJ revised the letter.

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Abstract

Duplicate publication can introduce significant bias into a meta-analysis if studies are inadvertently included more than once. Many studies are published in more than one journal to maximize readership and impact of the study findings. Inclusion of multiple publications of the same study within a meta-analysis affords inappropriate weight to the duplicated data if reports of the same study are not linked together. As studies which have positive findings are more likely to be published in multiple journals this leads to a potential overestimate of the benefits of an intervention. Recent advances in immunosuppression strategies following liver transplantation have led to many studies investigating immunosuppressive regimes including immunosuppression monotherapy. In this letter we focus on a recently published meta-analysis by Lan *et al* investigating studies assessing immunosuppression monotherapy for liver transplantation. The authors claim to have identified fourteen separate randomised studies investigating immunosuppression monotherapy. Seven of the references appear to relate to only three studies which have been subject to duplicate publication. Several similarities can be identified in each of the duplicate publications including similar authorship, identical immunosuppression regimes, identical dates of enrolment and citation of the original publication in the subsequent manuscripts. We discuss the evidence of the duplicate publication inclusion in the meta-analysis.

Key words: Liver transplantation; Immunosuppression; Meta-analysis; Duplicate publication; Bias

Core tip: The purpose of this letter to the editor is to comment on the potential inclusion of duplicate publications within the meta-analysis titled: "Efficacy of immunosuppression monotherapy after liver transplantation: A meta-analysis".

Fairfield CJ, Harrison EM, Wigmore SJ. Duplicate publication bias weakens the validity of meta-analysis of immunosuppression

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TO THE EDITOR

We read with interest the article titled "Efficacy of immunosuppression monotherapy after liver transplantation: A meta-analysis" by Lan *et al*[1]. The authors have performed a meta-analysis assessing the use of immunosuppression monotherapy after liver transplantation. The authors claim to have included fourteen randomised studies comparing monotherapy *vs* combination immunosuppression for liver transplanted patients and conclude that calcineurin inhibitor monotherapy is both effective and leads to fewer adverse events than combination therapy. The authors state that the review is the first meta-analysis to include multiple studies assessing the effect of immunosuppression with or without steroids on graft rejection after liver transplantation. Finally, the authors state that the strengths of their review include duplicate study elimination. For the following reasons, we do not agree with their results or their conclusions.

The authors claim to have included fourteen separate randomised studies. On closer inspection, the authors have included seven references relating to only three randomised studies and have not made adequate efforts to eliminate duplicate studies[2-8].

The first of these studies was performed in the United Kingdom and both publications share the same start date, protocol, several co-authors and the same recruitment centres[2,3]. The earlier publication appears to record preliminary results[2]. Manousou *et al*[3] appear to have included these preliminary results as a separate study. Furthermore, the main publication relating to this study clearly states that the findings are "similar to those in our preliminary report". The citation in support of this statement is identical to that included as a separate study in the meta-analysis by Lan *et al*[1].

The second of these studies was performed in Germany and both publications share the same enrolment dates, protocol, several co-authors, recruitment centre and numbers of patients allocated to each intervention arm[4,5]. Furthermore, the publication recording long-term follow-up for patients in this study explicitly states that the authors have previously published their study and that in the publication in 2010 they "present the results of a re-evaluation of our study patients"[5].

The third of these studies was performed in Italy and all three publications share the same enrolment dates, several co-authors, recruitment centre and protocol[6-8]. Furthermore, both duplicate studies with later publication dates explicitly state that the earlier publications are interim reports relating to the same study[6,7].

The authors also claim to have published the first meta-analysis assessing steroid-free immunosuppression in liver transplanted patients. Three meta-analyses[9-11] were published prior to the date of submission by Lan *et al*[1]. Two further meta-analyses have been published since this date[12,13]. In each case where any of the three studies discussed have been included in another meta-analyses the authors have concluded that the studies have been subject to duplicate publication.

The problem with inclusion of duplicated data in meta-analyses is that it creates bias with inappropriate weight being afforded to the duplicate data. The failure in Lan 2014 to adequately avoid duplicate publication bias may mean that the results of this meta-analysis are invalid.

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