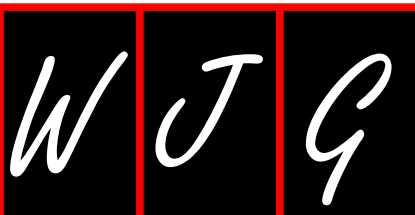


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Epidermal growth factor receptor and metastatic colorectal cancer: Insights into target therapies

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Panitumumab

Core tip: Metastatic colorectal cancer (mCRC) remains a challenge for oncologists worldwide. Despite a very aggressive disease profile, mCRC's outcomes are improving toward last decades. Target drugs, such as cetuximab and panitumumab, acquired a main role in this scenario whether phase III trials showed interesting results in overall survival and disease control. Thus, we will briefly in this paper discuss some issues and pitfalls concerning this framework.

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Abstract

Colorectal cancer (CRC) has high incidence and mortality worldwide. In 2012, CRC was the second most prevalent cancer among males (9%) and the third among females (8%). In recent decades, standard chemotherapy protocols combining 5-fluorouracil, leucovorin, irinotecan and oxaliplatin were important for improve survival in this set of patients. Further, biological drugs throughout epidermal growth factor receptor (EGFR) pathways showed interesting results in metastatic disease (mCRC) control when in association to standard chemotherapy regimens. Cetuximab and panitumumab are two cornerstones for mCRC treatment and are both approved in Europe and United States based on previous results phase III trials. This paper will briefly summarize those anti-EGFR therapies framework in mCRC and discusses some issues in this regard.

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Key words: Colorectal cancer; Epidermal growth factor receptor; *KRAS* mutation; Chemotherapy; Cetuximab;

INTRODUCTION

Colorectal cancer (CRC) has high incidence and mortality worldwide. In 2012, CRC was the second most prevalent cancer among males (9%) and the third among females (8%)^[1]. The survival rates, in advanced CRC remain low; therefore, the development of new therapeutic weapons becomes a real need. Targets therapies through epidermal growth factor (EGF) and its receptor (EGFR) and also *KRAS* pathways modulation acquired a main role whether in association with standard chemotherapy^[2]. Since its discovery, EGFR has been considered a good candidate for targeted cancer therapy^[3,4]. It is over expressed in many types of cancers, especially CRC^[5].

EPIDERMAL GROWTH FACTOR RECEPTOR

Although EGFR remains a controversial prognostic fac-

Table 1 Mainly clinical trial and target therapies

Study	Design	Median PFS (mo)	Median OS (mo)	Toxicity (grade 3/4)	Genetic analyses	Response rate
PACCE trial ^[18]	PMAB + Bev/Ox-CT	10	19.4	Skin rash, diarrhea, infections and pulmonary embolism	KRAS status was determined in 82% tumor samples. Mutations were found in 40%	46%
	PMAB + Bev/Iri-CT					
	Bev/Ox-CT	11.4	24.5			48%
	Bev/Iri-CT					
Peeters <i>et al</i> ^[22]	Panitumumab-FOLFIRI (in the WT KRAS subpopulation)	5.9	14.5 ¹	Toxicities associated with anti-EGFR therapy	KRAS status was available for 91% of patients: 597 (55%) with wild-type KRAS tumors, and 486 (45%) with mutant KRAS tumors	Improved to 35% vs 10% with the addition of panitumumab
	FOLFIRI (in the WT KRAS subpopulation)	3.9	12.5 ¹			
PRIME study ^[28]	Wild-type KRAS stratum	9.6	23.9 ¹			55%
	Panitumumab + FOLFOX (4)					
	FOLFOX(4)	8.0	19.7 ¹	Toxicities associated with anti-EGFR therapy	KRAS results were available for 1100 (93%) patients	48%
	Mutant KRAS stratum	7.3	15.5 ¹			40%
COIN trial ^[29]	Panitumumab + FOLFOX (4)	8.8	19.3 ¹			40%
	Ox and 5FU (arm A) in KRAS wild-type tumours	8.6 ¹	17.9 ¹	NA	565 (43%) had KRAS mutations	57%
	Ox and 5FU plus cetuximab (arm B) in KRAS wild-type tumours	8.6 ¹	17.0 ¹	Skin rash and gastrointestinal toxic effects		64%
	Standard Nordic FLOX (arm A)	7.9 ¹	20.4 ¹	The regimens were well tolerated	KRAS and BRAF mutation analyses were obtained in 498 (88%) and 457 patients (81%) respectively	41%
NORDIC-VII ^[20]	Cetuximab and FLOX (arm B)	8.3 ¹	19.7 ¹			49%
	Cetuximab combined with intermittent FLOX (arm C)	7.3 ¹	20.3 ¹			47%

¹Without statistical significance. PFS: Progression-free survival; OS: Overall survival; PMAB: Panitumumab; Bev: Bevacizumab; Ox:CT: Oxaliplatin-based chemotherapy; Iri-CT: Irinotecan-based chemotherapy; 5FU: 5-fluorouracil; FOLFOX/FLOX: Fluorouracil, leucovorin and oxaliplatin; FOLFIRI: Fluorouracil, leucovorin and irinotecan; NA: Not applicable.

tor, this expression-stage association may play a crucial role in the decision to initiate an adjuvant treatment toward *KRAS* mutation assessment^[6] as it will be discussed below.

However, not all patients have a good response to anti-EGFR monoclonal antibodies, and given the risks for adverse effects associated with their use and their substantial cost, there is particular interest in identifying predictors of treatment benefit or lack thereof^[2]. Genetic alterations may explain the resistance to anti-EGFR therapies^[7]. In current clinical practice, *KRAS* mutation (codon 12 and 13) is mainly responsible for primary resistance to the EGFR target drugs, particularly cetuximab and panitumumab^[8]. Thus the advantages of anti-EGFR monoclonal antibody treatment of colorectal cancer may be limited to *KRAS* wild-type patients^[9].

METASTATIC COLORECTAL CANCER

Currently, we know that many monoclonal antibodies has been approved by Food and Drugs Administration (FDA) and European Medicine Agency for the treatment of mCRC: cetuximab and panitumumab in *KRAS* wild-type patients^[5,9] and bevacizumab for those harbor codon 12 or 13 mutation^[10,11]. These drugs are used in association with

chemotherapy in patients with mCRC or maintenance therapies in chemorefractory tumors (Table 1). In overall, current guidelines advocate the use of the following regimens as initial standard chemotherapy for mCRC: fluorouracil, leucovorin, and oxaliplatin-based chemotherapy (FOLFOX), fluorouracil, leucovorin, and irinotecan-based chemotherapy (FOLFIRI), capecitabine plus oxaliplatin (CapeOx or XELOX)^[12,13], and fluorouracil, leucovorin, oxaliplatin and irinotecan-based chemotherapy (FOLFOXIRI)^[14]. The addition of a biological agent, such as anti-vascular endothelial growth factor (bevacizumab)^[15] or anti-EGFR (cetuximab or panitumumab), in *KRAS* wild-type, will depends on patients *KRAS* profile, fitness and related- clinical co-morbidities.

However, we should be aware for the toxicity profile. Most common anti-EGFR adverse events^[16] are the skin acneiform rash, xeroderma, hypomagnesemia, diarrhea and nausea^[17]. Hecht *et al*^[18] assessed panitumumab plus bevacizumab versus regular chemotherapy (oxaliplatin and irinotecan-based) as first line treatment for mCRC and showed no outcomes benefit, but only increase in toxicity profile, particularly diarrhea, infections and pulmonary embolism^[19]. The increased in the toxicity can be explicated by dual-pathway inhibition in combination with chemotherapy^[18]. In this study the patients were

enrolled onto one of two cohorts per investigator arm: a fluorouracil, leucovorin, and oxaliplatin-based chemotherapy (FOLFOX) cohort or a fluorouracil, leucovorin, and irinotecan-based chemotherapy (FOLFIRI) cohort, each with bevacizumab. Anyway, panitumumab given with FOLFOX or with FOLFIRI in the absence of bevacizumab appears to be well tolerated in other studies.

Tveit *et al.*^[20] evaluated in mCRC patients the efficacy of cetuximab plus bolus fluorouracil/folinic acid and oxaliplatin, administered continuously or intermittently as first line regimen. This study did not show significant benefit compared with the FOLFOX regime. For another hand in third-line treatment of patients with chemotherapy-refractory mCRC, cetuximab provides a substantial prolongation of progression-free-survival (PFS) and overall survival^[21]. Similarly, panitumumab plus FOLFIRI has shown significantly improved in PFS and was well-tolerated as second-line treatment in patients with wild-type *KRAS* mCRC^[22].

Plus, the combination of cetuximab plus FOLFIRI as first-line chemotherapy in wild-type *KRAS* tumors also can reduce the risk of progression of mCRC as compared with FOLFIRI alone^[23]. Moreover, we should note that the toxicity of FOLFIRI plus cetuximab combination was superior to FOLFIRI regimen alone (notably skin reactions). Notwithstanding, patients with *KRAS* wild-type locally advanced rectal cancer with the addition of cetuximab to chemoradiation regimen based on irinotecan plus capecitabine showed no benefit compared to the use of chemoradiation alone^[24]. Further, in spite of we have focused our attention to *KRAS* mutations; there are others biomarkers that are also implicated in colorectal cancer outcomes, such as *BRAF* mutation. *BRAF*-mutant tumors have worse prognosis^[25]. Recently *BRAF* inhibitor, vemurafenib, was approved by the FDA for treatment of patients with *BRAF*-mutant metastatic melanoma^[26]. It is expected that in the near future other *BRAF* inhibitors are developed and maybe directed to mCRC.

CONCLUSION

In summary, the most recent studies aim to demonstrate not only the efficacy and safety of the target molecules discussed above, in particular cetuximab and panitumumab, but also how these new agents act in conjunction with conventional chemotherapy. Currently, mCRC molecular profile assessments acquired a main role for oncologists worldwide due to the possibility of personalizing treatments approaches for mCRC patients and thus improve survival outcomes as well as quality of life^[27-29]. In addition, the choice to use bevacizumab, cetuximab or panitumumab in association with standard chemotherapy (FOLFOX or FOLFIRI) for mCRC framework run toward patients fitness, acceptable toxicities profiles, survival outcome and mainly pharmaco-economic evaluation of those drugs in this setting.

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Pain in chronic pancreatitis: Managing beyond the pancreatic duct

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Abstract

Chronic pancreatitis (CP) continues to be a clinical challenge. Persistent or recurrent abdominal pain is the most compelling symptom that drives patients to seek medical care. Unfortunately, in spite of using several treatment approaches in the clinical setting, there is no single specific treatment modality that can be earmarked as a cure for this disease. Traditionally, ductal hypertension has been associated with causation of pain in CP; and patients are often subjected to endotherapy and surgery with a goal to decompress the pancreatic duct. Recent studies on humans (clinical and laboratory based) and experimental models have put forward several mechanisms, including neuroimmune alterations, which could be responsible for pain. This might explain the partial or no response to single modality treatment in a significant proportion of patients. The current review discusses the recent concepts of pain generation in CP and evidence based therapeutic approaches (other than ductal decompression) to handle persistent or recurrent pain. We focus primarily on parenchymal and neural components; and discuss the role of antioxidants

and the existing controversies, drugs that interfere with neural transmission, pancreatic enzyme supplementation, celiac neurolysis, and pancreatic resection procedures. The review concludes with the treatment approach that we follow at our institute.

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Key words: Pain; Chronic pancreatitis; Nociception; Neuroplasticity; Antioxidant micronutrients; Pregabalin; Pancreatic enzymes

Core tip: Pain in chronic pancreatitis (CP) has multiple but simultaneously occurring mechanisms. Recent data have shown expression of nociceptors and neurotrophic factors in different neural locations. The expression of these and other neural chemokines (fractalkine) have positive correlation with pain. Pain also results from global sensitization. Among the therapeutic modalities, beneficial effects have been demonstrated with methionine containing antioxidant micronutrients supplements and pregabalin. Of the pancreatic enzymes, only non-enteric coated preparations might benefit a subgroup of patients. The threshold for performing celiac neurolysis should be high in view of variable response across clinical trials.

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INTRODUCTION

Pain in chronic pancreatitis (CP) is as enigmatic as the disease itself. There is currently no definitive cure for the illness; and treatment usually centers on pain relief and

management of exocrine and endocrine dysfunction. 85%-90% patient will have abdominal pain at presentation^[1], and in our experience over two-third of patients would present with ductal calculi and/or stricture (unpublished data). Traditionally, pain in CP has been largely associated with ductal hypertension. However, as evident from the literature, recurrence of pain is common even after ductal decompression in the form of extracorporeal shock wave lithotripsy (ESWL), endoscopic retrograde cholangiopancreatography (ERCP) or lateral pancreaticojejunostomy. Over the past several years, a host of pain mechanisms have been proposed and demonstrated directly or indirectly in humans^[2]. Most important among these are oxidative stress and inflammation induced pancreatic nociception, pancreatic neuropathy/neuroplasticity and central neuroplasticity. There appears to be significant cross talk among the different mechanisms, which could explain the partial or no success of single modality treatment approaches. This mandates a holistic and conceptualized approach to pain management in CP, aided by the little evidence available.

This review addresses the current concepts of genesis of pain in CP and evidence based management approaches focusing primarily on the parenchymal and neural components.

PAIN MECHANISMS

Nociception

Nociception refers to the perception of pain sensation as a result of activation of pain receptors (nociceptors). The proteinase-activated receptor 2 (PAR-2) and the transient receptor potential vanilloid 1 receptors are two discrete types of nociceptors that have been shown to be present in the pancreas specific sensory nerves and dorsal root ganglia^[3,4]. It is now known from experimental models that even sub-inflammatory doses of trypsin could bind to the PAR-2 receptor, thereby suggesting trypsin as a potential nociceptive stimulus, independent of its inflammatory role^[4]. Other nociceptive stimuli that have been proven or speculated to stimulate pancreatic nociceptors include trypsin, alcohol metabolites, protons, bradykinin, hydrogen sulphide, serotonin and calcium^[5]. The primary sources of intrapancreatic trypsin are the mast cells that infiltrate the pancreatic nerves in CP. The latter mediators are known to be released by injured acinar cells. Recently, another nociceptor namely the ligand-gated cation channel Transient receptor potential ankyrin 1 has also been shown to cause pancreatic inflammation and visceral hypersensitivity^[6].

Other than the above-mentioned ligands, several neurotrophic factors like nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glial-derived neurotrophic factor and artemin are expressed locally in the pancreas in response to inflammation and bind to specific receptors at different regions within the nerves (Figure 1)^[7-10]. These factors, after binding to the respective receptors cause nociceptive sensitization and neural

proliferation. Interestingly, the expression of TrkA, BDNF and artemin has been found to correlate with the severity of pain in patients with CP^[2].

Even though ductal hypertension had been considered to be a major cause of pain in CP, the mechanism was not known clearly. Recently, it was shown in experimental models that increase in pressure can activate pancreatic stellate cells (PSC), which in turn can generate oxidative stress^[11]. Furthermore, ethanol and smoking can by itself lead to oxidative stress within the PSCs^[12,13]. Oxidative stress is capable of generating a pro-inflammatory state by means of activating immune cells, increasing expression of pro-inflammatory cytokines, and activating cytokine receptors and transcription factors (*e.g.*, tumor necrosis factor alpha, NF- κ B)^[2]. This could indirectly or directly sensitize the intrapancreatic pain receptors. The response of pain in CP along with normalization of circulating oxidant stress markers after treatment with high-dose anti-oxidants is a testimony to this.

Once activated, the pain receptors generate an action potential in the first order sensory neurons of spinal levels T5 to T9. The action potential travel forward (antegrade) to the dorsal horn (gray matter) of the spinal cord where it results in the release of the neurotransmitters glutamate, calcitonin gene related peptide (CGRP) and substance P^[14], which subsequently excites the second order neurons in the dorsal horn *via* N-methyl D-aspartate, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and the neurokinin 1 (NK-1) receptors^[15]. The nociceptive transmission is then propagated through the ascending pathways in the spinal cord white matter to the thalamus, from where third order neurons relay to the sensory cortex, limbic system and the thalamus for cognitive and affective integration of pain. The sympathetic efferent cell body is the other sensory component to which the first order pancreatic nociceptive neurons project. This in turn relays to the celiac plexus *via* the greater splanchnic nerves and finally synapses with the second order sympathetic neurons. Axons of these sympathetic neurons then travel cephalad in the vagal trunks^[2].

Neural mechanisms

Several neuroimmune and neuroplastic mechanisms have been described in CP pain over the recent years in humans and experimental models. The most conspicuous neural changes in the intrapancreatic nerves include: (1) infiltration of inflammatory cells (especially mast cells and eosinophils)^[16]; (2) neural edema and perineural disruption^[17]; (3) Schwann cell (glial cell in peripheral nerves) proliferation^[18], and (4) neural hypertrophy and sprouting^[19], to name a few. The magnitude of these changes has been shown to correlate with the severity of pain, thereby ascribing them a causal role for neuropathic pain in CP. Possible factors that could be responsible for neural inflammation in the pancreatic nerves include glutamine, CGRP, substance P, and fractalkine. Some of these mediators can travel retrograde (antidromic) from the dorsal horn to the intrapancreatic nerve end-

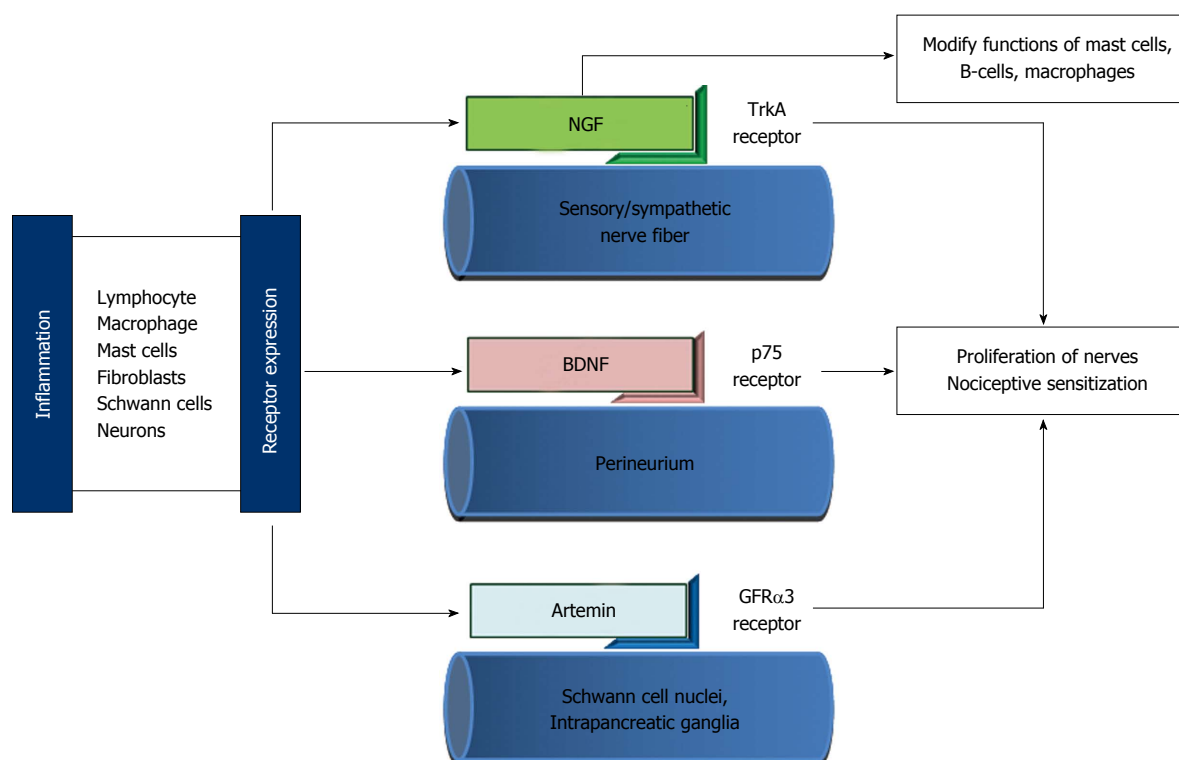


Figure 1 Schematic diagram representing neuroimmune mechanisms of pain in patients with chronic pancreatitis. The TrkA receptors (for nerve growth factor, NGF) are expressed on the sensory and sympathetic nerve fibres, p75 (for brain derived neurotropic factor, BDNF) on the perineurium and glial cell line-derived neurotrophic factor receptor $\alpha 3$ (GFR $\alpha 3$) (for Artemin) on the Schwann cell nuclei and intrapancreatic ganglia. The receptor expression is mediated by inflammation involving inflammatory cells and neural elements.

ing and induce chemotaxis of inflammatory cells^[2,20,21]. Furthermore, overexpression of two important markers, namely, nestin and growth-associated protein-43 has been demonstrated in pancreas of human CP^[19]. These two are markers of neuroplasticity/neural regeneration and are responsible for Schwann cell and neural growth. The composite of these findings and the associated pain clearly points towards profound neural remodeling within the pancreas (pancreatic neuroplasticity). These changes have important bearing on pain processing in central nervous system both at the level of the spinal cord and higher centers. Continuous sensitization of the intrapancreatic nociceptors due to persistent inflammation results in continuous stimulation of second order neurons present the dorsal horn of the spinal cord, a phenomenon called global sensitization^[22]. The clinical surrogate of global sensitization is an increase in the area of referred pain due to convergence of afferent fibres from different visceral and somatic organs on the same hyperexcitable secondary spinal neurons. This has been demonstrated recently in patients with CP, in whom the areas involved in referred pain in response to esophageal, gastric and duodenal stimulation were significantly higher than those in controls^[23]. Global sensitization results in two important phenomenon, mechanical allodynia (generation of pain after a physiological or non-noxious stimulus) and inflammatory hyperalgesia (amplified pain response to normal or minimal pain stimuli). The other manifestation of global sensitization is an increasingly painful response

to repetitive but isointense stimuli. This is known as temporal summation, which has been clearly demonstrated in patients with CP^[24,25]. It has also been demonstrated that early event-related brain potentials are altered in several key pain processing areas in the cerebral cortex in response to visceral stimulation in patients with CP^[23]. This, along with a posteromedial shift in the electrical dipoles indicates significant neural reorganization in the cerebral cortical pain processing architecture. This is central neuroplasticity. Other evidence of central neuroplasticity in CP has come from clinical studies that have shown increase in theta activity on electroencephalogram (EEG) and increased activity in the right secondary somatosensory area on magnetic resonance spectroscopy^[26,27]. Furthermore, abnormalities have been demonstrated in the descending inhibitory pathways from the cortex, which tilts the balance between these and ascending excitatory pathways in favour of more central pain processing abnormalities^[28].

Figure 2 depicts a conceptual model of pain in patients with CP.

CLINICAL EVALUATION

Even though different mechanisms for pain in CP have been proposed and demonstrated, there are currently no easily available and clinically validated tools to identify the type of pain. A clinical history of new or wider areas of referred pain could be an indicator of the develop-

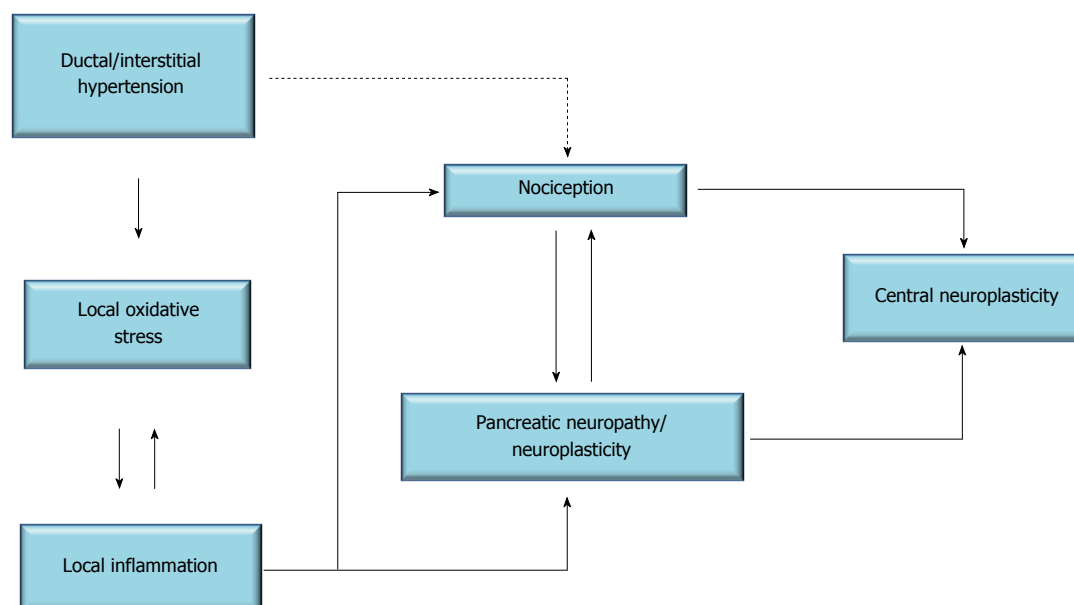


Figure 2 Schematic representation of the conceptual framework of pain mechanisms in chronic pancreatitis.

ment of neuropathy. An objective questionnaire based tool namely painDETECT has been used to evaluate neuropathic pain in the context of radiculopathy^[29]. This questionnaire evaluates components pertaining to neuropathic pain (for *e.g.*, burning/tingling nature of pain); and could have a potential use in patients with CP to assess the neuropathic component of the total pain. Persistence of theta wave on EEG is another proven feature of development of central neuroplasticity^[26]. However, this has not been tested and validated in large multicenter studies to be recommended for use in the routine clinical setting. Furthermore, even though few groups have used quantitative sensory testing for evaluation of pain in CP, this may not be feasible widely^[30].

PAIN MANAGEMENT BEYOND DUCTAL DECOMPRESSION

In routine pancreatology practice, usually three broad categories of CP patients with recurrent or persistent pain are encountered, namely those with ductal obstruction (with calculi or stricture), those after ductal decompression (post-endothecy/surgery) with a dilated duct and those with a non-dilated duct but only parenchymal changes. In the first category, ductal decompression in the form of endothecy (ESWL with/without ERCP and ductal stenting) is the current standard of care^[31,32]. Discussion of management of this group of patients is out of the scope of the current review. In the second category, it is important to rule out recurrent stones (which may be radiolucent), stricture, local complications (like inflammatory mass, biliary obstruction, pseudocysts), and cancer. In the absence of these, recurrent pain in this group of patients (and also in the third group) is most likely to be associated with predominant neural mechanisms resulting from interstitial hypertension, tissue

ischaemia, and neural inflammation. For the management of chronic and recurrent pain in CP, following treatment modalities have been practiced.

Analgesics

For short-term relief of pain in CP, the World Health Organization pain ladder, starting with an nonsteroidal anti-inflammatory drugs may be followed^[33]. It is a common practice in many western countries to use opiates long-term to ameliorate chronic and recurrent pain. However, even though high potency opiates like morphine and analogues are effective, they should be avoided as a first line drug as far as possible due to the risk of drug dependence and potentiation of side effects, including narcotic bowel syndrome. Furthermore, morphine and codeine can cause activation and degranulation of mast cells, thereby overriding the beneficial effect while worsening the inflammation and pain^[34]. Tramadol, though a low potency selective μ -opiate receptor agonist, has been shown to be as effective as higher potency narcotics but with a significantly better safety profile^[35]. Other effective alternatives for severe continuing pain include epidural buprenorphine and transdermal fentanyl (patch)^[36].

Antioxidant micronutrients

The primary aim of antioxidant micronutrient therapy in CP is to supply methyl and thiol moieties for the transsulfuration pathway, which is essential for protection against reactive oxygen species (ROS) mediated electrophilic stress^[37]. It has been demonstrated in clinical studies that there is a significant reduction in antioxidant defense in patients with CP. Studies from the United Kingdom, India and Spain have used a antioxidant cocktail consisting of methionine, organic selenium, ascorbic acid, β -carotene, and α -tocopherol; out of which 2-4 g/d of methionine (which preserves the transsulfuration path-

Table 1 Studies evaluating the role of antioxidant micronutrients on clinical outcomes, including pain, in patients with chronic pancreatitis

Ref.	Study type	Antioxidant micronutrients used	Indications; study duration	Outcomes
Uden <i>et al</i> ^[38] 1990	DB double dummy cross over	Vit C; Vit E; Beta carotene; Selenium; Methionine	<i>n</i> = 20 (ACP, ICP, IAP) 20 wk	↓ in VAS
De las Heras Castaño <i>et al</i> ^[39] 2000	Open label	Vit C; Vit E; Beta carotene; Selenium; Methionine	<i>n</i> = 19 (ACP, ICP, RAP) 12 mo	↓ in VAS, ↓ admission, ↑ exocrine fn
Dite <i>et al</i> ^[40] 2003	Open label	Vit C; Vit E	<i>n</i> = 70 (ACP, ICP) 12 mo	Pain abolished in 44%
Kirk <i>et al</i> ^[41] 2006	DB RCT cross over	Vit C; Vit E; Beta carotene; Selenium; Methionine	<i>n</i> = 19 (all ACP) 20 wk	↑ QOL
Bhardwaj <i>et al</i> ^[42] 2009	DB RCT	Vit C; Vit E; Beta carotene; Selenium; Methionine	<i>n</i> = 127 (ACP, ICP); 80% power; 6 mo	32% patients pain free ↓ No. of painful days ↓ analgesic need
Siriwardena <i>et al</i> ^[43] 2012	DB RCT	Vit C; Vit E; Beta carotene; Selenium; Methionine	<i>n</i> = 70 (ACP, ICP); 80% power; 6 mo	↔ pain ↔ QOL

↓: Indicates reduction; ↑: Indicates improvement; ↔: Indicates equivocal. DB: Double blind; RCT: Randomized controlled trial; QOL: Quality of life; Vit: Vitamin; ACP: Alcoholic chronic pancreatitis; IAP: Idiopathic acute pancreatitis; RAP: Recurrent acute pancreatitis; fn.: Function; ICP: Idiopathic chronic pancreatitis.

way in acinar cells) is believed to be the most important. Table 1 shows the clinical trials that have evaluated the effect of antioxidant micronutrient supplementation on pain relief in CP^[38-43]. The largest randomized controlled trial (from India) with 127 patients that used organic selenium (600 µg), ascorbic acid (0.54 g), β-carotene (6000 IU), α-tocopherol (270 IU) and methionine (2 g) for six months demonstrated a significant reduction in the number of painful days compared to placebo (7.4 ± 6.8 d *vs* 3.2 ± 4.0 d, respectively; $P < 0.001$) and use of analgesic tablets per month^[42]. There was also significant concomitant reduction in markers of oxidative/electrophilic stress like TBARS and improvement in antioxidant capacity. However, the clinical efficacy found in this trial was negated by the most recent randomized trial from Manchester (ANTICIPATE study), which concluded that even though micronutrients increased the antioxidant levels in blood, they did not produce adequate pain relief^[43]. It is important to note that in contrary to the Indian study, patients in the ANTICIPATE study were on high dose of narcotics, many continued to consume alcohol and many did not respond to other forms of therapy either^[44,45]. We believe that the optimal dose of antioxidant micronutrients confers definite benefit in terms of pain relief in carefully selected patients with CP. Even though it is advisable to monitor plasma glutathione and micronutrient concentrations, and titrate doses accordingly, in practice this may not be feasible.

In addition to the fixed dose antioxidant cocktail regimen, it is also important to give dietary advice on intake of anti-oxidant rich diet, and avoid practices that can adversely affect the bioavailability of dietary antioxidants (*e.g.*, vegetables cooked in high temperature)^[46]. Folate deficiency can hinder with methionine recovery for the transsulfuration pathway^[2,47]. Therefore folic acid supplementation could provide additional benefits especially to the alcoholic CP patients.

Drugs that interfere with neural transmission

In clinical practice, tricyclic antidepressants (like amitryp-

tiline) and serotonin-norepinephrine reuptake inhibitors (like duloxetine) are frequently used for refractory pain in CP, based on the observed benefits in other neuropathic states. Similarly, anticonvulsants like gabapentin and pregabalin, which are first line drugs for diabetic neuropathy have also been used. However, among all these agents, only pregabalin have been tested in a randomized controlled setting. A recent randomized controlled trial (RCT) evaluated the effect of increasing doses (150-600 mg/d) of pregabalin for three weeks on pain in 64 patients with CP; and demonstrated that there was significant reduction in pain score in the pregabalin arm [-36% (95%CI: -43 - -29)] *vs* -24% (95%CI: -31 - -16); $P = 0.02$]^[48]. Significant improvement was also observed in the patient's global impression of change at the end of the study. Ninety-one percent of patients had some adverse events in this trial, of which the most common were neurological (feeling drunk in 35%, and light-headedness in 12%). Rest of the adverse events was similar to those in the placebo arm. The number (proportion) of patients with serious adverse events in the pregabalin and placebo patients was 4 (12%) and 2 (7%) respectively; and this difference was not statistically significant. Pregabalin is α2δ receptor blocker that decreases presynaptic release of glutamate, noradrenaline and substance P; and has been shown to improve pain in CP by inhibiting central sensitization^[49]. Studies have shown that the inhibition of central sensitization is manifested by normalization of the theta band on EEG, particularly in the parietal lobe^[50]. These studies does give a comprehensive mechanistic insight of the beneficial role of pregabalin on chronic pain in CP. Further long-term follow-up studies would complement the current evidence and provide data on the long term efficacy of the drug.

Pancreatic enzymes

Pancreatic enzymes have been often used to control pain in CP. This is based on the hypothesis that proteases in the enzyme supplement would inhibit overstimulation of duodenal cholecystokinin (CCK) receptors, which will

in turn inhibit the food induced feedback loop thereby putting the pancreas to rest. However, meta-analysis of six double-blinded RCTs from 1983-1995 involving 186 patients concluded that pancreatic enzymes confer no benefit in pain relief^[51]. Similarly, a Cochrane Systematic Review of 10 RCTs (2 parallel design and 8 cross over) involving 361 patients found equivocal pain relief, fecal fat excretion and improvement in quality of life in the pooled analysis^[52]. However, the individual trials in the two studies that used non-enteric coated preparations did show significant improvement in pain^[53,54], thereby fitting into the notion of reducing post-prandial pancreatic secretion by CCK receptor inhibition. This does not happen with the enteric-coated preparations because the release of these enzyme preparations (which should happen at a pH of 5.5) in the duodenum is erratic and non-uniform due to reduced ductal bicarbonate secretion in CP. The enzymes from the enteric-coated preparations are generally released more distally in the jejunum and ileum. Unfortunately, almost all currently available enzyme preparations are enteric coated and should not be prescribed for pain relief as a sole indication. Non-enteric coated enzyme preparations (wherever available) can be of some benefit to a subgroup of patients with post-prandial pain. It is important to prescribe non-enteric preparations along with a proton pump inhibitor or H2 receptor blocker in order to prevent gastric acid mediated degradation of the enzymes in the stomach.

Celiac plexus block

Celiac plexus block with a local anesthetic (bupivacaine) with or without a combination of steroid (triamcinolone) is another modality for treatment for pain in chronic pancreatitis. Even though this can also be performed percutaneously, endoscopic ultrasound (EUS) based procedure has better results and negligible risk of developing paraplegia, which is associated with the percutaneous technique^[55]. However, the overall benefits of celiac plexus block are about 55% after 4-8 wk and a dismal 26% and 10% after 12 and 24 wk respectively^[56]. Therefore, this modality should be kept as rescue therapy for patients who do not respond to conventional medical and endoscopic therapy and are not ideal surgical candidates. EUS guided celiac ganglion neurolysis with absolute alcohol is another option, but is too extreme for a benign disease, especially in the presence of additional central mechanisms of chronic pain^[57].

Side-effects of celiac block are seen in 10%-33% of patients. The most common side effects include transient self-limiting diarrhea and orthostatic hypotension, owing to the sympathetic blockade with relatively unopposed visceral parasympathetic activity^[56,58]. Diarrhea usually settles in 48 h. Occasionally the patient may complain of an increase in the pain. Serious complications like retroperitoneal bleeding and peripancreatic abscess have infrequently been reported. An additional problem with the use of alcohol is the development of dense desmoplasia, which might make future pancreatic surgery difficult.

Surgery

Other than drainage procedures, surgical interventions for pain control in CP includes resectional procedures like classical (Kausch Whipple) or pylorus preserving (Traverso-Longmire) pancreaticoduodenectomy, distal pancreatectomy and total pancreatectomy. Pancreaticoduodenectomy is particularly useful in pain with an associated inflammatory head mass. Even though long-term pain relief has been demonstrated in 75%-95% of patients, this procedure is associated with worsening of exocrine and endocrine functions^[59,60]. Similarly, endocrine and exocrine insufficiency is seen in 80%-95% patients undergoing distal pancreatectomy^[61]; and is therefore currently performed only for recurrent pain with localized disease (such as a stricture in the upstream duct not amenable to endotherapy). Total pancreatectomy is also infrequently performed in view of the associated significant morbidity. However, with the development of islet transplantation, there has been a renewed interest in select centers in total pancreatectomy with autoislet transplantation in patients with end stage CP. However, it should be borne in mind that even after removing the entire pancreas, as high as 40% of patients could still require analgesics even after 2 years of follow-up^[62]. Many a time, a resectional procedure is combined with a drainage procedure, like Frey's, Beger's, Berne's and the V-shaped procedure, in order to provide the benefit of both ductal decompression and removal of a part of the inflamed pancreas (especially an inflammatory mass).

Bilateral thoracic splanchnicectomy is yet another infrequently used surgical modality that could ameliorate chronic pain in patients with CP; and have recently been shown to inhibit pain by predominantly impairing adrenomedullary function^[63].

Miscellaneous

Both short and long acting octreotide have been attempted in pain management in advanced CP in small-scale studies^[64,65]. Even though satisfactory pain relief has been documented, the results need to be verified in larger trials. Furthermore, whether the pain relief is better in patients with or without ductal obstruction also needs to be examined. Other than octreotide, secretin infusion has also been evaluated in a recent phase II trial with equivocal results^[66]. Few of the modalities that have been used as adjuncts to medical/surgical therapy include spinal cord stimulation^[67], cognitive-behavioral therapy, and other alternative approaches for chronic pain states. However, none of these are backed by sufficient good-quality evidence to be currently recommended specifically for pain in CP.

HURDLES IN MANAGING PAIN IN CP

Even though much have been understood on pain mechanisms in CP, there are still several hurdles in pain management in clinical practice. Firstly, several mechanisms might be simultaneously operating at any particular time

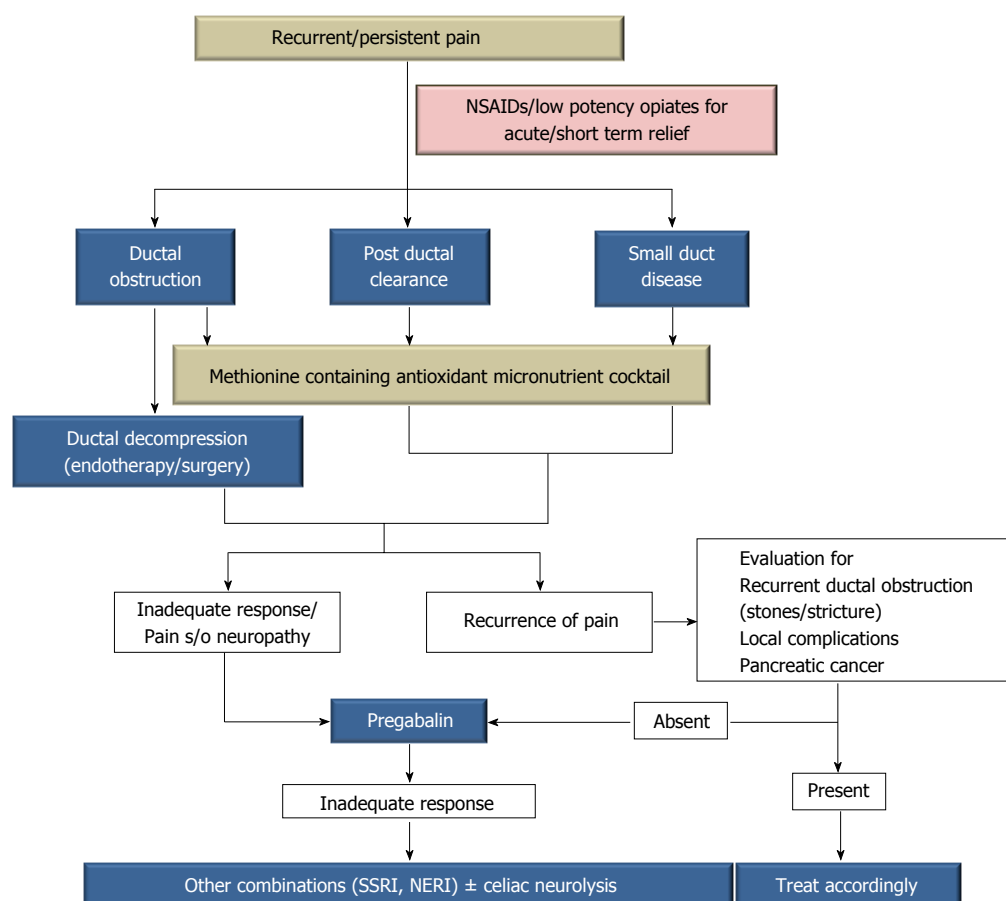


Figure 3 Management approach for recurrent and/or persistent pain in patients with chronic pancreatitis at the Asian Institute of Gastroenterology. SSRIs: Selective serotonin reuptake inhibitors; NERI: Norepinephrine reuptake inhibitor; NSAIDs: Nonsteroidal anti-inflammatory drugs.

point in a particular patient, thereby posing a question on selecting the most appropriate and optimal modality. Secondly, there are no validated objective tools that can identify the pain type, thereby precluding a fixed treatment regimen. Thirdly, there are no data to suggest an optimal duration of treatment with antioxidant and/or pregabalin in order to achieve long-term pain relief; as a result of which patients might run the risk of undertreatment or of building up excessive antioxidant micronutrients in circulation, which could impede with the physiological roles of ROS. Finally, there are no data on the efficacy or adversity of combination therapy for refractory pain.

APPROACH TO PAIN MANAGEMENT IN CP AT ASIAN INSTITUTE OF GASTROENTEROLOGY

Figure 3 shows the treatment approach that is followed at Asian Institute of Gastroenterology. This is a composite of clinical evidence; concepts build on experimental data; and clinical experience. Methionine containing antioxidants micronutrient cocktail is started early on after the diagnosis of CP. Dose and duration of treatment is titrated according to clinical response and patient's tolerance to treatment. Patient with recurrent pain with

ductal obstruction are subjected to ductal clearance by endotherapy (ESWL with or without ERCP) or surgery (in select patients). Pregabalin is added to the regimen for patients who do not show satisfactory response to antioxidant micronutrient therapy and ductal decompression; and in those who shows clinical signs suggestive of neuropathy. In patients who have recurrence of intractable pain are evaluated for recurrence of ductal obstruction, development of local complications or cancer; and treated with additional pregabalin in the absence of these. Patients who respond sub-optimally to these regimens are treated additionally with combination of SSRI and NERI like duloxetine with or without celiac plexus block. It is important to counsel the patients thoroughly on diet and lifestyle changes like quitting alcohol and smoking all along the treatment sessions.

CONCLUSION

Pain in CP is complex, and several independent and inter-dependent mechanisms may manifest simultaneously in a patient. Therefore, pain management in CP should be individualized for each patient rather than follow a fixed regimen. Recent laboratory data from human and experimental CP have opened up avenues to explore new and target specific antagonists against TRPV1, NGF, PAR2,

NK-1, CGRP and substance P.

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Liver elastography, comments on EFSUMB elastography guidelines 2013

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Core tip: The presented paper is intended to comment the "European Federation of Societies for Ultrasound in Medicine and Biology Guidelines and Recommendations on the Clinical Use of Ultrasound Elastography" and discuss the multivariate factors that have an influence on liver stiffness, and the current techniques of ultrasound elastography as well as magnetic resonance elastography.

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Abstract

Recently the European Federation of Societies for Ultrasound in Medicine and Biology Guidelines and Recommendations have been published assessing the clinical use of ultrasound elastography. The document is intended to form a reference and to guide clinical users in a practical way. They give practical advice for the use and interpretation. Liver disease forms the largest section, reflecting published experience to date including evidence from meta-analyses with shear wave and strain elastography. In this review comments and illustrations on the guidelines are given.

INTRODUCTION

Non-invasive methods for liver stiffness (LS) assessment have been researched over decades, often mirroring the development of new drugs in the treatment of chronic liver disease. So far, two main forms of elastography have become established in clinical practice. The first is known as quasi-static or strain elastography (SE). Imaging of strain and elastic modulus distributions in soft tissues based on external tissue compression, with subsequent computation of the strain profile along the transducer axis, was first described by Ophir *et al*^[1,2]. Strain imaging can be applied to the liver by inducing probe pressure^[3]. The temporal derivative of strain, *i.e.*, the strain rate, is a measure of the rate of deformation^[4]. Strain Rate Imaging is a Doppler-based method that can be used to mea-

sure strain of moving tissue^[5,6]. The second form is shear wave elastography (SWE). Shear waves are generated in the tissues when a directional force is applied to the tissue which causes shear deformation. Shear waves are rapidly attenuated by tissue, they travel much more slowly (between 1 and 10 m/s) and they are not supported by liquids of low viscosity^[7].

The use of different ultrasound methods to estimate liver fibrosis have been published, such as transient elastography (TE) (FibroScanTM)^[8-10], strain elastography (*e.g.*, Hitachi Aloka Medical)^[11-14] and SWE using acoustic radiation force impulse (ARFI) (Siemens *et al.*)^[14-16]. Other techniques including 2D-SWE (Supersonic, Siemens) and 3D-SWE (Supersonic) have since been introduced^[17-19].

Recently the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) Guidelines and Recommendations have been published assessing the clinical use of ultrasound elastography^[7,20]. The document is intended to form a reference and to guide clinical users in a practical way. The guidelines also give practical advice on its use and interpretation. Liver disease forms the largest section, reflecting the published experience to date, including evidence from meta-analyses with shear wave and strain elastography. This article comments on the EFSUMB elastography guidelines, discusses the multivariate factors that have an influence on LS, and the current techniques of ultrasound elastography as well as magnetic resonance elastography (MRE).

LS AS A DYNAMIC AND MULTIFUNCTIONAL PROCESS

LS (elasticity) is a dynamic and multifunctional process. This means that factors influencing the stiffness and elasticity of a healthy liver are different to the factors in advanced fibrosis. However, many studies have examined the grade of liver fibrosis as the sole indicator of LS^[21-25] (Table 1); a few others have evaluated more factors^[26-29] (Table 1).

In patients with chronic liver disease, the assessment of the patient should include age, liver-related comorbidity, aetiology and duration of the liver disease, grading (inflammation), fatty infiltration, risk of malignant transformation, fibrosis, general comorbidity and many other factors. Such factors are important as they guide management and indicate prognosis. Therefore, the assessment of liver fibrosis is only one of many other important factors to determine before treatment. However, the focus on the assessment of liver fibrosis seems to be overstated and many studies lack the design of multivariate analysis.

Factors influencing liver elasticity in healthy subjects depend mainly on blood volume and perfusion parameters that are reported by surgeons during daily routine. Studies have reported a positive correlation of LS with central venous pressure^[30], therefore knowledge of co-existing cardiac and pulmonary disease is necessary for interpretation of results.

In addition, it is also reported that food intake could

significantly increase the LS in adults^[31,32], children^[33] and the patients with chronic or resolved hepatitis C virus (HCV) infection^[34], therefore, elastography should be performed in fasting conditions. However, there is controversy on the influence of respiration on LS. Yun *et al.*^[35] reported that LS was significantly elevated during expiration especially in patients without liver cirrhosis while Goertz *et al.*^[32] did not find differences on the LS in deep inspiration, deep expiration and during Valsalva maneuver.

In liver cirrhosis, the degree and architecture of fibrosis is presumed to be the most important factor influencing LS (elasticity). The factors influencing liver elasticity in intermediate (significant) fibrosis are still not known in detail.

The factors influencing liver elasticity in patients with inflammatory disease (at least to some degree), independent of fibrosis, are acute hepatitis, any flare of transaminase values, acute-on chronic hepatitis^[36,37], cholestasis^[38] and acute liver failure^[39]. In a recent study of 104 patients with chronic hepatitis B (CHB) and 453 patients within chronic hepatitis C (CHC), histological necro-inflammatory activity was found to be an independent risk factor for the overestimation of LS in HCV and hepatitis B virus (HBV), while histological steatosis was a risk factor in HCV patients only^[40].

Other factors influencing liver elasticity in patients with fatty liver (hepatic steatosis) with or without inflammatory activity, with or without fibrosis, have also been described^[41-47].

The multivariate intercorrelation of factors influencing liver elasticity under different circumstances is not known. Since multiple factors have shown to influence LS measurements, interpretation of results has to be performed taking into account all these risk factors.

DIAGNOSIS OF LIVER FIBROSIS

Liver biopsy

Liver biopsy (LB) has been considered the “gold-standard” for grading and follow-up of necro-inflammatory activity and staging of fibrosis for more than fifty years^[48,49].

However, substantial limitations are obvious. Firstly, it is an invasive method with a significant complication rate^[50]. A review of the literature regarding possible complications has recently been published^[51]. Secondly, LB has shown some sampling variability^[52]. The specimen obtained by LB represents a very small part of the liver (about 1/50000) but inflammatory and fibrotic activity is known to be patchy within the liver. The sampling variability can be reduced by mini-laparoscopic guided biopsy with the ability to evaluate the liver surface^[53-57], however, it has been shown that the sampling error using mini-laparoscopic guided biopsy is still about 30%^[58]. LB has also shown some intra- and inter-observer variability^[58,59]. Thirdly, there is a high inter-observer variability during microscopic evaluation^[58].

Therefore, one difficulty for the evaluation of non-

Table 1 Examples of studies

Title	Comment	Ref.
Univariate approach		
Elastographic assessment of liver fibrosis in children: A prospective single center experience	Pearson's correlation	[21]
Is it better to use two elastographic methods for liver fibrosis assessment?	Spearman rank correlation	[22]
Is ARFI elastography reliable for predicting fibrosis severity in chronic HCV hepatitis?	Spearman rank correlation	[23]
Factors that influence the correlation of acoustic radiation force impulse, elastography with liver fibrosis	Spearman rank correlation	[24]
Liver stiffness measurement using acoustic radiation force impulse elastography and effect of necroinflammation	Pearson product-moment correlation	[25]
Multivariate approach		
Liver stiffness measurements in patients with different stages of non-alcoholic fatty liver disease: Diagnostic performance and clinicopathological correlation	Spearman's correlation (no attention paid to Bonferroni or alpha correction) 6 factors (higher age, serum albumin, serum AST, serum cholesterol, diabetes mellitus, LSM), LSM is the only independent predictor of advanced fibrosis (odds ratio = 1.47, 95%CI: 1.23-1.77, $P < 0.001$)	[26]
Assessment of liver fibrosis using transient elastography in patients with alcoholic liver disease	Spearman's correlation (with Bonferroni test). In multivariate analysis including fibrosis, HAH, and steatosis, fibrosis was the only histological parameter significantly correlated with LSM	[27]
FibroScan and ultrasonography in the prediction of hepatic fibrosis in patients with chronic viral hepatitis	Pearson correlation (no attention paid to Bonferroni or alpha correction) 12 factors. Multivariate analysis showed that LSM positively correlates with hepatic fibrosis, necro-inflammatory activity and ultrasound scores	[28]
Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis	Spearman's correlation (no attention paid to Bonferroni or alpha correction) 4 factors (fibrosis, ballooning, Lobular inflammation, steatosis). Multivariate analysis found fibrosis as the only factor influencing independently liver stiffness in NASH patients	[29]

LSM: Liver stiffness measurement; HAH: Hepatic abscess of hydatid origin; AST: Aspartate aminotransferase; NASH: Non-alcoholic steatohepatitis.

invasive markers of fibrosis is the use of LB as a reference method. Taking into account the limitations of LB, a perfect non-invasive method cannot be distinguished from an unacceptable fibrosis marker. Thus a new reference marker is needed. Studies have shown that non-invasive tests for liver fibrosis with FibroTest, enhanced liver fibrosis (ELF) and TE can predict 5-10 year survival of patients with CHC^[60-64]. However, more studies using liver related mortality as the endpoint are still awaited to identify the best non-invasive methods^[3].

Serum marker of liver fibrosis

One important non-invasive method for assessment of the severity of fibrosis includes serum markers^[65-68]. So far, many serum biomarkers, both direct and indirect, have been evaluated for their ability to stage liver fibrosis^[69-71]. Direct serum markers, reflecting either the deposition or the removal of extracellular matrix in the liver, include: (1) collagens such as type IV collagen, procollagen III N-peptide, collagenases; (2) inhibitors of collagens such as matrix metalloproteases and tissue inhibitory metalloprotease-1; and (3) glycoproteins such as serum hyaluronate, laminin, and YKL-40. So-called indirect markers include factors that can be measured from routine blood tests, such as platelet count, prothrombin index, and aspartate aminotransferase/alanine aminotransferase (AST/ALT), which indicate alterations in hepatic function. The usefulness of these markers has been assessed mostly in patients with CHC^[70-72] and hyaluronate has been the most extensively studied direct marker^[73,74]. These direct and indirect markers, when

used individually, are useful for the diagnosis or the exclusion of cirrhosis but have limited accuracy for the diagnosis of clinically significant fibrosis^[75]. Therefore, more sophisticated algorithms or indices combining the results of groups of markers have been developed to improve the diagnostic accuracy. The FibroTest™ (proprietary formula; Biopredictive, Paris, France) was the first algorithm that combined these data^[76]. Thereafter, several other indices, such as Fibrosure™ in the United States (LabCorp, Burlington, NC, United States), the Fibrometers™ (BioLiveScale, Angers, France), the FibroSpect II™ (Prometheus Laboratory Inc., San Diego, CA, United States), the ELF™ (Enhanced Liver Fibrosis Test, iQor Ltd, Southampton, United Kingdom) and the Hepascore™ (PathWest, University of Western Australia, Australia), have been developed. They are mainly for patients with CHC^[77-80], but can also be used in patients with hepatitis B^[81,82] and human immunodeficiency virus (HIV)-HCV co-infection^[83,84]. Among these indices, Fibrotest has been the one most extensively studied^[69].

In a prospective cohort of 537 HCV-infected patients, Fibrotest had a 5 year prognostic value (HCV-related complications and death) similar to that of LB^[61]. In a meta-analysis^[85] which included 6378 subjects with both FibroTest and biopsy (3501 HCV and 1457 HBV), the mean standardized area under the receiver operator curve (AUROC) for diagnosing significant fibrosis was 0.84 (95%CI: 0.83-0.86), without differences between HCV, 0.85 (95%CI: 0.82-0.87) and HBV, 0.80 (95%CI: 0.77-0.84). ELF has been evaluated in a recently published study^[86] that included 196 patients. The ELF panel

had an AUROC of 0.90 for distinguishing severe fibrosis, 0.82 for moderate fibrosis, and 0.76 for no fibrosis, and it was improved to 0.98, 0.93 and 0.84, respectively, by the addition of simple markers. The clinical utility model showed that 82% and 88% of liver biopsies could potentially be avoided for the diagnosis of severe fibrosis using ELF and the combined panel, respectively^[62,64].

Advantages and limitations

The practical advantages of analysing serum biomarkers to measure fibrosis include their high applicability and high inter-laboratory reproducibility^[87,88]. However, the direct markers of liver fibrosis are not routinely available in most hospital settings, and none of the serum markers are liver specific-their results can be influenced by comorbidity. For example, FibroTest and Hepascore produce false-positive results in patients with Gilbert's syndrome or haemolysis as these patients have hyperbilirubinaemia^[89]. Similarly, acute hepatitis can produce false-positive results in the marker measuring the level of aminotransferases, such as aspartate-to-platelet ratio index (APRI), Forns index, FIB-4, or Fibrometer tests.

Magnetic resonance elastography

In recent years, magnetic resonance elastography (MRE) has been developed as a non-invasive functional magnetic resonance imaging (MRI) method for assessing and staging liver fibrosis, using a modified phase-contrast method to image the propagation characteristics of shear waves in the liver^[90,91]. Elasticity is quantified by MRE and expressed in kilopascals (kPa) using a formula that determines the shear modulus, equivalent to one-third of the Young's modulus which is estimated with TE^[72,92]. So far, there is only limited data on the accuracy of MRE. Several studies^[92-96] have evaluated the usefulness of MRE for the assessment of LS among patients with chronic liver disease and have shown that increased shear stiffness measured on MRE is associated with increased severity of the fibrotic process. In addition, MRE has relatively high sensitivity and specificity for predicting the stage of hepatic fibrosis. It has shown at least equivalent diagnostic performance in fibrosis staging compared with TE with fewer limitations regarding its application in patients with a large amount of ascites or who are obese^[92-95]. Yin *et al.*^[95] reported sensitivity of 86% and 78%, and specificity of 85% and 96%, with cut-off values of 4.89 and 6.47 kPa, respectively. Huwart *et al.*^[93] showed similarly high sensitivity of 98% and 95%, and specificity of 100% and 100%, for discrimination, but lower cut-off values of 2.5 and 3.1 kPa were used. The reason for the difference in cut-off values obtained in the two studies may potentially be explained by the differently manufactured scanners used for MRE acquisition, case mixes, imaging protocols, and post-processing procedures. A meta-analysis has been recently published^[97].

Advantages and limitations

Compared with TE, dynamic MRE has the potential

to assess larger volumes (almost the entire liver) and to provide full three-dimensional information about the viscoelastic properties of tissues^[98], moreover, due to the theoretical advantages, MRE is capable of application to patients with obesity or ascites. However, MRE cannot be performed on the liver of patients with iron overload because of signal-to-noise limitations and it is too costly and time-consuming to use in routine practice^[72].

INTRODUCTION TO ULTRASOUND ELASTOGRAPHY

TE

TE (FibroScan[®]) was the first tool introduced for routine clinical use (Echosens, Paris, France) (Figure 1). TE does not display a conventional ultrasound image. TE has been mainly evaluated in patients with chronic viral hepatitis C and also in a few patients with HIV/viral hepatitis C co-infection and some other liver diseases (see below)^[99].

Technique

Basic principles: TE is an ultrasound-based non-invasive method. It is characterized by the material's strain response to external stress according to the principle of Hooke's law^[9]. Briefly, an ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency (50 Hz) are transmitted by the transducer from a right intercostal space, inducing an elastic shear wave that propagates through the liver. Pulse-echo ultrasound acquisition is used to follow the propagation of the shear wave and to measure its speed. This speed is proportional to the tissue stiffness, with faster wave progression occurring through stiffer material. The elastic modulus E is expressed as $E = 3qV^2$, where V is the shear wave speed and q is the material density (assumed constant for tissues): the stiffer the tissue, the faster the shear wave propagates^[100]. TE measures LS in a cylindrical volume approximately 10 mm wide and 40 mm long, between 25 and 65 mm below the skin surface with the standard M-probe, and between 35 and 75 mm for the recently developed XL probe, recommended for obese patients^[101,102]. This volume is at least 100 times bigger than a biopsy sample and it has been suggested, therefore, that the results compared to LB are more representative of the hepatic parenchyma. However, TE does not work for the left liver lobe or from a subcostal approach and the measurement is only feasible *via* a few intercostal spaces. Therefore, the technique is limited. Inter- and intra-observer variability depend on the intercostal space used, the presence of ascites, musculoskeletal habitus, depth of subcutaneous tissue, position of the patient, and many other factors^[47,103].

How to perform? The measurements with FibroScan[®] are taken from the right liver lobe *via* an intercostal space, while the patient lies flat on his/her back, with the right arm tucked behind the head to facilitate access to the liver parenchyma. The tip of the probe is covered with

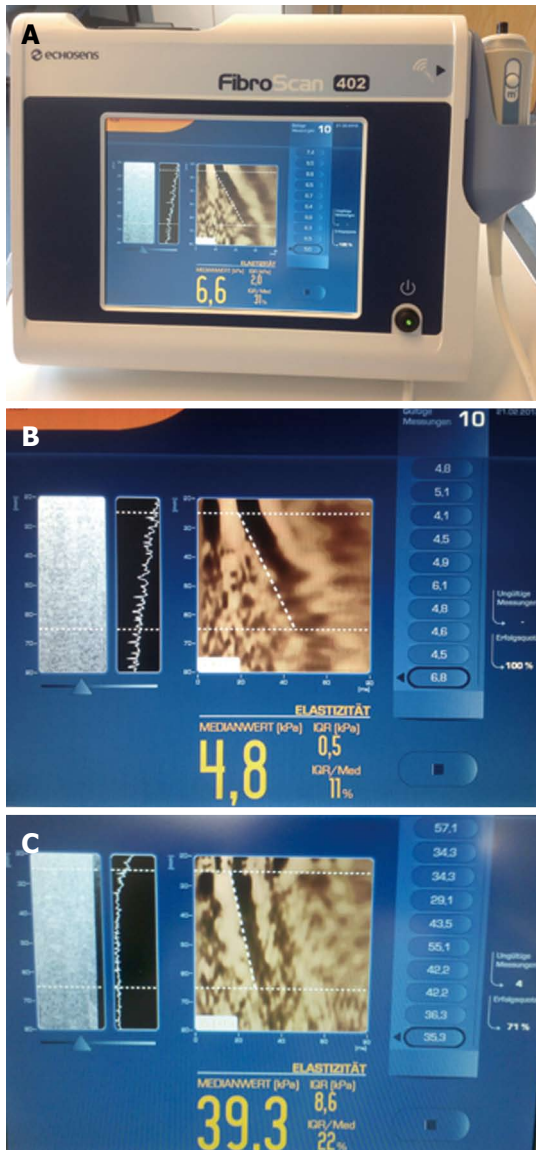


Figure 1 Transient elastography (FibroScan®, A) for the evaluation of normal liver (B) and liver cirrhosis (C).

coupling gel and placed on the skin between the ribs at the level of the right lobe where LB would be performed. Once the measurement area has been located, the operator presses the probe button (shot) to start an acquisition. When a shot is unsuccessful, the machine does not give a reading. Measurement of LS is measured in kilopascals (kPa) (range is between 2.5 and 75 kPa)^[100].

Advantages: TE with FibroScan® is a rapid procedure (less than 5 min), painless, and easy to perform even in the outpatient clinic or at the bedside. The results are immediately available^[103]. The examination can be performed by a nurse after a short learning curve (about 100 examinations)^[104]. In addition, TE analysis has excellent inter- and intra-observer agreement, which makes it suitable for widespread application in clinical practice^[103,105,106].

Limitations: TE provides only a regional elasticity measurement (determined by the width of the ultrasound beam and depth of the shear wave penetration), but no anatomical images or elastograms. Other drawbacks include limited depth (several cm), the inability of the shear wave to propagate beyond fluid collections (ascites) and difficulty in obtaining sufficient signal in obese patients. Recently, a new probe (XL probe; Echosens, Paris, France) has been proposed for overweight and obese patients^[107], and a so-called S-probe has been developed for patients with narrow intercostal spaces, especially children^[108]. However, it remains impossible to obtain TE results from patients with ascites^[105].

The validity of the TE result also depends on two important parameters: (1) the success rate (the ratio of the number of successful measurements to the total number of acquisitions) should be at least 60%; and (2) the interquartile range (IQR), which reflects the variability of the validated measurements, should not exceed 30% of the median value^[109] (Figure 1). Both the feasibility and reproducibility of the TE measurement may be affected by high body mass index (BMI). In a study with 13369 TE measurements, a failure rate of 3.1% was reported. Unreliable results were reported in 15.8% of measurements and were associated with a BMI > 30 kg/m², age > 52 years, female sex, operator experience and type 2 diabetes^[47].

The clinical interpretation of TE results should always be made by an expert clinician and with reference to the patient's history, disease aetiology and essential laboratory parameters Castera *et al.*^[110].

Intra- and inter-observer variability: Several studies^[103,105,106] have shown that the intra- and inter-observer reproducibility of TE measurements are good, at least in non-obese subjects. In the study by Sandrin *et al.*^[105] intra- and inter-observer variation in TE was investigated in 15 patients and was around 3%, but with a wide variation (2%-18%). The sample size of this study was small, and therefore inadequate to draw firm conclusions on host- and disease-related co-variables that may interfere with TE performance. Another study by Fraquelli *et al.*^[103] with a larger sample obtained similar results; 800 TE examinations were performed by two operators in 200 patients with various chronic liver diseases. Both inter- and intra-observer agreement was high and TE reproducibility was excellent, with an intraclass correlation coefficient (ICC) of 0.98. However, inter-observer agreement was significantly reduced in patients with mild hepatic fibrosis, and hepatic steatosis.

The probe location during the TE measurement may affect its reproducibility. In a recent study^[111] TE was performed on 625 consecutive patients with chronic liver disease at three different sampling sites. Sampling variability according to probe location was seen in approximately 30% of patients and it was suggested that TE should be performed from various sites to minimize the sampling error.

Review of the literature

Chronic viral hepatitis: For patients with CHC, LS values > 6.8 – 7.6 kPa are indicative of significant fibrosis ($F \geq 2$) using the gold standard of LB, and the cut-off values for predicting complete cirrhosis ($F = 4$) range between 11.0 and 13.6 kPa^[20,112,113]. TE is able to distinguish mild fibrosis from advanced liver fibrosis and cirrhosis, which is important for decision making^[114]. In contrast, TE does not allow differentiation between the contiguous stages of liver fibrosis. In a meta-analysis including 40 studies^[114], the pooled sensitivity and specificity of TE was 79% and 78% for the diagnosis of significant fibrosis; 82% and 86% for diagnosing severe fibrosis; and 83% and 89% for the diagnosis of liver cirrhosis.

It might be of interest to remember that conventional ultrasound techniques can also distinguish between liver cirrhosis and early liver disease in approximately 70% of patients with high specificity but low sensitivity^[115-124]. However, TE had an acceptable diagnostic accuracy for detecting early compensated cirrhosis in patients with CHB who did not fulfil the clinical and ultrasound criteria for cirrhosis^[125]. Conventional ultrasound techniques are helpful in the detection of complications of liver cirrhosis including portal hypertension^[126,127] and can also give important information about fatty infiltration^[128-132] and inflammation^[133-136]. In a study with 90 patients with suspected liver cirrhosis, liver surface nodularity on conventional ultrasound and TE showed comparable results for diagnosis and exclusion of liver cirrhosis, with the best results when both methods were combined. Liver surface nodularity was better for the diagnosis of liver cirrhosis, while TE was better at ruling out cirrhosis^[137].

The performances of TE, when compared, have been shown to be similar between patients with HBV and HCV^[138]. Several studies have investigated the performance of TE in an Asian population with CHB^[125,139-144] and concluded that TE is a promising and accurate tool for the early detection of cirrhosis. It is demonstrated that the optimal cut-off values for diagnosing HBV-related cirrhosis were between 9.0 and 10.1 kPa in the Asian population^[125,140,142,145], which is lower than that in patients with CHC^[146,147]. Since there is an increasing number of evidence on the usefulness of TE in patients with CHB, especially in the Asian population, TE should also be recommended in patients with CHB, though the evidence is more limited compared to CHC. Future and updated guidelines have to include this recommendation.

It would be interesting to know in what percentage of patients TE can give important additional information which is of relevance to the treatment, over and above sophisticated ultrasound technology in the hand of an expert hepatologist^[43,148].

EFSUMB recommendations

TE can be used to assess the severity of liver fibrosis in patients with chronic viral hepatitis, provided that confounding factors are taken into account, and especially to distinguish patients with nil/mild fibrosis from those with

significant fibrosis, and to identify those with cirrhosis. TE is useful for assessment of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD), alcoholic liver diseases, and in patients co-infected with HIV and HCV. Other types of chronic liver disease might also have been investigated, but the evidence is more limited. TE is useful for assessment of liver fibrosis in patients with post-transplant recurrence of CHC. TE has some value for predicting the occurrence of complications of liver cirrhosis, portal hypertension, hepatocellular carcinoma (HCC) and liver-associated mortality. It cannot replace upper gastrointestinal endoscopy for identifying patient with varices^[20].

POINT SHEAR WAVE ELASTOGRAPHY WITH ACOUSTIC RADIATION FORCE IMPULSE IMAGING

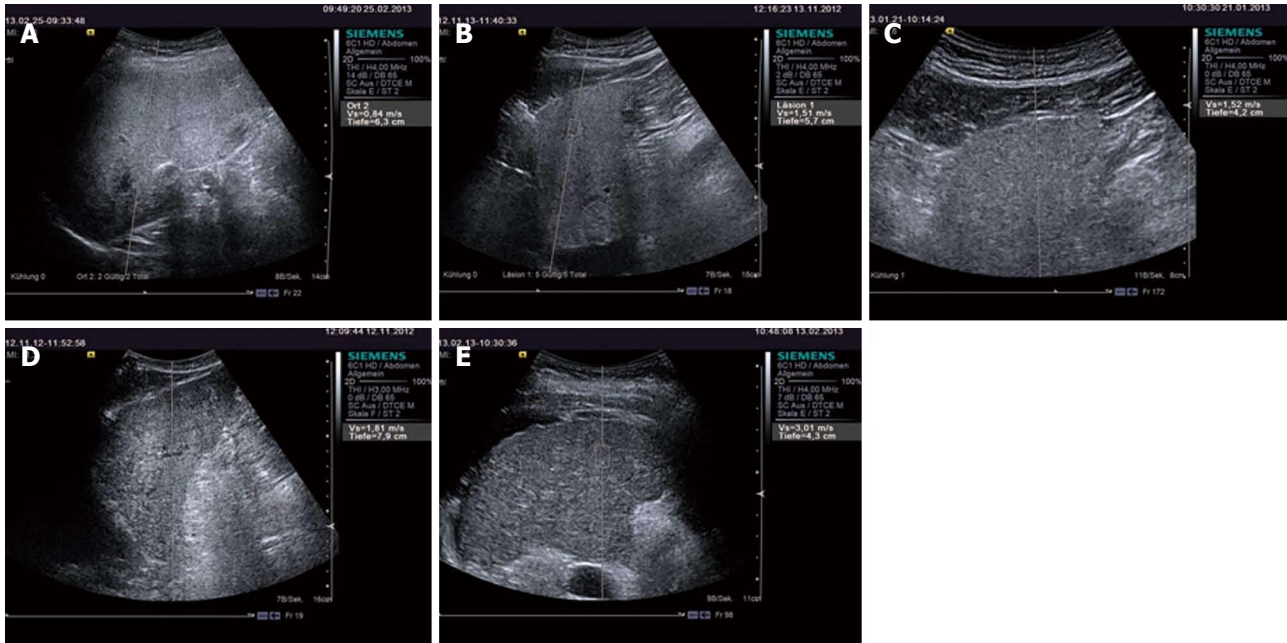
Point shear wave elastography (pSWE) has been introduced by different companies, each currently at different stages of development^[7,20]. Acoustic radiation force impulse (ARFI) was the second method to be introduced as a tool for liver fibrosis assessment in a clinical setting. ARFI has a significant advantage over TE in that it simultaneously displays a conventional ultrasound image. The accuracy of both methods has been shown to be similar in the differentiation of normal liver parenchyma from liver cirrhosis^[15,149,150]. ARFI has been mainly evaluated in patients with chronic viral hepatitis C and in a few other liver diseases.

Technique

Basic principles: ARFI quantification has been developed by two companies (Siemens and Philips) according to the guidelines^[7,20], almost all reported studies were done with a conventional high-end ultrasound machine (Siemens S2000). It uses a region of interest (ROI) cursor to interrogate the elastic properties of a specific anatomic region, while real-time B-mode imaging of the abdomen being performed. Short-duration acoustic pulses with a fixed transmit frequency of 2.67 MHz, are generated in the vicinity of the ROI and the subsequent mechanical excitation of the tissues results in tissue displacement and the formation of shear waves that propagate away from the region of excitation. Ultrasound tracking beams laterally adjacent to the single push-beam are used to estimate the shear wave speed in the tissue by the measurement of the time to peak displacement at each lateral location^[151]. The shear wave speed is estimated in the central window 5 mm long by 4 mm wide within a graphically displayed ROI of size 10 mm long by 6 mm wide. The results are expressed in meters per second (m/s) (range: 0.5–4.4 m/s with $\pm 20\%$ accuracy over the range), the shear wave propagation speed being proportional to the square root of the tissue elasticity^[152,153]. The ARFI imaging examination takes approximately 5 min. Unlike FibroScan®, ARFI can be utilized in patients with ascites. No limitations

Table 2 Mean shear wave velocities (VirtualTouch values) of the left and right liver lobes (mean \pm SD)

Ref.	n	Subjects	Left lobe (m/s)	Right lobe (m/s)
Karlas <i>et al.</i> ^[158]	50	Healthy individuals	1.28 \pm 0.19	1.15 \pm 0.17
Karlas <i>et al.</i> ^[158]	23	Patients with F1, F2 fibrosis	2.1 \pm 0.73	1.75 \pm 0.89
Toshima <i>et al.</i> ^[159]	103	24 healthy volunteers, 79 patients with chronic liver disease	1.90 \pm 0.68	1.61 \pm 0.51
Piscaglia <i>et al.</i> ^[157]	14	Healthy individuals	1.29 (1.00-1.60)	1.15 (0.80-1.74)
Piscaglia <i>et al.</i> ^[157]	114	Patients with chronic liver disease	1.79 (0.80-4.00)	1.67 (0.45-3.76)

**Figure 2** Point-shear wave elastography with acoustic radiation force impulse for the evaluation of histologically proved liver fibrosis and cirrhosis. A: F0; B: F1; C: F2; D: F3; E: F4 = cirrhosis.

concerning measurement are known^[154].

Tips and tricks: When scanning the right lobe (especially segment VIII), an optimal window should be used. To reduce the variance of the measurement, it is recommended to apply minimal scan pressure and for the patient to minimize breathing, the influence of cardiac motion should also be avoided. In general, the best and most consistent results will occur when the “normal” state of the liver is measured. When scanning intercostally, no pressure should be applied to the liver and the patient should be asked to just stop breathing for a moment (instead of deep inspiration and breath hold).

In difficult patients, several measurement attempts are needed to “average” out the readings, and data that varies significantly should be excluded. It is recommended to put the patient in a left lateral decubitus position with right arm behind the head in order to get better access to the liver without excessive pushing or the need for breath holds^[155]. However, it may still not be possible to get reliable readings in 5.3 % of patients^[156].

Intra- and inter-observer variability: Reproducibility of ARFI is also an important pre-requisite for its widespread application in clinical practice. Good inter-observer vari-

ability has been reported^[157]. Since ARFI allows different measurement sites, comparison of measurements in the right and left liver lobes have been made, and have shown a trend toward higher values in the left lobe^[157-159]. However, results in the right lobe revealed higher diagnostic accuracy compared to the left (AUROC: for diagnosis of F1, F2, F3, F4, right lobe: 0.92; 0.83; 0.86; 0.80; left lobe: 0.77; 0.71; 0.78; 0.84; sensitivity, specificity, positive predictive value and negative predictive value of right lobe: 0.88; 0.81; 0.74; 0.92; left lobe: 0.80; 0.75; 0.87; 0.68)^[159] (Table 2).

Clinical applications

Chronic viral hepatitis: In patients with significant fibrosis ($F \geq 2$) the ARFI cut-off values published have been between 1.21-1.34 m/s (AUROCs 0.85-0.89)^[23,149,150] and in patients with cirrhosis, 1.55-2 m/s (AUROC's 0.89-0.93)^[15,23,149,160] (Figure 2). Similar to TE, SWE has not proved accurate enough to distinguish between contiguous stages of fibrosis^[20].

Other liver diseases: ARFI has also been evaluated in patients with NAFLD and NASH^[161,162] and in patients after liver transplantation^[163].

Meta-analysis: Friedrich-Rust *et al.*^[154] published a meta-

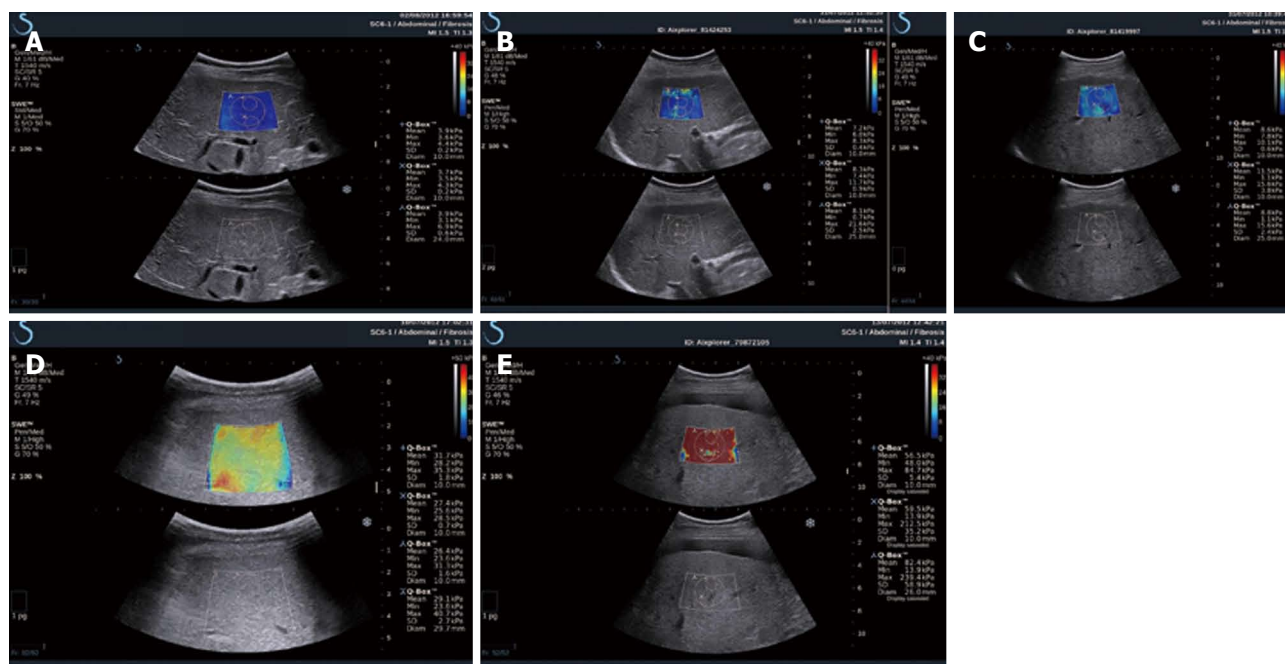


Figure 3 2D-shear wave elastography with supersonic shear imaging for the evaluation of histologically proved liver fibrosis and cirrhosis. A: F0; B: F1; C: F2; D: F3; E: F4 = cirrhosis.

analysis which included 9 studies with a combined total of 518 patients with chronic liver disease and evaluated the diagnostic performance of ARFI imaging for the staging of liver fibrosis. The diagnostic accuracy of ARFI quantified by the AUROC was 87% for predicting significant fibrosis ($F \geq 2$), 91% for the diagnosis of severe fibrosis ($F \geq 3$) and 93% for the diagnosis of liver cirrhosis. The meta-analysis revealed good diagnostic accuracy for ARFI in the diagnosis of significant liver fibrosis and excellent diagnostic accuracy for the diagnosis of liver cirrhosis.

It was also shown that a comparison of ARFI with TE in the four studies that included 312 patients, resulted in comparable diagnostic accuracies for both methods in the diagnosis of severe fibrosis, and slightly, but significantly, higher diagnostic accuracies of TE for the diagnosis of significant fibrosis and liver cirrhosis. However, a recent study showed superior results for ARFI elastography^[150]. Future multicentre studies are necessary to compare the different methods before any conclusions can be drawn.

Advantages and limitations

In contrast to TE, ARFI has been shown to be less influenced by obesity and ascites^[151]. One study showed that valid LS measurement (LSM) were obtained in all 23 patients with morbid obesity (mean BMI was higher than 44 kg/m²)^[164]. In addition, it can be easily added to a commercial ultrasound machine.

However, in contrast to TE values, ARFI values have a narrow measurement range (0.5-4.4 m/s), which limits the definition of cut-off values required for decisions on patient management. In addition, inflammatory activity

and elevated aminotransferase levels may lead to overestimation of ARFI-LS values^[15,25] as has been shown for TE. Moreover, since this is a new technique, the quality criteria are not yet well-defined.

EFSUMB recommendations

pSWE with ARFI can be used to assess the severity of liver fibrosis in patients with chronic viral hepatitis, especially with hepatitis C. pSWE with ARFI is promising for liver fibrosis assessment in patients with NAFLD, and post-transplant patients^[7,20].

2D SWE

SWE [Aixplorer®, SuperSonic Imagine (SSI), France] has been introduced as a 2D and also 3D-technique. So far, only 2D-SWE has been evaluated in studies on the liver. The studies of 3D-SWE have mainly focused on the breast^[165,166].

Technique

This technique is based on the combination of a radiation force induced in the tissues by focused ultrasonic beams and very high frame rate (up to 5000 f/s) ultrasound imaging capable of catching, in real time, the transient propagation of the resulting shear waves^[167,168]. The local shear wave speed is recovered using a dedicated time-of-flight estimation technique and enables the 2-D quantitative mapping of elasticity. This imaging modality can be performed using a conventional ultrasound probe, during a standard intercostal ultrasound examination. Three supersonic shear wave imaging sequences are applied successively to the left, middle and right parts of

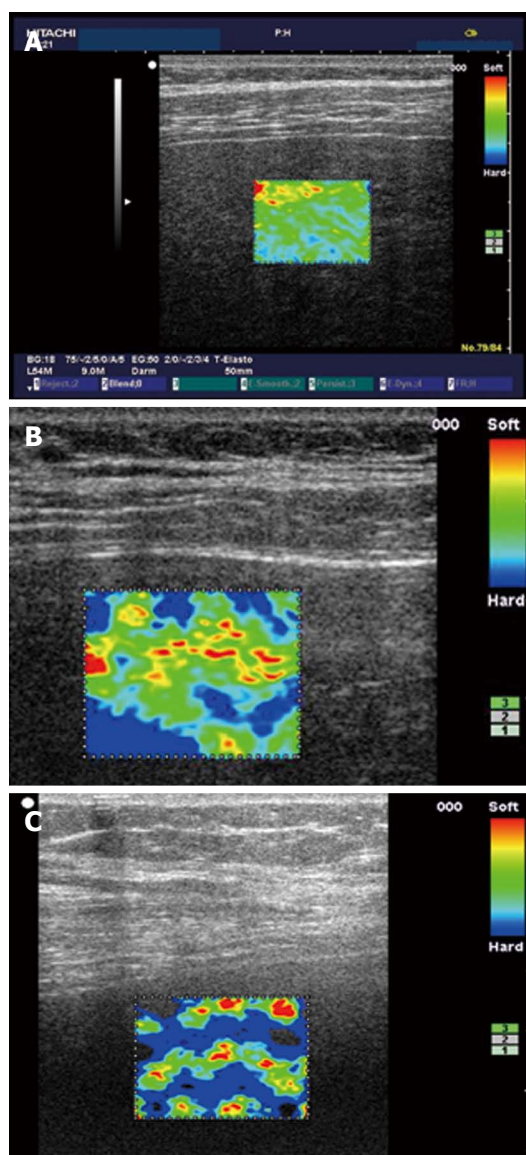


Figure 4 Strain elastography for the evaluation of histologically proved liver fibrosis and cirrhosis. A: F0; B: F2; C: F4 = cirrhosis.

the 2-D ultrasound image. The resulting elasticity images in the three regions are concatenated to provide the final image covering the entire region-of-interest. The ability of the SWE technique to provide a quantitative and local estimation of liver shear modulus with a millimetric resolution has been proven in a pilot study in 15 healthy volunteers^[18]. Liver moduli extracted from *in vivo* data from healthy volunteers, were consistent with those reported in the literature (Young's modulus ranging from 4 to 7.5 kPa). Moreover, LSM using the SWE mode was fast (less than one second), repeatable (5.7% standard deviation) and reproducible (6.7% standard deviation)^[3].

Intra- and inter-observer variability: To date, there has only been one study^[169] aimed at assessing the intra- and inter-observer precision of 2D-SWE measurements in the evaluation of liver elasticity. It was reported that the

reproducibility was good with high intra- and inter-observer agreement. In this study, 2D-SWE was performed on 60 volunteers (42 cases with 10 consecutive measurements, 18 cases with 2 measurements) on 2 different days by 2 operators (one expert and one novice). The intra-observer agreement between measurements performed in the same subject on the same day (day 1 or day 2) showed intraclass correlation coefficient (ICC) values of 0.95 and 0.93 for the expert operator and novice, respectively, and the ICC values for intra-observer agreement between measurements performed in the same subject on different days were 0.84 and 0.65, respectively. The inter-observer agreement was 0.88. Therefore, real-time 2D-SWE has been shown to be a reproducible method to measure liver elasticity, but the novice operator showed lower measurement reproducibility over time than the expert operator.

Clinical application

CHC: 2D-SWE might be used in assessing liver fibrosis for patients with CHC, as has been proved in two large studies. Ferraioli *et al.*^[170] assessed the accuracy of 2D-SWE in comparison with transient elastography in 121 patients with CHC using LB as the reference standard, and found that LS values increased in parallel with the degree of liver fibrosis both with 2D-SWE and TE. The AUROC was 0.92 for 2D-SWE and 0.84 for TE ($P = 0.002$); 0.98 for 2D-SWE and 0.96 for TE ($P = 0.14$); 0.98 for 2D-SWE and 0.96 for TE ($P = 0.48$), when comparing F0-F1 *vs* F2-F4, F0-F2 *vs* F3-F4, and F0-F3 *vs* F4, respectively (Figure 3). Therefore, the real-time 2D-SWE was more accurate than TE in assessing significant fibrosis ($\geq F2$). In the other study which included 113 hepatitis C virus patients, a good agreement was shown between 2D-SWE and TE, the AUROC for elasticity values assessed by 2D-SWE were 0.948, 0.962 and 0.968 for patients with predicted fibrosis levels $F \geq 2$, $F \geq 3$ and $F = 4$, respectively. However, LB was only available in 39 patients^[17].

EFSUMB recommendations

2D-SWE can be used to assess the severity of liver fibrosis in patients with chronic viral hepatitis, especially with hepatitis C^[7,20].

STRAIN ELASTOGRAPHY

Strain elastography (SE), also termed as quasi-static strain imaging, has been developed by several manufacturers, however, only Hitachi ultrasound system has been evaluated for use in liver.

Technique

SE is based upon the fact that soft tissue can be more easily compressed than hard tissue. When subtle compression is applied with probe, SE shows the relative degree of tissue strain, but not demonstrates the physical elasticity directly. SE calculates the strain response of the tissue

Table 3 Advantages and disadvantages of non-invasive methods to evaluate liver fibrosis

Parameters	Transient elastography	ARFI	2D-SWE	MR Elastography	Serum biomarkers
Advantages	High and rapid performance Reproducibility Easy to learn	High and rapid performance Reproducibility Easy to learn	High and rapid performance Reproducibility Easy to learn, large ROI	High performance (applicability) Reproducibility Examination of the whole liver Combined with conventional MRI obesity and ascites are not limiting	Availability Reproducibility Low cost
Disadvantages	Technical requirements (equipment) without additional use Intermediate cost Limited recognition of intermediate stages of fibrosis Blind selection of region of interest Restricted value in obese patients and ascites False positive values in patients with acute hepatitis, cholestasis, and heart failure	Combined with conventional ultrasound Obesity and ascites are not limiting Technical requirements (ultrasound equipment) Intermediate cost Limited recognition of intermediate stages of fibrosis Narrow range of values, small ROI Quality criteria not well defined	Combined with conventional ultrasound Ascites are not limiting Technical requirements (ultrasound equipment) Intermediate cost Limited recognition of intermediate stages of fibrosis Quality criteria not well defined	Technical requirements (MRI equipment) Extremely high cost, time consuming Limited recognition of intermediate stages of fibrosis Not applicable in case of iron deposition	Non-specific (hyperbilirubinemia, hemolysis, inflammation, others) Relatively high cost, limited availability (patent) Limited recognition of intermediate stages of fibrosis Results not immediately available

ARFI: Acoustic radiation force impulse; SWE: Shear wave elastography; ROI: Region of interest; MRI: Magnetic resonance imaging.

to stress (relative tissue elasticity) and displays it as a colour overlay [ranges from red (soft) to blue (hard)] on the B-mode image^[171]. The echo signals could be captured in real-time by incorporating a high speed algorithm, in addition, both the B-mode image and corresponding tissue elasticity image could be simultaneous displayed^[172]. Semi-quantitative elastography techniques are based on quantification of the strain distribution within a defined ROI.

Because the pressure generated by the operator's compression may influence both the image of elasticity and the resulting elasticity score, Hitachi medical system has recently developed an elastography method that did not require extra external stress. The required liver distortion for future analysis would be achieved from the rhythmic pulsations of the abdominal aorta or the heart.

Clinical application

In 2007, Frederick-Rust *et al*^[11] reported the clinical application of SE in the liver. They developed an elasticity score by assessing the colour-coded strain image using the computer program Matlab. The diagnostic accuracy for F2, F3 and F4 were 0.75, 0.73 and 0.69, respectively. In 2009, the same group^[173] compared SE with TE (Fibroscan) and serum fibrosis marker (Fibrotest), and concluded that SE in its evaluated format could not replace TE for non-invasive assessment of liver fibrosis at the time of the study. After the software for elastography was developed by Hitachi medical systems, good results were published by several studies. Morikawa *et al*^[174] transferred the pixel data in the ROI into a histogram and a binary

image for semi quantification with a devised system, and found that the mean value on the histogram and the percentage of hard tissue may directly represent liver elasticity. The diagnostic accuracy of SE for liver fibrosis was also compared with TE, the author felt SE compared favourably with TE and suggested SE could potentially be used as a routine imaging tool to evaluate liver fibrosis. A Chinese group^[175] utilized a new Hitachi ultrasound system (HI VISION Preius) and concluded that there was a strong positive correlation ($r = 0.81$) between the elasticity index and fibrosis stage. Diagnostic accuracies of SE for the diagnosis of F1, F2, F3, F4 were 0.93, 0.92, 0.84 and 0.66, respectively. Koizumi *et al*^[176] performed a semi-quantitative analysis using the elastic ratio method (ratio of strain distribution in two selected ROI) on 70 patients with CHC and with the hepatic vein as the internal control and found that the AUROC curves for elastic ratio were superior to serum fibrosis markers and scores of fibrotic change based on blood results (Figure 4).

More studies including meta-analysis about the use of SE for the evaluation of liver fibrosis are required to establish a protocol for accurate imaging and to standardize analysis.

Advantages

The main advantage of this technique is the relatively large region of interest that can be interrogated in the right liver lobe, plus the quantification method that can measure the change from the diffuse soft uniform architecture of the liver to a patchy hard pattern as hepatic

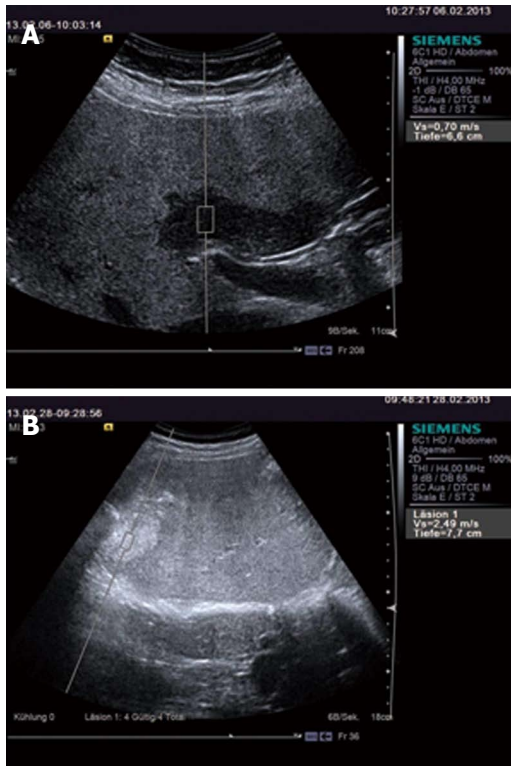


Figure 5 Point-shear wave elastography with acoustic radiation force impulse for evaluation of focal liver fatty lesion (A) and liver metastasis (B).

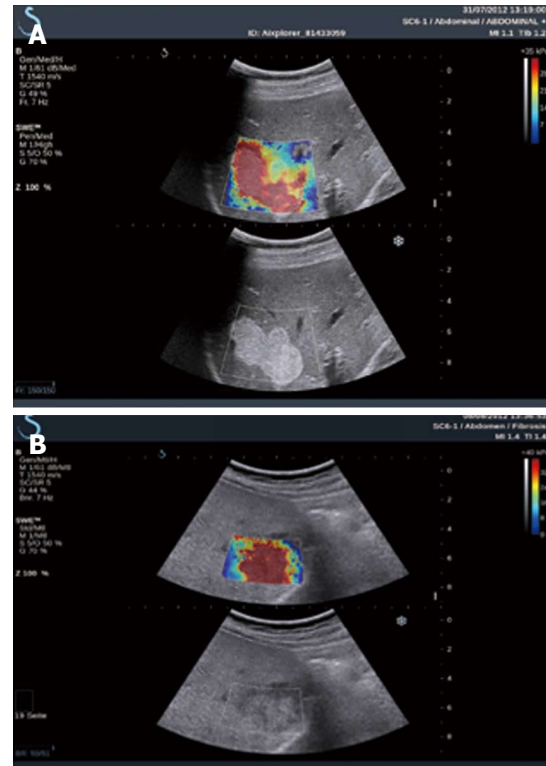


Figure 6 2D shear wave elastography with supersonic shear imaging for the evaluation of liver hemangioma (A) and metastasis (B).

Table 4 Performance of acoustic radiation force impulse in the identification of malignant focal liver lesions

No. of FLL	Rate of malignancy	Reference standard	Lesion types	ARFI cut-off (m/s)	QUADAS score	Ref.
105	64.8%	Biopsy, imaging	Haemangioma, FNH, focal fatty sparing, focal fat deposits adenomas, HCC, metastasis	2.7	11	[182]
60	71.7%	Biopsy, CT/MRI	haemangioma, HCC, CCC, metastasis	2	10	[183]
128	53.1%	Biopsy, surgery, imaging	Haemangioma, FNH, focal fatty change, abscess, adenoma, solitary necrotic nodule, HCC, metastasis, CCC	2.2	10	[184]
42	64.3%	Biopsy	Haemangioma, lymphoma, FNH, sarcoid, abscess, focal fatty sparing, HCC, metastasis	2.5	12	[185]
45	22.2%	Biopsy, CT/MRI	Haemangioma, metastasis	2.5	8	[186]

QUADAS: Quality assessment of diagnostic accuracy studies; HCC: Hepatocellular carcinoma; FNH: Focal nodular hyperplasia; CCC: Cholangiocarcinoma; FLL: Focal liver lesions; CT: Computed tomography; MRI: Magnetic resonance imaging.

fibrosis progresses.

Intra- and inter-observer reproducibility

The intra-observer variability and intra-observer agreement of SE for the assessment of liver fibrosis have been criticized in several studies^[173,177,178]. In a more recent study, a Japanese group^[176] used a semi-quantitative method (elastic ratio) and found that the measurements obtained from four separate locations had no observed variation between the two operators ($K = 0.835$, $ICC = 0.966$).

EFSUMB recommendations

The evidence with this approach is still too limited to allow recommendation for its clinical use, at least in European patients^[7,20].

ADVANTAGES AND DISADVANTAGES OF CURRENT NON-INVASIVE METHODS IN EVALUATING LIVER FIBROSIS

Advantages and disadvantages of currently available non-invasive methods in patients with chronic viral hepatitis C are summarized in Table 3.

ELASTOGRAPHY FOR DETECTION AND CHARACTERIZATION OF FOCAL LIVER LESIONS

Elastography methods have been also applied for detection and characterisation of focal liver lesions (FLL).

Although the method so far cannot be applied to all segments and the limited depth of penetration is so far disappointing, several studies have evaluated the performance of ARFI to differentiate FLL, and the results are encouraging. ARFI has shown a high accuracy for the identification of malignant FLL. In a meta-analysis by Ying *et al.*^[179] including 590 lesions in eight studies, the summary sensitivity and specificity for identification of malignant liver lesions were 0.86 and 0.89, respectively. The hierarchical summary receiver operating characteristic (HSROC) was 0.94. However, one paper showed that ARFI did not permit differentiation between benign and malignant FLL because high ARFI values occur in benign as well as in malignant lesions^[180]. In another study by Gallotti A, the mean shear wave speed of HCC, haemangioma, adenoma, metastasis, FNH was 2.17, 2.30, 1.25, 2.87, 2.75 m/s, respectively. Adenoma showed similar stiffness to the surrounding liver, and was significantly softer than the other four types of lesion. FNH showed different stiffness to HCC and metastasis, however, haemangioma showed no difference to HCC, metastasis and FNH^[181] (Figures 5 and 6). The performance of ARFI is summarized in Table 4.

EFSUMB recommendations

Although promising results have been reported, more research is needed, especially in comparison to CEUS, before recommendations on its use in clinical practice can be made. So far, elastography cannot be recommended for the differential diagnosis of benign from malignant liver lesions^[7,20].

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Hepatectomy for bile duct injuries: When is it necessary?

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Abstract

Iatrogenic bile duct injuries (IBDI) are still a challenge for surgeons. The most frequently, they are caused by laparoscopic cholecystectomy which is one of the commonest surgical procedure in the world. Endoscopic techniques are recommended as initial treatment of IBDI. When endoscopic treatment is not effective, surgery is considered. Different surgical biliary reconstructions are performed in most patients in IBDI. Roux-Y hepaticojejunostomy is the commonest biliary reconstruction for IBDI. In some patients with complex IBDI, hepatectomy is required. Recently, Li *et al* analyzed the factors that had led to hepatectomy for patients with IBDI after laparoscopic cholecystectomy (LC). Authors concluded that hepatectomy might be necessary to manage early or late complications after LC. The study showed that proximal IBDI (involving hepatic confluence) and IBDI associated with vascular injuries were the two independent risk factors of hepatectomy in this series. Authors distinguished two main groups of patients that require liver resection in IBDI: those with an injury-induced liver necrosis necessitating early intervention, and those in whom liver resection is indicated for treatment of liver atrophy following long-term cholangitis. In this commentary, indications for hepatectomy in patients with IBDI are discussed. Complex biliovascular injuries as indications for hepatectomy are presented. Short- and long-term results in patients fol-

lowing liver resection for IBDI are also discussed. Hepatectomy is not a standard procedure in surgical treatment of IBDI, but in some complex injuries it should be considered.

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Key words: Hepatectomy; Bile duct injury; Cholecystectomy; Laparoscopic cholecystectomy

Core tip: Different surgical biliary reconstructions are performed in most patients with iatrogenic bile duct injuries (IBDI). Roux-Y hepaticojejunostomy is the commonest biliary reconstruction. However, in some patients with complex IBDI involving disruption of hepatic confluence and injuries associated with concomitant vascular damage, hepatectomy is required. In this commentary, indications for hepatectomy in patients with IBDI are discussed. Complex biliovascular injuries as indications for hepatectomy are presented. Short- and long-term results in patients following liver resection for IBDI are also discussed. Hepatectomy is not a standard procedure in surgical treatment of IBDI, but in some complex injuries it should be considered.

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COMMENTARY ON HOT TOPICS

I have read with great interest the recent article Li *et al*^[1] analyzing the factors that had led to hepatectomy for patients with bile duct injuries after laparoscopic cholecystectomy (LC). I would strongly recommend this article to the readers.

Iatrogenic bile duct injuries (IBDI) are still a chal-

lenge for surgeons. The most frequently, they are caused by laparoscopic cholecystectomy which is one of the commonest surgical procedure in the world^[2,3]. The early and proper diagnosis of IBDI is very important for surgeons and gastroenterologists, because unrecognized IBDI lead to serious complications such as biliary cirrhosis, hepatic failure and death^[3,4]. Endoscopic techniques are recommended as initial treatment of IBDI. When endoscopic treatment is not effective, surgery is considered. Different surgical biliary reconstructions are performed in most patients in IBDI. Roux-Y hepaticojejunostomy is the commonest biliary reconstruction for IBDI. However, in some patients with complex IBDI, hepatectomy is required. Complex IBDI involve disruption of hepatic confluence and injuries associated with concomitant vascular damage^[1,5]. The incidence of arterial injury in patients in IBDI ranges between 12% and 47%^[6,7]. Distal IBDI are accompanied by damage of axial arteries (10%-15%) and proximal IBDI are usually associated with damage of the proper hepatic artery and its branches (most frequently right hepatic artery) (40%-60%)^[2,6]. The incidence of complex biliovascular injuries has risen since the introduction of LC^[8]. According to Li *et al*^[1], most complex IBDI such as Strasberg types E4 and E5 IBDI, either as an isolated condition or in combination with injury to vascular structures, can be effectively repaired by Roux-Y hepaticojejunostomy. However, in some patients with liver necrosis or lobar atrophy and fibrosis, hepatectomy is required^[1].

In their study, Li *et al*^[1] analyzed the medical records of 76 patients who had received surgery for IBDI following LC from April 1998 to September 2007. Hepatectomy was performed in 10 of 76 patients (13.2%), with IBDI either as isolated damage or in combination with vascular injury (VI). Proximal IBDI (defined as disruption of the biliary confluence) and injury to the right hepatic artery were found to be independent risk factors of hepatectomy. When both injuries occurred, 72.7% (8/11) of their referred patients required hepatectomy. Five patients required early liver resection (within 5 wk post-LC) to control sepsis caused by confluent liver necrosis or bile duct necrosis. In five patients, hepatectomy was indicated during long-term follow-up (over 4 mo post-LC) to effectively manage recurrent cholangitis and liver atrophy. Based on their own observation, authors distinguished two groups of patients with IBDI that require hepatectomy: those with an injury-induced liver necrosis necessitating early intervention, and those in whom liver resection is indicated for treatment of liver atrophy following long-term cholangitis.

Another interesting aspect of this study is analysis of short- and long-term results in 10 patients undergoing hepatectomy. Authors noted high postoperative morbidity (60%) and mortality (10%), and satisfactory long-term results (with median follow-up of 34 mo) with either no or only transitory symptoms in 67% of the patients^[1].

There are a number of publications regarding the

use of hepatectomy in surgical treatment of complex IBDI^[5-22]. Hepatectomy is one of therapeutic possibilities in patients with complex IBDI. Right hepatectomy is the most frequently performed liver resection in patients with IBDI because of the highest incidence injuries of the right hepatic artery^[5,6,22]. Li *et al*^[1] presented the following indications for hepatectomy in patients with IBDI: vascular injury causing liver necrosis without the possibility of vascular reconstruction, uncontrolled bile leakage due to a destructed segmental or sectional hepatic duct without the possibility of biliary reconstruction and recurrent cholangitis (more than four episodes) refractory to endoscopic management and not effectively amenable to bilioenteric anastomosis due to imaging evidence of atrophy or cirrhotic changes of the liver parenchyma. These indications are similar to literature data^[5-22]. Most frequently, liver atrophy or necrosis, sepsis, and unreconstructable hepatic ducts, as complications of complex IBDI, and multiple failed previous repairs are indications for hepatectomy. Liver resection removes the fibrotic, atrophic segment and the diseased biliary confluence and allows good access to the remnant bile duct for a safe healthy anastomosis^[5,6].

Laurent *et al*^[5] presented aims of hepatectomy in patients with complex IBDI involving biliary confluence (Bismuth IV, Strasberg E4). The aim of partial liver resection in patients with complex IBDI was to remove fibrotic and atrophic liver parenchyma with a high risk of secondary complications because of vascular or septic lesions. The other aim of hepatectomy was to remove completely the biliary stricture at the early stages of the disease for preventing progressive liver damage and potential malignancy caused by bile stasis and repeated cholangitis. Authors presented the following indications for hepatectomy: simultaneous ipsilateral portal and arterial injuries, stenosis of the hilar confluence involving secondary biliary confluence; presence of liver atrophy and presence of metallic stent.

Mercado *et al*^[9], based on their 20 years experience in surgical treatment of IBDI including 512 patients with complex IBDI (Strasberg E), performed major hepatectomy in patients with chronic biliary obstruction, liver atrophy, and persistent or recurrent cholangitis, with 1 to 3 previously failed attempts of surgical repair before arriving at their hospital. In remaining patients, Roux-Y hepaticojejunostomy without major liver resection was possible to perform. Authors pointed that in 2 patients, acute recurrent cholangitis, with pericholangitic abscess involving one hemi-liver that had not responded to medical and radiologic treatment, was the indication for major liver resection. In these cases, the liver parenchyma was not possible to rehabilitation, in spite of absence of disruption of hepatic influence. Based on the above mentioned two studies^[8,9], complex IBDI involving hepatic influence without vascular injury can be managed successfully with Roux-Y hepaticojejunostomy without major liver resection.

According to Thomson *et al*^[10], most patients are managed successfully with a Hepp-Couinaud hepaticojejunostomy because the left hepatic duct remains readily accessible. However, complications such as hepatic infarction, sepsis, anastomotic stricture, and intrahepatic stone formation can require hepatic resection, or even transplantation. Authors presented the following indications for hepatectomy in patients with IBDI: vascular injuries leading to partial liver devascularization, major injuries to the right hepatic duct that could not be repaired by conventional methods, and severe atrophy or sepsis of the hepatic lobe resulting from vascular injury or prolonged biliary obstruction that could not be drained effectively by Roux-Y hepaticojejunostomy. Liver transplantation was performed in combined biliary and vascular injuries leading to acute liver failure and in secondary biliary fibrosis with chronic liver failure.

In studies conducted in 1994 by Madariaga *et al*^[14], and in the year 1996 by Majno *et al*^[15], they indicated hepatectomy in cases of liver-infected necrosis. Sauvanet *et al*^[16] described the following indications for hepatectomy: injuries from the confluence or higher with unilateral portal injury, right pedicle destruction, and liver atrophy. de Santibañes *et al*^[21] presented algorithm for management of lobar atrophy including patients with IBDI. In asymptomatic cases of lobar atrophy, authors recommended control. Liver resection was indicated for symptomatic lobar atrophy caused by vascular injury, combined vascular and biliary injury, and biliary stenosis not responded to balloon dilatation. According to Truant *et al*^[22] large review, the presence of a Strasberg type E4 or E5 BDI associated with hepatic artery injury was an independent risk factor for hepatectomy. Based on the analyzing studies in PubMed database authors presented the following indications for hepatectomy: recurrent biliary sepsis, biliary strictures caused by continuous cholangitis, intrahepatic abscesses, non-visualization and/or unsuitability of the proximal stump of the injured bile duct(s) for anastomosis, intrahepatic injuries of an aberrant right hepatic duct, anastomotic strictures and intrahepatic lithiasis, right hepatic lobar atrophy, secondary biliary cirrhosis, primary non-diagnosed Klatskin tumor.

It should be emphasized that immediate arterial reconstruction of biliovascular injuries recognized intraoperatively or at least within 4 d is recommended in order to avoid liver devascularization^[8]. In another study, Li *et al*^[11] presented hepatic rearterialization, with vascular reconstruction with or without vascular graft, when it was technically possible. Authors carried out hepatic resection only in patients with partial liver atrophy or necrosis. Only early recognition of vascular injury allows to perform rearterialization. In late recognized injuries, partial hepatectomy is required.

Partial hepatectomy is not only performed in patients with damaged liver parenchyma, such as liver ischemia, atrophy and necrosis. A minor partial hepatectomy is

used in order to improve biliary reconstruction in high intrahepatic IBDI. Mercado *et al*^[23-27] described partial liver resection of segments IV and V that allowed adequate exposure of the bile duct at its bifurcation with an anterior approach of the ducts in order to perform a high quality anastomosis. The first partial-segment IV resection was performed in 1994.

The interesting phenomenon described by Li *et al*^[11] was a higher incidence of vascular injuries, involving a right hepatic artery, in proximal IBDI compared to distal ones. In this series, 11/33 high injuries were associated with arterial injury but only 9/43 distal injuries were. Authors explained this phenomenon by the fact that the high biliary injury involving the confluence was more likely to damage the hilar component of the choledochal hilar plexus and thus prevent compensatory flow from the left artery. A high risk of concomitant vascular injury in patients with proximal IBDI has been also reported in other studies on LC-related complications^[11,12,28-32]. Two arterial plexuses play important roles in adequate vascularization of the extrahepatic biliary system. One is the arterial plexus on the surface of the common bile duct and the common hepatic duct, connecting the posterosuperior pancreaticoduodenal artery and the right hepatic artery (most frequently, the 3 o'clock and 9 o'clock axial arteries). The other one (described by Vellar^[33]) is located within hilar plate on the inferior surface of the hilum of the liver. It is formed by the collateral vessels coming from the right hepatic left hepatic arteries^[33,34]. In patients with an occluded right hepatic artery combined with major bile duct injury, the arterial plexus on the bile duct is totally transected, and the hilar plate plexus might be jeopardized. In these cases, necrosis of the bile duct, dehiscence and stenosis of the biliary anastomosis, or a syndrome of multiple peripheral strictures within the biliary tree with jaundice and recurrent cholangitis, can occur^[11].

In the commented study, high morbidity and mortality rates and good long-term results were reported. It is associated with presence of serious complications in patients with complex IBDI requiring hepatectomy, such as peritonitis, sepsis with multi-organ insufficiency, and liver failure. Good long-term results show that liver resection should be considered in patients with complex IBDI that do not respond to other treatment. According to literature, hepatic resections in patients with IBDI can be performed successfully with low (0%) mortality, although with significant morbidity (50%-60%), and with excellent long-term success of 94%^[6].

In conclusion, depending on the time of surgical intervention, two groups of indications (early and late) can be distinguished. In the early postoperative period after cholecystectomy, hepatectomy is necessary in patients with liver necrosis or abscesses and bile leakage, in order to control peritonitis and sepsis. In remaining patients, hepatectomy is required in cases of recurrent cholangitis, that do not respond to standard therapy, and

symptomatic lobar atrophy. Although hepatectomy is not a standard procedure for patients with IBDI, it should be considered as a part of the surgical armamentarium for the repair of a selected group of patients in post-cholecystectomy injuries.

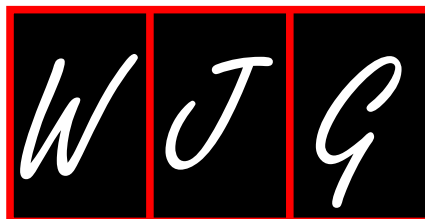
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WJG 20th Anniversary Special Issues (7): Liver transplant

Right hepatic lobe living donation: A 12 years single Italian center experience

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to obtain a graft for adult to adult living related liver transplant. During this 10 years period some changes, herein highlighted, have occurred to our surgical techniques. This study reports the largest Italian experience with RHL, focused on surgical technique evolution over a 10 years period. Donor safety must be the first priority in right-lobe living-related donation: the categorization of complications of living donors, specially, after this "highly sensitive" procedure, reflects the need for prompt and detailed reports.

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Key words: Adult-to-adult living-related liver transplantation; Liver regeneration; Liver resections; Liver transplantation; Liver surgery

Core tip: A 12 years Italian single center experience is herein reported, focusing on the live donors who underwent conventional open right hepatectomies for adult to adult living related liver transplantations. In light of this experience we individualized three area of interest where we accomplished remarkable goals over this time period: donor nutritional status and rescue of steatotic donors; analysis of post hepatectomy liver regeneration; surgical technical developments.

Abstract

Mini invasive techniques are taking over conventional open liver resections in the setting of left lateral segmentectomy for living liver donation, and hydride procedure are being implemented for the living related right hepatectomy. Our center routinely performs laparoscopic left lateral segmentectomy for pediatric recipient and has been the first in the Europe performing an entirely robotic right hepatectomy. Great emphasis is posed on living donor safety which is the first priority during the entire operation, then the most majority of our procedures are still conventional open right hepatectomy (RHL), defined as removal of a portion of liver corresponding to Couinaud segments 5-8, in order

Gruttadauria S, Pagano D, Cintorino D, Arcadipane A, Traina M, Volpes R, Luca A, Vizzini G, Gridelli B, Spada M. Right hepatic lobe living donation: A 12 years single Italian center experience. *World J Gastroenterol* 2013; 19(38): 6353-6359 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6353.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6353>

INTRODUCTION

In July 2013 we have been communicated by the edito-

rial board of the *World Journal of Gastroenterology* that our paper “Analysis of Surgical and Perioperative Complications in Seventy-five Right Hepatectomies for Living Donor Liver Transplantation” published in May 2008^[1], had been cited, up to now, more than 27 times in the world English literature. This figure supported by data banks such as Scopus and ISI web of Science, made this scientific article entering in the 1% most cited papers of ever. Prompted by this achievement we reviewed our 12 years single Italian center experience with hepatic right lobe living donation. Indeed, we were particularly glad to contribute with this retrospective clinical report, to the special number of the *World Journal of Gastroenterology*, published to celebrate the 50th year’s anniversary of liver transplantation^[2].

Although in the last 6 years the number of procedures performed each year has been dramatically fallen down, our center is still, by far, the busiest living related liver transplant program in Italy (Table 1).

Herein, we will focus our attention on live donor of conventional right lobe (Coineaud segment 5-8) and only marginally on live donor of open left lateral segments (Coineaud segments 2-3)^[3,4]. To further improve the outcome of these complex procedures, refinements in the surgical technique and better comprehension of the interrelations between post resectional liver regeneration, and donor nutritional status is, in our opinion, needed.

We individualized three area of interest where we accomplished remarkable goals over a 12 years period: nutritional status and rescue of steatotic donors^[5]; analysis of liver regeneration in donors^[1]; technical developments in conventional open resection for living donation^[6].

PATIENTS POPULATION

At the “Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione” in Palermo, Italy, from January 2002 to April 2013, we performed an overall of 107 live donor hepatectomy of which in details: 95 for adult patients and 12 for pediatric recipients. One additional case of potential right hepatectomy was aborted after the beginning of the surgery because of an abnormal venous outflow discovered at the intra-operative ultrasound and not previously detected at the pre operative imaging.

In the early phase of our experience two of the adult patients received a full left lobe (Coineaud segment 2-4), while the others adult recipients received a right lobe (Coineaud segments 5-8); in one case the right hepatic graft was harvested using a totally robotic procedure of retrieval. This case, the first performed in Europe and the second in the world, will not be treated here.

The pediatric recipients always received an anatomical left lateral segmentectomy (Coineaud segments 2, 3); in 8 cases we have performed entirely laparoscopic harvesting procedures and details of these procedures will not be discussed here^[7].

No living donor mortality, neither administration of heterologous blood transfusion were reported; 25 (27.1%)

Table 1 Living liver donor potential candidates and type of surgical procedures performed at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies, and the University of Pittsburgh Medical Center in Italy *n* (%)

	Statistics
Exclusion criterion ¹	
Donor-related reasons	158 (44.0)
Donor withdrawal	43 (12.0)
Donor death	1 (0.3)
Exclusion at work-up:	
ABO incompatible	11 (3.1)
Psychology	18 (5.0)
Clinical/biochemistry	27 (7.5)
At imaging	
CT scan	30 (8.4)
MRCP scan	10 (2.8)
At liver biopsy	17 (4.7)
Other	1 (0.3)
Recipient-related reasons	93 (25.9)
Death awaiting LDLT	21 (5.8)
Drop-out (HCC progression)	21 (5.8)
LDLT refusal	12 (3.3)
Unsuitable to LDLT	10 (2.8)
Cadaveric OLT	29 (8.1)
Total excluded	251 (69.9)
Partial liver living graft ²	
For adult recipients	
Full left lobe	2 (0.6)
Right lobe	94 (26.2)
For pediatric recipients	12 (3.3)
Left lateral segment	12 (3.3)
Total included	108 (30.1)
Overall	359 (100)

¹Potential donors; ²Suitable donors. CT: Computed tomography; MRCP: Magnetic resonance cholangiopancreatography; HCC: Hepatocellular carcinoma; LDLT: Living donor liver transplant; OLT: Orthotopic liver transplant.

living donors presented 1 or more episodes of complication in the post-operative period.

One case of HCV infection was revealed after donation^[8,9].

Regarding recipients, the patient and graft survival at 1, 3 and 5 year after right hepatectomy (RHLT) were 89.3%, 83.2%, 77.8% and 83.4%, 77.3%, 71.9% respectively.

NUTRITIONAL STATUS AND RESCUE OF STEATOTIC DONORS

In our center a comprehensive step-by-step donor work-up protocol is designed to ensure donor and recipient safety.

Donors with hepatic steatosis > 30% are excluded from donation, due to reported impairment of both graft and patient survival after living donor liver transplantation (LDLT)^[10].

Donors with body mass index (BMI) ≥ 30 kg/m², a significant correlation between BMI and overall grade of steatosis is well known^[11] and/or steatosis at imaging

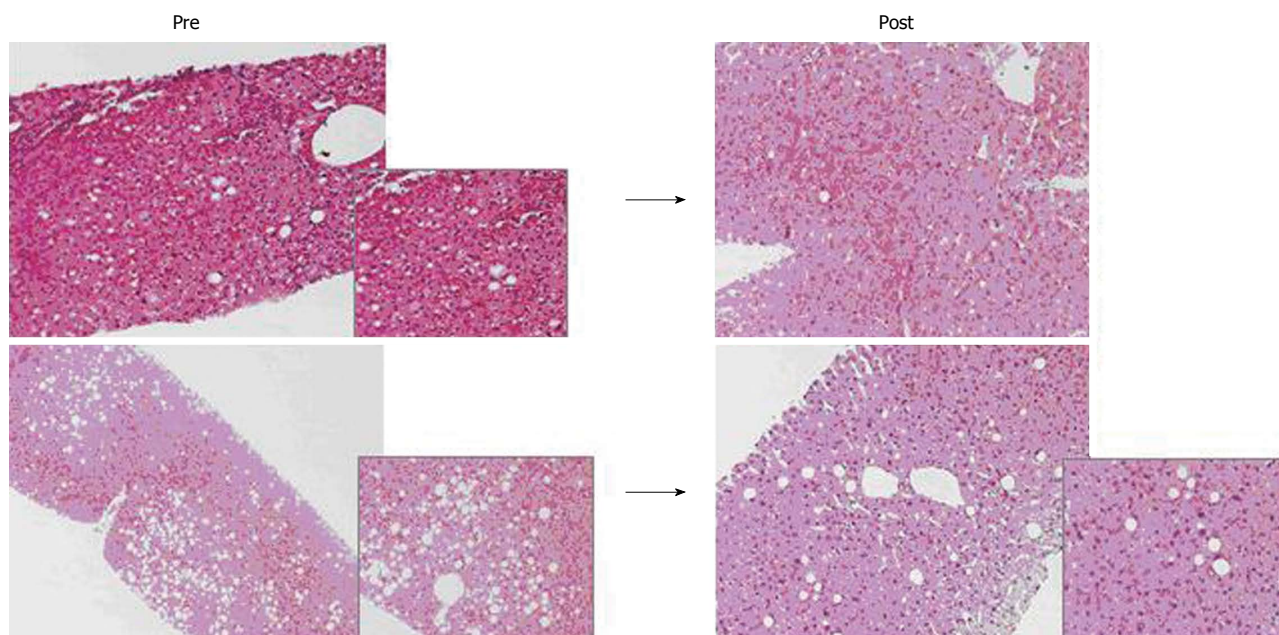


Figure 1 Two histologic examinations of liver parenchyma with steatosis, before and after intensive dietary treatment in the same donor. 10/18 donors successfully completed the 3-mo treatment (body mass index < 30 kg/m²). In all 10 treated donors, hepatic macrosteatosis < 30% was found (range: 2%-15%) and therefore they were considered eligible for donation.

(ultrasonography, computed tomography or magnetic resonance imaging) or at histology underwent dietitian consult and then re-evaluated within 3 mo.

After nutritional assessment [nutritional and dietary anamnesis, life-style evaluation and resting energy expenditure (REE) calculation] the dietitian arranged a personal diet (carbohydrates 55%-57%, proteins 17%-19%, and lipids 24%-27%) and encouraged the donor to do physical activity.

Dietetic compliance of the donor was monthly reassessed.

Acceptable monthly weight loss was considered 2-4 kg, with a final gained BMI of < 30 kg/m².

Eighteen potential living donors (age 27-59 years, male/female 14/4) were treated with diet. Nine out of 18 donors didn't complete the 3-mo dietary follow-up for donor-unrelated reasons. In 9 donors who successfully completed the 3-mo treatment a liver biopsy was performed. In all cases a hepatic steatosis degree < 30% was found and they became eligible as donors (Figure 1).

After LDLT, none of them experienced life-threatening complications or died. Liver function in both remnant liver donors and transplanted grafts showed a good outcome, with no differences (in terms of hospital length of stay, liver function parameter normalization after resection, and liver regeneration) between them and other LDLT without hepatic steatosis.

Surprisingly, in a very recent multicentric study, according to data maintained in the LiverMetSurvey database, a paradoxical survival advantage was observed in patients with steatosis undergoing liver resection for colorectal liver metastases (CLM)^[12].

This data has generated a fascinating hypothesis that

of excess body adiposity has a survival protective effect, concept which warrants further research.

DONOR LIVER REGENERATION

Based on the evidence that an exposure of a small graft to persisting hyperdynamic circulation and high portal blood inflow may induce impairment of liver regeneration, and hepatic dysfunction^[13,14], we have translated in a population of 70 donors who underwent right hepatectomy the analysis of the impact of the donor regeneration predictors on post hepatectomy outcomes^[1].

Liver regeneration was evaluated with multidetector computed tomography (MDCT) at a mean of 61.07 d after surgery.

We have examined the possible impact of pre-surgical variables (*e.g.*, age, weight, height, BMI, liver function tests, creatinine levels, platelet counts, international normalized ratio, and glucose levels) and variables detected with preoperative MDCT imaging [*e.g.*, main portal vein diameter, steatosis, original liver volume, and spleen volume (SV)]. The future remnant liver volume (FRLV) was preoperatively calculated with a virtual surgical cut. Donor BMI was 23.7 ± 2.9 kg/m², and was used to physiologically assess nutritional status.

In 26 of the 70 donors analyzed (37.14%), 100% or greater hepatic regeneration had occurred at 2 mo.

There was no association between the clinical outcome and the liver regeneration rate. A stepwise multiple regression analysis showed that a higher BMI (coefficient = 0.035, $P < 0.0001$) and preoperative parameters such as a smaller FRLV (coefficient = -0.002, $P < 0.0001$) and a

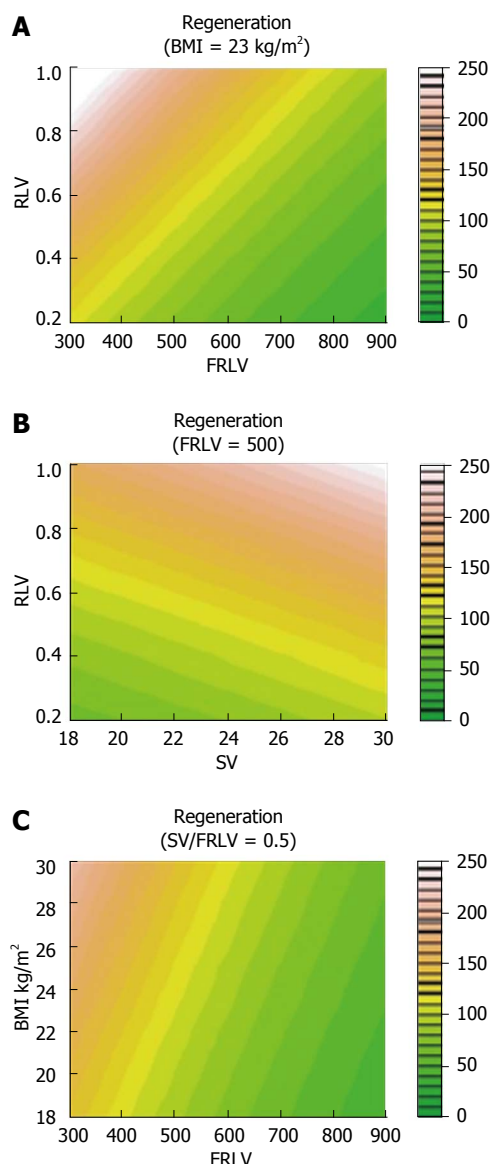


Figure 2 Locally Weighted Scatterplot Smoother graphics of the overall population, evidencing the association of percentage of liver regeneration with levels of body mass index (A), future remnant liver volume (B) and spleen volume/future remnant liver volume (C). FRLV: Future remnant liver volume; SV: Spleen volume; BMI: Body mass index.

greater SV/FRLV ratio (coefficient = 1.196, $P < 0.0001$) were predictors of greater liver regeneration (Figure 2).

TECHNICAL UPDATES

The most majority of our procedures are still conventional open RHLT, defined as removal of a portion of liver corresponding to Couinaud segments 5-8, in order to obtain a graft for adult to adult living related liver transplant.

While in the setting of the left lateral segment procurement open procedures are reserved only to rare cases presenting vascular anomalies.

During this 12 years period some changes, herein highlighted, have occurred to our surgical techniques. In

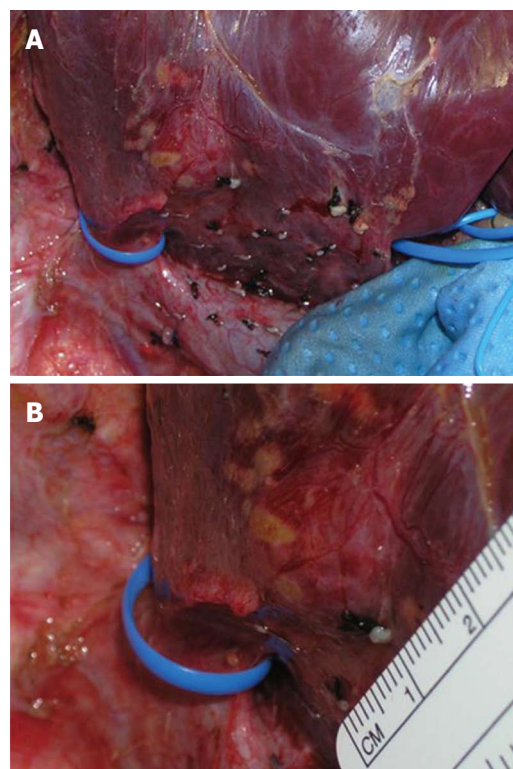


Figure 3 A large accessory hepatic vein draining the right lobe along with the right hepatic vein is encircled using a vessel loop (A). The measurement of the accessory hepatic vein [major of 5 mm, (B)] was performed for decision making to transect it with a vascular stapler, only when transections of liver parenchyma and of the structures of hepatic pedicle were almost complete.

particular we felt that the modifications we adopted concerning the transection of the accessories veins and final severing of the vascular stumps contributed to fasten, ameliorate and make safer the entire procedure.

Those technical development were possible using tools, strategies and experiences gained in laparoscopic surgery.

The operation is lately (after March 2008) being performed (last 10 cases, 9.2%) with a right renal flap incision while we were used to start with a bilateral subcostal incision, with upper midline extension (Mercedes incision). In the setting of the left lateral donation even an upper midline incision has been employed. For the right hepatectomy our original technique has been described elsewhere^[15].

We later adapted our technique to all type of anatomic variants. In the case of a right dominant hepatic vein, when a tributary of the hepatic venous system larger than 5 mm was encountered in the transection plane a test clamp was performed in order to see whether the liver parenchyma became dusky, after which a decision was made as to whether to preserve the branch.

This practice was lately substituted by the preoperative use of the MEVIS Hepavision[®] system in order to obtain a more detailed analysis of the outflow venous drainage.

In case of preservation we severed the accessory he-

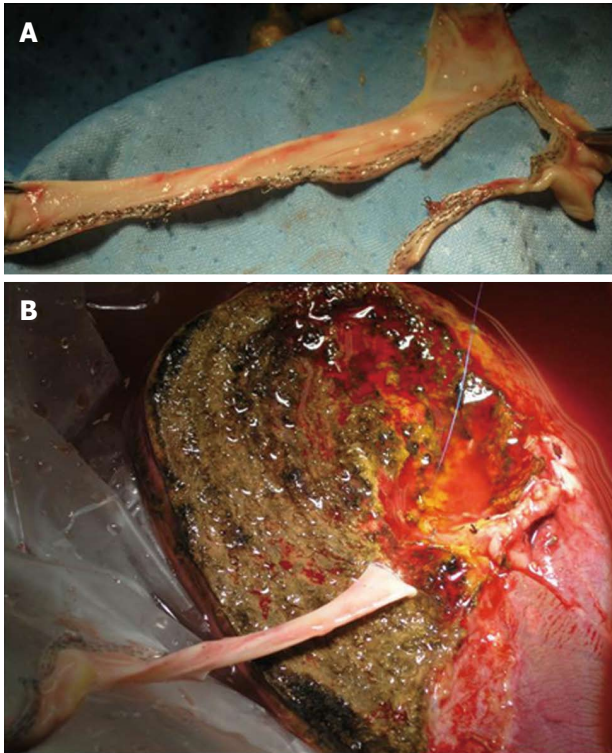


Figure 4 Usage of several vascular staplers were crucial to shapes and employees a heterologous venous conduit (A), previously harvested from a deceased donors, for a back-table reconstruction of a large accessory hepatic vein (B) to be connected to the recipient inferior vena cava in the LRLT.

patic vein, regardless was anterior or posterior, with the Endopath vascular staples (35 mm long, 12.3 mm wide; Ethicon Inc., Somerville, NJ) (Figure 3).

This step was originally taken using straight pediatric Pott vascular clamp and then suturing the two stumps of the vein.

This ultimate practice allowed us to avoid a complex manual suture especially in case of medial tributary when the parenchyma is not yet completely transected. Subsequently, once the parenchyma had been completely divided, the vascular stumps of the right branch of the portal vein, of the right hepatic vein and of the ipsilateral hepatic artery were sectioned with the Endopath vascular staples (35 mm long, 12.3 mm wide; Ethicon Inc., Somerville, NJ), while before the traditional Satinsky and Pott vascular clamps with subsequent manual suture were used.

In the setting of the left lateral segmentectomy, after mobilization of the left anatomic lobe and after performing the parenchymal transection as described above for the right counterpart, we lately substituted the conventional use of vascular Satinsky clamps with endo-vascular stapler.

In particular, while the vascular stumps of the left portal vein and of the left hepatic artery were treated with the Endopath vascular staples (35 mm long, 12.3 mm; Ethicon Inc., Somerville, NJ), the left or the common middle and left stumps of the hepatic veins was

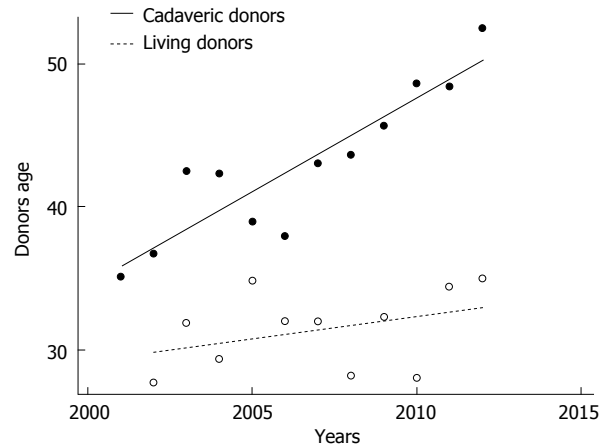


Figure 5 Linear regression models were used for explaining differences in terms of age between deceased and living liver donors over the last decade at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies, and the University of Pittsburgh Medical Center in Italy. In our series of 677 cadaveric liver transplantation and 105 living donor liver transplantation, the deceased donor age has been increasing with a significant difference of average values between deceased and living liver donor age (95%CI: 0.9-1.3), $P < 0.0001$.

treated with Endo GIATM Universal 12 mm vascular stapler (Covidien, New Haven, CT).

Eventually we like to report on the utility of vascular endo-stapler into the open transplantation surgery, in the setting of the use of vascular graft. Indeed in few cases, the usage of several vascular staplers were crucial to shapes and employees a heterologous venous conduit, previously harvested from a deceased donors, for a back-table reconstruction of a large accessory hepatic vein which was connected to the recipient inferior vena cava of a LRLT (Figure 4).

DISCUSSION

Deceased donor quality is worsening in Italy, the average of donor age in our center is much better in the living counterpart (Figure 5). However living donation carries a special risk and donor safety must be the first priority in liver living-related donation.

Indeed, the categorization of complications, the developments of new surgical tips, the changes matured over time in terms of donor selection and match needs to be promptly reported in details^[16].

Herein, we separately analyzed three area of interest in live donor hepatectomy that have been exposed to some changes and ameliorations since the beginning of our practice.

We found that the reduction of hepatic steatosis to a values of $< 30\%$, could be obtained with a strategic nutritional assessment and arranging an adequate personal diet. This concept is guiding our strategy in order to expand the live donor pool without affecting donor and recipient safety.

At this regard, the paradoxical survival advantage observed in patients with steatosis undergoing liver resec-

tion for CLM might create a new fascinating scenario, in which overweight living donor could be suitable for transplantation^[12].

However it is too early to conclude that peri-diagnosis overweight is a good prognosticator after a major liver resection, because its impact upon long-term survival is less well documented.

Further clinical studies with large series, comparing patients and potential live donors with different BMI, grade of liver steatosis and nutritional marker, will be necessary to obtain convincing evidence.

Assuming that preoperative nutritional status is one of the key points for successful resection in living-related liver donors, we have recently put emphasis not only on the evaluation of the ratio between donor and recipient liver volume but also on the predictors of optimal early liver regeneration in the donors.

Historical series suggested that in adult-to-adult living related liver transplantation one of the most challenging tasks is to match an optimal size graft, balancing the clinical condition of the sick recipient and the safety of the healthy donor. In this setting, particularly care must be taken in the pre operative imaging evaluation of liver and spleen volume^[17-19].

Eventually, in the scenario of the surgical refinements obtained over a 12 years period the adoption into the open conventional surgery of tools created for laparoscopic surgery such as the endo-mechanical stapler for vascular structures allowed us to make safer a unique surgical operation such as the living donor hepatectomy.

At this regard, we like to mention that traditionally one of the most stress full point of the operation is the positioning of the vascular clamp around the right hepatic vein or the common trunk of the middle and left hepatic vein and the subsequent manual suture of a potentially long vascular stump. Indeed, no matter how safely the clamp is placed the length of remnant vein to be sutured in the donor side might be short, and any moment the clamps could be displaced with detrimental consequences.

On the other hand, using the vascular stapler will make this step faster and safer with no consequence on the recipient side, once the stumps are opened on the back-table and the graft is flushed.

Adult-to-Adult Living-related Donor Liver Transplantation remains the greatest most recent and challenging evolution of liver transplantation, both from a technical and ethical point of view, which has contributed to reduce donor shortage^[20]. Stringent criteria of donor selection criteria and peri-operative care were implemented following one of the first reported case of living donor death in 2002, and a smaller number of centers with large experience refined the surgical technique, selection and clinical management of both donors and recipients^[21].

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Quality of care delivered to hospitalized inflammatory bowel disease patients

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Core tip: Hospitalized patients with inflammatory bowel disease are at risk of harm and increased utilization of healthcare resources. Variation in the care delivered to these patients is common. There is room for improvement in the quality of care focusing on reducing admissions and identifying patients at risk for inpatient complications such as venous thromboembolism and *Clostridium difficile* infection. This review outlines several aspects of inpatient care in need of improvement and discusses a number of improvement strategies that have been implemented with potential to benefit both patients and providers.

Abstract

Hospitalized patients with inflammatory bowel disease (IBD) are at high risk for morbidity, mortality, and health care utilization costs. While the literature on trends in hospitalization rates for this disease is conflicting, there does appear to be significant variation in the delivery of care to this complex group, which may be a marker of suboptimal quality of care. There is a need for improvement in identifying patients at risk for hospitalization in an effort to reduce admissions. Moreover, appropriate screening for a number of hospital acquired complications such as venous thromboembolism and *Clostridium difficile* infection is suboptimal. This review discusses areas of inpatient care for IBD patients that are in need of improvement and outlines a number of potential quality improvement initiatives such as pay-for-performance models, quality improvement frameworks, and healthcare information technology.

Weizman AV, Nguyen GC. Quality of care delivered to hospitalized inflammatory bowel disease patients. *World J Gastroenterol* 2013; 19(38): 6360-6366 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6360.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6360>

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic gastrointestinal condition characterized by relapsing inflammation. Most patients with IBD are managed in the outpatient setting, however as disease severity progresses and complications arise, hospitalization is often required. Patients admitted to hospital are at increased risk for a variety of complications including venous thrombotic events (VTE), hospital acquired infections, *Clostridium difficile*, and death^[1-5]. Moreover, hospitalized patients are more likely to require surgery^[5,6]. There have been

conflicting reports on trends in hospitalization rates for IBD over the last decade and the literature has revealed significant variation in care and disease outcomes among hospitalized IBD patients. The heterogeneous nature of IBD severity, location, and phenotype as well as limited evidence to guide some therapeutic domains make standardization of IBD care delivery difficult. However, given that hospitalized patients are at the highest risk for morbidity, mortality, and health care utilization costs, quality improvement initiatives aimed at reducing variation, a known surrogate marker of poor performance, are well suited to this subset of patients^[7,8]. This review outlines recent trends in rates of hospitalization for IBD and highlights areas of inpatient care that are in need of improvement.

HOSPITALIZATION RATES FOR IBD

Most IBD care is delivered in the ambulatory setting. However, a significant proportion of patients will require hospitalization at some point in their disease course. Reports on overall trends in hospitalization rates for IBD over the past two decades are conflicting. Among a large cohort of patients followed across an integrated care network in Northern California, Herrinton *et al*^[8] noted a 33% decline in hospitalization rates for Crohn's disease ($P = 0.02$) and a 29% decline among those with ulcerative colitis ($P = 0.0009$) from 1998-2005. However, a report using the National Hospital Discharge Survey (NHDS) showed that between the years 1970-2004, the rates of hospitalization for both Crohn's disease and ulcerative colitis in the United States increased^[9]. Moreover, readmission is not uncommon, as demonstrated by Bernstein *et al*^[10], whereby 20% of patients with IBD were readmitted within the same calendar year. The most important advance in IBD care over the last ten years has been the increasing use of anti-tumour necrosis factor (TNF) therapy. The true impact of this on hospitalization rate may not have been completely captured in all these reports, thus more data is needed to evaluate the impact of anti-TNF on recent hospitalization trends.

While the literature on hospitalization rates is conflicting, most studies clearly show variation in practice patterns among hospitalized IBD patients. For example, in the cohort from Northern California discussed above, variability in surgery rates and immunomodulator use depending on the number of gastroenterologists and colorectal surgeons at each site was noted among the 16 medical centers included in the study^[8]. Similarly, Spiegel *et al*^[11] demonstrated significant variation among community and expert gastroenterologists in a number of care areas including patients admitted to hospital with severe ulcerative colitis. Expert gastroenterologists had a lower threshold to consult a surgeon for patients with severe steroid refractory disease. Outcomes following colectomy based on surgical volumes have also been shown in several studies, with high volumes centers having lower mortality rates^[12,13]. Differences in outcomes based on the

type of admitting physician have also been demonstrated. Murthy *et al*^[14] showed that patients with ulcerative colitis admitted to non-gastroenterologists had higher in-hospital mortality rates compared to those admitted under the care of a gastroenterologist (1.1% *vs* 0.2%, $P < 0.0001$). Colectomy rates have also shown to be subject to geographic variation across the United States, with rates in the Midwest and West regions being three fold higher than those in the Northeast^[15]. These studies underscore the need for improvement efforts focused on minimizing variation and bridging the gap between ideal and true performance in caring for the hospitalized inpatient with IBD.

VENOUS THROMBOEMBOLISM PROPHYLAXIS

The risk of venous thromboembolism (VTE) has been shown to be increased among patients with IBD. Multiple studies have shown patients with IBD have a 2-3.5 fold increased risk for VTE compared to the general population and a recent meta-analysis confirmed a relative risk of 2.2 (95%CI: 1.83-2.65)^[1,16-18]. In fact, one study showed that among 17 chronic illnesses, only heart failure and cancer carried a greater risk of VTE than IBD^[19]. Moreover, it appears the prevalence of VTE among this group of patients is rising^[1]. A number of risk factors for VTE among IBD patients have been identified. In a review of the Nationwide Inpatient Sample (NIS) between 1998-2004, Nguyen *et al*^[11] identified increasing age, co-morbidities, ulcerative colitis (as opposed to Crohn's disease), surgery, and the need for public health assistance as important risk factors for the development of VTE. Disease activity has also been shown to be an important predictor, with one study showing a 4.5 fold increased risk of developing VTE during times of disease flare compared to remission^[20]. Hospitalized IBD patients, particularly those with ulcerative colitis, appear to be at very high risk of VTE. Hospitalized IBD patients have been shown to have nearly a 6 fold increased absolute risk of VTE compared to an ambulatory IBD population^[17] and an increased adjusted odds ratio of 1.85 (95%CI: 1.7-2.1) compared to those non-IBD patients admitted to hospital^[1]. Moreover, VTE has been shown to be a marker of worse outcomes and higher health resource utilization. A review of a large database of hospital discharges in the United States found an odds ratio (OR) of 2.5 (95%CI: 1.83-3.43) for in-hospital mortality compared to IBD patients without VTE^[1]. Mortality rates for ulcerative colitis were particularly high (37.4 per 1000 hospitalizations *vs* 9.9 per 1000 hospitalizations, $P < 0.0001$). Patients with IBD and VTE also had a longer average length of stay (11.7 d *vs* 6.1 d, $P < 0.0001$) and higher hospital charges compared to IBD patients without VTE.

Given the morbidity and mortality associated with inpatient VTE, the utility of VTE prophylaxis to prevent this complication is clear. Prophylaxis with heparin has been shown to significantly and safely decreased

the incidence of deep-vein thrombosis and pulmonary embolism^[21]. However, despite the efficacy and ease of administering VTE prophylaxis, a significant percentage of IBD patients admitted to hospital are not receiving it and remain at risk. In a retrospective review of a tertiary IBD center in the United States, Tinsley *et al.*^[22] noted that the overall prophylaxis rate was only 67.6%. Variation was noted depending on the admitting service, with significantly higher rates noted among those admitted to a surgical service compared to a medical service (93.5% *vs* 57.4%). Even among those in which VTE prophylaxis was ordered, up to 34% of doses were not given. The lower prevalence for prophylaxis of IBD patients may in part be due to lack of awareness of their increased risk, as they are often young and mobile. This was suggested by a survey of gastroenterologists who were members of the American Gastroenterological Association^[23]. Only 45% of respondents were aware that guidelines recommending VTE prophylaxis were published and a third surveyed reported working in a hospital with no protocols for VTE prophylaxis. Significant variation in practice was noted. However, contributors other than lack of awareness are suggested by studies of IBD experts. At a large Canadian tertiary IBD center, rates of VTE prophylaxis were lowest for patients admitted to the gastroenterology run IBD service compared to those admitted to general internal medicine or surgery^[24]. Moreover, a survey of Canadian IBD experts found that almost 20% did not routinely use VTE prophylaxis and there was inconsistency among respondents regarding the indication for prophylaxis for patients in remission^[25]. These studies underscore tremendous variation and suboptimal quality of care in preventing this morbid IBD related extra-intestinal manifestation. Given the uniform increased risk among hospitalized IBD patients, the presence of readily available and safe prophylactic agents, and the identification of important predictors for lack of prevention, this area of IBD care is a “low hanging fruit” that is very amenable to quality improvement initiatives.

CLOSTRIDIUM DIFFICILE TESTING

A substantial body of evidence has emerged to implicate IBD as an important risk factor for *Clostridium difficile* infection (CDI). IBD patients have been shown to have higher infection rates with CDI compared to non-IBD patients. In an analysis of administrative data using a large registry of hospital discharges in the United States, Nguyen *et al.*^[4] noted that patients with ulcerative colitis (UC) had a prevalence of CDI that was 8 times that of non IBD patients admitted with a gastrointestinal problem (37.3 cases/1000 discharges *vs* 4.8 cases/1000 discharges, $P < 0.001$). This finding was supported by a systematic review of 42 articles that showed CDI was more common among IBD patients than non IBD controls^[26]. In addition to the higher prevalence of CDI among IBD patients, the incidence of CDI appears to be increasing over the last decade, particularly among hospitalized IBD patients. A review of discharges

among hospitalized IBD patients showed that the percentage of IBD admissions complicated by CDI had increased from 1.4% to 2.9% between the years 1998 and 2007 ($P < 0.001$)^[27]. This increase was most marked for the subset with UC in which CDI complicated 5.3% of admissions. Similarly, in a retrospective review of hospitalized patients, Rodemann *et al.*^[28] showed that while CDI rates doubled among Crohn's disease patients between the years 1998 and 2004, they tripled among those with UC.

Not only does the literature support a true rise in CDI incidence and prevalence among individuals with IBD, but CDI also may confer worse outcomes. In-hospital mortality was four fold higher among IBD patients with CDI compared to those with IBD alone in a retrospective review of the NIS^[27]. Similarly, a retrospective cohort study from Ontario, Canada showed a higher in-hospital mortality rate among hospitalized UC patients with CDI compared to those with UC alone (3.3% *vs* 0.38%, $P < 0.0001$)^[29]. This increased mortality rate persisted out to five years of follow up in which the cumulative 5 years mortality rate was 27% for the CDI group and 14% for those with UC alone ($P = 0.0073$). CDI has also been shown to increase length of stay and hospitalization costs among those with concomitant IBD. A review of a large administrative database of hospital discharges from the United Kingdom showed that median length of stay was 26 d among those with both CDI and IBD compared to only 5 d for those with IBD alone, a difference that was statically significant^[30]. This translates into increased health care costs as shown by Nguyen *et al.*^[4], whereby average hospital charges were \$35606 for a UC patient with CDI compared to \$23856 for those with UC alone ($P < 0.0001$). The impact of CDI on colectomy is less clear. Jen *et al.*^[30] showed an increased risk of in-hospital colectomy among hospitalized UC patients with CDI as compared to UC alone (OR = 1.7, 95%CI: 1.4-2.1). This conflicts with the finding of Nguyen *et al.*^[4], who showed a lower risk of colectomy in IBD patients with CDI (OR = 0.44, 95%CI: 0.34-0.55). Studies evaluating long term risk of colectomy after CDI are also conflicting. Navaneethan *et al.*^[31] showed that one year following hospitalization for UC, the colectomy rate was 35% for those with CDI during that hospitalization compared to 9.9% for those without infection ($P < 0.001$). This was in keeping with a study from a large, tertiary IBD center in which one year colectomy rates for those with IBD and CDI were higher compared to those with IBD alone (44.6% *vs* 25%, $P = 0.04$)^[32]. However, no difference in the risk of colectomy at 5 years was seen in the Canadian study cited above^[29].

The literature supports the finding that CDI among patients with IBD is a significant and increasingly prevalent problem, particularly for those with UC. Moreover, CDI confers increased short and long term mortality risk and increased health care utilization costs and may increase short and long term risk of colectomy. The majority of CDI is diagnosed within 48 h of admission, suggesting most patients acquire CDI in the community^[28]. Given the high incidence and potential poor outcomes

associated with CDI and the fact that it is most often acquired before admission, routine testing of patients presenting with exacerbation of IBD for *Clostridium difficile* is a reasonable and potentially powerful intervention. In fact, a single center study showed a reduction in the number of colectomies after routine testing on admission was introduced^[33]. While more evidence evaluating the benefits of routine testing is indicated, the literature thus far supports its use. Nonetheless, it appears routine testing is not widespread. A study of 34 European countries found tremendous variation in the incidence of CDI across hospitals and suggested difference in testing behavior was most likely responsible for these results^[34]. Moreover, despite the rising prevalence of CDI, there is variation in approaches management in terms of antibiotic selection and practices regarding IBD specific immunosuppressive therapy. A survey of gastroenterologists in Canada and the United States found that nearly half of respondents add antibiotics to ongoing immunosuppressive therapy while the other half routinely held all immunosuppressants during antibiotic treatment^[35]. The lack of consensus even among IBD experts highlights the need for more studies aimed at bringing clarity to the commonly encountered clinical “grey area”.

INTERVENTIONS AIMED AT IMPROVEMENT

In order to adequately address gaps in care, an understanding of the contributing factors to the target problem is essential. It is important to tailor a quality improvement (QI) initiative to the local context and implement according to the resources, infrastructure, and QI culture available. A variety of methods to improve identified deficiencies in the quality of care of hospitalized IBD patients are already underway and discussed in detail below.

Pay-for-performance program

Guidelines have outlined algorithmic approaches for following this complex group of patients. However, the uptake of IBD guidelines by gastroenterologists has been shown to variable^[36,37]. Therefore, other improvement approaches are necessary. A pay-for-performance (P4P) funding model has been advocated by some, whereby hospital and/or physician reimbursement is tied to meeting certain predetermined care benchmarks. This model is increasingly being used, although its impact on patient outcomes remains controversial. A review of over 7000 primary care physicians in the United Kingdom Quality and Outcomes Framework Pay for Performance Program found significant improvements in outcomes of a number of chronic diseases such as diabetes and coronary artery disease^[38]. Similarly, a large study from the National Health Services in England compared mortality in a region of the country that had uniformly adopted a P4P model in all hospitals to the remainder of the country which did not use this model^[39]. In the 24 hospitals that did use the P4P model, an absolute reduction in

Table 1 American Gastroenterology Association Physician Quality Reporting System inflammatory bowel disease measures

1	IBD type, location and activity all documented
2	Corticosteroid sparing therapy after 60 d
3	Bone loss assessment
4	Influenza immunization
5	Pneumococcal immunization
6	Testing for latent tuberculosis before initiating anti-TNF therapy
7	Assessment of Hepatitis B status before initiating anti-TNF therapy
8	Tobacco use: screening and cessation intervention

IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor.

mortality of 1.3% (95%CI: 0.4-2.1, $P = 0.006$) and a relative reduction of 6% (95%CI: 260-1500) was observed. However, an American study evaluating the impact of the Centers for Medicare and Medicaid Services strategy that relies primarily on financial penalties through not providing hospitals with additional payment for health care-acquired or preventable complications found no significant changes in performance before or after this policy was adopted^[40]. Therefore, while P4P programs hold promise, more study is needed before there is universal adoption of these models. Moreover, there is a need to evaluate the impact of these programs on IBD patient, given their complexity and unique needs. The American Gastroenterology Association has developed IBD specific quality indicators eligible for reimbursement through the Physician Quality Reporting System (PQRS) (Table 1)^[41]. The impact of the PQRS on improving the quality of inpatient IBD care needs to be further characterized.

While not designed for the purposes of a reimbursement program, the Crohn's and Colitis Foundation of American have recently sponsored the publication of a set of quality indicators^[42]. Both process and outcome indicators were developed that encompass a variety of domains in IBD care including treatment, surveillance, and health care maintenance. A number of inpatient IBD care process indicators are defined such as “IF a hospitalized patient with severe colitis is not improving on intravenous steroids within 3 d, THEN sigmoidoscopy with biopsy should be performed to exclude cytomegalovirus, AND surgical consultation should be obtained” as well as “IF a patient in whom a flare of IBD is suspected with new or worsening diarrhea THEN the patient should undergo *Clostridium difficile* testing at least once” and inpatient related outcomes measures including: (1) Number of days per year in the hospital attributable to IBD; and (2) Number of emergency room visits per year for IBD. It is important for gastroenterologists to become familiar with these quality indicators as they can be expected to become increasingly incorporated into the accreditation processes of health care institutions.

Quality improvement frameworks

As the quality improvement movement continues to build momentum, there are increasing calls for innovative changes to the way health care is delivered. System rede-

sign is a fundamental principal in QI and there has been a particular focus on healthcare provided in the hospitalized setting as this is associated with significant morbidity and cost. Examples of new frameworks in IBD care are increasing. For example, a program in Australia implemented a new model of care consisting of a designated IBD service aimed at reducing hospitalizations^[43]. The service consisted of a team of gastroenterologists, a designated weekly IBD clinic, a joint gastroenterology-surgery clinic, and a nurse practitioner (NP). The NP performed a variety of tasks including standardized protocols for monitoring patients on immunomodulator and biologic therapy, a 24-h help line, routine post-discharge follow up phone calls, and a routine education session at discharge. Outcomes were compared before and after adopting this framework. Following the implementation of the IBD service, the mean number of admissions per patient, mean length of stay, and total cost for inpatient care decreased. While this simple before and after design does not clearly control for biases, it does highlight the potentially valuable role of designated chronic care teams, particularly the role of the NP. NPs have been shown to improve outcomes in other chronic diseases, however their use in IBD has lagged behind other fields^[44-46]. More studies are needed to evaluate their role in participating in IBD care.

Centralizing care delivery of certain disease into designated tertiary centers of excellence has also become a model employed by some jurisdictions. A number of large studies using administrative data have shown outcomes may be improved in high volume IBD referral centers. For example, United States hospital discharges were reviewed using the Nationwide Inpatient Sample between 1998-2004^[6]. IBD patients admitted to high volume centers had lower in-hospital mortality compared to non-high volume hospitals. Similarly, Ananthakrishnan *et al*^[13] found that patients admitted to high volume centers were more likely to undergo IBD surgery and had lower post-operative mortality rates compared to those in average volume hospitals. These studies support the designation of IBD centers of excellence whereby complicated IBD patients can be referred to for expert opinion and management. However, these centers must have the resources in place to handle such a complex cohort of patients and to be able to accommodate a large number of referrals to be seen in a timely fashion by gastroenterology and/or surgery.

Advancing healthcare information technology

Hospitals have been increasingly incorporating healthcare information technology (HIT) into patient care. Many QI experts link HIT with improved quality, safety, efficiency, and coordination of care^[47]. Hospitalized patients are at increased risk of harm in the form of hospital acquired infections, preventable complications (*e.g.*, VTE), medication errors, and lapses in communication at discharge regarding follow-up. Therefore, initiatives aimed at reducing these harms are needed, and HIT is one avenue that may

achieve improvements. If designed well and appropriately adapted to the context of a given institution, an electronic health record has the potential to improve efficiency, safety, and communication. Computerized provider order entry has the potential to decrease medication errors, link providers to clinical decision support, and address the underuse or overuse of certain resources^[47]. For example, standardized admission order sets involve a collection of orders or investigations that when designed well, are effective through improving efficiency, decreasing variation, enhancing workflow, and improving communication of evidence based practices^[48,49]. Fields can be customized to an admitting service (*e.g.*, general surgery, gastroenterology, *etc.*) or disease specific (*e.g.*, IBD). An IBD admission order set has the potential to address areas in which the quality of care is suboptimal. For example, including *Clostridium difficile* testing on the admission order may be expected to increase the rates of screening for IBD patients presenting to hospital with new or worsening diarrhea. While the impact of such initiative on IBD outcomes is not yet known, it would increase adherence to recently defined QI benchmarks and potentially identify a high risk group for bad outcomes^[42]. Similarly, an electronic order set that automatically defaults to ordering VTE prophylaxis on admission may improve the underuse of VTE prophylaxis outlined above. The physician would deliberately have to remove this order if it is not desired. These “forcing functions” are regarded among the most effective patient safety interventions available^[50]. This strategy has been shown to be effective in increasing prophylaxis rates in several studies of non-IBD patients and overcomes barriers to ordering VTE prophylaxis such as the knowledge gaps outlined above^[51,52]. However, other barriers to VTE prophylaxis have also been identified that may not be adequately addressed by an order set. Moreover, evidence in support of VTE order sets in IBD is lacking. This underscores the importance of a clear understanding of the local context before implementing an initiative and to ensure that it is well tailored to the patients, resources, and providers at a given institution. Nonetheless, the theory behind order set effectiveness is sound and more study is needed to evaluate their impact on IBD outcomes.

CONCLUSION

In summary, hospitalized patients with inflammatory bowel disease are at risk of harm and increased healthcare utilization resources. More attention needs to be placed on reducing hospital admissions and re-admissions and preventable inpatient complications such as VTE. A number of potential improvement strategies may benefit both patients and providers including pay-for-performance programs, quality improvement frameworks, nurse practitioners, and healthcare information technology. While the true impact of these interventions on IBD outcomes still needs to be elucidated, quality indicators are expected to become increasingly measured in all aspects

of clinical care and it is therefore important that IBD providers familiarize themselves with these concepts.

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Geoffrey C Nguyen, MD, PhD, FRCPC, Series Editor

Quality improvement in pediatric inflammatory bowel disease: Moving forward to improve outcomes

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Abstract

In recent years, pediatric health care has embraced the concept of quality improvement to improve patient outcomes. As quality improvement efforts are implemented, network collaboration (where multiple centers and practices implement standardized programs) is a popular option. In a collaborative network, improvement in the conduct of structural, process and outcome quality measures can lead to improvements in overall health, and benchmarks can be used to assess and compare progress. In this review article, we provided an overview of the quality improvement movement and the role of quality indicators in this movement. We reviewed current quality improvement efforts in pediatric inflammatory bowel disease

(IBD), as well as other pediatric chronic illnesses. We discussed the need to standardize the development of quality indicators used in quality improvement networks to assess medical care, and the validation techniques which can be used to ensure that process indicators result in improved outcomes of clinical significance. We aimed to assess current quality improvement efforts in pediatric IBD and other diseases, such as childhood asthma, childhood arthritis, and neonatal health. By doing so, we hope to learn from their successes and failures and to move the field forward for future improvements in the care provided to children with IBD.

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Key words: Inflammatory bowel disease; Colitis; Ulcerative; Crohn's disease; Child; Adolescent; Quality of health care; Review

Core tip: This review article provides an overview of the quality improvement movement and the role of quality indicators. Active quality improvement efforts in pediatric inflammatory bowel disease are discussed, and the need for standardizing the development of quality indicators across all fields of healthcare is emphasized. This article also discusses the importance of incorporating validation techniques when developing and selecting quality indicators. Examples of quality improvement efforts in other areas of pediatric chronic illnesses are presented, with important lessons highlighted to guide future quality improvement initiatives.

Quach P, Nguyen GC, Benchimol EI. Quality improvement in pediatric inflammatory bowel disease: Moving forward to improve outcomes. *World J Gastroenterol* 2013; 19(38): 6367-6374 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6367.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6367>

INTRODUCTION

The inflammatory bowel disease (IBD) are a group of chronic gastrointestinal diseases caused by inflammation of the gastrointestinal tract and resulting in malabsorption of nutrients, failure to thrive, abdominal pain, and extraintestinal manifestations^[1]. They consist of two main subtypes: Crohn's disease (CD) and ulcerative colitis (UC), and patients who do not fall into either subtype are deemed IBD type unclassified (IBD-U)^[1]. Adult and pediatric onsets of IBD differ in some regards, with one of them being in regards to the degree of psychosocial burden. Quality of life is significantly affected, with children being frequently affected by psychosocial issues as a result of stunted growth, weight gain from drug therapy and the inability to feel confident around peers due to associated bowel issues^[2].

Incidence and prevalence of pediatric IBD have been increasing worldwide. A recent systematic review with the aim of describing international trends for pediatric IBD rates found that 60% and 20% of relevant publications reported statistically significant increases in CD and UC incidence, respectively^[3]. The findings represented data from 32 countries, thereby providing evidence that pediatric IBD has become a global disease affecting a multitude of countries^[3]. Several developed countries had released reports characterizing incidence rates within their pediatric population. In Ontario, Canada, there was a 5% and 7.6% increase per year in incidence for children aged 0-4 years and 5-9 years, respectively^[4]. Similar increases have been demonstrated in Spain and Northern California, United States^[5,6].

With increasing incidence and prevalence comes greater economic burden, both on the healthcare system and on patients' families. Based on 2003-2004 data, the direct healthcare costs of IBD in the United States was \$3.1 billion for CD and \$2.1 billion for UC^[7]. Children had the highest cost of direct medical care, and lengths of hospital stay were also high, with an average of 8.1 d for CD patients who were ≥ 5 years of age^[8]. While the average pediatric patient with IBD costs significantly more in direct medical costs than the average adult, a high degree of variability in care and outcomes has been noted in the literature^[7]. A study from the United States demonstrated variation in care provided to children in a network of pediatric IBD centers, including a large degree of variation in use of immunosuppressive medications at diagnosis^[9]. Similarly, we have previously described variation in surgical outcomes in Canadian children based on family income, despite a universal access healthcare environment^[10]. In addition, we described a high degree of variability in medication prescription rates in children with IBD from three countries^[11]. This variation in care may be unwarranted, and indicate room for improvement in the quality of care^[12]. The description of unwarranted variation in care has therefore spurred quality improvement efforts in pediatric IBD^[13].

In addition, with increasing burden of pediatric IBD,

the issue of quality of care becomes more important. Improved quality of care should lead to improved outcomes, and therefore lower long-term burden as well as medical and psychosocial benefits. While the motivation for improving processes involved in providing high quality medical care is clear, such quality improvement efforts should be based in evidence and undergo validation to ensure efficient resource allocation.

Recognizing the disparities present in modern-day healthcare systems, the Institute of Medicine released two reports highlighting current issues affecting quality^[14,15]. Both reports have argued that quality of care is sub-optimal across all aspects of health regardless of disease type, and have proposed that healthcare systems be reformed to prevent mis-use of healthcare services^[16]. As a result of these reports, many providers, including pediatric IBD specialists, have worked with quality improvement experts to improve the quality of care for their patients by implementing quality improvement programs. We have reviewed current published quality improvement efforts in pediatric IBD, and the evidence that they have improved outcomes. In addition, we have examined evidence from quality improvement work in other fields to inform future pediatric IBD efforts and improved their likelihood of success.

WHAT IS QUALITY IMPROVEMENT?

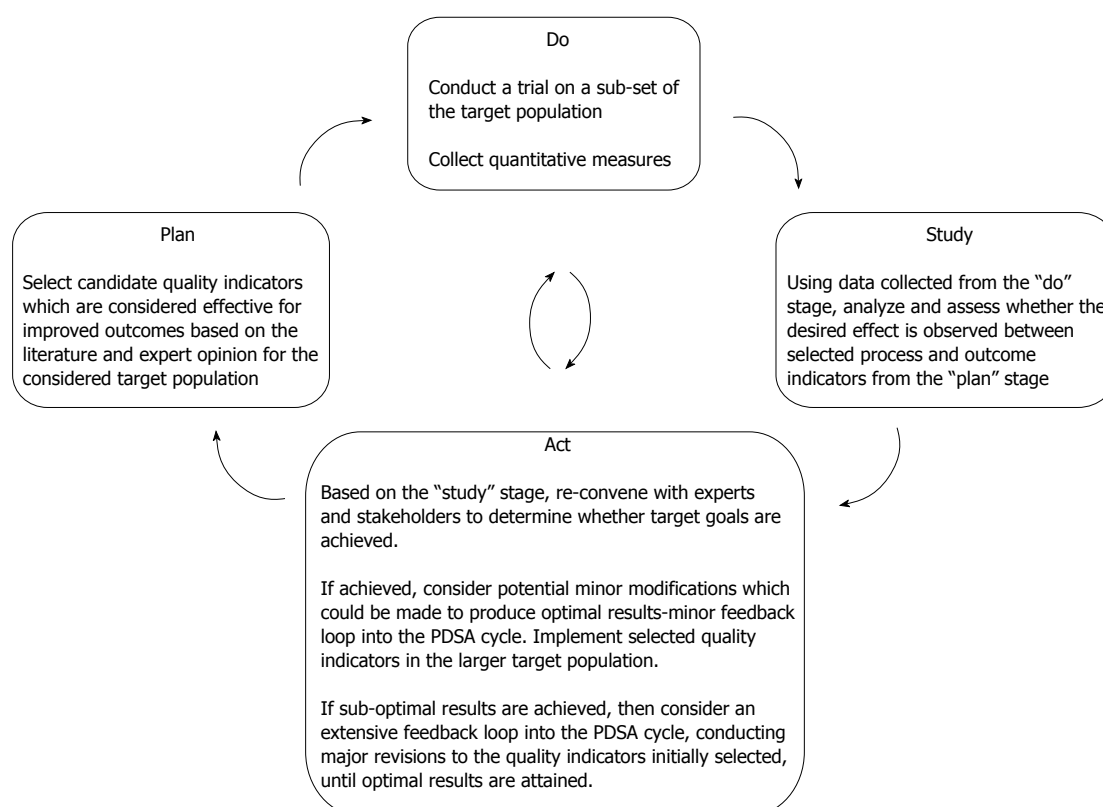
Quality improvement in medicine is defined as the effort to change care using an evidence-based approach in order to make tangible positive changes to the delivery healthcare^[17]. With origins stemming from the field of industry and production, quality improvement efforts have slowly been introduced into the field of healthcare delivery over the past few decades. Definitions used to describe common quality improvement terms can be found in Table 1.

The plan-do-study-act (PDSA) has been used as a paradigm for quality improvement efforts^[18,19]. With this framework, at the plan stage, quality indicators are developed to measure the quality of care provided. During the do stage, these indicators are implemented into practice and quantitative measures are collected. At the study stage, the statistics gathered in the previous stage are used to evaluate the progress that this action has on healthcare delivery. At the act stage, a feed-back loop is utilized such that quality indicators which have produced sub-optimal results are re-examined and cycled back through the PDSA cycle. Quality indicators which have improved quality of care are also re-examined to ensure that additional modifications cannot be added to ensure optimal care is being provided^[20]. The National Health Services(NHS) in the United Kingdom has recommended that the PDSA be used in trial phase and then implemented fully once outcomes have been satisfied^[20]. A modified PDSA cycle can be found in Figure 1, and is adapted from Langley *et al*^[19].

In general, many efforts for quality improvement have been unsuccessful due to the lack of a trial phase,

Table 1 Definitions of common quality improvement terms^[17,18,22,23]

Term	Definition
Quality improvement	The overall framework used to describe the process of implementing evidence-based interventions to bridge the disparities currently present in various healthcare systems
Quality indicators	A set of measures used to assess the appropriateness and quality of health care. Quality indicators are considered the fundamental building blocks of quality improvement efforts
Structural indicators	Indicators having to do with the structure of the healthcare system (<i>e.g.</i> , staffing, equipment, environment, electronic health records)
Process indicators	Indicators having to do with the process of providing care (<i>e.g.</i> , investigations, treatments, interaction with patients)
Outcome indicators	Indicators having to do with assessing the outcome of patients (<i>e.g.</i> , mortality, morbidity, quality of life, patient satisfaction)

**Figure 1** Modified plan-do-study-act cycle. PDSA: Plan-do-study-act.

and the lack of feed-back and change. Too often, quality indicators are developed and measures are extracted, but the process does not extend further beyond that point^[21].

WHAT ARE QUALITY INDICATORS?

The process of quality improvement of medical care requires markers of adequate and inadequate care. The essential building blocks for quality improvement efforts are the proper identification and implementation of effective quality indicators^[22]. These quality indicators are measurable elements of practice performance for which there is evidence or consensus that they may be applied to assess and improve the quality provided^[23]. The types of quality indicators have been broadly categorized as follows: (1) Structural measures-[indicators to do with the structure of the health system (*e.g.*, staffing, equipment, electronic medical records)]; (2) Process measures-[indicators to do with the process of providing care (*e.g.*,

investigations, treatment, interactions with patients)]; and (3) Outcomes measures-[indicators which assess the outcome of patients (*e.g.*, mortality, morbidity, quality of life, patient satisfaction)]^[18]. While improvement in all categories of indicators is desirable, process measures have garnered the majority of the attention, as they are most easily modified. To serve their intended purpose, process measures should predict facility-level outcomes, predict patient-level outcomes, and specify changes in care that are supported by the scientific evidence while being acceptable to patients and clinical staff^[24].

QUALITY IMPROVEMENT IN PEDIATRIC IBD

Understanding the benefits associated with standardized quality improvement efforts, an initiative called ImproveCareNow (ICN) was implemented amongst several

centers in the United States, and is rapidly expanding^[25]. It consists of a network of IBD centers engaged in a well-designed quality improvement program with an overall aim to determine whether measuring and decreasing variability would improve remission rates and other outcomes^[25]. Patient details and center practices are inputted prospectively into a registry, with quality indicator compliance rates fed back to centers on a regular basis. This feedback mechanism forms the basis of well-planned quality improvement efforts, including comparative reports, knowledge sharing activities, and clinical pre-visit planning mechanisms. The participating centers can then use their own results as benchmarks and compare future results as markers of improvement. They can also compare their performance to other participating centers^[26]. Initial results from ICN activities are promising, with improved compliance and remission rates demonstrated in the earliest years of the program. Crandall *et al*^[27] reported improvement in adherence to the selected quality indicators based on prospectively collected data from 6 participating centers. This was associated with a higher proportion of patients with inactive disease by Physician Global Assessment (PGA). However, improvements were relatively modest (13% improvement in remission rates for CD, 11% improvement for UC, based on statistical process control methods). These improvements were associated with a decreased proportion of patients with mild active disease. The proportion of patients with moderate or severely active disease remained stable over time. In addition, improvements in remission rates measured by the more objective short Pediatric Crohn's Disease Activity Index (sPCDAI) were smaller than those measured with PGA^[13,28]. This raises the issue of disease activity measurement in IBD. As evidence grows that clinical remission is insufficient to predict long-term prognosis, the use of measures which correlate strongly with mucosal healing and complete remission becomes especially important^[29].

In another study, Cincinnati Children's Medical Centre, one of the original participating centers in ICN with a long history of quality improvement efforts, published preliminary results of their quality improvement program in a separate report^[30]. As with ICN, a registry was developed, and indicators and outcomes were measured. To assess remission rates, PGA was used, along with patient-reported symptoms. Other variables measured included use of azathioprine and corticosteroids. They also assessed the use of vitamin D supplementations and serum 25-hydroxyvitamin D levels. Process and outcome indicators were chosen based on available guidelines and expert consultation. The institution reported improved remission rates of 59% to 76%, ($P < 0.05$), and a decreased use of repeated steroid courses of 17% to 10%, ($P < 0.05$). Investigators also found significant associations between decreased disease activity and vitamin D supplementations as well as disease activity and serum 25-hydroxyvitamin D levels ($P = 0.02$), although there was no control for confounders such as overall medica-

tion adherence and frequency of clinic visits^[30].

While ICN has become the first large-scale pediatric IBD quality improvement network to demonstrate successful changes in practice, some lessons can be learned from their methods (as well as those of quality improvement efforts in other pediatric patient groups) to further increase the likelihood of success in future quality improvement efforts.

QUALITY INDICATOR DEVELOPMENT AND VALIDATION

The indicators developed by ICN formed the basis of the measurement and feedback system, and therefore were developed with the assumption that improvement in the care provided and outcomes achieved would follow improved compliance with these indicators.

The initial set of indicators developed by ICN were not considered adequate and were revised^[25]. The initial 19 measures initially deemed appropriate for improving pediatric IBD quality were implemented amongst multiple centers. As these measures were being used in routine practice, it became obvious that several quality indicators needed further clarification, and some measures were not appropriate or feasible for inclusion^[25]. Flexibility is therefore required in the development and implementation of a quality improvement network, and the allowance for revision is an important part of the quality improvement process.

A pilot phase, as conducted by ICN is also important to ensure that intervention in the population being studied will produce a desirable effect. While quality indicators in quality improvement efforts are typically derived using RAND appropriateness methodology, which integrates expert opinion and review of the evidence, the literature may not be representative of the centers involved in the network^[24]. For example, a quality improvement network could consist of centers whose patients are mostly from low income neighborhoods. Measurement and control for these confounding factors is paramount. Without a pilot phase, and assessment of confounding, a formal quality improvement network may use imprecise process measures, leading to wasted resources and possibly misleading information^[24]. Following development of a second set of indicators for ICN, various mechanisms were put into place to provide clarification (such as a manual detailing strategies for accurate and complete measurement by participating centers). Of the 19 quality indicators developed, the quality indicators assessed by Crandall *et al*^[25,27], through ICN can be found in Table 2.

Both sets of ICN quality indicators were developed using RAND appropriateness methodology. Briefly, experts convene twice, before and after a meeting to rate importance of items derived from existing medical literature^[31]. Median scores are calculated and a final list is developed^[32]. Although reliability, feasibility and validity of indicators using the RAND appropriateness method have been established, improvement in the performance of

Table 2 ImproveCareNow quality indicators assessed in Crandall *et al*^[25,27] (of 19 total indicators developed)

Original set of quality indicators	Modified set of quality indicators	Results of quality improvement
Process: Diagnostic evaluation, disease phenotype, disease severity, body mass index including height and weight are all presented as separate measures under the domain titled: "Initial Diagnostic Evaluation"	Process: Assessing disease phenotype, disease severity, body mass index including height and weight were combined into a single "bundled" domain titled: Model classification	Increase in complete disease classification through the "bundled" measures: CD 38% ^b increase, UC 27% ^b increase
Outcome: Nutritional and growth status (those "at risk" with evaluation plans and those currently experiencing "failure" with treatment plans) are presented as separate domains	Outcome: Nutritional and growth status (those "at risk" and those currently experiencing "failure") are combined into the same domain, with no reference to further intervention plans based on the assessed status	Nutritional status: No changes in BMI z-scores for CD, however there was a 0.11 decrease in BMI z-score for UC ($P = 0.01$) Growth status did not change for CD and UC
Process: Treatment measures listed consist of measuring TPMT levels to ensure appropriate doses of thiopurine are prescribed	Process: Several other treatment quality indicators were included under the domain titled Treatment Measures which were not included in the original set such as anti-TNF therapy, skin test, screening for tuberculosis, appropriate infliximab and methotrexate dosage, among several others Outcome: Remission as an outcome measure was added (overall remission, prednisone free remission and sustained remission) The absence of prescribing prednisone was also an added outcome measure	Improved compliance with TPMT status assessment before prescribing thiopurines: CD 20% ^b increase, UC 23% increase Improvement in appropriate dose: CD 8% ^b increase, UC 41% ^b increase Only those with mild disease had significant changes to disease activity for CD and UC Remission rate (sPCDAI) increased 4% ($P < 0.0001$) Proportion with inactive disease improved: CD 13%, UC 11% Proportion who were not on prednisone increased by 4% for CD

^b $P < 0.01$. CD: Crohn's disease; UC: Ulcerative colitis; BMI: Body mass index; TPMT: thiopurine methyltransferase; TNF: Tumor necrosis factor; sPCDAI: Short pediatric Crohn's disease activity index.

the selected indicators do not necessarily correlate with improved outcomes^[33].

The typical indicator development process does not include a validation stage to ensure that the effects on outcomes are desirable. An alternative to the RAND appropriateness method incorporating a validation stage was proposed by Harris *et al*^[24] in the context of an alcohol addiction program. First, outcomes were collected and compared from pre- and post-treatment in a large sample of the target population. The goal of this stage was to determine whether implementing an effort to improve the completion of selected quality indicators would improve scores from baseline^[24]. A candidate set of quality indicators were selected from available literature, and association between selected indicators and outcomes were evaluated, using statistical methods and controlling for important confounding variables. As several predictors were tested for effects, true positives were maximized and false positives were minimized to avoid detection of spurious associations^[24]. Finally, those indicators which demonstrated the highest statistical correlation with outcomes were cross-validated with another subset of patients from the target population to determine whether the effect is sustained. Lastly, expert consultation was re-convened and indicators were re-evaluated^[24]. This approach may result in indicators that are more closely correlated with outcomes, thereby maximizing the cost-benefit ratio of implementing a formal quality improvement network.

Ideally process measures which indicate quality should be associated with both facility-level and patient-outcomes^[24]. Outcomes chosen by ICN as important

measures of success include remission rates (as measured by both PGA and PCDAI), nutritional status [measured by body mass index (BMI) z-score], linear growth velocity, and steroid-free treatment rates^[27]. Some of the indicators chosen would not directly correlate with these outcomes. For example, thiopurine methyltransferase (TPMT) genotype status would dictate safety of use of azathioprine or 6-mercaptopurine and risk of adverse events, but may not directly affect remission or growth velocity. In addition, completion of TPMT genotype is restricted to certain regions with some centers preferring TPMT phenotypic expression testing, and others preferring to monitor complete blood count and/or serum azathioprine metabolite levels. Therefore, TPMT genotype measurement may predict avoidance of serious adverse events, but may not be associated with either patient-level or facility-level outcomes^[34].

In summary, while ICN has successfully demonstrated improved documentation and compliance with select indicators, only modest benefits in patient outcomes have been achieved. Rigorous pilot work, with assessment and validation of correlation between indicators and outcomes could improve success. Elimination of indicators that are unassociated with outcomes would reduce the burden on participating centers and improve the cost benefit balance of a quality improvement network.

LESSONS LEARNED IN OTHER PEDIATRIC QUALITY IMPROVEMENT PROGRAMS

As the idea of quality improvement in health care has

become increasingly significant, several network collaboratives have been created with the overall goal of improving child health. A recent review by Billett *et al*^[35] highlighted five well-established and impactful regional and national pediatric quality improvement networks in the United States. The networks were in the fields of IBD (ICN), childhood asthma care, perinatal care, patient safety, and central line associated blood stream infection prevention in intensive care patients.

Although the review identified five examples of successful collaboratives, there are many other collaboratives in existence which have been able to demonstrate successes in their endeavors as well. The Canadian Neonatal Network (CNN) is a large network which includes upwards of 30 neonatal centers across Canada, with the goal of improving care in intensive care units, and therefore improving neonatal outcomes. Information on patients are collected in a database, which is then subsequently used to inform selection of indicators and to benchmark progress. Quality improvement is a priority of the CNN, as demonstrated by the creation of evidence-based practice for improving quality (EPIQ) cluster randomized controlled trial^[36]. EPIQ aimed to reduce nosocomial infections and bronchopulmonary dysplasia (BPD). Results demonstrated significantly reduced nosocomial infections and BPD in the quality improvement intervention group compared with control centers^[36]. In EPIQ, evidence based literature is used to inform the selection of quality indicators and information collected from the database is used to inform the use of the most appropriate indicators^[37]. Based on the EPIQ trial to assess the association between indicators and outcomes, the collaborative is now confident that these indicators can be used to determine high quality care in all centers involved in the CNN.

Another pediatric collaborative network aimed to improve the quality of care received by children with asthma presenting to emergency departments^[38]. Process and outcome quality indicators were chosen from an existing adult quality initiative, where associations between the selected process indicators and outcomes were observed^[39]. Unfortunately, preliminary results from the pediatric collaborative did not find an association between these process indicators and outcomes, indicating the importance of validation of indicators in the specific patient group to which they will be applied prior to their widespread application^[38].

Another quality improvement network in pediatric asthma based their quality improvement efforts on the chronic care model^[40,41]. Indicators were selected from existing guidelines, a pilot study was conducted to collect data before and after the intervention in cases and controls, and results of the pilot study were used to refine the processes used for quality improvement. After the rigorous initial process, the network was expanded to additional centers. Initial research from this network demonstrated significant improvement in the completion of processes, which resulted in improved outcomes^[41].

IBD practitioners and researchers are certainly not

the only specialists dealing with these issues in chronic inflammatory conditions. No fewer than four sets of quality indicators have been developed for arthritis care^[42-45], including one set for juvenile idiopathic arthritis^[43]. While quality improvement efforts are planned for arthritis care (including by the eumusc.net network), care providers also struggle with issues of measurement and validation^[46].

CONCLUSION

The increased availability of routinely-collected health data (including disease registries, electronic health records, and health administrative data) has resulted in a spotlight on unnecessary variability in the medical care of children with IBD. Quality improvement efforts have therefore never been more relevant, and reduction in negative outcomes in children with chronic diseases such as IBD could save healthcare costs and improve long-term quality of life for patients and families. While quality improvement programs in pediatric IBD are more advanced than those in other pediatric diseases, continually learning from the successes and limitations of networks such as ICN will allow for more rapid improvement in outcomes. The development and validation of quality indicators that are more strongly associated with outcomes will allow for more efficient implementation of quality improvement efforts, thereby reducing costs while improving the quality of life of children with IBD. We are at the beginning of a revolution in health care improvement, and we must therefore continuously learn from and improve upon the methods currently employed by our current quality improvement efforts.

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Interventions and targets aimed at improving quality in inflammatory bowel disease ambulatory care

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Core tip: Over the past decade, there has been increasing focus on improving quality in healthcare. This has led to the reinvigoration of the quality improvement movement. Inflammatory bowel disease is a complex, chronic condition with associated morbidity, health care costs, and reductions in quality of life. The condition is managed primarily in the outpatient setting. The delivery of high quality care is suboptimal in several ambulatory IBD domains. This review outlines current gaps in performance in IBD outpatient care and provides potential initiatives aimed at improvement.

Weizman AV, Nguyen GC. Interventions and targets aimed at improving quality in inflammatory bowel disease ambulatory care. *World J Gastroenterol* 2013; 19(38): 6375-6382 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6375.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6375>

Abstract

Over the past decade, there has been increasing focus on improving the quality of healthcare delivered to patients with chronic diseases, including inflammatory bowel disease. Inflammatory bowel disease is a complex, chronic condition with associated morbidity, health care costs, and reductions in quality of life. The condition is managed primarily in the outpatient setting. The delivery of high quality of care is suboptimal in several ambulatory inflammatory bowel disease domains including objective assessments of disease activity, the use of steroid-sparing agents, screening prior to anti-tumor necrosis factor therapy, and monitoring thiopurine therapy. This review outlines these gaps in performance and provides potential initiatives aimed at improvement including reimbursement programs, quality improvement frameworks, collaborative efforts in quality improvement, and the use of healthcare information technology.

INTRODUCTION

Over the past decade, there has been increasing focus on improving the quality of healthcare. Much of this interest was inspired through the publication of *To Err is Human* by the Institute of Medicine (IOM) in 2000, that painted a portrait of a health care system full of preventable morbidity and mortality in desperate need for change^[1]. This has led to the reinvigoration of the quality improvement (QI) movement, the foundation of which had developed over the last century.

QI is defined by the IOM as "the degree to which health services for individuals and populations increase

the likelihood of desired health outcomes and are consistent with current professional knowledge^[2]. Fundamental principles of the study of QI include reflection on individual and peer performance in delivering high quality care, transparency in reporting performance, and implementing changes to improve deficiencies with the ability to measure successes and failures. Variation in practices may also be a marker of suboptimal performance. This had led to the resurrection and refinement of measures, study designs, and statistical analyses that are uniquely suited to QI.

Chronic disease management has become a significant focus of QI initiatives given their associated morbidity and cost. Some of this may be due to gaps in delivering evidence-based care. This was demonstrated in a landmark trial that showed that only 57% of outpatients regularly receive recommended standard of care for a variety of conditions^[3]. As a result, there has been significant focus on improving delivery of evidence based care and preventative measures to patients with chronic disease in order to decrease complications, hospitalizations, and death. Moreover, quality indicators are increasingly becoming incorporated in the accreditation and funding models of healthcare institutions. Inflammatory bowel disease (IBD) is a chronic gastrointestinal condition characterized by relapsing inflammation. Crohn's disease (CD) and ulcerative colitis (UC) are the major subtypes of IBD. In North America, the incidence of CD ranges from 3.1-20.2 cases per 100000 population and 2.2-19.2 cases per 100000 population for UC^[4,5]. While the incidence is less in Asia and the Middle East, the incidence and prevalence have been noted to be rising in many different regions of the world^[5]. As in other chronic diseases, IBD patients are at increased risk of morbidity due to symptoms, hospitalizations, and complications of disease or therapy^[6]. Moreover, there are significant health care costs and reduction in quality of life associated with IBD^[7,8]. The economic burden of IBD is significant, with high disability rates among this young cohort of patients^[9] and one cost analysis of eight European cohorts showing a mean total health care cost of 1871 euros per patient-year over 10 years^[10]. Patients requiring hospitalization had 10 fold higher costs. Most patients with IBD are managed in the outpatient setting. However as disease severity progresses and complications of disease or therapy arise, hospitalization is often required. Unlike some other chronic conditions, IBD is a heterogeneous disease with a wide spectrum of disease phenotypes and management strategies. This makes disease wide QI strategies particularly challenging. Nonetheless, there are several areas of IBD care that are amenable to QI study and change. This review outlines current gaps in quality in a number of outpatient domains and provides potential initiatives aimed at improvement.

ASSESSMENT OF DISEASE ACTIVITY

A challenging management issue in patients with IBD is how to best assess disease activity. This assessment has

traditionally been based on clinical symptoms. However, with the increasing number of more objective tools to assess disease activity now available, such as serum inflammatory markers and fecal calprotectin, the use of symptoms alone may no longer be the best approach to follow these patients. Reliance solely on symptoms can potentially miss ongoing inflammation that may not be clinically apparent. In a Groupe d'Etudes Therapeutiques des Affections Inflammatoires Digestives (GETAID) study of 121 patients with CD, there was weak correlation between clinical symptoms and endoscopic activity^[11]. This puts patients at risk of disease complications and may make treatment more difficult once symptoms ultimately develop. Alternatively, active inflammation may not always be the cause of persistent gastrointestinal symptoms in patients with IBD. A meta-analysis of 13 studies containing 1703 IBD patients found the pooled prevalence for symptoms meeting criteria for IBS was 39%, with an OR compared to healthy controls of 4.89 (95%CI: 3.43-6.98)^[12]. Similarly, a pediatric study found significant overlap between functional abdominal pain and CD, with almost half of the patients meeting criteria for functional pain classified as having active IBD according to the Pediatric CD activity index^[13]. This often leads to patients being inappropriately treated with immunosuppressants, with a low likelihood of improvement in symptoms and exposure to unnecessary risk. Therefore, there is a clear need for routine objective assessments of patients with IBD both at diagnosis and during follow up. While regular endoscopic evaluation, the gold standard to assess disease activity, has well established barriers such as cost and invasiveness, incorporating other objective tools such as erythrocyte sedimentation rate, C-reactive protein, and fecal calprotectin may facilitate more accurate and targeted approaches to managing these patients. A recent comparison of these tools noted a sensitivity and specificity of C-reactive protein > 6 mg/L of 68% and 72%, respectively as compared to a sensitivity and specificity of fecal calprotectin of 91% and 90%, respectively for the detection of endoscopically active disease^[14]. More studies such as this are needed to provide more insight on the most valuable and cost-effective non-invasive approach to monitor disease activity in patients.

STERIODS SPARING AGENTS

Corticosteroids are effective in inducing remission among patients with CD and UC^[15]. However, they have not been shown to be helpful in long-term maintenance^[16]. Moreover, their poor safety profile and tolerability makes avoidance of prolonged use a priority. Nonetheless, a significant proportion of patients treated with corticosteroids remain on extended courses. A retrospective review of patients referred to a tertiary IBD center in the United States found that over 75% of patients had been on corticosteroids for over 3 mo, including patients classified as having "mild" disease^[17]. There was no attempt to consider steroid sparing medications, such as immunomod-

lators, in almost 60% of patients. Similarly, in a study of time trends in therapy among 16 medical centers between the years 1998 and 2005, there was a 27% increase in prolonged corticosteroid use (defined as > 120 d) among patients with UC^[18]. Significant variation in the use of steroid-sparing agents was noted among centers. This was also demonstrated among 10 North American pediatric centers whereby the use of immunomodulators as a steroid sparing-agent varied significantly, ranging from 30%-95% of patients followed at the center^[19]. Corticosteroids are a well-established risk factor for osteoporosis and as such, patients on extended courses should undergo bone density measurement. Despite this recommendation, a practice audit at a large tertiary IBD center found that almost 80% of patients referred had not received the appropriate screening for metabolic bone disease^[17]. Clearly there is significant variation in practice patterns regarding the recognition of the need to minimize steroid exposure and highlights the underuse of steroid-sparing agent such as immunomodulators and anti-TNF therapy.

SCREENING PRIOR TO ANTI-TUMOR NECROSIS FACTOR THERAPY

Anti-tumor necrosis factor therapy (anti-TNF) has emerged as an effective treatment for IBD^[20-23]. It, however, carries risk of infection due to immunosuppression. The incidence of reactivation of latent tuberculosis infection (LTBI) has been shown to be increased among individuals treated with anti-TNF. A review of the United States Food and Drug Administration Adverse Event Reporting System found an incidence of 24 cases of tuberculosis per 100000 per year among those treated with anti-TNF, which translates into a 4 fold increased risk^[24]. Similarly, the incidence of hepatitis B virus (HBV) reactivation is also increased among these patients^[25-27].

In order to minimize this risk, screening measures have been recommended prior to initiating anti-TNF therapy. Screening for LTBI and HBV prior to treatment has been recommended by the United States Food and Drug Administration, Health Canada, and all gastrointestinal societies^[28-31]. Screening is effective in reducing infections complications, is easy to perform, and has minimal risks to patients^[32-34]. This involves tuberculin skin testing and chest-X-ray for LTBI and a panel of three serological blood tests for HBV (HBsAg, HBsAb, HBcAb). Adherence to screening with tuberculin skin testing and chest x-ray has been shown to reduce the risk of tuberculosis by 78%-90%^[32,33]. Screening for HBV with subsequent vaccination or chemoprophylaxis if indicated has also been shown to be effective^[34].

Despite these recommendations, cases of severe and sometimes fatal infection with tuberculosis or hepatitis B have been described, and many of these can be attributed to lack of screening^[34-36]. A retrospective review of over 200 patients followed at a large United States academic IBD center revealed only 65% of patients were appropriately screened for tuberculosis and 25% screened for

hepatitis B^[37]. Similarly, a study from Australia showed that only 50% of gastroenterologists were routinely screening for HBV prior to starting anti-TNF^[38]. This underscores the problem in provider's adherence to screening. The development of tuberculosis or hepatitis B while on anti-TNF has the potential for high morbidity and mortality. Given the ease and effectiveness of screening and the consequences of lack of screening, one can argue that anti-TNF screening rates less than 100% are unacceptable.

There is growing literature exploring contributors to this safety problem. In their review of 287 IBD patients starting anti-TNF, Vaughn *et al.*^[37] identified factors most often associated with lack of screening for tuberculosis: previous exposure to anti-TNF [OR = 5.3 (95%CI: 2.8-10.3)], health care providers in practice for more than 10 years [2.5 (95%CI: 1.4-4.5) and treatment at a non-IBD center [1.9 (95%CI: 1-3.4)]. The factors contributing to lack of HBV screening were the same. These reasons highlight the role of lack of knowledge, as physicians in practice longer or those at a non-IBD center may be less likely to be up to date with current guidelines. Previous exposure to anti-TNF may falsely reassure the prescribing physician that the appropriate work up had already been completed. This highlights the contribution of confusion as to who is responsible for screening. Uncertainty as to how and when to screen is also an important contributor, as evident in a gastroenterology practice audit that showed that while most knew that screening was indicated, there was significant heterogeneity in the type and timing of screening^[38]. Thus, knowledge gaps as to the need for screening, confusion surrounding responsibility for screening, and details regarding how to screen appear to be major contributors to this problem.

MONITORING THIOPURINE THERAPY

Thiopurines, including azathioprine and 6-mercaptopurine, are commonly used in patients with IBD. While most patients tolerate these medications with minimal side effects, ongoing monitoring is required once therapy commences. Regular complete blood counts (CBC) are recommended by all published guidelines to monitor for myelosuppression^[39-42], for example weekly CBC within the first month of therapy, every other week for the following two months, and every 3 mo thereafter. While the routine checking of thiopurine S-methyltransferase (TPMT) genotype and phenotype status prior to therapy remains controversial, it is strongly recommended by the United States Food and Drug Administration and has recently been listed as a quality indicator^[31,43]. Regular monitoring of liver chemistries is also recommended by some, although the frequency of which is less clear^[42]. Despite tremendous experience with this class of medication that has been available for over 5 decades, variation in monitoring patients while on this medication is significant and lapses in many best-practice recommendations are noted. A survey of members of the Canadian Association of Gastro-

Table 1 American Gastroenterology Association Physician Quality Reporting System inflammatory bowel disease measures

1	IBD type, location and activity all documented
2	Corticosteroid sparing therapy after 60 d
3	Bone loss assessment
4	Influenza immunization
5	Pneumococcal immunization
6	Testing for latent tuberculosis before initiating anti-TNF therapy
7	Assessment of Hepatitis B status before initiating anti-TNF therapy
8	Tobacco use: screening and cessation intervention

IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor.

enterology revealed that while all providers acknowledged the need to monitor blood counts, there were differences in the frequencies of monitoring^[44]. Forty-two percent of those surveyed checked CBC weekly after starting therapy while 26% said they checked monthly and 23% biweekly during the initial period of treatment. Moreover, only 62% of respondents routinely monitored liver chemistries. In terms of routine TPMT testing, an international questionnaire sent to experts in the use of thiopurines in IBD found that only 30% and 43% routinely ordered genotype and phenotype testing, respectively^[45]. Lack of reimbursement for testing was the most important predictor of not ordering the test, and almost half of respondents felt that they would incorporate routine testing into their practice if it was reimbursed.

More recently, an association with thiopurine use and non-melanoma skin cancer (NMSC) has been noted. In a review of over 1000 South African IBD patients, a strong association was noted between thiopurine exposure and NMSC (OR = 5.0, 95%CI 1.1-22.8)^[46]. This was similar to the association noted by Peyrin-Biroulet *et al*^[47] in which ongoing thiopurine use had a hazard ratio for NMSC development of 5.9 (95%CI: 2.1-16.4). Lifelong, regular dermatologic screening has therefore been recommended^[48]. Nonetheless, a recent survey of dermatologists and gastroenterologist found that only 46% of gastroenterologists were aware of the association between NMSC and immunosuppression^[49]. This implies that at least half of IBD patients are not receiving the recommended screening.

INTERVENTIONS AIMED AT IMPROVEMENT

In order to adequately address gaps in care, an understanding of the contributing factors to the target problem is essential. While the evidence in support of a potential intervention is often regarded as the most important factor when choosing between potential initiatives focused on improving care, there is often limited supporting research available. As a result, other factors also need to be considered when choosing QI interventions including the prevalence and severity of the problem, the potential for undesirable outcomes as a result of the intervention, cost, complexity, and the ability to generate momentum

for future related initiatives^[50]. Moreover, once an intervention has been selected, continuous measurement is essential in order to know if an observed change represents an improvement. Thus, prior to implementing an initiative, well defined measures need to be developed and measured continuously. This will provide support that the initiative is responsible for any observed improvements in performance or alternatively, negative outcomes and unattended consequences.

Reimbursement programs

Guidelines have outlined algorithmic approaches for following this complex group of patients. However, the uptake of IBD guidelines by gastroenterologists has been shown to variable^[51,52]. Therefore, other improvement approaches are necessary. In 2006, the American Gastroenterology Association began to develop quality indicators that would be eligible for reimbursement through the Physician Quality Reporting System (PQRS)^[53]. Recently, IBD specific measures have been added to this growing list of indicators, and documentation of disease activity was the first such IBD indicator implemented. Other IBD indicators eligible for reimbursement through this program include recommending steroid-sparing therapy after 60 d of corticosteroid, assessment of tuberculosis and hepatitis B status prior to anti-TNF therapy, vaccinations, bone loss assessment, and addressing tobacco cessation (Table 1). While the impact of the PQRS on increasing objective assessment of disease activity is not yet known, data extrapolated from other disease states shows promise for the potential beneficial impact of similar reimbursement programs^[54]. Nonetheless, prior to implementing such an intervention, careful study is required as the literature showing the benefits of reimbursement programs on quality are conflicting and some studies identifying unintended consequences, such as providers avoiding the most severely ill patients, a phenomenon known as “adverse selection”^[54-57].

Although not designed for the purposes of a reimbursement program, the Crohn's and Colitis Foundation of American have recently sponsored the publication of a set of quality indicators^[43]. Both process and outcome indicators were developed that encompass a variety of domains in IBD care including treatment, surveillance, and health care maintenance. A number of corticosteroid related indicators are defined such as “IF a patient with IBD requires at least 10 mg prednisone (or equivalent) for 16 wk or longer, THEN an appropriately dosed steroid-sparing agent or operation should be recommended” and steroid related outcomes measures including; (1) proportion of patients with steroid-free clinical remission for a 12 mo period; and (2) the proportion of patients currently taking prednisone. Screening for latent tuberculosis and hepatitis B prior to therapy with anti-TNFs and TPMT testing prior to thiopurine therapy are also included. As more quality indicators develop and become increasingly incorporated into the accreditation processes of health care institutions, it is likely that more reimbursement models, or alternatively citations and pen-

alties for under performance, can be expected over the coming years.

Quality improvement frameworks

It does not appear that knowledge gaps are solely responsible for barriers in delivering high quality, evidence-based care. In terms of the underuse of steroid sparing agents, for example, the avoidance of prolonged corticosteroid and the importance of transitioning to steroid-sparing agents are not new concepts, have been endorsed by all gastrointestinal societies, and have been highlighted in guidelines for many years. This was borne out in a survey of gastroenterologists from 36 countries whereby 100% of those surveyed agreed that there is minimal evidence for continuing high dose corticosteroids for more than 3 wk and that steroid-sparing agents should begin to be considered after 2-4 wk of therapy^[58]. Therefore other contributors beyond physician knowledge base need to be addressed. Patients often initiate or modify steroid doses on their own without consultation with their health care provider. This may be due to poor access to a timely visit to a gastroenterologist when symptoms first present or when disease activity flares. Early referral to a specialist has been shown to improve IBD outcomes and initiatives aimed at improving access to gastroenterology have been shown to reduce steroid use and increase the use of early steroid-sparing therapy^[59]. A Swedish gastroenterology unit implemented a quality improvement framework whereby a registry of quality metrics was established and performance tracked^[60]. All routine visits were initiated by the clinic, rather than the patients and regular reminders to contact a designated IBD nurse for problems was provided. The program resulted in 98% of patients receiving regular IBD follow up visits, less than 3 wk between primary care referral and specialist visit, and less than 2 d to schedule an acute patient visit during disease flares. This experience highlights that implementing well designed frameworks, which are common place in other chronic diseases, has the potential to improve quality of care in IBD^[61]. Frameworks need to be developed with the appropriate local context in mind with and some have argued that frameworks do nothing to improve quality but rather improve documentation alone^[62]. This underscores the importance of continuous measurement after implantation to ensure the effort and costs associated are translating to improvements.

Collaborative efforts in quality improvement

Another potential motivator for change is collaborative efforts between institutions. These involve multiple sites working together towards a common improvement goal through receiving training in quality improvement methods, defining QI metrics, tracking performance, and transparency in reporting^[63]. While the use of improvement collaboratives in inflammatory bowel disease lags behind other chronic diseases, early outcomes of such initiatives have been promising. The ImproveCareNow Network consists of 51 pediatric hospitals across the United States and Europe that adopted the Chronic Ill-

ness Care Model and developed standardized practices and measures^[64,65]. Process and outcomes measures were prospectively collected and shared between sites. Early data has shown significant improvements in processes of care and patient outcomes in a variety of care areas. The use of a classification bundles to assess disease location, phenotype, activity, and nutritional/growth parameters at every visits has allowed for standardization between sites. Not only does this improve care, but also allows for collaborative clinical research efforts. Other outcomes already reported by the network include a decrease in the number of patients with CD on corticosteroids and an increase in the number of patients starting thiopurines with TPMT activity measured. These improvements in process measures are likely responsible for the increased remission rates noted in the participating sites. While more data on the efficacy of this and other such collaboratives are needed, given that an overarching theme of QI is to improve care delivery throughout the entire health care system, more widespread adoption of such broad, multi-site quality improvement initiatives should be considered.

Advancing healthcare information technology

The widespread incorporation for healthcare information technology (HIT) has been identified as essential in order to improve quality, safety, efficiency, and coordination of care by many leaders in the field of QI and patient safety^[66]. Many of the organizations regarded as leaders in the field of QI, such as the Veterans Affairs (VA) system in the United States or the Intermountain Healthcare Network in Utah attribute their success to the early adoption of electronic medical records and ongoing refinement of HIT resources^[67]. Providers delivering care to IBD patients have the potential to benefit from a variety of HIT related interventions including an electronic health record, computerized provider order entry (CPOE), and clinical decision support. If designed well and appropriately adapted to the context of a given institution, an electronic health record has the potential to improve efficiency, safety, and communication. It also has the potential to engage patients as platforms in which patients are able to access their own health record are increasingly being developed^[66]. This is important in IBD as patients are often young and may travel or move frequently for school and work. An electronic record also lends well to automated reminders which could address many areas of care that have been shown to have suboptimal performance such as monitoring blood work on thiopurines and bone density assessments^[63]. CPOE is another HIT intervention that in addition to decreasing medication errors, has the potential to enable drug interaction warnings, monitoring tests, and linkage to decision support systems^[66]. For example, order sets involve a collection of orders or investigations at one location that when designed well, are effective through improving efficiency, decreasing variation, enhancing workflow, and improving communication of evidence based practices^[68,69]. Traditionally, order sets have been paper-based, but

electronic order sets have become increasingly popular and have already been evaluated extensively in the patient safety literature. Compared to traditional paper order sets, electronic order sets have been shown to be more readily accessible, easier to link with other relevant order sets, and can be updated in real time^[70]. A number of areas within IBD patient care may be improved with electronic order sets, such as pre anti-TNF screening. While the evidence for order sets improving anti-TNF screening is lacking, examples in other fields support their utility. A pediatric study showed that an order set improved adherence to evidenced based asthma medication behaviors by almost 25%^[71]. While these results are encouraging, the quality of most studies evaluating order sets is not high and often employ simple before and after designs with poor control of biases^[72]. Moreover, some studies have shown unintended consequences of HIT. For example, one study aimed at using an electronic reminder to improve adherence to colon cancer found that following the intervention was unveiled, colon cancer screening adherence actually declined as a result of ineffective reminders and increased fecal occult blood screening rather than colonoscopy^[73]. Nonetheless, the theory behind order set effectiveness is sound and addressed several of the contributors to the anti-TNF safety problem identified above including knowledge gaps and confusion with details as to how to screen.

CONCLUSION

Caring for patients with IBD can be challenging due to the heterogeneous nature of the disease and the lack of consensus in many areas of practice. Variation in practice is therefore unavoidable and does not necessarily imply deficiencies in quality. Nonetheless, there are several aspects of IBD care whereby suboptimal performance has been documented and may be amenable to quality improvement initiatives including regular objective assessments of disease activity, recommending steroid sparing therapy, and appropriate monitoring of patients initiating and ongoing immunomodulator and anti-TNF therapy. Reimbursement programs, chronic disease frameworks, QI collaboratives, and health information technology resources are several potential interventions that may benefit IBD patient care. Quality performance indicators are expected to increasingly become incorporated into accreditation and funding models and it is therefore important that gastroenterologists become familiar with QI concepts and consider implementing initiatives where warranted.

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Personalizing therapies for gastric cancer: Molecular mechanisms and novel targeted therapies

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Abstract

Globally, gastric cancer is the 4th most frequently diagnosed cancer and the 2nd leading cause of death from cancer, with an estimated 990000 new cases and 738000 deaths registered in 2008. In the advanced setting, standard chemotherapies protocols acquired an important role since last decades in prolong survival. Moreover, recent advances in molecular therapies provided a new interesting weapon to treat advanced gastric cancer through anti-human epidermal growth factor receptor 2 (HER2) therapies. Trastuzumab, an anti-

HER2 monoclonal antibody, was the first target drug in the metastatic setting that showed benefit in overall survival when in association with platinum-5-fluorouracil based chemotherapy. Further, HER2 overexpression analysis acquired a main role in predict response for trastuzumab in this field. Thus, we conducted a review that will discuss the main points concerning trastuzumab and HER2 in gastric cancer, providing a comprehensive overview of molecular mechanisms and novel trials involved.

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Key words: Gastric cancer; Human epidermal growth factor receptor 2; Biomarkers; Target therapies; Trastuzumab; Lapatinib; Pertuzumab; Trastuzumab-DM1; Afatinib

Core tip: Advanced gastric cancer is a very aggressive disease though the standard chemotherapy protocols available. In 2010, Trastuzumab for Gastric Cancer trial showed that the combination of trastuzumab, could be considered a new standard option for patients with human epidermal growth factor receptor 2 (HER2) positive advanced gastric and gastro-esophageal junction cancer. Thus, a new era emerged for those patients due to the interesting possibility in prolong survival in a personalized setting (HER2 positive). Our manuscript will provide an overview of the molecular mechanisms involved and also promising targeted therapies in this field.

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INTRODUCTION

Gastric cancer has been described since 3000 BC in ancient Egypt. One of the first epidemiologic studies on cancer, with data spanning from 1760 to 1839, pointed gastric cancer as the most common and lethal. In modern times, it remains one of the most important forms of cancer, with different geographic, ethnic and socioeconomic distributions. Incidence is particularly high in Japan, China, South Korea, Chile and Costa Rica. The large regional variations in incidence possibly reflect different prevalences of *Helicobacter pylori* infection, which is responsible for more than 60% of gastric cancer globally. Globally, gastric cancer is the 4th most frequently diagnosed cancer and the 2nd leading cause of death from cancer, with an estimated 990000 new cases and 738000 deaths registered in 2008^[1]. The human epidermal growth factor receptor 2 (HER2) protein, a 185 kDa protein (p¹⁸⁵) encoded by a gene located on chromosome 17q21 is a transmembrane tyrosine kinase receptor with an extracellular ligand-binding domain; a short transmembrane domain and an intracellular domain with kinase activity (Figure 1). It belongs to the epidermal growth factor receptor (EGFR) family of growth factors comprising four structurally related members, HER1 or ErbB1, also known as EGFR, HER2 or ErbB2, HER3 or ErbB3 and HER4 or ErbB4. Activation occurs through homo- or heterodimerization induced by ligands. HER2 is designated an orphan receptor which is believed to homodimerize independently of a ligand or to heterodimerize with another ligand-bound member of the EGFR family. Activation triggers a cascade of events that involves autophosphorylation and activation of the tyrosine kinase domain, Ras/Raf/mitogen-activated protein kinase pathway, phospholipase C- γ and phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) (Figure 2). HER2 receptors have also been found in nuclear localization, where they act as transcription factors for cyclin D1 and p53^[2,3]. Therefore, *HER2* (also known as *c-erbB-2/neu*) acts as an oncogene involved in the regulation of cell proliferation, differentiation, motility and apoptosis^[4-8]. Heterodimers of HER2 with other members of the HER family, particularly with HER3, are the most mitogenic dimers and HER2 increases the affinity of EGFR, HER3 and HER4 to their ligands^[9-12].

Analysing the molecular structure of HER2 allows new insights into approaching it as a potential therapeutic target (Figures 1 and 3). The extracellular domain of the receptor is subdivided into four subdomains. Whereas subdomains II and IV are involved in the process of dimerization, subdomains I and III are the binding sites for pertuzumab and trastuzumab respectively, two of the most well studied HER2 inhibitors which will be discussed further on. The transmembrane domain of HER2 plays an important role in the process of dimerization and oncogenic mutations in this region were described. The intracellular domain contains the active enzyme site which activates different downstream pathways by phosphorylation^[13-16].

The importance of addressing HER2 as a therapeutic target is underscored by a number of molecular and pathological findings. Amplified HER2 relates to processes of carcinogenesis and adverse pathologic features such as tumor size, invasion and metastatic spread; the level of *HER2* gene expression is much higher in cancer cells than that in non-malignant adult cells^[17]. HER2 overexpression has been reported in breast, lung, salivary gland, ovary, colon, prostate and pancreatic cancers^[18,19].

About 10%-34% of invasive breast cancers present HER2 overexpression. Trastuzumab has shown survival advantage in early and metastatic disease and is now a part of standard care. HER2 overexpression stands as a poor prognosis marker for chemo- and endocrine therapy but at the same time as a positive predictive marker for treatment with trastuzumab. Furthermore, trastuzumab proved to be effective as adjuvant treatment in breast cancer with HER2 overexpression, with different chemotherapy regimens^[20-26]. In gastric cancer, the prognostic role of HER2 overexpression remains controversial. The most important prognostic factor for gastric cancer is the tumor node metastasis (TNM) stage^[20,27]. Initial works addressing the prognostic significance of HER2 overexpression reported a negative effect on overall survival (OS)^[28,29]. However, conflicting results regarding the prognostic value of HER2 have been published more recently. Some studies found a negative effect of HER2 on prognosis with reduction in OS^[17,20,29-36], others found no relationship^[37-40] and a trend towards improved survival was found in one cohort^[41]. A comprehensive review by Jørgensen *et al.*^[42] found that the majority of publications that fulfilled the selection criteria for the analysis, associated HER2-positive status with poor survival and clinicopathological characteristics such as serosal invasion, lymph node metastases, disease stage or distant metastases. Chua *et al.*^[43] recently reviewed 49 studies with data regarding the relation of HER2 with clinicopathological variables and survival and concluded that HER2 overexpression is associated with poor survival; results pertaining other variables were not conclusive. HER2 overexpression has also been suggested as a molecular abnormality in the development of intestinal type gastric cancer and HER2 expression increases with disease progression, leading to the suggestion that the initial timing of this event probably occurs in early stages. Barros-Silva *et al.*^[20] found overexpression and amplification in both components of mixed tumors (intestinal and diffuse components) and *HER2* amplification in early stages, supporting this idea of amplification in an early stage of carcinogenesis. Further support arises from the high levels of concordance between primary tumors and paired metastatic sites found by some authors, suggesting HER2 amplification as an early event and not acquired at a later moment by cells with metastatic potential^[44]. Kataoka *et al.*^[45] found no HER2 positivity in the diffuse component of mixed type cases, but also found HER2 overexpression in early TNM T1a cases, pointing towards an early event^[30,46]. Although these data tend to establish

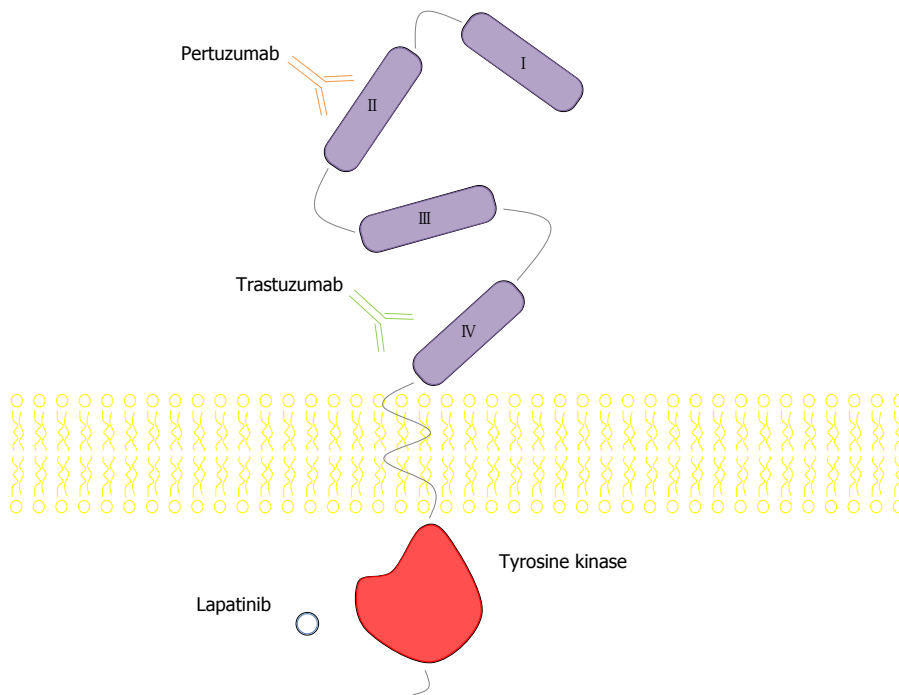


Figure 1 Human epidermal growth factor receptor 2 and binding sites. I-IV: Epidermal growth factor receptor 1-4.

HER2 as a potential negative prognostic factor in gastric cancer, the relation seems not to be as consistent as in breast cancer^[42]. In fact, more recent studies demonstrate no significant prognostic relationship. In a study involving 381 metastatic gastric cancer patients, Yanjigian *et al*^[47] found that patients with HER2-positive gastric cancer had longer median OS compared with HER2-negative gastric cancer patients, but on multivariate analysis HER2 status was not an independent prognostic factor. Terashima *et al*^[48] found no correlation with OS in 829 stage II / III resected gastric cancer patients. Hsu *et al*^[49] investigated 1036 gastric cancer patients undergoing curative-intent resection. Although HER2 positivity emerged as a favourable prognostic factor for stage III-IV gastric cancer on univariate analysis, it did not on multivariate adjustment.

Despite these conflicting results, it seems likely that HER2 is not associated with an adverse prognosis in gastric cancer in an extent similar to breast cancer; nevertheless, inhibition of the HER2 pathway in patients with HER2 amplification demonstrated clinical benefits. In this review, we will address the main advances in the treatment of advanced gastric cancer, focusing on the novel biomarkers and target therapies concerning HER2 signalling pathways.

HER2 TESTING IN GASTRIC CANCER

A precise analysis is necessary in order to address the status of HER2 expression in gastric cancer, which constitutes an essential step to select the patients feasible to treatment with HER2 target therapies. Techniques include primarily immunohistochemistry (ICH) and *in situ* hybridization (ISH), which constitute standard techniques

also used in the current practice of HER2-status determination in breast cancer. Current evidence suggests the need to adopt the methods used in breast cancer in order to address HER2 expression in gastric cancer^[50]. Considering the different biologic origin of the tissue, the high density of glandular structures needs to be understood in its context. In gastric tissue, ICH staining for HER2 occurs typically on the basolateral membrane and less so on the luminal aspect of the cell, conferring an U-shaped appearance to the staining whereas completeness of the membrane staining is the rule for higher scores in breast cancer. Another difference concerns the heterogeneity of immunostaining which is rare in breast, but frequent in gastric tumors. ICH should be used as primary test; cases with ICH score 3+ are candidates for HER2 directed therapy, 2+ scoring cases should be re-tested using ISH; in the case of ISH positivity patients are eligible for these therapeutic modality^[51].

HER2-DIRECTED THERAPIES IN GASTRIC CANCER

In January 2010, the European Medicines Agency granted approval to trastuzumab plus chemotherapy in the treatment of with IHC 3+ or 2+/metastatic adenocarcinoma of the stomach or gastro-esophageal junction (GEJ)^[52]. The United States Food and Drug Administration approved trastuzumab for HER2 overexpressing patients, without further specification^[53].

Trastuzumab is a fully humanized monoclonal antibody that binds to the extracellular domain of the receptor, acting by blockage of the HER2 receptor cleavage, inhibition of dimerization, as well as by the induction

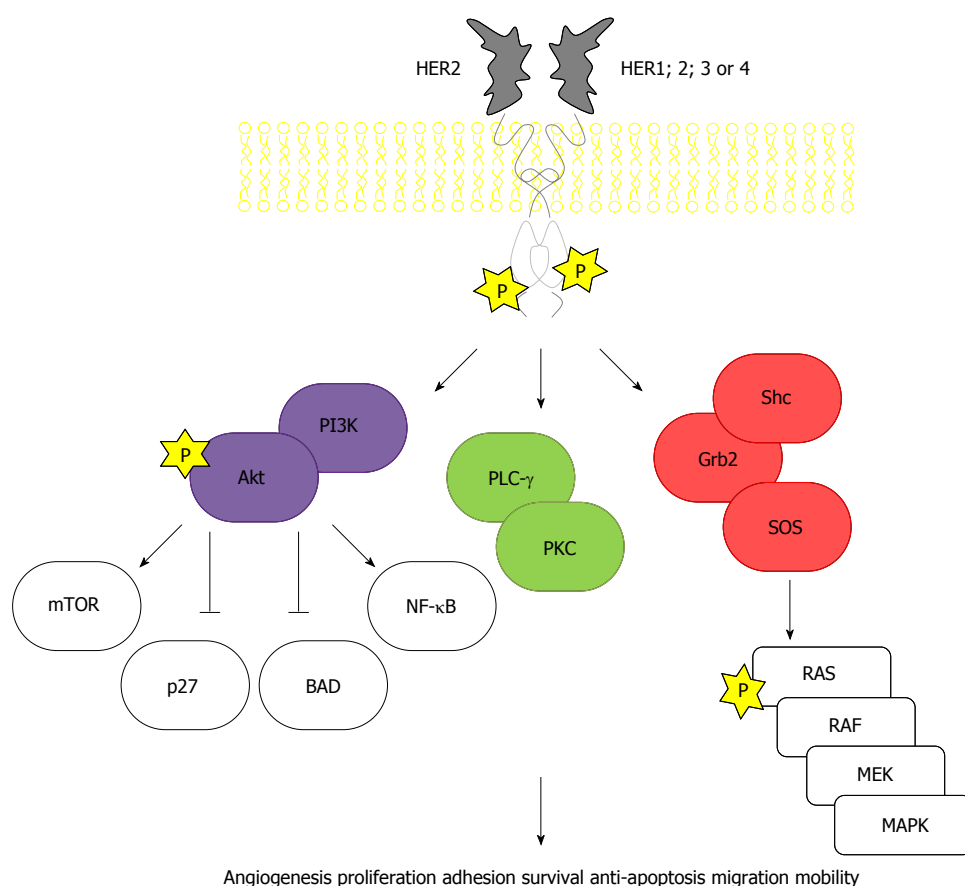


Figure 2 Human epidermal growth factor receptor 2 signalling pathways. HER: Human epidermal growth factor receptor; PI3K: Phosphoinositide 3-kinase; BAD: Bcl-2-associated death promoter protein; NF- κ B: Nuclear factor κ B; PLC- γ : Phospholipase C gamma 1; PKC: Protein kinase C; Grb2: Growth factor receptor-bound protein 2; SOS: Son of Sevenless; MEK: Mitogen-activated protein kinase 1; MAPK: Mitogen-activated protein kinase; mTOR: Mammalian target of rapamycin; RAS: Rat sarcoma viral oncogene; RAF: Rapidly accelerated fibrosarcoma.

of antibody-dependent cellular cytotoxicity (ADCC), increasing endocytosis of the receptor and possibly through anti-angiogenic effects^[54-56]. It was developed in the 1990s, after murine monoclonal antibodies directed to the extracellular domain of HER2 were produced and evaluated in cell lines and xenografts^[57-59].

Preclinical data

Overexpression of HER2 in gastric cancer cells was first reported in 1986 by Sakai *et al.*^[60] and Fukushima *et al.*^[61]. Preclinical models of gastric cancer were successful in demonstrating the inhibitory effect of trastuzumab on human gastric cancer cell lines *in vitro* and in mice xenografts *in vivo*, with additive and synergistic antineoplastic effects in combination with chemotherapy^[59,62-65]. A study by Tanner *et al.*^[17] points out a gastric cancer cell line that was as sensitive to trastuzumab as a breast cancer cell line, both of them with amplified *HER2*, while Matsui *et al.*^[63] reported suppression of tumor growth in a xenograft model. Enhanced antineoplastic effects were observed with capecitabine, cisplatin, docetaxel, paclitaxel and irinotecan^[62], and a further synergistic effect with cisplatin has been found by Kim *et al.*^[64].

Clinical data

Although information on the specific pathways involved

is scarce, *HER2* has been shown to be amplified in gastric cancer and *HER2* is progressively regarded as an important biomarker and driver of cancerization in gastric cancer, with studies pointing out amplification or overexpression in 7%-34% of tumors, mainly in the intestinal type and in GEJ and proximal tumors^[17,27,66].

Cortés-Funes *et al.*^[67] presented preliminary results of a phase II study involving 21 chemotherapy-naïve patients with *HER2* overexpressing locally advanced or metastatic gastric cancer. Trastuzumab at a loading dose of 8 mg/kg and maintenance dose of 6 mg/kg and cisplatin 75 mg/m² were administered every 21 d until progression, unacceptable toxicity or withdrawal of consent. Response rate was of 35%, with 17% of patients achieving stabilization. The tolerability profile was favourable; no grade 4 toxicity was observed and most the frequent grade 3 events were asthenia, nausea or vomiting, diarrhea, hyporexia and neutropenia. Data from another preliminary phase II study involving 16 gastric cancer patients were presented by Egamberdiev *et al.*^[68]. Trastuzumab 6 mg/kg was administered once in addition to cisplatin 100 mg/m² during 3 d + fluorouracil (5-FU) 1000 mg/m² 3 d + leicovirin 100 mg/m² 3 d, every 3 wk. Authors reported an objective response rate of 54.5% in the combined therapy group *vs* 33.3% in the chemotherapy-only group and a median remission duration of 8.3 mo *vs* 5.2 mo. In a recent phase

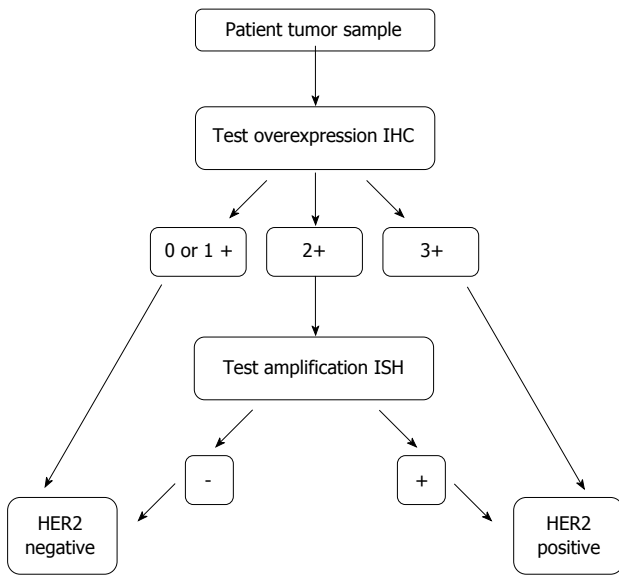


Figure 3 Human epidermal growth factor receptor 2 testing algorithm. IHC: Immunohistochemistry; HER2: Human epidermal growth factor receptor 2; ISH: *In situ* hybridization.

II study carried out by Grávalos *et al.*^[69], chemo-naïve patients with non-resectable advanced or metastatic gastric or GEJ adenocarcinoma overexpressing HER2 were treated with trastuzumab 8 mg/kg as loading dose and 6 mg/kg in subsequent cycles + cisplatin 75 mg/m² every 3 wk. Twenty-two out of 228 patients (9.6%) enrolled had HER2 overexpression. An overall response rate of 32% was found, with disease control achieved in 64% of patients; median time to progression was 5.1 mo. No grade 4 toxicities occurred, whereas most frequent grade 3 adverse events were asthenia, neutropenia, anorexia, diarrhoea and abdominal pain. Interestingly, higher baseline HER2 extracellular domain levels associated with better response to therapy.

In more recent studies, HER2 overexpression was found to be lower than previously reported, especially in distant gastric cancers^[70]. Resectable gastric cancer has reported HER2-positive ratios of 8.1% and 11.7%, suggesting that in resectable gastric cancer HER2 positive status might be less frequent than in advanced gastric cancer^[71,72].

The phase III ToGA trial constitutes a milestone, establishing trastuzumab as the first biological therapy that demonstrated survival benefits in gastric cancer^[62,63]. ToGA was a multicenter, international trial, undertaken in 24 countries^[73]. It evaluated the combination of trastuzumab with standard chemotherapy (cisplatin + either capecitabine or 5-FU) in advanced (inoperable locally advanced, recurrent or metastatic) HER2-positive gastric cancer as a first-line therapy *vs* chemotherapy alone. Patients were treated with six cycles of chemotherapy in both treatment arms, with patients in the experimental arm continuing to be treated with trastuzumab until disease progression. Cisplatin 80 mg/m² was given on day 1 by intravenous infusion. Capecitabine 1000 mg/m² was given orally twice a day for 2 wk followed by a 1-wk rest

or 5-FU 800 mg/m² per day was given by continuous infusion on days 1-5 of each cycle. Trastuzumab was given intravenously at a loading dose of 8 mg/kg on day 1 of the first cycle, followed by 6 mg/kg afterwards.

The primary objective of the study was to compare OS in both arms, and the secondary objectives were to compare progression-free survival (PFS), time to progression, overall response rate, disease control, duration of response and quality of life between the two treatment arms. Among 3665 tumor tissue specimens screened for HER2 positivity, 22% were HER2 positive (34% of the intestinal type *vs* 6% of diffuse and 20% of mixed types). Assessment was done with IHC and fluorescence ISH (FISH), according to Figure 3. The highest rate was observed in 34% of GEJ cancer and 20% of gastric cancer samples^[74], which is in conformity with other studies where positivity rates for the GEJ are between 24%-35% and in gastric cancer samples comprise 9.5%-21.0%^[17,27,75-77].

The combination of trastuzumab with chemotherapy in advanced HER2-positive cancer patients led to significantly better OS compared to the same chemo-therapeutic regimen alone (median OS in the combination therapy group was 13.8 mo against 11.1 mo in the chemotherapy-alone group). This effect was observed in patients with intestinal type gastric cancer but not in those with diffuse type gastric cancer^[73,78]. Median PFS (6.7 mo *vs* 5.5 mo) and radiological response rate (47% *vs* 35%), also improved with trastuzumab therapy. Exploring these data further, a sub-group analysis of the ToGA study which excluded patients with IHC 0-1+ FISH+ disease, found a main gain in median survival of 4.2 mo, comparable to the figures in breast cancer^[28]. In fact, patients with strongest HER2 expression (IHC 3+ FISH+) gained the greatest benefit, with a median survival of 17.9 mo in patients treated with trastuzumab *vs* 12.3 mo with chemotherapy alone.

A summary of selected clinical trials of trastuzumab in gastro-esophageal cancer can be found in Table 1.

Adjuvant treatment

In this behalf, it is important to consider the possible benefits of trastuzumab in the adjuvant setting for earlier stages of the disease; however activity of targeted therapeutics in advanced disease should not automatically be extrapolated into the adjuvant setting, as results may be misleading^[79]. Trials have been initiated which intend to investigate anti-HER2 therapeutics in this setting^[80,81]. Early onset gastric cancer (presenting at or under the age of 45) seems to have lower HER2 overexpression than in late onset cases, with possible different molecular genetic pathways^[82-84]. Ongoing clinical trials with trastuzumab can be found in Table 2.

Maintenance therapy

From a clinical perspective, data known from breast cancer suggest that trastuzumab administration after disease progression might have benefits in OS^[23,85]. In an observational study of 623 patients, median time to progression was longer in patients who continued trastu-

Table 1 Selected clinical trials of trastuzumab in gastro-esophageal cancer

Ref.	Phase	Treatment	n	OS (mo)	PFS (mo)	RR	CR	PR
Bang <i>et al</i> ^[73]	III	5-FU + cisplatin or capecitabine + cisplatin	290	11.1	5.5	34.50%	NA	NA
		Trastuzumab + 5-FU + cisplatin or trastuzumab + capecitabine + cisplatin	294	13.8	6.7	47.30%	NA	NA
Cortés-Funes <i>et al</i> ^[67]	II	Trastuzumab + cisplatin	21	NA	NA	41.10%	5.80%	35.00%
Egamberdiev <i>et al</i> ^[68]	II	Trastuzumab + leucovorin + cisplatin + 5-FU	16	NA	8.3	54.50%	NA	NA
		Leucovorin + cisplatin + 5-FU	18	NA	5.2	33.30%	NA	NA
Grávalos <i>et al</i> ^[69]	II	Trastuzumab + cisplatin	22	NA	5.1	32.00%	NA	NA

OS: Overall survival; PFS: Progression-free survival; 5-FU: 5-fluorouracil; RR: Response rate; CR: Complete response; PR: Partial response; NA: Not available.

Table 2 Clinical trials with trastuzumab-based combination therapies

Setting, therapy line	ID	Phase	n	Treatment combined with trastuzumab	Primary EP	Status
Operable disease	NCT01196390	III	480	Carboplatin, paclitaxel, radical radiotherapy	PFS	Recruiting
	NCT01472029	II	53	5-FU, leucovorin, docetaxel, oxaliplatin	Rate of CR	Recruiting
	NCT01130337	II	45	Oxaliplatin, capecitabine	PFS	Active, not recruiting
Advanced first line	NCT01450696	III	400	Cisplatin, capecitabine	OS	Recruiting
	NCT01503983	II	51	Oxaliplatin, capecitabine	OS	Recruiting
	NCT01461057	II	30	Cisplatin, capecitabine, pertuzumab	Safety	Active, not recruiting
	NCT01396707	II	56	Oxaliplatin, capecitabine	RR	Recruiting
	NCT01364493	II	51	Oxaliplatin, capecitabine	RR	Recruiting
	NCT01359397	II	80	Docetaxel, oxaliplatin, capecitabine, bevacizumab	PFS	Recruiting
	NCT01228045	II	30	Cisplatin, S-1	RR	Unknown
	NCT01191697	II	36	Oxaliplatin, capecitabine, bevacizumab	RR	Recruiting
	NCT01402401	II	48	AUY922	RR	Terminated
Advanced second line						

ID, NCT identification (information available at <http://clinicaltrials.gov>, as accessed June 28, 2013). OS: Overall survival; PFS: Progression-free survival; 5-FU: 5-fluorouracil; RR: Response rate; CR: Complete response; PR: Partial response; NA: Not available.

zumab beyond progression than in those who stopped (10.2 mo *vs* 7.1 mo)^[86]. Data from an interventional study involving 156 patients revealed OS rates of 20.4 mo *vs* 25.5 mo and response rates of 27.0% *vs* 48.1% in patients who stopped and continued trastuzumab beyond progression, respectively. Continuation of trastuzumab beyond progression was not associated with increased toxicity^[87]. However, the issue is still a matter of debate, as increasing therapeutic options pose a challenge on the best possible sequencing and combinations of these interventions^[88-90].

Perioperative treatment

Perioperative chemotherapy regimens have shown promising results in gastric cancer. The MAGIC trial randomized over 500 patients to either surgery alone or perioperative chemotherapy consisting of epirubicin, cisplatin and fluorouracil (3 cycles before and 3 cycles after surgery). This triplet therapy demonstrated a decrease in tumor size and improved PFS and OS in comparison with surgery alone^[91,92]. In addition, some data indicate that response to neoadjuvant treatment is a major predictive factor of survival after curative surgical resection^[93].

Although there is no trial so far reporting results on the role of trastuzumab in the neoadjuvant setting, a number of case reports with trastuzumab-containing neoadjuvant chemotherapy regimens have been published, with interesting outcomes; complete pathological

responses were attained in 2 cases and a partial response with tumor mass reduction allowing for an extensive surgery in another case^[94-96].

Pharmacokinetics and pharmacodynamics

Most data regarding the pharmacokinetic and pharmacodynamic profiles of trastuzumab stem from studies in breast cancer. A low systemic clearance (5.15 ± 2.45 mL/kg per day) and volume of distribution (44 mL/kg) have been described. Serum minimum concentrations of 10 µg/mL are needed to attain anti-proliferative effects and ADCC. With the usual loading dose of 4 mg/kg followed by 2 mg/kg per week, trastuzumab achieves and maintains serum minimum concentrations greater than 20 µg/mL. Recent results demonstrate that trastuzumab 6 mg/kg every 3 wk lead to the same plasma trough levels as trastuzumab 2 mg/kg weekly. Trastuzumab has been found not to exhibit dose-related nonlinear pharmacokinetics and the value of half-life of trastuzumab has an estimated value of 28.5 d^[97,98]. No relevant drug interactions have been reported to date and elimination pathways remain largely unknown^[99]. An extensive review about the pharmacodynamic and pharmacokinetic profiles, tolerability and dosage of trastuzumab in gastric cancer has been elaborated by Croxtall *et al*^[100]. Targeted delivery systems involving anti-HER2 antibody mediated nano-scaled systems, drug conjugates, and fusion proteins are under active investigation^[4,101,102].

Safety

The most commonly described adverse events with trastuzumab are infusion-related, described as fever, rigors, chills, nausea, dyspnea, and hypotension, and are present in about 40% of patients after the first administration and in 5% with subsequent treatment^[103]. Trastuzumab has been extensively evaluated in breast cancer with a wide range of chemotherapeutic agents showing no significant overlapping toxicity, with one important exception, regarding an increased risk of cardiotoxicity. Trastuzumab-related cardiac dysfunction is largely reversible on withdrawal of the antibody. However, significant cardiopathy such as valvular heart disease, angina pectoris, previous transmural infarction and heart failure with left ventricular ejection fraction (LVEF) < 50% are generally regarded as counter-indications for trastuzumab use^[28]. With the chemotherapy doublet regimen evaluated in the ToGA trial, trastuzumab contributed with little added toxicity; no increase in chemotherapy related grade 3-4 toxicities (68% both arms) or cardiac events (6% both arms) were found. Nonetheless the number of patients with cardiac dysfunction (considered a $\geq 10\%$ drop in LVEF to an absolute value < 50%) was low in both arms (5% trastuzumab + chemotherapy *vs* 1% chemotherapy alone). The European Society for Medical Oncology^[104], issued a statement regarding the cardiac monitoring of patients receiving trastuzumab. Clinical evaluation and assessment of cardiovascular risk factors and comorbidities should be performed in every patient proposed for treatment with trastuzumab^[105]. While screening algorithms for trastuzumab-induced cardiomyopathy provide guidance, patient-based strategies of surveillance remain important. Many clinical trials involving patients with metastatic breast cancer include a screening study to document the baseline LVEF, followed by serial monitoring at 8- to 16-wk intervals^[106].

In the ToGA trial, serious adverse events were reported in 32% of patients treated with trastuzumab + chemotherapy and 28% in the chemotherapy group; with treatment-related mortality of 3% and 1% respectively. The adverse events were similar between both groups, with no difference in the overall rate of adverse events. Nausea, neutropenia, vomiting, and anorexia were the most frequently reported adverse events. Patients treated with trastuzumab + chemotherapy had slightly higher rates of diarrhoea, stomatitis, anemia, thrombocytopenia, fatigue, chills, weight loss, pyrexia, mucosal inflammation, and nasopharyngitis^[73]. In a phase II study with trastuzumab and cisplatin as first-line therapy in GEJ and gastric cancer, trastuzumab showed a favourable toxicity profile^[69].

Resistance to trastuzumab

Whilst data regarding mechanisms of resistance to trastuzumab in gastroesophageal cancer is scarce, important information can be retrieved from previous knowledge in the treatment of breast cancer. Primary resistance to single-agent trastuzumab in HER2-overexpressing

metastatic breast carcinomas is described in 66%-88% of cases, with resistance eventually ensuing after a relatively short treatment period; in fact, the majority of patients who achieve an initial response to trastuzumab-based regimens develop resistance within 1 year (PFS between 6.7 and 7.4 mo)^[85,107-109].

Proposed resistance mechanisms include aberrations in the PI3K/AKT/mTOR pathway with or without loss of the phosphatase and tensin homologue protein (PTEN) tumor suppressor gene, accumulation of truncated forms of the HER2 receptor that lack the extracellular trastuzumab-binding domain (collectively known as p95HER2), loss of phosphatase, activation of other tyrosine kinase receptors including the insulin-like growth factor receptor (IGF-1R), increased expression of membrane-associated glycoprotein (MUC1 and MUC4) and cyclin E overexpression^[85,109-111].

PTEN inhibits PI3K, thereby inhibiting the PI3K/AKT/mTOR pathway. Loss of this tumor suppressor gene leads to at least partial resistance to trastuzumab. Indeed, both PIK3 mutations and PTEN loss were associated with inferior PFS and OS in a retrospective study of 256 HER2-positive metastatic breast cancer patients treated with trastuzumab^[112]. A potential role for PI3K, AKT or mTOR inhibitors seems to exist, since these agents preclude the constitutive activation of this pathway, reversing PTEN loss-induced trastuzumab resistance^[113-116].

Truncated forms of HER2 which arise through the proteolytic shedding of the extracellular domain of full-length HER2 or by alternative translation initiation from two methionine residues are the predominant HER2 forms in some tumors. The biological function of p95HER2 has not been fully characterized, though overexpression of p95HER2 has been shown to lead to growth of tumor xenografts in nude mice. The p95HER2 protein has kinase activity, and this activity is required for tumor growth; however, the mechanisms involved and its possible relationship with those used by full-length HER2 are still unknown. Importantly, since p95HER2 lacks the binding site for trastuzumab, it conveys resistance to this antibody. p95HER2 is expressed in approximately 30% of HER2-positive breast tumors and is correlated with poor disease-free survival and increased nodal metastasis when compared with patients that express full-length HER2^[110,117]. p95HER2 can therefore be seen as a prognostic and predictive biomarker in breast cancer. In one study analysing 93 metastatic breast tumors, patients overexpressing p95HER2 were found to have a higher incidence of lung metastases and had significantly shorter PFS and OS with trastuzumab treatment in comparison with patients expressing only the full-length receptor^[118]. Tumors that express p95HER2 may be resistant to trastuzumab but sensitive to the inhibitory effects of lapatinib, a low-molecular-weight dual tyrosine kinase inhibitor (TKI) of HER1/2 that has activity in patients with HER2-expressing tumors that are resistant to trastuzumab. Combination of trastuzumab

with lapatinib has been evaluated in women with HER2-positive, trastuzumab-refractory metastatic breast cancer. Lapatinib with trastuzumab was superior to lapatinib alone in clinical benefit: complete response, partial response, and stable disease for ≥ 24 wk was observed in 24.7% of patients in the combination arm *vs* 12.4% in the monotherapy arm^[119,120]. According to some authors this combination could provide a chemotherapy-free option after first line chemotherapy + trastuzumab^[109].

Increased signalling through other receptor TKIs including EGFR, HER3, MET and IGF-1R has been found in cells resistant to HER2-targeting treatments^[109]. PI3K/AKT/mTOR pathway activation through up-regulation of HER3 signalling was demonstrated after exposure of breast cancer cells to HER TKIs^[121]. On the other hand, pertuzumab, a HER2-HER3 dimerization inhibitor has demonstrated activity against trastuzumab resistant breast cancer cells^[122]. Taking this findings into account, HER3 seems to play an important role in the mechanism of trastuzumab resistance.

In preclinical studies, co-expression of HER2 and IGF-1R in breast cancer cells resulted in loss of sensitivity to trastuzumab, conversely, inhibiting ligand-mediated activation of IGF-1R restored sensitivity to trastuzumab, therefore pointing towards a possible strategy to reduce or delay trastuzumab resistance^[123,124].

Overcoming resistance to trastuzumab

Strategies to overcome trastuzumab resistance imply the important fact that many HER2-positive gastric tumors retain dependency on downstream signalling *via* the HER2 pathway. Therefore, besides other anti-HER2 agents (described in the following section), a focus on targeting these downstream signalling molecules has emerged^[125,126]. Implied targets include mTOR inhibitors, HSP90 inhibitors and MET inhibitors; particularly interesting data exists concerning the possibility to combine some of these agents with anti-HER2 agents on which a patient has progressed, as the potential to reverse resistance to trastuzumab has been demonstrated^[127-129].

OTHER ANTI-HER2 AGENTS

Lapatinib

Lapatinib is a dual TKI active on both EGFR and HER2, with known activity in trastuzumab resistant advanced breast cancer; data suggests that there is no cross-resistance with trastuzumab and lapatinib restored trastuzumab sensitivity in preclinical models^[28,130,131]. Wainberg *et al*^[132] evaluated the effect of lapatinib in HER2-amplified cell lines and xenograft models, concluding that lapatinib inhibits the growth of HER2-amplified cancer cell lines, induces cell cycle arrest and apoptosis and acts synergistically with trastuzumab.

It is approved as combination therapy with capecitabine for patients with HER2-overexpressing breast cancer with prior progression on trastuzumab, an anthracycline and a taxane^[133]. In a phase II trial conducted by Galsky

et al^[134], patients with HER2 amplified gastro-esophageal, bladder, ovarian, or uterine tumors were enrolled into a double-blinded randomized discontinuation study of lapatinib 1500 mg *per os* a day. Of a total of 141 patients screened, 32 patients with HER2 amplified tumors were enrolled in the study. At 3 mo, 1 (3%) patient had a complete response (CR), 9 (28%) had stable disease, 20 (63%) had progressive disease, and 2 (6%) were unknown. Unfortunately, due to low response rate and slow enrolment, the study had to be closed early. Concerning gastro-esophageal cancer, a modest CR rate of 6.25% was reported. A phase II study with lapatinib as first-line therapy in 47 patients with advanced gastric cancer showed modest single-agent activity, with 12% response rate, 20% disease stabilization, 7% of patients experiencing partial response and a median OS of 5 mo, less than that seen with conventional cytotoxic chemotherapy^[135]. Another phase II study of lapatinib monotherapy in patients with HER2-overexpressing GEJ or esophageal cancer reported limited single-agent activity, with no objective responses and stable disease in 8% of patients^[136]. Lapatinib in conjunction with capecitabine in the first line treatment of HER2 positive metastatic gastric cancer setting was addressed in a multicenter phase II trial, reporting a response rate of 22% and stable disease rate of 45%^[137]. In another phase II trial, partial response of 24% and stable disease in 34% of patients was reported with lapatinib + capecitabine. Most frequent grade 3 and 4 side effects were anorexia, hand-foot syndrome, anemia and nausea; no significant cardiotoxicity was reported^[138]. Two phase III studies evaluating the role of lapatinib in combination with chemotherapy in advanced esophago-gastric cancer are currently being conducted, the LOGIC trial^[139,140] (combination of lapatinib with oxaliplatin and capecitabine as first-line treatment) and the TYTAN trial^[141,142] (lapatinib in combination with weekly paclitaxel in second-line setting). OS results from the LOGIC trial are expected in 2014. Data from the TYTAN trial were presented at ASCO GI 2013. 430 patients were randomized, with an OS of 11 mo for the experimental arm *vs* 8.9 mo for the paclitaxel-alone arm; the subgroup of patients with HER2 3+ expression score attained an OS of 14 mo.

As previously stated, dual blockade with lapatinib and trastuzumab in metastatic breast cancer patients that progressed on trastuzumab-containing regimens improved PFS and clinical response rate^[120]; a clinical case reported durable stable disease in a patient treated with this strategy despite progression during prior chemotherapy with trastuzumab^[143].

Pertuzumab

Pertuzumab is a monoclonal antibody targeting HER2 in domain II (Figure 1), preventing formation of the highly mitogenic HER2/HER3 dimer. Available data stem mostly from breast cancer. As with trastuzumab, the antibody is not effective in patients without amplification of HER2^[144]. In the phase III CLEOPATRA

Table 3 Clinical trials with other anti-human epidermal growth factor receptor 2 agents

Setting, therapy line	ID	Phase	n	Treatment	Primary EP	Status
Operable disease	NCT00450203	III	370	Lapatinib, epirubicin, cisplatin, capecitabine	OS	Recruiting
Advanced first line	NCT00680901	III	535	Lapatinib, oxaliplatin, capecitabine	OS	Active, not recruiting
	LOGiC					
	NCT01395537	II	43	Lapatinib, carboplatin, paclitaxel	Safety, RR	Active, not recruiting
	NCT01123473	II	192	Lapatinib, epirubicin, cisplatin, capecitabine, 5-FU	PFS	Unknown
	NCT00526669	II	67	Lapatinib, capecitabine	RR	Active, not recruiting
Advanced second line	NCT00486954	III	273	Lapatinib, paclitaxel	OS	Completed
	TYTAN					
	NCT01522768	II	27	Afatinib	RR	Recruiting
	NCT01152853	II	28	Dacomitinib	PFS	Unknown
	NCT01145404	II	76	Lapatinib, capecitabine	RR	Active, not recruiting

OS: Overall survival; PFS: Progression-free survival; 5-FU: 5-fluorouracil; RR: Response rate; CR: Complete response; PR: Partial response; NA: Not available.

study, 808 patients with HER2-positive metastatic breast cancer received placebo + trastuzumab + docetaxel (control group) or pertuzumab + trastuzumab + docetaxel (pertuzumab group). Median PFS was 12.4 mo in the control group *vs* 18.5 mo in the pertuzumab group. The hazard ratio for the addition of pertuzumab to docetaxel + trastuzumab for PFS was 0.62, with moderate toxicity added by the second antibody^[145]. Pre-clinical results show potentiation of trastuzumab antitumour activity when combined with pertuzumab^[146]. Pertuzumab is currently under investigation in a phase II study, in the first line gastric setting in combination with trastuzumab and platinum-fluoropyrimidine based chemotherapy^[147].

Trastuzumab emtansine

Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate, which combines trastuzumab with the targeted delivery of the cytotoxic agent DM1, a derivative of maytansine, and a potent antimicrotubule agent. As systemic therapy, gastrointestinal toxicity limits the therapeutic usefulness of the agent^[109]. In xenograft models, T-DM1 was found more effective than trastuzumab alone, with positive results independent of the tumor burden at therapy initiation, or preceding treatment with trastuzumab^[101]. In a phase II study by Burris *et al.*^[148], T-DM1 had robust single-agent activity in patients with heavily pre-treated, HER2-positive metastatic breast cancer, with a favourable toxicity profile. In breast cancer, the EMILIA trial assigned patients with HER2-positive advanced breast cancer, previously treated with trastuzumab and a taxane, to T-DM1 or lapatinib + capecitabine. Median PFS was 9.6 mo with T-DM1 *vs* 6.4 mo with lapatinib plus capecitabine; with an objective response rate of 43.6% for T-DM1^[149]. Taken together, results from preclinical studies and breast cancer clinical trials point out T-DM1 as a promising agent to be evaluated in gastro-esophageal cancer. Currently, a phase II-III study is ongoing to evaluate T-DM1 *vs* taxane in patients with previously treated locally advanced or metastatic HER2+ gastric and GEJ cancer.

Pan-HER TKIs

Irreversible small molecule pan-HER TKIs causes tumor

regression in HER2-overexpressing human gastric cancer xenograft models. They act by inhibition of HER family receptor phosphorylation and blocking of hetero-dimerization among them. Pre-clinical data reveal a synergistic effect with other molecular targeted agents (including trastuzumab) and chemotherapeutic agents. Currently investigated pan-HER TKIs include dacomitinib and afatinib^[150,151].

Selected ongoing clinical trials exploring other anti-HER2 agents can be found in Table 3.

OTHER HER2-DIRECTED STRATEGIES

HER2 vaccines, both DNA and peptide-based, are actively researched in the field of breast cancer and results indicate a possible future role for this modality in combination with other HER2 targeted therapies. A phase I study carried out by Hamilton *et al.*^[152] combined HER2 immunization with lapatinib found this combination to be safe and immunogenic, however, the anticancer activity of immunization-induced antibodies is still not well characterized. Successful repression of the HER2 gene by the means of adenovirus constructs rises expectations for possible applications in cancer treatment^[153]. Radioimmunotherapy is another possible application of HER2 directed homing, with authors currently evaluating 212Pb immunoconjugates with trastuzumab in intraperitoneal application after preclinical studies showed interesting results^[154,155].

CONCLUSION

Now, times are changing. New strategies had been developed and implemented for advanced gastric cancer treatment. HER2 acquired a main role in gastric cancer management and current is also mandatory in order to predict trastuzumab response in association with standard platinum-based chemotherapy. Furthermore, others drugs are in developing to overcome resistance to trastuzumab, serious treatment-related toxicities and also help oncologists to improve treatments approaches. In future, genomic profiles will probably be part of clinical routines

for personalizing therapies and improve outcomes of those patients.

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New insights in bilirubin metabolism and their clinical implications

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Abstract

Bilirubin, a major end product of heme breakdown, is an important constituent of bile, responsible for its characteristic colour. Over recent decades, our understanding of bilirubin metabolism has expanded along with the processes of elimination of other endogenous and exogenous anionic substrates, mediated by the action of multiple transport systems at the sinusoidal and canalicular membrane of hepatocytes. Several inherited disorders characterised by impaired bilirubin conjugation (Crigler-Najjar syndrome type I and type II, Gilbert syndrome) or transport (Dubin-Johnson and Rotor syndrome) result in various degrees of hyperbilirubinemia of either the predominantly unconjugated or predominantly conjugated type. Moreover, disrupted regulation of hepatobiliary transport systems can explain jaundice in many acquired liver disorders. In this review, we discuss the recent data on liver bilirubin handling based on the discovery of the molecular basis of Rotor syndrome. The data show that a substantial fraction of bilirubin conjugates is primarily secreted by

MRP3 at the sinusoidal membrane into the blood, from where they are subsequently reuptaken by sinusoidal membrane-bound organic anion transporting polypeptides OATP1B1 and OATP1B3. OATP1B proteins are also responsible for liver clearance of bilirubin conjugated in splanchnic organs, such as the intestine and kidney, and for a number of endogenous compounds, xenobiotics and drugs. Absence of one or both OATP1B proteins thus may have serious impact on toxicity of commonly used drugs cleared by this system such as statins, sartans, methotrexate or rifampicin. The liver-blood cycling of conjugated bilirubin is impaired in cholestatic and parenchymal liver diseases and this impairment most likely contributes to jaundice accompanying these disorders.

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Key words: Hyperbilirubinemia; Hereditary jaundice; UGT1A1; ABCC2; Organic anion transporting polypeptide 1B1; Organic anion transporting polypeptide 1B3

Core tip: Experiments with *Oatp1a/1b*-null mice and *Oatp1a/1b*; *Abcc3* combination knockout mice plainly demonstrated that even under physiologic conditions a substantial portion of bilirubin glucuronides is not excreted directly into bile but is transported back to the blood by *Abcc3*. *Oatp1a/1b* activity accentuated in downstream (centrizonal) hepatocytes allows efficient reuptake of bilirubin conjugates, with a subsequent possibility being safely eliminated by excretion into bile. This and molecular findings in Rotor syndrome suggest that human transporters MRP3 and OATP1Bs form a sinusoidal liver-to-blood cycle which mediates shifting (hopping) of bilirubin and other substrates from periportal to centrizonal hepatocytes (References 18, 19, 22, 125).

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INTRODUCTION

Bilirubin is the end product of heme breakdown. About 80% of bilirubin originates from degradation of erythrocyte haemoglobin in the reticuloendothelial system; the remaining 20% comes from inefficient erythropoiesis in bone marrow and degradation of other heme proteins^[1-4]. Water insoluble, unconjugated bilirubin (UCB) bound to albumin is transported to the liver where it is removed from the plasma. The exact mechanism of UCB uptake is unknown; however, passive transmembrane diffusion seems to be combined with active transport mediated by several sinusoidal transporters (see below). Within the cytoplasm of hepatocytes, bilirubin is bound to ligandin and transported to endoplasmic reticulum where conjugation with glucuronic acid takes place. Conjugation is catalysed by the enzyme uridine diphosphate glucosyltransferase 1A1 (UGT1A1; EC2.4.1.17), a member of an enzyme family in the endoplasmic reticulum and nuclear envelope of hepatocytes^[5-8]. In addition to the liver, UGT activity has also been detected in the small intestine and kidney^[9,10]. *UGT1A1* gene (ID: 54658) is a part of a complex locus encoding 13 UDP-glucuronosyltransferases^[11]. The locus contains a series of thirteen unique alternate promoters and first exons, followed by four common exons No. 2-5. Theoretically, each of the unique first exons is spliced to the first of the four shared exons. The unique first exons encode different substrate binding domains whereas the other functional domains encoded by the shared exons 2-5 are the same^[11-15]. In reality, only 9 of the 13 predicted *UGT1As* are active genes encoding functional enzymes; four are nonfunctional pseudogenes.

The excretion of conjugated bilirubin into bile is mediated by an ATP-dependent transporter identified as the multidrug resistance-associated protein MRP2/cMOAT and, to a lesser extent, also by ATP-binding cassette (ABC) efflux transporter ABCG2. MRP2 is encoded by *ABCC2* and expressed under physiologic conditions at the apical (canalicular) membrane of hepatocytes and, to a much lesser extent, in the kidney, duodenum, ileum, brain and placenta^[16]. Since the MRP2 mediated export represents an important step in detoxification of many endogenous and exogenous substrates, the absence of functionally active MRP2 prevents the secretion of these conjugates into bile. Absence of MRP2 mediated transport is followed by upregulation of the basolateral MRP2 homologues at the sinusoidal membrane of hepatocytes and conjugated bilirubin flow is redirected into sinusoidal blood^[17]. Aside from MRP2 mediated transport of conjugated bilirubin into bile, recent studies have shown that a significant fraction of the bilirubin conjugated in the liver is, under physiologic conditions, secreted into sinusoidal blood and subsequently reuptaken by hepatocytes for fi-

nal biliary excretion^[18,19]. The process is mediated by sinusoidal transporters MRP3 and organic anion-transporting polypeptides OATP1B1 and OATP1B3. OATP1B transporters facilitate sodium-independent uptake of numerous endogenous and exogenous substrates^[20,21]. Since expression of OATP1Bs is higher in centrilobular hepatocytes, the MRP3-OATP1B1/3 loop is likely responsible for shifting (hopping) of conjugated bilirubin and other substrates from the periportal to the centrilobular zone of the liver lobule (Figure 1). Such intralobular substrate transfer may protect periportal hepatocytes against elevated concentrations of various xenobiotics^[22]. In addition, the OATP1B proteins mediate hepatic clearance of bilirubin conjugated in splanchnic organs and may represent an important alternative pathway in enterohepatic circulation^[18].

OATP1Bs may also contribute to liver uptake of UCB since complete absence of both OATP1Bs in Rotor syndrome (RS, see below) is associated with elevated levels of UCB and single nucleotide polymorphisms in genes encoding OATP1B proteins have been shown to influence serum bilirubin level^[23,24]. Furthermore, results of functional studies demonstrate that OATP1B3, but not OATP1B1, may play an important role in the carrier-mediated uptake of foetal UCB by the placental trophoblast and contribute to elimination of UCB across the placental barrier^[25,26].

Mild or moderately elevated serum bilirubin seems to be beneficial: Bilirubin is known as a strong antioxidant^[27,28] and the protective effects of bilirubin on atherogenesis and cancerogenesis have been demonstrated in both *in vitro* and *in vivo* studies^[29-33]. On the other hand, patients with profound unconjugated hyperbilirubinemia are at risk for bilirubin encephalopathy (kernicterus)^[34,35]. The toxic effects of bilirubin are explained by inhibition of DNA synthesis^[36]. Bilirubin may also uncouple oxidative phosphorylation and inhibit adenosine triphosphatase (ATPase) activity of brain mitochondria^[37,38]. Bilirubin mediated inhibition of various enzyme systems, RNA synthesis and protein synthesis in the brain and liver, and/or alteration of carbohydrate metabolism in the brain can also contribute to its toxicity^[39-43]. The accumulation of bilirubin in plasma and tissues results in characteristic yellow discoloration of tissues known as icterus or jaundice.

Inherited disorders of bilirubin excretory pathway played the key role in understanding the individual steps of the bilirubin excretory pathway. Disrupted regulation of hepatobiliary transport systems explained jaundice in many acquired liver disorders^[44-48]. Additional information was obtained from a number of animal models of hereditary jaundice. These include the Gunn rat and *Ugt1*(-/-) mouse mimicking the Crigler-Najjar syndrome type I^[49-51], the Bolivian population of squirrel monkeys mimicking Gilbert syndrome (GS)^[52,53] and mutant TR or GY (Groningen yellow) rats with organic anion excretion defect (TR -/-), Eizai hyperbilirubinuria rats (EHBR), mutant Corriedale sheep, and *Mrp2*(-/-) mice, all modelling the Dubin-Johnson syndrome (DJS)^[54-58].

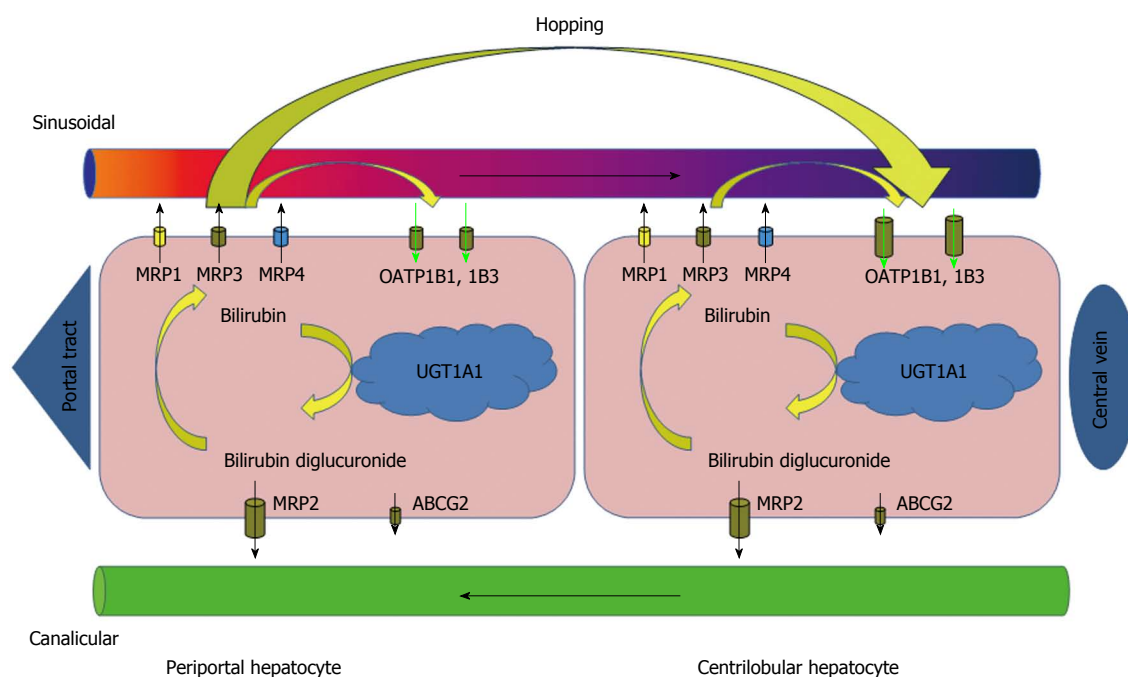


Figure 1 Liver cycle of conjugated bilirubin. Bilirubin conjugated in endoplasmic reticulum of hepatocytes is secreted into the bile. This process is mediated by MRP2/ABCC2 with possible minor contribution of other transporters (ABCG2) at the canalicular membrane of hepatocytes. In addition, even under physiologic conditions, a fraction of bilirubin conjugates is secreted by MRP3 across the sinusoidal membrane into the blood, from where they can be subsequently reuptaken by sinusoidal membrane-bound OATP1B1 and OATP1B3 transporters. The highest overall expression of OATP1Bs has been demonstrated at the centrilobular hepatocytes. The process of substrate shifting (hopping) from periportal to centrilobular hepatocytes may act as a protection of the periportal hepatocytes against elevated concentrations of various xenobiotics. MRP: Multidrug resistance-associated protein; OATP: Organic anion transporting polypeptide; UGT: Uridine diphosphate glucuronosyl-transferase; ABC: ATP-binding cassette.

HEREDITARY PREDOMINANTLY UNCONJUGATED HYPERBILIRUBINEMIA

Conjugation of bilirubin in endoplasmic reticulum is catalysed by the enzyme UGT1A1. Mutations in *UGT1A1* can lead to decreased expression or partial or even complete inactivation of the enzyme^[59]. By contrast, expression of *UGT1A1* can be increased by phenobarbital (PB) administration. PB response activity is delineated to a 290-bp distal enhancer module sequence (-3483/-3194) glucuronosyltransferase phenobarbital response enhancing motif (gtBPREM) of the human *UGT1A1*^[59,60]. gtBPREM is activated by the nuclear orphan receptor, human constitutive active receptor (hCAR). CAR is a cytoplasmic receptor which, after treatment with activators such as PB, translocates into the nucleus, forms a heterodimer with the retinoid X receptor and activates the PB response enhancer element.

Three types of inherited, predominantly unconjugated hyperbilirubinemia with different levels of UGT1A1 activity are recognised: Crigler-Najjar syndrome type I (CN1), type II (CN2) and GS.

CN1 (MIM#218800), the most deleterious form, described in 1952 by Crigler and Najjar^[61], is characterised by complete or almost complete absence of UGT1A1 enzyme activity with severe jaundice^[62]. Icterus occurring shortly after birth is complicated by bilirubin encephalopathy (kernicterus). Until the introduction of phototherapy and plasmapheresis, kernicterus was fatal in almost all cases during the first two years of life or caused seri-

ous brain damage with permanent neurologic sequelae. Intermittent phototherapy is lifelong and it results in a thorough elimination of water-soluble photoisomers of unconjugated bilirubin *via* bile. The effectiveness of phototherapy may decrease gradually with age and patients are at higher risk of sudden brain damage^[63].

Although new treatment modalities such as hepatocyte or hepatic progenitor cell transplantation have already been used to treat CN1 patients, liver transplantation is still considered to be the only definitive treatment for CN1^[63-67]. Gene therapy seems to be a promising therapeutic possibility for the patients with CN1 in the near future^[68,69].

CN2 (Arias syndrome, MIM #606785), described by Arias in 1962^[70], is characterised by reduced UGT1A1 enzyme activity with a moderate degree of nonhemolytic jaundice. Bilirubin levels do not exceed 350 $\mu\text{mol/L}$ and CN2 is only rarely complicated by kernicterus^[71]. Virtually all the mutations responsible for the syndrome are autosomal recessive, as in CN1, but several observations have also suggested the possibility of autosomal dominant pattern of inheritance^[72-74].

An important clinical difference between CN type I and type II is the response to PB treatment, with no effect in type I (complete loss of the UGT1A1 enzyme activity) and a decrease of serum bilirubin levels by more than 30% in CN type II (some residual UGT1A1 activity is preserved). Moreover, bilirubin glucuronides are present in bile in CN2. However, the method of choice for the diagnosis of CN syndrome is mutation analysis of

UGT1A1^[75].

GS (MIM #143500), described in 1901 by Gilbert and Lereboullet^[76], is characterised by fluctuating mild, unconjugated nonhemolytic hyperbilirubinemia < 85 µmol/L without overt haemolysis, usually diagnosed around puberty, and aggravated by intercurrent illness, stress, fasting or after administration of certain drugs^[77,78]. Physical examination and the results of routine laboratory tests are normal apart from elevated serum bilirubin and jaundice. The clinical diagnosis of GS can be established if patients have a mild, predominantly unconjugated hyperbilirubinemia and normal activity of liver enzymes. The reduced caloric intake test and phenobarbital stimulation test have low diagnostic specificity in GS subjects^[79]. Histological findings in GS are mild, with a slight centrilobular accumulation of pigment with lipofuscin-like properties^[80]. Ultrastructurally, hepatocytes reveal hypertrophy of smooth endoplasmic reticulum^[81,82]. Since the morphological picture of GS is completely non-specific and the disorder is benign, liver biopsy is not indicated.

GS is characterised by reduced levels of *UGT1A1* activity to about 25%-30% caused by homozygous, compound heterozygous, or heterozygous mutations in the *UGT1A1* with autosomal recessive transmission^[80].

GS is the most frequent hereditary jaundice affecting nearly 5%-10% of the Caucasian population^[83]. The genetic basis of GS was first disclosed in 1995^[84] as presence of the allele *UGT1A1**28, characterised by insertion of TA in the TATAA box (A[TA]-TAA) in the proximal promoter of *UGT1A1*. *UGT1A1**28 has been identified as the most frequent mutation in Caucasian GS subjects^[85]. The insertion is responsible for reduction of transcription of *UGT1A1* to 20% from normal and for a decrease of hepatic glucuronidation activity by 80% in a homozygous state^[86]. In Caucasians and African Americans, the frequency of *UGT1A1**28 allele is about 35%-40%, but it is much lower in Asians, including Koreans (13%), Chinese (16%), and Japanese (11%)^[87-89]. Moreover, in the majority of Caucasian GS subjects, expression of *UGT1A1* is further decreased by the presence of the second mutation T>G in gtpBREM^[59,60]. In addition to the mutations in the promoter, GS may be caused by mutations in structural regions of the *UGT1A1*. In Asians, other variants, such as *UGT1A1**6 characterised by a missense mutation involving G to A substitution at nucleotide 211 (c.211G>A) in exon 1 (also known as p.G71R), *UGT1A1**7 (p.Y486D), *UGT1A1**27 (p.P229Q), and *UGT1A1**62 (p.F83L) have been detected^[60,87-90].

In addition to biochemical defect leading to reduced glucuronidation, other factors, such as impaired hepatic (re)uptake of bilirubin (see Rotor syndrome below for the possible mechanism) or an increased load of bilirubin, seem to be necessary for clinical manifestation of GS^[86,91,92].

GS is benign and GS carriers present with no liver disease. However, the mutations in the *UGT1A1* identical to those recognised in GS subjects may contribute to the

development of prolonged neonatal hyperbilirubinemia in breast-fed infants^[93,94].

Moreover, since the process of glucuronidation is an important step in elimination of numerous endogenous and exogenous substrates, GS subjects may be more susceptible to the adverse effects of some drugs metabolised by *UGT1A1*, such as indinavir, atazanavir^[95-99] or irinotecan^[100-102].

HEREDITARY PREDOMINANTLY CONJUGATED HYPERBILIRUBINEMIA

Two types of hereditary conjugated jaundice are known as Dubin-Johnson and Rotor syndrome. Both are characterised by the presence of mixed, predominantly conjugated hyperbilirubinemia, with conjugated bilirubin more than 50% of total bilirubin.

DJS (MIM # 237500), a benign autosomal recessive disorder described in 1954 by Dubin *et al.*^[103] and Sprinz *et al.*^[104], is characterised by fluctuating mild, predominantly conjugated hyperbilirubinemia, with typical manifestation in adolescence or young adulthood. Most patients are asymptomatic except of occasional slight abdominal pain and fatigue. Urine excretion of total coproporphyrin in 24 h is normal, but 80% are represented by coproporphyrin I. Biliary excretion of anionic dyes including bromo-sulfophthalein (BSP), indocyanine green and cholescintigraphy radiotracers is delayed with absent or delayed filling of the gallbladder^[105]. BSP clearance in DJS subjects is normal at 45 min with the second peak at 90 min^[106]. Liver histology in DJS shows an accumulation of distinctive melanin-like lysosomal pigment in an otherwise normal liver that gives the organ a characteristic dark pink or even black colour. The pigment is positive in PAS and Masson-Fontana reaction with marked autofluorescence. In contrast to melanin, DJS pigment does not reduce neutral silver ammonium solution^[103,107]. The amount of pigment may vary and possible transient loss may occur in coincidence with other liver diseases^[108,109]. The molecular mechanism in DJS is absence or deficiency of human canalicular multispecific organic anion transporter MRP2/cMOAT caused by homozygous or compound heterozygous mutation in *ABCC2* (gene ID: 1244) on chromosome 10q24^[110-114]. The *ABCC2* mutation alters not only MRP2-mediated transport of conjugated bilirubin but also transport of many anionic substrates as well as a wide range of drugs, such as chemotherapeutics, uricosurics, antibiotics, leukotrienes, glutathione, toxins and heavy metals. Absence of MRP2/cMOAT may result in impaired elimination and in subsequent renal toxicity of the substrates mentioned above^[115-120].

A rare type of hereditary mixed hyperbilirubinemia caused by the simultaneous presence of mutations characteristic for DJS and GS has been classified as dual hereditary jaundice^[121]. Serum direct bilirubin concentrations in dual hereditary jaundice reach only 20%-50% of total bilirubin.

RS (MIM #237450), described in 1948 by Rotor *et al.*^[122],

is characterised by mild, predominantly conjugated hyperbilirubinemia with delayed excretion of anionic dyes without re-increase of their concentration. Total urinary coproporphyrin excretion is significantly increased and the proportion of coproporphyrin I in urine is approximately 65% of the total in homozygotes and 43% in heterozygotes^[123,124]. By histopathological examination, the liver tissue does not display any marked architectural or cytomorphological abnormalities and pigment is not present.

The presence of homozygous mutations in both *SLCO1B1* and *SLCO1B3* neighbouring genes located on chromosome 12 with complete and simultaneous deficiency of proteins OATP1B1 and OATP1B3 has recently been identified as the molecular mechanism of the syndrome^[125]. The complete absence of both transporters OATP1B1 and OATP1B3 has been confirmed by immunohistochemistry in all studied Rotor subjects. Interestingly, the presence of a single functional allele of either *SLCO1B1* or *SLCO1B3* prevented the jaundice.

RS does not require any therapy but, with regard to the impact of OATP1B transporters on pharmacokinetics of a broad spectrum of commonly used drugs such as penicillins, statins, sartans, rifampicin, methotrexate and many others, it is assumed that RS subjects and also those with the deleterious mutations in either of the *SLCO1B* genes, even without full clinical expression of the syndrome, may be at increased risk for drug toxicity^[125-129].

BILIRUBIN HANDLING PROTEINS IN CHOLESTASIS

Animal models of obstructive and intrahepatic cholestasis help us to discover and understand the main principles of acquired defects in hepatobiliary transport of bile salts and other organic anions. Up and down regulation of these mechanisms can explain impaired liver uptake and excretion of the biliary constituents resulting in the cholestasis and icterus which accompanies many common acquired liver disorders^[48,130,131]. A general pattern of response to cholestatic liver injury is initiated by down-regulation of the basolateral membrane bound transporters NTCP and OATP1B1. The expression of several canalicular export pumps is relatively unaffected [bile salt export pump (BSEP), multidrug resistance protein 2 (MDR2)] or even upregulated (MDR1). Decreased expression of MRP2 in sepsis or in obstructive cholestasis is followed by upregulation of several MRP homologues at basolateral membrane of hepatocytes that may extrude bile salts back to the sinusoidal blood and systemic circulation. Most of these changes are believed to help prevent an accumulation of potentially toxic bile components and other substrates in the liver.

Similar patterns of expression of the bilirubin and bile salts handling proteins and mRNA are observed in cholestatic liver diseases in humans. At the stage I and II of primary biliary cirrhosis (PBC), expression and localisation of OATP1B1, OATP1B3, NTCP, MRP2, MRP3

and MDR3 are unchanged. At stage III, immunostaining intensities of the sinusoidal uptake transporters and their mRNA levels decrease. Irregular MRP2 immunostaining suggests redistribution of MRP2 into intracellular structures in the advanced stages of PBC; however, at stage III and IV, basolateral uptake transporters NTCP and OATP1B1 are downregulated. Expression of the canalicular export pumps for bile salts (BSEP) and bilirubin (MRP2) remains unchanged and the canalicular P-glycoproteins MDR1 and MDR3 and the basolateral efflux pump MRP3 are upregulated^[132-135].

At the early-stages of cholestasis in extrahepatic biliary atresia, BSEP, MDR3, MRP2, NTCP/SLC10A1, SLC10A2 and nuclear receptor farnesoid X receptor are downregulated. At the late-stages of cholestasis, farnesoid X receptor and BSEP levels returns to normal, MDR3 and MDR1 are upregulated and MRP2 is down-regulated^[136].

In primary sclerosing cholangitis, the level of OATP1B1 mRNA in liver tissue has been demonstrated to represent 49% of controls and the level of MRP2 mRNA dropped to 27% of controls^[137].

CONCLUSION AND PERSPECTIVES

Over the last decades, molecular basis of hyperbilirubinemia syndromes has been elucidated and mutations affecting the basolateral and apical membrane transporters responsible for accumulation of either conjugated or unconjugated bilirubin have been identified.

Except for GS, the majority of inherited hyperbilirubinemia syndromes are rare autosomal recessive disorders with a low prevalence in the general population and, apart from CN syndrome type I and some cases of CN type II in neonatal period, mostly not requiring further therapy. Nonetheless, the enzyme and transport systems involved in bilirubin metabolism may play an important role in the elimination and disposition processes of many other endogenous and exogenous substrates including hormones, drugs, toxins and heavy metals^[102,138]. Dysfunction or absence of these systems, including selected ABC transporters and OATPs, may alter pharmacokinetics and pharmacodynamics of many biologically active agents, affect penetration of the substrates into various tissues and lead to their intracellular accumulation with a subsequent increase of organ toxicity^[126,127,128]. In addition, the absence of the functional transport proteins involved in hepatobiliary and enterohepatic circulation may involve drug disposition, drug-drug or drug-food interactions and result in decreased effectiveness or even resistance to a diverse spectrum of chemotherapeutic agents and xenobiotics^[139-141]. Individuals with mutations in the responsible gene or genes with the fully expressed phenotype of the corresponding hyperbilirubinemia syndrome, as well as subjects carrying mutations without clinical manifestation of hyperbilirubinemia under normal conditions, may be more susceptible to the adverse effects of some drugs and metabolites^[142,143].

Clarifying the molecular genetic basis of hereditary hyperbilirubinemia syndromes together with the discoveries of the major systems essential for the metabolism and transport of bilirubin and other endogenous and exogenous substrates represent a substantial contribution to the current knowledge of the heme degradation pathway. Further investigation of how bilirubin transport proteins and their variations affect pharmacokinetics of drugs may be of significant clinical importance.

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Irritable bowel syndrome and organic diseases: A comparative analysis of esophageal motility

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Abstract

AIM: To assess the esophageal motility in patients with irritable bowel syndrome (IBS) and to compare those with patients with autoimmune disorders.

METHODS: 15 patients with IBS, 22 with systemic lupus erythematosus (SLE) and 19 with systemic sclerosis (SSc) were prospectively selected from a total of 115 patients at a single university centre and esophageal motility was analysed using standard manometry (Mui Scientific PIP-4-8SS). All patients underwent esophago-gastro-duodenoscopy before entering the study so that

only patients with normal endoscopic findings were included in the current study. All patients underwent a complete physical, blood biochemistry and urinary examination. The grade of dysphagia was determined for each patient in accordance to the intensity and frequency of the presented esophageal symptoms. Furthermore, disease activity scores (SLEDAI and modified Rodnan score) were obtained for patients with autoimmune diseases. Outcome parameter: A correlation coefficient was calculated between amplitudes, velocity and duration of the peristaltic waves throughout esophagus and patients' dysphagia for all three groups.

RESULTS: There was no statistical difference in the standard blood biochemistry and urinary analysis in all three groups. Patients with IBS showed similar pathologic dysphagia scores compared to patients with SLE and SSc. The mean value of dysphagia score was in IBS group 7.3, in SLE group 6.73 and in SSc group 7.56 with a P -value > 0.05 . However, the manometric patterns were different. IBS patients showed during esophageal manometry peristaltic amplitudes at the proximal part of esophagus greater than 60 mmHg in 46% of the patients, which was significant higher in comparison to the SLE (11.8%) and SSc-Group (0%, $P = 0.003$). Furthermore, IBS patients showed lower mean resting pressure of the distal esophagus sphincter (Lower esophageal sphincter, 22 mmHg) when compared with SLE (28 mmHg, $P = 0.037$) and SSc (26 mmHg, $P = 0.052$). 23.5% of patients with SLE showed amplitudes greater as 160 mmHg in the distal esophagus (IBS and SSc: 0%) whereas 29.4% amplitudes greater as 100 mmHg in the middle one (IBS: 16.7%, SSc: 5.9% respectively, $P = 0.006$). Patients with SSc demonstrated, as expected, in almost half of the cases reduced peristalsis or even aperistalsis in the lower two thirds of the esophagus. SSc patients demonstrated a negative correlation coefficient between dysphagia score, amplitude and velocity of peristaltic activity at middle and lower esophagus [$r = -0.6$, $P <$

0.05].

CONCLUSION: IBS patients have comparable dysphagia-scores as patients with autoimmune disorders. The different manometric patterns might allow differentiating esophageal symptoms based on IBS from other organic diseases.

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Key words: Irritable bowel syndrome; Systemic lupus erythematosus; Systemic sclerosis; Esophageal manometry; Dysphagia

Core tip: This is the first comparative study concerning esophageal motility among functional and autoimmune disorders. Patients in irritable bowel syndrome (IBS)-group showed comparable dysphagia-scores as patients with systemic lupus erythematosus and systemic sclerosis. Nevertheless, different manometric patterns between the three examined groups were observed, which might allow differentiating esophageal symptoms based on IBS from other organic diseases.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract characterized mainly by symptoms as diarrhoea, constipation and diffuse abdominal pain^[1]. The prevalence of IBS in the western population varies between 15% and 20%^[2-7] with an overall 2:1 female predominance^[5]. In 2006 the Rome III criteria for diagnosis and classification of IBS were established^[8]. According to these criteria IBS is defined as recurrent abdominal pain or discomfort associated with altered defecation.

Dyspepsia and dysphagia are commonly reported of IBS patients. However, there are no comparative data available dealing with esophagus motility in IBS patients compared to autoimmune disorders. Analyses of peristaltic changes of the esophagus in patients with IBS have led to controversial findings^[9-13]. Reduced resting pressure and relaxation of the lower esophageal sphincter (LES)^[9,11], abnormal contractions up to 150 mmHg^[10] as well as normal peristaltic motility in patients with IBS^[12] have been described.

Systemic sclerosis (SSc) and systemic lupus erythematosus (SLE) show a variety of visceral manifestations. Skin, joints, lungs, nervous system and internal organs

can be affected. Dysfunction of esophagus motility is reported in about 70%-90% of patients with SSc^[14-18]. In patients with SLE the percentage varies between 1.5% and 25%^[19]. Aim of the present study was to assess the esophageal motility function in patients with IBS compared to organic diseases (SLE and SSc).

MATERIALS AND METHODS

Subjects

Patients were prospectively invited to participate at this single centre study. Patients were identified based on the database of our outpatient clinic of gastroenterology and rheumatology at the University Mainz. Patients with IBS, SLE and SSc were able to participate. The database showed 115 patients with one of the mentioned diseases. 74 patients were screened and 56 patients agreed to participate in the study and were included (15 patients with IBS, 22 with SLE and 19 with SSc).

All SLE and SSc patients met the criteria established by the American Rheumatism Association for autoimmune diseases, whereas the patients in IBS group were included on the basis of Rome III process^[8,20,21]. Patients with uncertain diagnosis, with known other severe diseases of the upper gastrointestinal tract and pregnant and lactating women were excluded from the study. All patients gave their written consent. The study was approved by the local ethics committee of Rheinland-Pfalz (No. 837.432.09).

Methods

Besides thorough history and physical examination, the following diagnostic methods were performed:

Blood biochemistry analysis: A complete blood biochemistry examination including blood count and biochemical analysis were performed in each patient. Furthermore, the following parameters were examined: Complement factors C3 and C4, ANA, ENA, dsDNA, CRP and ESR.

Upper endoscopy: All patients underwent esophago-gastro-duodenoscopy (EGD) before entering the study. Only patients with normal endoscopic findings were included in the current study.

Esophageal manometry: Esophageal manometry was performed after 8 h of fasting. All medications which potentially could affect the esophageal motility were paused 48 h before manometry.

The measurements were performed using a 60 cm long, 8 channel lumen catheter (Sierra Scientific Instruments, Germany) with 5 distal openings separated 1 cm vertically and 3 proximal openings distributed at 5 cm distance apart.

Each of the catheter lumens was perfused with distilled water at a rate of 1.36 mL/min. The catheter was connected to an infusion system (Mui Scientific, Canada) with attached pressure converters. The catheter was ini-

Table 1 Demographic data of the three examined groups *n* (%)

IBS			SLE	SSc
	Diarrhoea	Constipation	Diarrhoea + Constipation	
Total	8 (53.3)	4 (26.7)		
Male	3 (100)	0	2 (9.01)	3 (15.8)
Female	8 (66.7)	1 (8.3)	3 (25)	20 (90.9)
Age (yr)	42 ± 16	56 ± 15	34 ± 18	48 ± 10

IBS: Irritable bowel syndrome; SLE: Systemic lupus erythematosus; SSc: Systemic sclerosis.

tially inserted transnasally into the patient's stomach. The patients remained in a sitting position during insertion of the catheter.

After the lumen reached the stomach, patients were brought to a horizontal position and then the pressure of the LES was measured by using the station and rapid pull-through technique^[22,23].

Subsequently, the catheter was slowly withdrawn at 1 cm intervals with wet (10 mL water) and dry swallows at each level, so that a complete analysis of esophagus motility could be obtained. Ineffective swallows were not included in detailed measurements of manometric parameters.

The resulting esophageal parameters were: position, length, resting pressure and relaxation of the LES und upper esophageal sphincter (UES), mean peristaltic pressure, simultan or retrograde contractions, duration and velocity of the peristaltic waves at the proximal, medial and distal third of esophagus.

Questionnaires

Patients were asked to complete the following questionnaires:

SLEDAI: The disease activity in patients of SLE was assessed *via* the SLEDAI-Index^[24].

Rodnan score: The disease activity in patients with SSc was assessed *via* the modified Rodnan score^[25].

Dysphagia score: The intensity and frequency of the esophageal symptoms was assessed as an accumulation score as described before^[26]. Specifically all patients were evaluated for symptoms such as odynophagia, difficulty in swallowing, chest pain, dysphagia etc in relation to frequency, need for treatment and weight loss.

Statistical analysis

The statistical analysis was conducted using SPSS program (version 19.0). Data were analyzed using the Mann-Whitney *U*-test to compare group means. A *P*-value < 0.05 was considered to represent a significant difference. Sample size estimation: Distinct sample size estimation could not be performed because of lack of comparative data. However, we hypothesised that IBS patients have at least 30% different manometric outcome parameters

Table 2 Disease activity scores in patients with systemic lupus erythematosus and systemic sclerosis *n* (%)

Modified Rodnan score (average score)				SLEDAI (average score)		
No activity		0	7 (39)	No activity	0	4 (19)
Activity	Mild	7.2	10 (55)	Mild	3	10 (48)
	Moderate	18	1 (6)	Moderate	7.4	5 (24)
	Severe	-	-	Severe	11	2 (9)
	Total	5.2	18 (100)	Total	4.2	21 (100)

SLEDAI-score: 0: No activity; 2-5: Mild activity; 6-9: Moderate activity; 10-12: Severe activity. Modified Rodnan score: 0: No activity; 1-14: Mild activity; > 14: Moderate-severe activity.

compared to patients with autoimmune disorders, which lead to a sample size of 15 patients per group (Power 80%).

RESULTS

Lab analysis

There was no statistical difference in the standard lab values in all three groups. Patients with SLE and SSc showed as expected higher incidence in expression of autoimmune antibodies such as ANA, ENA, dsDNA or altered complement concentrations.

Patient's characteristics

Patients with IBS showed higher prevalence of diarrhoea compared to constipation or to the combination of diarrhoea and constipation (Table 1). Patients with SLE and SSc reported a great variety of symptoms including weakness, difficulty in swallowing or non-specific musculoskeletal tenderness that can be explained due to secondary fibromyalgia. Gender and age showed no statistical significant changes within the three groups (Table 1).

Disease activity in SLE and SSc

The disease activity of SLE and SSc are shown in Table 2. SSc patients tend to have a milder disease activity compared to SLE patients. However, dysphagia score was similar in all three groups without any statistical difference. The mean value of dysphagia score was in IBS group 7.3, in SLE group 6.73 and in SSc group 7.56 with a *P*-value > 0.05 (Figure 1).

Manometric analysis

In the manometric studies we observed significant differences concerning the quality of the peristaltic waves (amplitude, duration and velocity) among the three groups (Figure 2). IBS patients showed increased peristalsis in the lower two thirds of esophagus in comparison to patients with SSc who in almost 50% of the cases manifested wide peristalsis with reduced amplitude and velocity (Figure 3). There was no significant difference between the amplitude, duration and velocity of the peristaltic movements in the lower two thirds of esophagus among

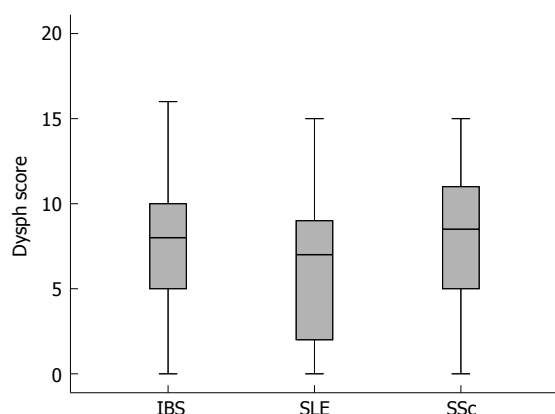


Figure 1 Dysphagia score in patients with irritable bowel syndrome, systemic lupus erythematosus and systemic sclerosis. All groups showed similar abnormal dysphagia scores. The mean value of dysphagia score was in IBS group 7.3, in SLE group 6.73 and in SSc group 7.56. IBS: Irritable bowel syndrome; SLE: Systemic lupus erythematosus; SSc: Systemic sclerosis.

patients with IBS and patients with SLE. Nevertheless 23.5% of patients with SLE showed distal amplitudes greater as 160 mmHg and 29.4% middle amplitudes greater as 100 mmHg. At the proximal esophageal part patients with IBS showed significant higher peristaltic waves also when compared with patients in the SLE group (Table 3, Figure 2). Particularly, 45.5% of IBS patients showed amplitudes greater as 60 mmHg whereas in the SLE and SSc group the rates were 11.8% and 0% respectively (Figure 3). Measurements concerning the LES showed that patients with IBS had significant lower resting pressure in comparison to patients with autoimmune disorders (Table 3). Though, no significant difference could be observed when other manometric measures between the 3 groups were examined, such as length and relaxation of the lower esophageal sphincter as well as duration of distal and middle peristalsis. Regarding the UES we found a significant higher resting pressure and length by patients in IBS group in comparison to SLE and SSc (Table 3). Interestingly, 58.8% of patients with SLE and 56.35% of patients with SSc showed resting pressure less than 40 mmHg (Figure 3).

Correlation coefficient tests revealed a negative relation between dysphagia score, amplitude and velocity of peristaltic activity at middle and lower esophagus in SSc patients ($r = -0.6$, $P < 0.05$). A connection between dysphagia and peristaltic abnormality in IBS and SLE groups was not observed. Furthermore, there was no association between the three subgroups of IBS, the score of dysphagia and manometric findings, as well as between the presence of autoantibodies and dysphagia among patients with SLE and SSc (data not shown).

DISCUSSION

We conducted a comparative analysis of the peristalsis of the esophagus between patients with IBS and patients with SLE and SSc. The groups consisted in 66.7% IBS, 91% SLE and 84.2% SSc of female patients mainly be-

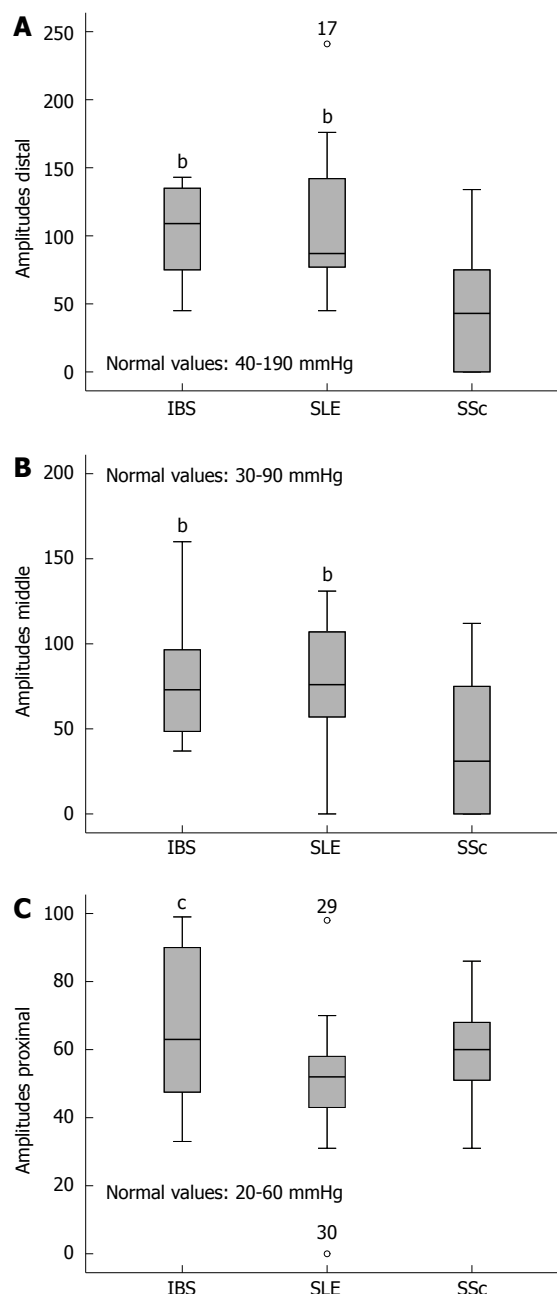


Figure 2 Amplitudes of the peristaltic waves throughout esophagus. The mean value of amplitudes in patients with SSc was significant lower comparing to subjects with SLE and IBS in the middle (B) and distal (A) esophagus ($^bP < 0.01$ vs SSc). In the proximal part (C) IBS patients showed higher peristaltic amplitudes in comparison to the other groups ($^cP = 0.05$ vs SLE). Normal values as previously described. IBS: Irritable bowel syndrome; SLE: Systemic lupus erythematosus; SSc: Systemic sclerosis.

tween 40 and 60 years, which is completely in accordance with the epidemiology of the examined diseases^[27-29].

We found significant different manometric patterns in IBS patients compared to those with autoimmune disorders. It was interesting to notice that patients in the IBS group, as seen in the derived dysphagia scores, showed the same intensity and frequency of swallowing problematic as subjects in the two other categories. Specifically, patients with IBS complained about difficulties in swallowing, retrosternal sore and heartburn as frequent as pa-

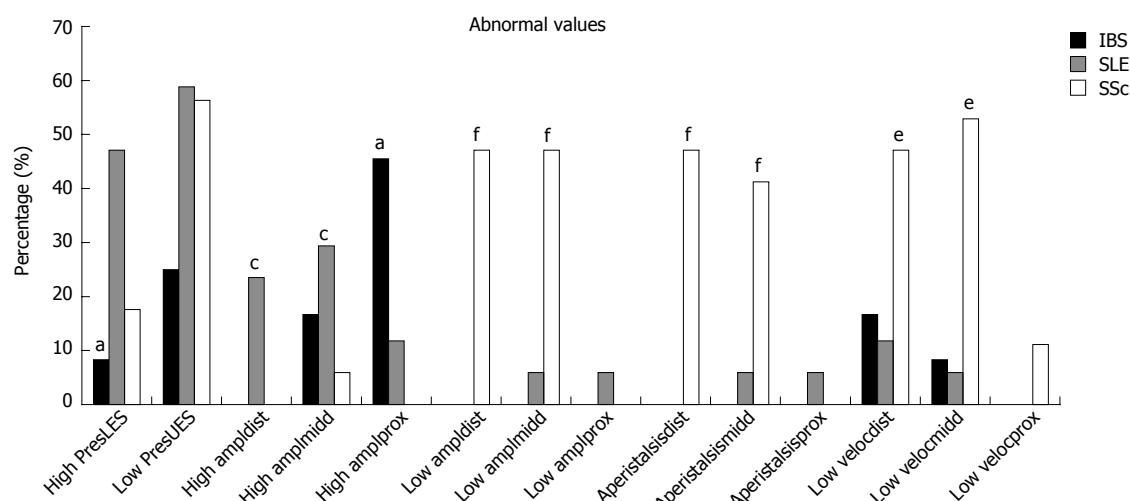


Figure 3 Comparative analysis of pathological manometric parameters in patients with irritable bowel syndrome, systemic lupus erythematosus and systemic sclerosis. 47.1% of SLE patients showed pressure of lower esophagus sphincter > 30 mmHg. 58.8% of SLE patients and 56.35% of SSc patients showed resting pressure of the upper esophagus sphincter < 40 mmHg. 23.5% of patients with SLE showed distal amplitudes > 160 mmHg and 29.4% middle amplitudes > 100 mmHg, whereas in the proximal part 45.5% of IBS patients had amplitudes > 60 mmHg. The majority of SSc patients showed as expected reduced peristaltic activity in the lower two thirds of esophagus. ^a*P* < 0.05 vs SLE and SSc; ^b*P* < 0.05 vs IBS and SSc; ^c*P* < 0.05, ^d*P* < 0.01 vs IBS and SLE. High PresLES: Pressure lower esophageal sphincter (LES) > 30 mmHg; Low PresUES: Pressure upper esophageal sphincter (UES) < 40 mmHg; High amplitist: Distal amplitude > 160 mmHg; High amplitmid: Middle amplitude > 100 mmHg; High amplitprox: Proximal amplitude > 60 mmHg; Low amplitdist: Distal amplitude < 50 mmHg; Low amplitmid: Middle amplitude < 40 mmHg; Low amplitprox: Proximal amplitude < 30 mmHg; Low velocist: Distal velocity < 2 cm/s; Low velocmid: Middle velocity < 2 cm/s; Low velocprox: Proximal velocity < 2 cm/s. IBS: Irritable bowel syndrome; SLE: Systemic lupus erythematosus; SSc: Systemic sclerosis.

Table 3 Comparative manometric findings in patients with irritable bowel syndrome, systemic lupus erythematosus and systemic sclerosis

	IBS	SLE	SSc	<i>P</i> value
Lower esophageal sphincter				
Pressure (mmHg)	22 ± 5	28 ± 8.9	26 ± 5.2	<i>P</i> < 0.05 ^{1,2}
Length (cm)	3.6 ± 1.3	3.4 ± 1.1	3.6 ± 1	
Relaxation (%)	84.8 ± 15.4	89.5 ± 13.2	83.4 ± 10.4	
Distal esophagus				
Amplitude (mmHg)	103.6 ± 33.7	107.3 ± 53.3	43.3 ± 48.2	<i>P</i> < 0.001 ^{2,3}
Duration (s)	4.3 ± 1.4	4.7 ± 1.4	2.9 ± 2.9	
Velocity (cm/s)	3 ± 1.6	2.6 ± 1	1.4 ± 2	<i>P</i> < 0.05 ^{2,3}
Middle esophagus				
Amplitude (mmHg)	78.3 ± 35.7	75.9 ± 35	39.2 ± 42.4	<i>P</i> < 0.01 ^{2,3}
Duration (s)	3.3 ± 1.3	3.4 ± 1.3	2.3 ± 2.2	
Velocity (cm/s)	3.5 ± 1.4	3.9 ± 3.4	1.3 ± 3.7	<i>P</i> < 0.05 ^{2,3}
Proximal esophagus				
Amplitude (mmHg)	67.3 ± 23.3	50.6 ± 19.8	58.3 ± 15.9	<i>P</i> = 0.05 ¹
Duration (s)	2.5 ± 0.7	2.2 ± 0.7	2.8 ± 0.6	
Velocity (cm/s)	2.5 ± 1.1	3.6 ± 2.3	3.0 ± 2.4	
Upper esophageal sphincter				
Pressure (mmHg)	70.5 ± 22.3	52.7 ± 20	50.2 ± 17.8	<i>P</i> < 0.05 ^{1,2}
Length (cm)	3.6 ± 1.0	2.8 ± 1.0	2.7 ± 0.9	<i>P</i> < 0.05 ^{1,2}
Relaxation (%)	94.7 ± 8.0	85.2 ± 12.7	87.1 ± 12	

¹Between IBS and SLE; ²Between IBS and SSc; ³Between SLE and SSc. IBS: Irritable bowel syndrome; SLE: Systemic lupus erythematosus; SSc: Systemic sclerosis.

tients with SLE and SSc (Figure 1). It was speculated that esophageal symptoms are mainly caused due to esophageal reflux^[30,31]. However, in our study only patients with normal EGD were included and retrosternal burning was treated with PPI prior entering our study.

The most significant finding in our manometric study was that IBS patients showed very high peristaltic amplitudes in the proximal esophageal part and reduced

resting pressure of the lower esophagus sphincter (Figure 3) which was statistically significant different to SSc and SLE patients.

The analysis of esophageal peristaltic activity in patients with IBS showed controversial results. Diffuse peristaltic dysfunction with amplitudes > 150 mmHg and duration > 7 s^[10,32], simultan peristaltic^[11] or also normal findings^[12,33,34] have been reported. Reduced resting pres-

sure of LES has already been confirmed by others studies^[9,11,30]. A pathophysiologic explanation or underlying pathomechanism is not known to date, however a correlation between small bowel or colonic dysfunction has been suggested^[33,35].

Our study clearly shows that IBS patients have pathologic motility patterns which are comparable to organic disorders (like SLE or SSc). However, the distribution of changes is different in IBS patients compared to SLE and SSc. A hypermotility of the proximal esophagus > 60 mmHg was seen in almost 50% of the patients. Our data suggest that altered esophageal motility is a common feature in IBS. Taken into consideration the small invasiveness of this method, esophageal manometry may have a place in the diagnostic work up of patients with suspected IBS, especially in the presence of dysphagic symptoms.

SLE patients showed significantly higher incidence of pathological manometric measurements concerning the resting pressure of LES (> 30 mmHg), the amplitudes of distal and middle peristalsis (> 160 mmHg and > 100 mmHg respectively) and the resting pressure of UES (< 40 mmHg) in comparison to IBS group. These findings, with exception to the resting pressure of the UES, are in contrast with the manometric findings in SSc, where most of the patients showed reduced peristaltic activity in the lower two thirds of esophagus (Figures 2 and 3).

Hyperperistalsis has been described from Peppercorn *et al.*^[36] reporting cases of SLE with manometric features similar to diffuse esophageal spasm. In our study, we noticed peristaltic motility similar to nutcracker esophagus with biphasic waves and amplitudes up to 241 mmHg. Hypoperistalsis or aperistalsis, as previously described^[37,38], even in a small percentage, were not observed. These findings are consistent with Gutierrez *et al.*^[38]. They described that such abnormalities are more often in SSc and mixed connective tissue diseases than in SLE.

In conclusion, this is - to the best of our knowledge - the first comparative study concerning esophageal motility among functional and autoimmune disorders. Although IBS, SLE and SSc patients showed different motility patterns, the intensity and frequency of dysphagia were comparable.

The esophageal peristalsis in patients with IBS appears to be more affected in the proximal part, where as in the autoimmune disorders in the middle and distal one. Thus, smooth muscle changes might be associated with autoimmune diseases whereas striated muscles might be more affected in patients with IBS as suggested previously^[39,40]. However, the absence of direct correlation between dysphagia score and manometric parameters in patients with IBS implies that, apart from motor dysfunction, visceral hypersensitivity plays an additional role to the pathology in IBS. In deed, visceral hypersensitivity in IBS patients has been documented in older and recent studies^[34,41-45] pointing various lines of evidence for its relevance in the pathophysiology of IBS. Future studies are needed to further verify our data and to evaluate whether

different motility patterns can be used to diagnose IBS related motility changes of the esophagus.

COMMENTS

Background

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract with a still unclear pathophysiology. Although patients with IBS complain predominantly about manifestations concerning the lower gastrointestinal tract, esophageal symptoms are not uncommon.

Research frontiers

Esophageal dysmotility is frequent in patients with autoimmune diseases, such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). However, there is no comparative data available concerning esophageal motility between IBS patients and those with autoimmune disorders.

Innovations and breakthroughs

This is the first comparative study concerning esophageal motility among functional and autoimmune disorders. Patients with IBS appear to have similar grade of dysphagia in comparison to patients with autoimmune disorders such as SLE and SSc. This study shows that IBS patients have pathologic esophageal motility patterns which are comparable to organic disorders.

Applications

Esophageal manometry might have a place in the diagnostic work up of patients with suspected IBS.

Terminology

Irritable bowel syndrome: Functional disorder of the gastrointestinal tract characterized mainly by symptoms as diarrhoea, constipation and diffuse abdominal pain. Systemic lupus erythematosus and systemic sclerosis are autoimmune diseases with esophageal and multiple other visceral manifestations.

Peer review

The authors compared the esophageal motility between patients with IBS and patients with autoimmune disorders, such as SLE and SSc, at a single university prospective study. The outcome was calculating correlation coefficient between amplitudes, velocity and duration of the peristaltic waves throughout esophagus and patients' dysphagia for all three groups. It revealed that IBS patients showed similar pathologic dysphagia scores but were characterized from different motility patterns when compared to patients with autoimmune diseases. The results are interesting and suggest that esophageal manometry may have a place in the diagnostic work up of patients with suspected IBS, especially in the presence of dysphagic symptoms.

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Diabetic neuropathy: An evaluation of the use of quercetin in the cecum of rats

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Abstract

AIM: To investigate the effect of quercetin supplementation on the myenteric neurons and glia in the cecum of diabetic rats.

METHODS: Total preparations of the muscular tunic were prepared from the ceca of twenty-four rats divided into the following groups: control (C), control supplemented with quercetin (200 mg/kg quercetin body weight) (CQ), diabetic (D) and diabetic supplemented with quercetin (DQ). Immunohistochemical double staining technique was performed with HuC/D (general population)/nitric oxide synthase (nNOS), HuC/D/S-100 and VIP. Density analysis of the general neuronal population HuC/D-IR, the nNOS-IR (nitrergic subpopulation) and the enteric glial cells (S-100) was performed, and

the morphometry and the reduction in varicosity population (VIP-IR) in these populations were analyzed.

RESULTS: Diabetes promoted a significant reduction (25%) in the neuronal density of the HuC/D-IR (general population) and the nNOS-IR (nitrergic subpopulation) compared with the C group. Diabetes also significantly increased the areas of neurons, glial cells and VIP-IR varicosities. Supplementation with quercetin in the DQ group prevented neuronal loss in the general population and increased its area ($P < 0.001$) and the area of nitrergic subpopulation ($P < 0.001$), when compared to C group. Quercetin induced a VIP-IR and glial cells areas ($P < 0.001$) in DQ group when compared to C, CQ and D groups.

CONCLUSION: In diabetes, quercetin exhibited a neuroprotective effect by maintaining the density of the general neuronal population but did not affect the density of the nNOS subpopulation.

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Key words: Diabetes; Myenteric plexus; Neuroprotection; Neuronal nitric oxide synthase; Vasoactive intestinal polypeptide; Enteric glia

Core tip: The present study is the first to report a neuroprotective effect of the flavonoid quercetin in the general population of enteric neurons in the cecum of rats with experimental diabetes mellitus. Quercetin did not reduce the loss of nitrergic neurons in the diabetic rats. This observation suggests that selective changes in the neurochemical code of enteric neurons occur in the presence of quercetin. We propose a causal link between the area and number of glial cells and the size of VIP-IR (reduction in varicosity population) varicosities. Although this link is not fully understood, these observations provide a basis for further studies to clarify the link between glia and VIP-IR varicosities.

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INTRODUCTION

Diabetes affects the gastrointestinal tract causing changes in its motility, such as diarrhea, constipation and gastroparesis^[1]. These symptoms are related to damage to the Enteric Nervous System (ENS) caused by diabetic neuropathy. Diabetes affects subpopulations of enteric neurons differently^[2,3], causing changes in neuronal size and density^[2-6] and altering neurochemical code and neurotransmitters release^[7]. The etiology of diabetic neuropathy is complex; hyperglycemia, which through various metabolic pathways including mitochondrial dysfunction and osmotic stress, can induce toxicity in neurons^[8]; and oxidative stress, which results in a decrease in antioxidant capacity^[9] through glucose oxidation, protein glycosylation and a decrease in the formation of reduced glutathione^[10], are among the causative factors. Reductions in the levels of non-enzymatic antioxidants such as ascorbic acid^[11] and vitamin E enhance oxidative stress^[12].

Antioxidants are promising therapies for preventing and alleviating the debilitating clinical symptoms of diabetic neuropathy. Flavonoids, a family of polyphenolic compounds with a high antioxidant capacity^[13], might effectively protect against the pathology of diabetes. Quercetin is a flavonoid that is naturally present in various foods, such as onions, apples, broccoli, tea and red wine^[14,15]. Quercetin has shown several beneficial pharmacological properties, such as antiperoxidative, anticarcinogenic, anti-inflammatory and antioxidant activities^[16]. Ganglion neurons and their bundles of nerve fibers in the ENS are surrounded by numerous glial cells^[17]. These glial cells play an important role in gastrointestinal physiology and pathophysiology, contributing to intestinal homeostasis, serving as a link between the nervous and immune systems^[18] and influencing neurochemical phenotype^[19]. The number of glial cells and neurons in the ENS are reduced by diabetes^[2].

The use of a potent antioxidant, such as quercetin, could mitigate this damage and alleviate the clinical symptoms of the disease. Because diabetes affects enteric neurons subpopulations differently^[2,3], the aim of this study was to investigate the potential for quercetin to mitigate of neuropathy. This aim was achieved by comparing the areas of the varicosities of vasoactive intestinal polypeptide (VIP)-containing neurons and the distributions of neuronal nitric oxide synthase (nNOS) and HuC/D (general population) containing neurons and glial cells in the ceca of diabetic and control rats.

MATERIALS AND METHODS

Animals

All the procedures involving animals were conducted in accordance with the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA) and were reviewed and approved by the Ethics Committee on Animal Experiments (CEEa) at the Universidade Estadual de Maringá (State University of Maringá). For the present study, twenty-four adult male Wistar rats (Central Animal Facility at the State University of Maringá) were used. At 88 d of age, weighing 360 g on average, the animals were transferred to the vivarium Sector of the Morphological Sciences Department, where they were housed in individual cages maintained under controlled environmental temperature conditions of $(22 \pm 2^\circ\text{C})$ and light/dark cycle (12/12 h) with *ad libitum* access to a water fountain and food (Nuvilab[®]).

After a 2-d period of adaptation to the new environment, rats were weighed and the 120-d trial period monitoring began. At this time, rats were divided randomly into four groups, each containing 6 animals, that received the following treatments: control (C), control supplemented with quercetin (200 mg/kg quercetin body weight) (CQ), diabetic (D) and diabetic supplemented with quercetin (DQ).

To induce diabetes, rats from the D and DQ groups were fasted for fourteen hours and then received an intravenous injection (penile vein) of streptozotocin (STZ) (Sigma, St. Louis, MO) at a dose of 35 mg/kg body weight, dissolved in citrate buffer 10 mmol/L (pH 4.5). Four days after the induction, blood glucose was measured (Accu-Chek Active, Roche Diagnostics GmbH, Mannheim, BW, Germany) to confirm the establishment of experimental diabetes. All the animals in the D and DQ groups had glucose levels above 210 mg/dL.

Starting on the fourth day of the experiment, animals in the CQ and DQ groups were weighed weekly and their water intake was measured. These measurements were used to calculate the dilutions required to ensure each animal in the CQ and DQ groups received 200 mg/kg per day of quercetin in their drinking water. Animals in C and D groups received water without supplementation. After 120 d (210-d-old), the rats were euthanized following anesthesia with Thiopental[®] (40 mg/kg *ip*; Abbott Laboratories, Chicago, IL). Blood was collected by cardiac puncture and blood glucose concentration measured using the glucose oxidase method^[20].

Cecum collection and processing

The ceca were removed, washed in phosphate buffered saline (PBS; 0.1 mol/L pH 7.4) and filled with and immersed in Zamboni fixative solution^[21] for 18 h at 4 °C. Following fixation, ceca were opened along their mesenteric borders and washed with 80% alcohol until the excess fixative was removed. Then, dehydration was performed in 95% and 100% EtOH, followed by diaphanization in xylene and sequential rehydration in 100%, 90%, 80% and 50% EtOH and finally PBS. Individual

Table 1 Primary and secondary antibodies used for immunohistochemistry

Primary	Host	Dilution dose	Company	Secondary	Dilution dose	Company
HuC/D	Mouse	1:500	Molecular Probes, Invitrogen	Anti-mouse Alexa Fluor 488	1:500	Molecular probes, Invitrogen
nNOS	Rabbit	1:500	Zymed	Anti-rabbit Alexa Fluor 546	1:500	Molecular probes, Invitrogen
S-100	Rabbit	1:500	Molecular Probes, Invitrogen	Anti-rabbit Alexa Fluor 546	1:500	Molecular probes, Invitrogen
VIP	Rabbit	1:500	Península Laboratories, Inc.	Anti-rabbit Alexa Fluor 546	1:500	Molecular probes, Invitrogen

HuC/D: General population; nNOS: Neuronal nitric oxide synthase; VIP: Vasoactive intestinal polypeptide.

Table 2 Circumference area of the cecum and the correction factor used to calculate the neuronal density (mean \pm SEM)

Groups	n	Area (cm ²)	Correction factor
C	6	8.1 \pm 1.0	Not applicable
CQ	6	7.5 \pm 0.5	0.9
D	6	13.1 \pm 0.7 ^b	1.6
DQ	6	10.9 \pm 1.2	1.3

^b*P* < 0.01 vs C group. C: Control; CQ: Control supplemented; D: Diabetic; DQ: Diabetic supplemented with quercetin.

ceca were cut into small segments (approximately 2 cm²) that were subsequently microdissected under a stereomicroscope to remove the mucosa and submucosa and reveal the tunica muscularis.

Immunohistochemistry

Three tissues sections per animal underwent immunohistochemical staining. One section was double-labeled to reveal immunoreactivity for HuC/D (general population) and nNOS. A second section was stained for HuC/D and S-100 (a glial protein), and a third section was stained to reveal immunoreactivity for vasoactive intestinal peptide (VIP). Tissues were washed twice in PBS containing 0.5% Triton X-100 for 10 min followed by one hour incubation in a blocking solution of PBS containing 2% BSA and 10% goat serum at room temperature under constant agitation. Tissues were then incubated for 48 h under agitation at room temperature in solutions of PBS containing primary antisera at the dilutions indicated in Table 1, 2% BSA, 0.5% Triton X-100 and 2% goat serum. Tissues were washed in PBS containing 0.5% Triton X-100 and incubated for 2 h at room temperature in solutions of PBS containing the appropriate secondary antisera at the dilutions indicated in Table 1, 2% BSA, 0.5% Triton X-100 and 2% goat serum. Tissues were then washed in PBS containing 0.5% Triton X-100 three times for 10 min and mounted on slides with 10% PBS in glycerol.

Quantitative analysis of immunoreactive myenteric neurons

Analysis was performed by sampling the antimesenteric

basal region^[22]. High-resolution micrographs of stained tissue were captured using an AxioCam MRC camera (Carl Zeiss, Jena, Germany) coupled to an Axioshop Plus fluorescence microscope (Carl Zeiss, Jena, Germany) with Axio Vision software (v. 4.6). Images were subsequently analyzed using Image-Pro Plus (v. 4.5.029; Media Cybernetics, Silver Spring, MD) to quantify the neurons and glia. For each animal, all the neurons and glial cells present in 30 images captured at $\times 20$ magnification were manually identified and counted. The area of each image was approximately 0.2041 mm² and the total quantified area was 6.123 mm². The results were expressed per square centimetre.

Neuronal density correction

According to Cowen *et al.*^[23], pathological processes can change organ size, which can scatter the neurons. Therefore, the results of the neuronal and glial quantification were corrected for the changes in cecum size caused by diabetes (Table 2). For this correction, the cecum of each animal was outlined on cardboard and the images were transferred to the Image-Pro Plus software to measure the perimeter of each animal's cecum. The average area in cm² of the cecum in each group was used to calculate the correction factor and the factor was then applied to the quantitative results for each animal in the CQ, DQ and D groups (Tables 2 and 3).

Morphometric analysis of immunoreactive myenteric neurons

Images of ganglia were captured using a 20 \times objective for HuC/D-, nNOS- and S-100-immunoreactivity and a 40 \times objective for VIP-immunoreactivity. Morphometric analyses were performed using the image analysis software Image Pro-Plus. The areas of 100 neuronal cell bodies (HuC/D-IR, nNOS-IR) and glial cells (S-100-IR) were measured per animal. For VIP, the areas of 400 varicosities per animal were measured. Varicosity measurements were performed using a digital zoom of $\times 800$, maintaining the original calibration of the captured image.

Statistical analysis

Statistical analysis of the quantitative data was performed

Table 3 Neuronal and glial density in the myenteric plexus of the cecum (mean \pm SEM)

Groups	HuC/D	nNOS	S-100	Ratio HuC-D/nNOS	Ratio HuC-D/S-100
C	5492 \pm 81	2028 \pm 102	6343 \pm 367	2.7 \pm 0.09	1.2 \pm 0.05
CQ	5104 \pm 132	1826 \pm 124	8615 \pm 318 ^b	2.9 \pm 0.20	1.7 \pm 0.07 ^{a,c}
D	4121 \pm 325 ^b	1511 \pm 162 ^a	5708 \pm 322	2.8 \pm 0.18	1.4 \pm 0.16
DQ	5060 \pm 25 ^c	1500 \pm 59 ^a	5998 \pm 269	3.4 \pm 0.16 ^a	1.2 \pm 0.04 ^c

^a $P < 0.05$, ^b $P < 0.01$ vs C group; ^c $P < 0.05$ vs D group; ^e $P < 0.05$ vs DQ group. C: Control; CQ: Control supplemented; D: Diabetic; DQ: Diabetic supplemented with quercetin.

using Statistica 7.1 and GraphPad Prism 3.1, with data expressed as the means \pm SEM. Morphometric data were set in delineation blocks and analyzed by Tukey's test. For the other results, we performed one-way analysis of variance (ANOVA) followed by Tukey's test. The level of significance was 5%.

RESULTS

Diabetes was induced by STZ administration in the D and DQ groups, as demonstrated by the assessment of blood glucose (Table 4). Hyperglycemia was accompanied by a significant reduction in the body weights in the D and DQ groups when compared with the control (C) group. Other symptoms typical of diabetes, such as polydipsia (increased water intake) and polyuria (increased urine output), were also observed (Table 4). In addition, the D group demonstrated a significant dilatation (61%) of the cecum compared with the C group ($P < 0.01$). Treatment with quercetin helped to reduce the diabetes-associated dilation of the cecum in DQ group to 34% of the controls (Table 2), which was not significantly different from the C group ($P > 0.05$). However, quercetin treatment did not affect the hyperglycemia, polydipsia or polyurea induced by STZ (Table 4).

Morphology of HuC/D, nNOS, S-100 and VIP immunoreactivity in the cecum myenteric plexus

HuC/D-IR and nNOS-IR neuronal cell bodies were observed within the ganglia and along the interganglionic nerve tracks. The intensity of the immunofluorescence in neurons was heterogeneous within the four groups studied (Figure 1). We observed that the nitrergic population was usually located peripherally in the ganglion. Glial cells were present in all ganglia and along the nerve tracks. However, more glial cells were found in the nerve tracks of the CQ group than the other groups (Figure 2E and F). The VIP-IR varicosities were distributed throughout the tunica muscularis. However, in the D and DQ groups a reduction in the number of nerve fibers was observed (Figure 3C and D).

Neuronal and glial density

After correction for cecum dilatation (Table 2), we ob-

Table 4 Animal parameters (mean \pm SEM)

Groups	Starting weight (g)	Final weight (g)	Blood glucose (mg/dL)	Water intake (mL/d)	Feed intake (g/d)	Urine volume (mL/d)
C	341 \pm 9	517 \pm 13	138 \pm 5	42 \pm 3	37 \pm 3	11 \pm 2
CQ	366 \pm 14	510 \pm 10	146 \pm 8	45 \pm 3	34 \pm 5	12 \pm 2
D	333 \pm 4	301 \pm 21 ^a	518 \pm 19 ^a	113 \pm 16 ^a	44 \pm 6	66 \pm 10 ^a
DQ	368 \pm 6	292 \pm 8 ^a	532 \pm 35 ^a	103 \pm 22 ^a	30 \pm 7	53 \pm 13 ^a

^a $P < 0.05$ vs C group. C: Control; CQ: Control supplemented; D: Diabetic; DQ: Diabetic supplemented with quercetin.

served a reduction in the general population (HuC/D) of myenteric neurons in the ceca of diabetic rats compared with the C group ($P < 0.001$). The CQ and DQ groups were not significantly different from the C group ($P > 0.05$) (Table 3). There was a significant reduction in nNOS-IR neurons in D and DQ groups compared with the C group ($P < 0.05$). Quercetin supplementation did not alter the cell densities between the D and DQ groups in this subpopulation ($P > 0.05$). Double labeling showed that the ratio of HuC-D/nNOS was similar in the C, D and CQ groups but was significantly reduced in the DQ group (Table 3). Quantitative analysis of glia (S-100-IR) showed a significant increase in the CQ group compared with the C group ($P < 0.001$). The D and DQ groups were not different from the C group ($P > 0.05$). The ratio of glial cells to neurons (HuC-D/S-100) were similar in groups C, D and DQ, but were significantly increased in the CQ group when compared with the C and DQ groups ($P < 0.05$) (Table 3).

Morphometric analysis

Neuronal population HuC/D-IR and nNOS-IR and glial cells: The average HuC/D-IR and nNOS-IR neuronal areas in the C and CQ groups were not significantly different ($P > 0.05$). However, there was an increase in the neuronal area in the D group when compared with the C group ($P < 0.05$) and an even larger increase in the average area of the DQ group when compared with the D group ($P < 0.05$). The mean areas of the HuC/D-IR and nNOS-IR neuron cell bodies are shown in Table 5. We observed a significant increase in the average area of the glia in the D group compared with the C, CQ and DQ groups, and a significant decrease in the DQ group compared with the C, CQ and D groups (Table 5).

VIP-IR varicosities: Fluorescence intensity was lower in VIP-IR varicosities in the DQ group compared with the other groups (Figure 3). We found significant differences in the diameter of the varicosities among the four groups studied; the CQ group had an increase in the average area of varicosities compared with the C group ($P < 0.01$), a larger increase was observed in the D group compared with the C and CQ groups ($P < 0.01$). In contrast, a decrease in the area of varicosities was found in the DQ group compared with all the other groups ($P < 0.001$) (Table 5).

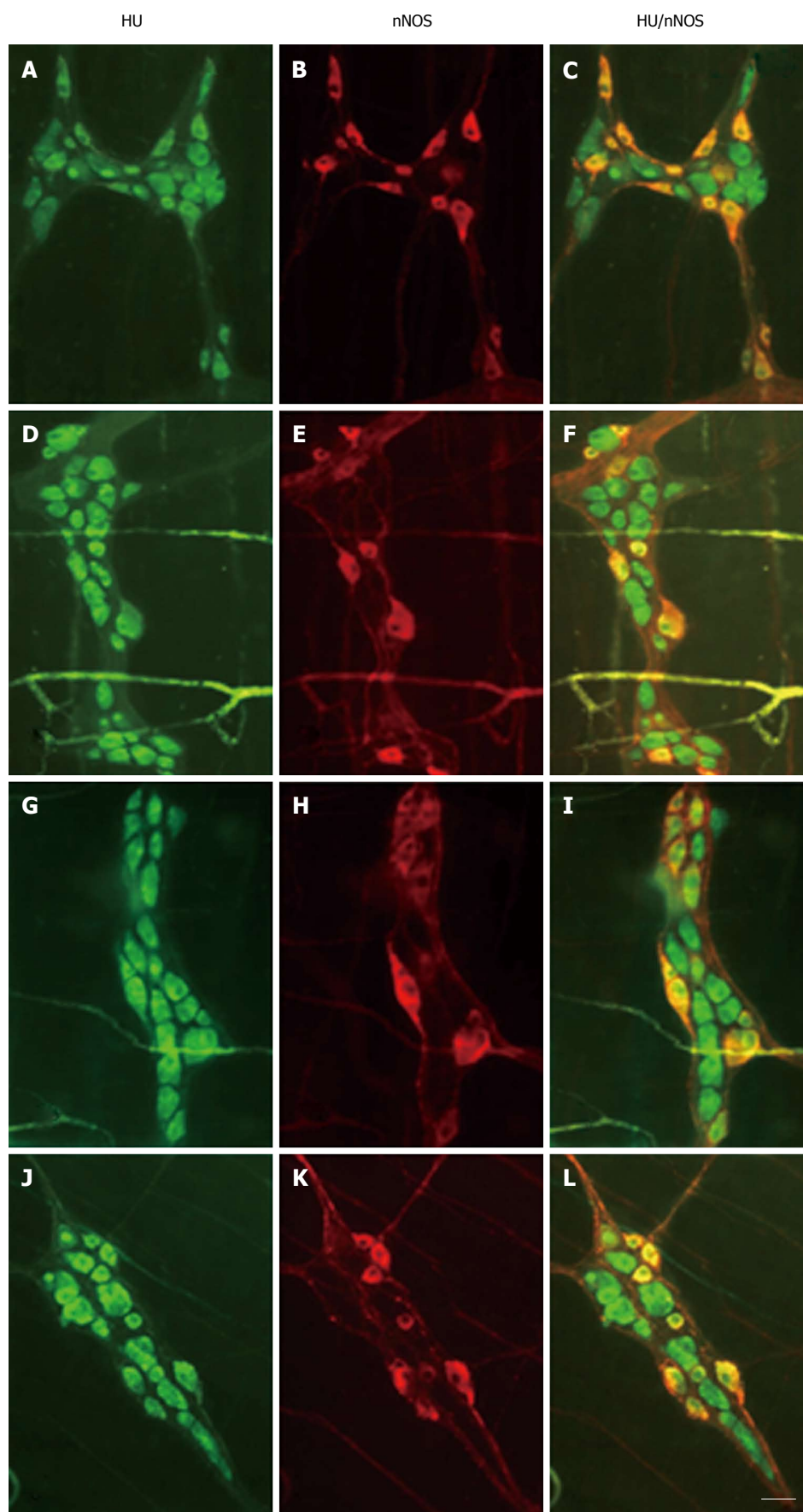


Figure 1 Representative micrographs showing immunoreactivity to general (green) and nitrergic (red) in the myenteric plexus of the rat cecum: A-C: Control group; D-F: Quercetin supplemented control group; G-I: Diabetic group; J-L: Quercetin supplemented diabetic group. Scale bar = 50 μ m. nNOS: Neuronal nitric oxide synthase.

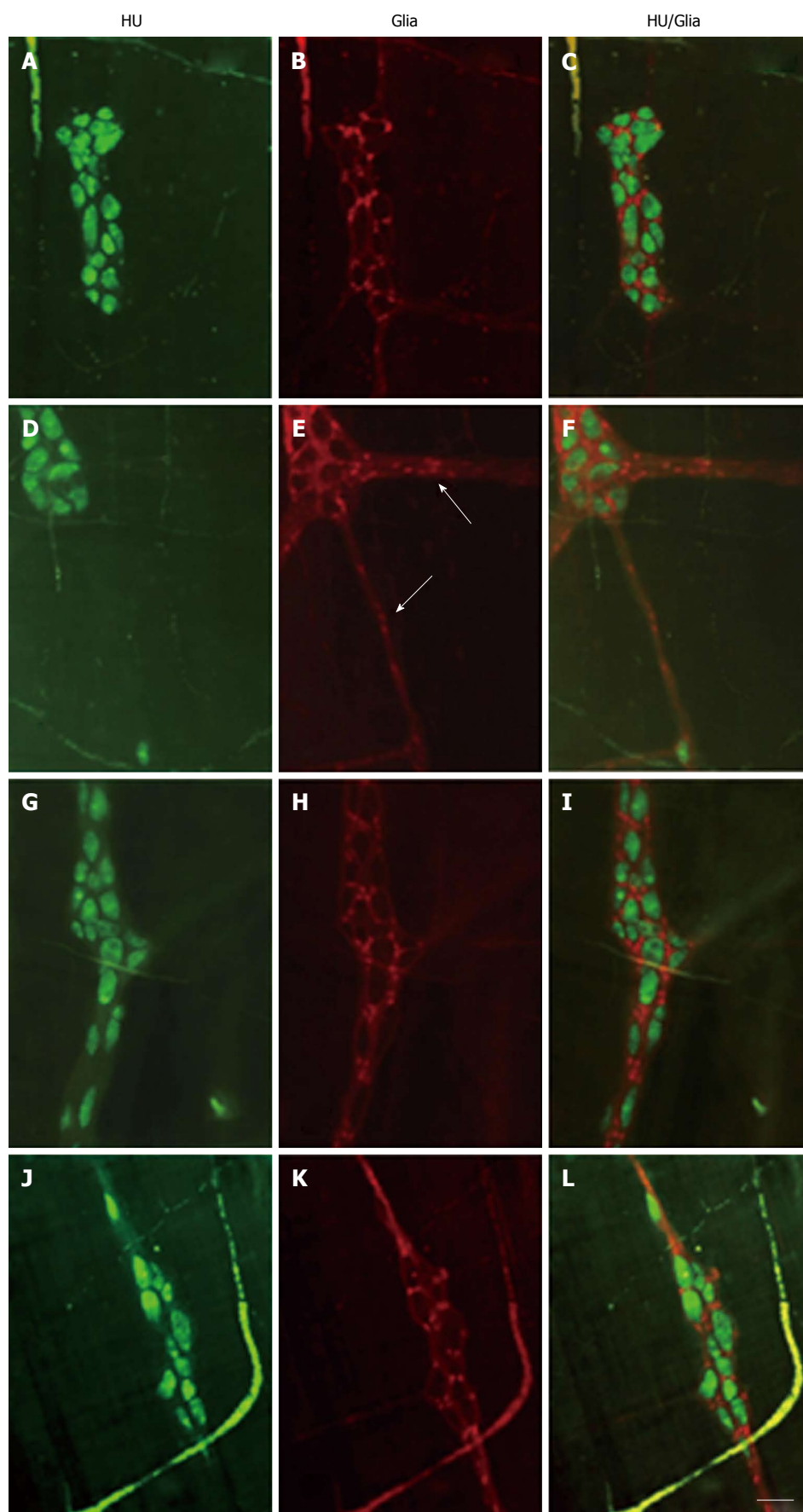


Figure 2 Representative micrographs showing immunoreactivity to HuC/D (green) and S-100 (red) in the myenteric plexus of the rat cecum. A-C: Control group; D-F: Quercetin supplemented control group; G-I, Diabetic group; J-L: Quercetin supplemented diabetic group. White arrows indicate glial cells present in nerve fibers. Scale bar = 50 μ m.

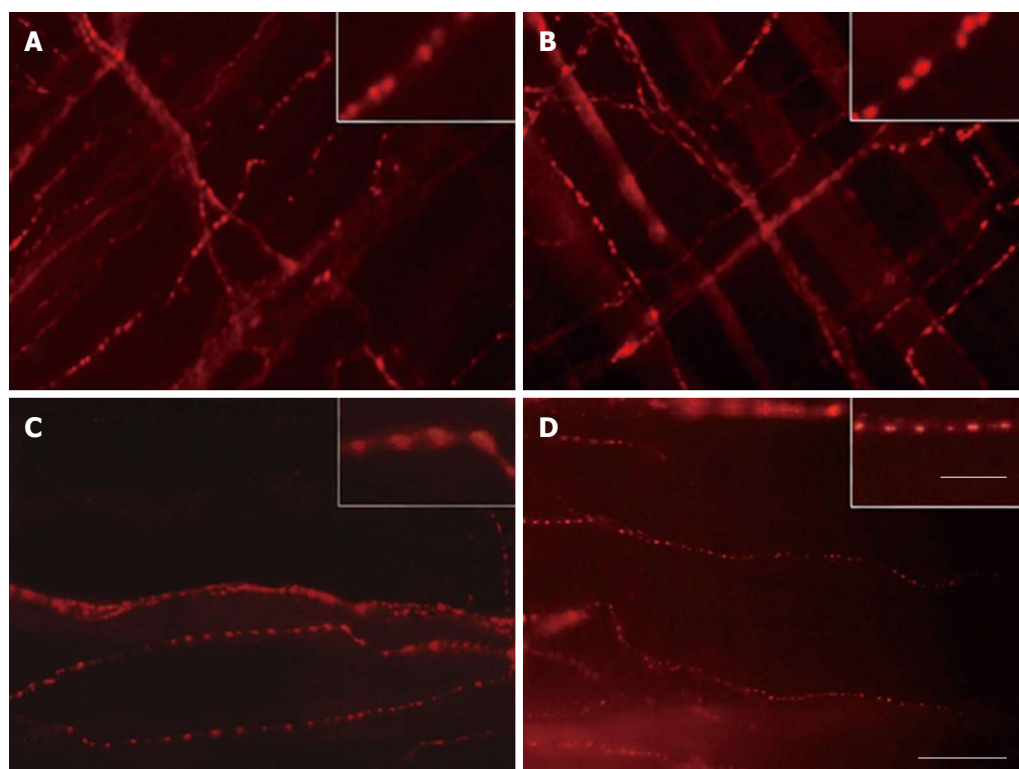


Figure 3 Representative micrographs showing immunoreactivity to vasoactive intestinal peptide in the myenteric plexus of the rat cecum. A: Control group; B: Quercetin supplemented control group; C: Diabetic group; D: Quercetin supplemented diabetic group. Magnified inserts show varicosities of the individual nerve fibers. Note the enlarged appearance of the varicosities in the diabetic group (C) and the reduced varicosities in the quercetin diabetic group (D) compared with the controls (A and B). Scale bars = 50 μ m for main panels, 15 μ m for inserts.

Table 5 Mean cell body area of the neurons, glia and vasoactive intestinal polypeptide-IR varicosities (mean \pm SEM)

Groups	HuC/D	nNOS	S-100	VIP
C	505 \pm 11	455 \pm 10	35.1 \pm 0.5	4.63 \pm 0.03
CQ	501 \pm 12	430 \pm 11	34.8 \pm 0.4	4.88 \pm 0.03 ^b
D	561 \pm 13 ^a	539 \pm 12 ^a	36.9 \pm 0.5 ^a	5.12 \pm 0.04 ^b
DQ	644 \pm 16 ^{b,c}	606 \pm 15 ^{b,c}	29.6 \pm 0.4 ^b	3.97 \pm 0.03 ^b

^a $P < 0.05$, ^b $P < 0.01$ vs C group; ^c $P < 0.05$ vs D group. C: Control; CQ: Control supplemented; D: Diabetic; DQ: Diabetic supplemented with quercetin; HuC/D: General population; nNOS: Neuronal nitric oxide synthase; VIP: Vasoactive intestinal polypeptide.

DISCUSSION

STZ administration to animals in the D and DQ groups promoted typical characteristics of diabetes mellitus, including hyperglycemia, polyuria, polydipsia and weight loss. Quercetin treatment did not affect these measurements in control or diabetic rats suggesting that supplementation with this antioxidant did not influence metabolic pathways linked to weight gain or the mobilization of energy substrates in these animals.

Rats in the D group exhibited 61% cecum dilation compared with animals in the C group, so we used a correction factor to quantify neuronal density. The correction factor was required to ensure the results presented reflected real changes in the neuronal/glial population

density rather than the dispersion of these populations as a consequence of the dilation. Previous studies have shown that diabetes promotes dilation of the small intestine^[6,24] and the large intestine^[4,25,26].

A reduction (25%) in the density of myenteric neurons that were immunoreactive to HuC/D in the D group compared with the C group was observed. Earlier studies in our laboratory showed neuronal loss in different gastrointestinal segments, including the stomach^[27], duodenum^[28], ileum^[6], proximal colon^[29] and cecum^[4,26]. These alterations may be attributed to the reduction in antioxidant defenses and the concomitant intensification of oxidative stress in the cells^[10]. Free radicals can react with DNA, proteins and lipids and these reactions could cause nerve damage^[30], which results in the gastrointestinal motility disorders that are typical of diabetes^[31].

In the DQ group, quercetin promoted a preservation of neuronal density (HuC/D-IR) of 18% compared with the D group. The density in the DQ group was similar that observed in the C group. This preservation may be attributed to the antioxidant potential of quercetin^[16], which would minimize oxidative stress, preventing cell death by necrosis or apoptosis^[32]. The ability of quercetin to reduce superoxide anions ($O_2^{\cdot-}$), singlet oxygen and hydroxyl radicals (HO^{\cdot}), could also prevent lipid peroxidation caused by these molecules^[33]. Finally, quercetin may induce the gene expression of antioxidant enzymes, increasing glutathione levels (GSH) and conferring neuroprotection^[34]. In the present study, we

found a significant reduction in the density of nitrergic neurons in the cecum of the rats from the D group (25%) and the DQ group (26%) compared with the C group. These decreases in nitrergic density may be attributed to the duration of the diabetes (17 wk). Previous studies have shown an accumulation of advanced glycation end-products, which result in oxidative stress and in neuronal apoptosis, begins in the twelfth week of diabetes^[35,36]. Reductions in the number of neurons and/or nNOS activity were also observed in the stomach of diabetic rats^[37,38]. However, depending on the model, the duration of diabetes and the techniques used to assess these changes, we can find an increase^[39], a decrease^[35,37], or no change^[6] in the number of nitrergic neurons and/or nNOS levels and activity in different regions of the gastrointestinal tract. According to Shotton and colleagues^[3], these inconsistencies may be explained by regional or neuronal subpopulation differences and/or by the existence of multiple stages in the development of neuropathy. In the current study, quercetin treatment did not prevent the reduction of nitrergic neurons compared with D group as it did for the general neuronal population (HuC/D-IR). These findings are of great interest, as quercetin seems not only to prevent neuronal loss but also to direct the chemical coding of the neurons it protects.

In the CQ group, we observed an increase in the VIP-IR varicosity areas compared with the C group ($P < 0.01$), similar data were obtained by Alves *et al.*^[40] who studied the effect of supplementation with L-glutamine in the jejunum of normoglycemic and diabetic rats. VIP is an inhibitory neuropeptide that has an important role in regulating glial cell proliferation, modulating cell plasticity, stimulating the release of neuroprotective factors and secreting gliotransmitters/gliopeptides that are involved in intercellular communication^[41]. In the present study, there was a significant increase (36%) in the number of glial cells in the CQ group compared with the C group ($P < 0.001$). In the CNS, VIP promotes astrocytic proliferation^[41,42]; a quercetin-induced increase in VIP may cause an increase in enteric glia in the same way. Furthermore, VIP is capable of stimulating the production of neurotrophic factors by glia in the CNS^[43]. An interesting finding in the present study was that there was a greater increase in the number of glia within the fiber tracts rather than within the ganglia in the CQ group. Fiber tracts also contain numerous VIP-IR varicosities.

However, the observation that diabetic rats (group D) demonstrated an increase in the area of VIP-IR varicosities compared with the C group ($P < 0.01$), without a concomitant increase in the density of glial cells argues against the hypothesis of a direct causal relationship between VIP and glia. These seemingly conflicting observations may be explained by other factors that might be present in the diabetic state. There are published reports of increased expression and release of interleukin (IL)-1 beta in STZ-induced diabetic rats^[44] and in human monocytes treated *in vitro* with different concentrations of glu-

cose^[45]. Studies by Rühl and colleagues^[46] demonstrated a combined response of IL-1 beta and IL-10 that lead to a reduction in glial cell proliferation. Thus, despite an increase in IL-10 resulting from increased VIP expression the diabetic state could also be promoting an increase of IL-1 beta and together these two cytokines would inhibit glial cell proliferation in the D group. Regardless of the relationship between VIP and glia, which requires further study, the increase in the size of the VIP-IR varicosities in the diabetic rats may be explained by an increased expression of VIP as a compensatory effect due to neuronal loss^[47], or it may be a reflection of neuronal plasticity to maintain the survival of the neurons in response to the pathophysiological conditions of diabetic neuropathy^[48]. This conclusion could be supported by studies demonstrating an important role for VIP in neuroprotection^[49,50], perhaps by scavenging reactive oxygen species as demonstrated *in vitro*^[49] and *in vivo*^[51].

Morphometric analysis of the general neuronal population (HuC/D-IR) and the nitrergic (nNOS-IR) subpopulation showed a significant increase in the neuronal area in the D group compared with the C group. An increase in neuronal area is a frequent finding in diabetic animals^[4,6]. Hypertrophy was also observed in subpopulations of enteric neurons in the diabetic rats, including the VIP-IR neurons^[40,47], nNOS-IR^[5] and NADH diaphorase-positive neurons^[52]. In the present study, we observed a significant increase in the neuronal area of the general (HuC/D) and nitrergic (nNOS-IR) populations in the DQ group when compared with the other studied groups (Table 5). This event could be explained a the reduction in glial function and metabolism, which was suggested by the decrease in glial cell area and which could reduce the production of neurotrophic factors leading to a loss of control over the processes of synthesis, potentially changing the neurochemical phenotype of the neurons. Evidence that enteric glial cells can produce neurotrophic factors, such as nerve growth factor, brain derived neurotrophic factor and neurotrophin 3, that modulate neuronal gene expression and possibly the enteric neuropenotype has been observed^[53]. Additionally, neurotrophic factors play a critical role in regulating the synthesis of neurotransmitters and neuropeptides and in influencing neuronal morphology^[54].

In summary, we concluded that in diabetic rats, quercetin exhibited a neuroprotective effect due to its antioxidant action. This action is independent of diabetes-induced changes in hyperglycemia, polydipsia, polyurea and weight loss. Interestingly, while quercetin was able to reduce the loss of myenteric neurons, it did not reduce the loss of nitrergic neurons suggesting a selective change in the neurochemical coding of the enteric neurons during quercetin treatment. Quercetin treatment increased the area of VIP-IR varicosities and concurrently increased the density of enteric glia in control animals. In diabetic rats, there is a disconnection between these observations: quercetin does not increase glial density, but does decrease VIP-IR varicosity area. While there are

data in the literature to support a causal link between VIP and glia and to suggest a connection to neuronal loss and changes in chemical coding, the molecular mechanisms and the relationships between these observations remain to be elucidated.

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COMMENTS

Background

Hyperglycemia from diabetes mellitus (DM) may cause long-term neuropathic abnormalities that affect the autonomic nervous system. In the gastrointestinal tract, neurodegeneration and morphological changes in the neurons and glia cells of the enteric nervous system (ENS) are observed. These changes are related to the oxidative stress of diabetes. Hyperglycemia is responsible for an increase in oxidative stress and a decrease in antioxidant capacity. In this context, the use of flavonoids, a family of polyphenolic compounds with a high antioxidant capacity, might be effective in protecting against the pathology of diabetes. Quercetin is a flavonoid that is present in various foods, such as grapes, and their derivatives. Several studies have revealed the beneficial pharmacological effects of quercetin in biological systems, including its potent antioxidant effect. Thus, the use of quercetin is a promising therapy for the prevention of neurological disorders and can reduce the pathological conditions of diabetes.

Research frontiers

Currently, diabetes is considered an epidemic that affects more than 300 million people worldwide. Its chronic nature combined with the severity of its complications and the necessary means to control them, makes diabetes a very costly disease for patients and for the healthcare system. Autonomic neuropathies, which are among the complications of diabetes, can trigger a wide range of gastrointestinal problems, such as nausea, vomiting, diarrhea, constipation and fecal incontinence, that cause discomfort and deeply affect the quality of life of patients with DM. In this context, studies evaluating therapeutic strategies that have the potential to improve or mitigate the degenerative damage to the enteric nervous system, such as the use of the flavonoid quercetin, may eventually contribute to an improved quality of life for these patients.

Innovations and breakthroughs

Previous studies in their research group, using simple neuronal marking techniques, reported the absence of an effect of antioxidants on the cecum of rats with experimental DM. In this study, using immunohistochemical techniques, the authors could observe an effect of antioxidant treatment on the cecum in a rat DM model. In addition, using these techniques we observed this effect in both the general neuronal population and in specific neuronal subpopulations of the cecum ENS. Although, studies suggest a neuroprotective effect of quercetin in the central nervous system, there are only a few studies examining the effect of quercetin in the ENS of rats with experimental DM.

Applications

The present study shows that quercetin could improve antioxidant capacity and thus protect the enteric nervous system in the cecum of streptozotocin-induced diabetic rats *in vivo*. When these effects are confirmed by further research, future application of quercetin as a therapeutic in the treatment of diabetic neuropathy may be merited. Another aspect of the present study is the identification of an apparent causal relationship between VIP and glia. This observation could provide a basis for the clarification of other research.

Terminology

ENS is made of sensory neurons, interneurons and motoneurons and is divided into two major plexuses in the gastrointestinal tract: the myenteric and submucosal. VIP and the enzyme Nitric Oxide Synthase are neuronal sub-

populations of the ENS that express inhibitory neurotransmitters and are non-adrenergic and non-cholinergic. Enteric glia: a set of cells, which are similar to the astrocytes of the CNS, that are adhered to the ENS ganglion neurons and their nerve fiber bundles.

Peer review

This paper concerns an interesting issue, however introduction is repetitive, and discussion is too long and should be reduced.

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Full robot-assisted gastrectomy with intracorporeal robot-sewn anastomosis produces satisfying outcomes

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Abstract

AIM: To evaluate the feasibility and safety of full robot-assisted gastrectomy with intracorporeal robot hand-sewn anastomosis in the treatment of gastric cancer.

METHODS: From September 2011 to March 2013, 110 consecutive patients with gastric cancer at the authors' institution were enrolled for robotic gastrectomies. According to tumor location, total gastrectomy, distal or proximal subtotal gastrectomy with D2 lymphadenectomy was fully performed by the da Vinci Robotic Surgical System. All construction, including Roux-en-Y jejunal limb, esophagojejunal, gastroduodenal and gastrojejunal anastomoses were fully carried out by the intracorporeal robot-sewn method. At the end of surgery, the specimen was removed through a 3-4 cm incision at the umbilicus trocar point. The details of the surgical technique are well illustrated. The benefits in terms of

surgical and oncologic outcomes are well documented, as well as the failure rate and postoperative complications.

RESULTS: From a total of 110 enrolled patients, radical gastrectomy could not be performed in 2 patients due to late stage disease; 1 patient was converted to laparotomy because of uncontrollable hemorrhage, and 1 obese patient was converted due to difficult exposure; 2 patients underwent extra-corporeal anastomosis by minilaparotomy to ensure adequate tumor margin. Robot-sewn anastomoses were successfully performed for 12 proximal, 38 distal and 54 total gastrectomies. The average surgical time was 272.52 ± 53.91 min and the average amount of bleeding was 80.78 ± 32.37 mL. The average number of harvested lymph nodes was 23.1 ± 5.3 . All specimens showed adequate surgical margin. With regard to tumor staging, 26, 32 and 46 patients were staged as I, II and III, respectively. The average hospitalization time after surgery was 6.2 d. One patient experienced a duodenal stump anastomotic leak, which was mild and treated conservatively. One patient was readmitted for intra-abdominal infection and was treated conservatively. Jejunal afferent loop obstruction occurred in 1 patient, who underwent re-operation and recovered quickly.

CONCLUSION: This technique is feasible and can produce satisfying postoperative outcomes. It is also convenience and reliable for anastomoses in gastrectomy. Full robotic hand-sewn anastomosis may be a minimally invasive technique for gastrectomy surgery.

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Key words: Robotic surgery; Gastric cancer; Total gastrectomy; Esophagojejunal anastomosis

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INTRODUCTION

Although laparoscopic gastrectomy has been widely performed worldwide, its role is still a matter of debate due to inherent difficulties in specific node dissection and intracorporeal anastomosis^[1,2]. Recently, robotic surgery has been demonstrated to overcome the intrinsic limitations of a traditional laparoscopic approach, where the anatomical and operative conditions are similar to those encountered during gastric resection^[3,4]. Several recent retrospective studies have reported that robotic surgery for the treatment of gastric cancer is feasible and can produce satisfying postoperative outcomes^[5-7]. However, most studies have reported that anastomosis after robotic gastrectomy was carried out by extracorporeal hand-sewn sutures or an intracorporeal stapler.

Wristed instruments that allow seven degrees of freedom, tremor filtering, the ability to scale motions, and stereoscopic vision improve the surgeon's dexterity when fine manipulation of tissues in a close, fixed operating field or when hand-sewn sutures and knot tying are required^[8]. In robotic surgery for other complex robotic procedures, such as urethral anastomosis in radical prostatectomy or valve replacement in cardiac surgery, several studies have reported that robot hand-sewn anastomosis was possible within a narrow space due to these distinct advantages^[9-11]. Therefore, we believe that a robotic approach would also be relevant for laparoscopic D2 dissection and intracorporeal anastomosis by a full robot hand-sewn method.

To the best of our knowledge, no study has assessed the reliability of this hand-sewn technique or described its technical details, although it is a classic and feasible method. The current study aimed to assess the feasibility and safety of full robot-assisted total and subtotal gastrectomy with extended lymphadenectomy and intracorporeal robot-sewn anastomosis.

Here, we present the results of a preliminary study in which anastomosis after gastrectomy was successfully achieved by a robot-sewn technique. All procedures, including lymph node dissection and anastomosis, were completed by the robot, the so-called "full robot-assisted gastrectomy", which was different from previous robotic surgery for the treatment of gastric cancer.

MATERIALS AND METHODS

All procedures were performed by the da Vinci Surgical System (Intuitive Surgical, Inc, Mountain View, CA, United States). We began using this system for gastric cancer surgery in May 2010 at Jinglin Hospital, affiliated to Nanjing University, China. From September 2011 to

March 2013, we conducted a prospective evaluation of the feasibility and safety of robot-assisted gastrectomy with intracorporeal robot hand-sewn anastomosis. During this time, all patients with histologically proven gastric cancer without organ invasion (T4) underwent preoperative work-up and examination. One hundred and ten consecutive patients diagnosed with gastric cancer were enrolled in this trial (details in Figure 1). Robotic anastomosis was performed by a surgeon (Dr. Jiang ZW) who had been involved with more than 100 cases of robotic-assisted gastrectomy before this trial. We obtained informed consent from all patients for administration of this robotic surgery anastomotic method.

Perioperative management was performed by adopting the measures of fast track surgery^[12,13]. Preoperative short-time fasting and carbohydrate loading were introduced. Nasogastric decompression tubes were abandoned in all patients, unless absolutely necessary. When able, patients were given water from postoperative day 1, liquid diet was started on postoperative day 2, and soft diet was started on postoperative day 3. After 1 d of soft diet without complications, patients were discharged.

All data were collected prospectively. Operative time was calculated as the time between pneumoperitoneum induction and port-site closure. Intraoperative blood loss was measured by subtraction. Tumor staging and lymph node harvest rate were assessed by the pathology department. Surgical and oncologic outcomes were well documented. Patients were evaluated weekly with clinical examinations during the 30 d after discharge and then followed-up every 3 mo. We evaluated feasibility and safety of the procedure with the Clavien-Dindo classification, which categorizes surgical complications from grade 1 to 5 based on the invasiveness of the treatment required. Grade 1 requires no treatment; grade 2 requires medical therapy; grade 3a requires surgical, endoscopic, or radiologic intervention, but not general anesthesia; grade 3b requires general anesthesia; grade 4 represents life-threatening complications that require intensive care; and grade 5 represents death of the patient.

Patient and robot position, port placement

The patient is moved to the 20° reverse Trendelenburg position under general anesthesia. The camera port (C) is inserted into the infra-umbilical area for a 12 mm trocar. After establishing 12-mmHg pneumoperitoneum, the other four ports are placed with the aid of camera visualization.

Two 8-mm Intuitive cannulae for robotic devices are placed under direct visualization 2-3 fingerbreadths below the costal margin at the right and left anterior axillary line, respectively (Figure 2, trocar A and E). The last 8-mm Intuitive cannula (B) is placed in the right paraumbilical area below the level of port A and at least one handbreadth away from the camera port.

One 12-mm trocar (D) is placed along the left mid-clavicular line, in the left paraumbilical area and at least

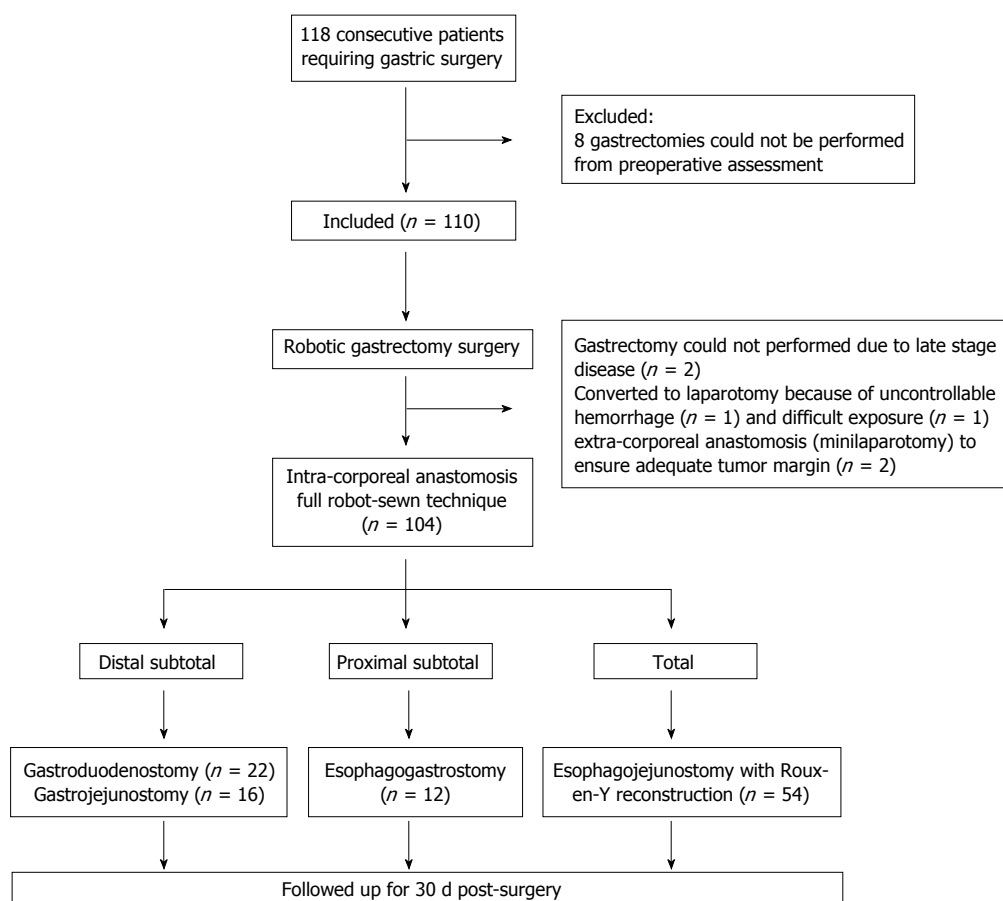


Figure 1 Flow diagram.

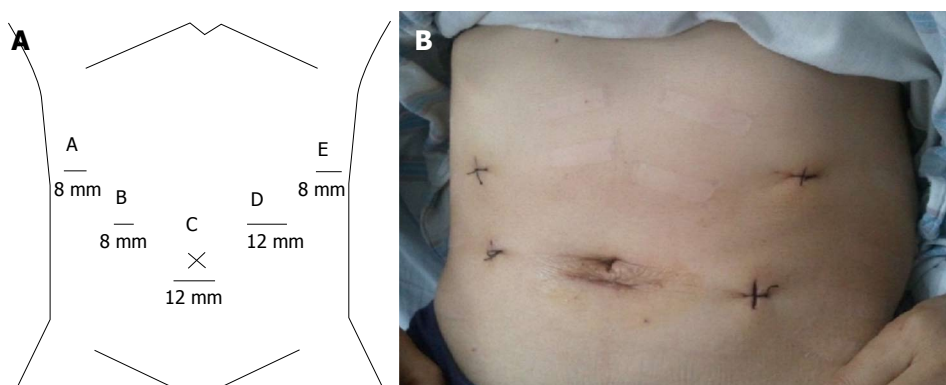


Figure 2 Placement of surgical ports. For A, B and E 8-mm ports were used. For C and D 12-mm ports were used. Port C was extended to 3 cm for specimen extraction from the abdominal cavity.

one handbreadth away from the camera port (C). The assistant who works on the patient's left side uses this port (D) to aid the surgeon during the robotic operation, such as insertion of an endo-stapler for resection of the duodenum, the stomach, or the abdominal esophagus and for placement of gauze or a suction device for clearing the operative field (Figure 2). After port placement, the robotic cart is installed from the patient's head.

In Japan and Europe, extended lymph node dissection (D2) is the standard of care for gastric cancer^[14-16].

Robotic gastrectomy with D2 lymph node dissection were performed according to the rules of the Japanese Research Society for Gastric Cancer^[17,18]. Total gastrectomy, distal or proximal subtotal gastrectomy was decided according to tumor location. The lymphatic tissues are removed *en bloc* along the hepatic, splenic, left gastric artery and celiac trunk using an ultrasonic shear. The origins of these arteries are clearly identified and skeletonized, and the lymphatic tissue dissected away from the adventitia. The left gastric artery is then clipped or tied at its origin

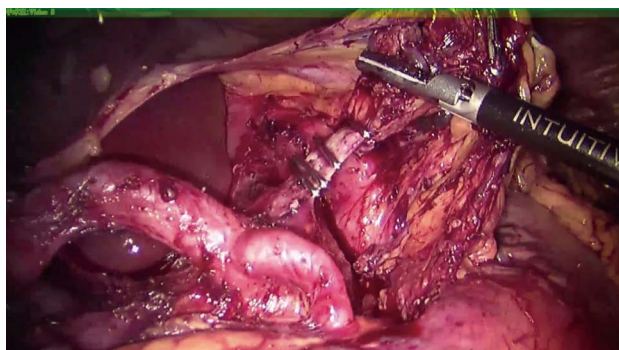


Figure 3 Lymphatic tissues are removed *en bloc* along the hepatic, splenic, left gastric artery and celiac trunk using an ultrasonic shear. The origins of these arteries are clearly identified and skeletonized, and the lymphatic tissue dissected away from the adventitia. The left gastric artery is then clipped or tied at its origin.

(Figure 3). Once the lymphadenectomy is complete, the assistant divides the stomach, intestine or esophagus using multiple endostapler applications (Ethicon Endo-Surgery, Cincinnati, United States) from the 12 mm trocar D. The specimen, including the stomach, omentum, and the lymphatic tissue, are wrapped by an endobag. The specimen was extracted from the abdominal cavity through the intraumbilical port site extended to 3 cm.

Distal subtotal gastrectomy with gastroduodenostomy (Billroth I) and gastrojejunostomy (Billroth II)

After distal subtotal gastrectomy, 38 patients underwent gastroduodenostomy or gastrojejunostomy reconstruction. For gastroduodenostomy, the duodenum was resected by an endo-linear stapler inserted into the assistant's 12 mm trocar D. The remnant portion of the lesser curvature was resected by an endo-linear stapler, and the completely resected stomach was then wrapped by an endobag. The posterior walls of the duodenum and the stomach were approximated by continuous seromuscular sutures (Figure 4B and C); the duodenal stump was then opened by an ultrasonic shear. A continuous suture with interlocking of the full intestinal layers of the posterior and anterior wall of the duodenum and the stomach was then made (Figure 4D and F). Finally, the anterior wall of the anastomosis was reinforced by interrupted seromuscular sutures (Figure 4G). Sometimes, the duodenum was not transected until the posterior wall suturing of the gastroduodenostomy was finished. This method can facilitate pulling up the duodenum for anastomosis. The duodenum was totally dissected using an ultrasonic scalpel (Figure 4C and E). For gastrojejunostomy reconstruction, the jejunum which is about 20 cm away from the Treitz was brought up just below the remnant stomach for antecolic end-to-side anastomosis, which was achieved using the hand-sewn technique in the same manner.

Proximal subtotal gastrectomy with esophagogastrostomy

For esophagogastrostomy reconstruction after proxi-

mal subtotal gastrectomy, the remnant distal stomach in which the gastroepiploic arcade was preserved was brought up just below the dissociated esophagus for end-to-end anastomosis. Robotic interrupted suturing was performed to fix the distal gastric-remnant and esophagus together. A continuous suture with interlocking of the full layers of the posterior and anterior wall of the esophagus and the stomach was then made (Figure 5). Finally, the anterior wall of the anastomosis was reinforced by interrupted seromuscular sutures.

Total gastrectomy with esophagojejunostomy and Roux-en-Y reconstruction

Fifty-four esophagojejunostomies were performed using methods similar to those described above (Figure 6). After the total stomach was divided, the assistant aids the console surgeon in manipulating the bowel to identify the ligament of Treitz. The small bowel which is 15-20 cm away from the Treitz was brought up just below the dissociated esophagus for antecolic end-to-side anastomosis. Robotic needle holders are loaded with 3-0 absorbable sutures and interrupted suturing is performed to fix the jejunum and esophagus together. Then continuous interlocking suturing is performed between the posterior esophageal wall and seromuscular layer of the jejunum. A 2-3 cm incision is made in the jejunum to be anastomosed. The posterior wall of the esophagus is dissected for the half ring at about 1-2 cm above the cardia. The posterior esophageal and jejunal walls are sutured by a continuous interlocking suture (Figure 6).

Sometimes, especially when the tumor is small or adjacent to the cardia, the tumor cutting edge must be clearly identified. The esophagus is not transected until the posterior wall suturing of the esophagojejunostomy is finished (Figure 6K and L). This strategy can facilitate not only identification of the tumor cutting edge, but also pulling down the esophagus for anastomosis. The remaining half ring of the anterior wall of the esophagus is dissected using an ultrasonic scalpel.

After the anterior wall is clearly exposed, a continuous interlocking suture anterior of the anastomosis wall is performed. Finally, the anterior esophageal wall and anterior seromuscular layer of the jejunum are sutured using interrupted sutures (Figure 6D and E). The proximal jejunum 5 cm away from the esophagojejunal anastomotic stoma, is then transected by the assistant using a 45-mm cartridge endostapler (gold loads, Ethicon Endo-Surgery). The side-to-side jejunojunctionostomy and jejunal stump are achieved using the hand-sewn technique in the same manner (Figure 6F-J).

RESULTS

Of the 110 patients enrolled in this trial, two patients could not undergo radical gastrectomy due to late stage disease; 1 patient was converted to laparotomy because of uncontrollable hemorrhage, and 1 obese patient was

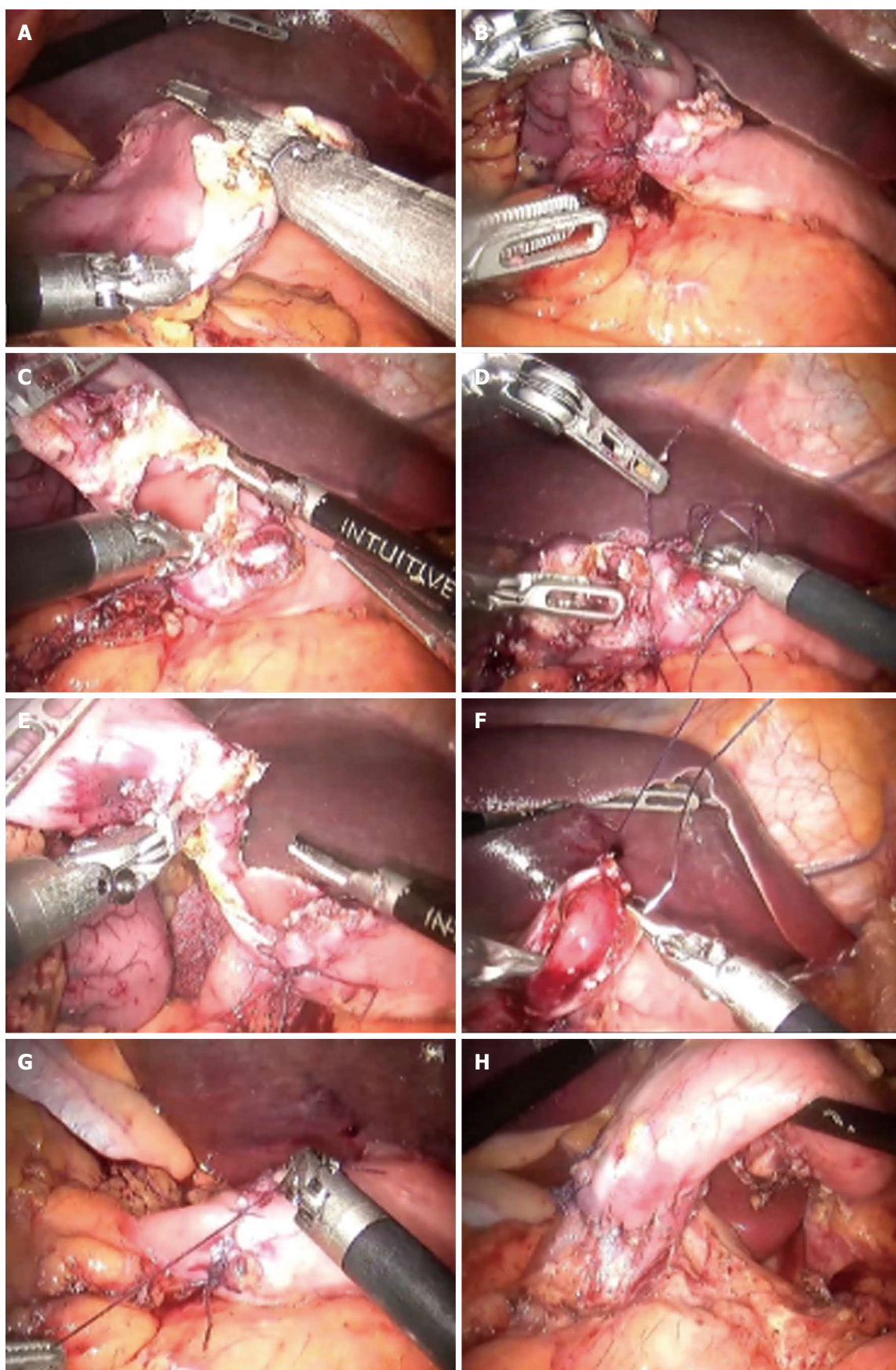


Figure 4 Distal subtotal gastrectomy with gastroduodenostomy (construction type of Billroth I). A, B: Robotic anastomosis for gastroduodenostomy; C: Continuous seromuscular suture; D, E: Continuous interlocking suture for posterior wall; F: Continuous interlocking suture for anterior wall; G: Interrupted sero-muscular suture; H: Complete anastomosis.

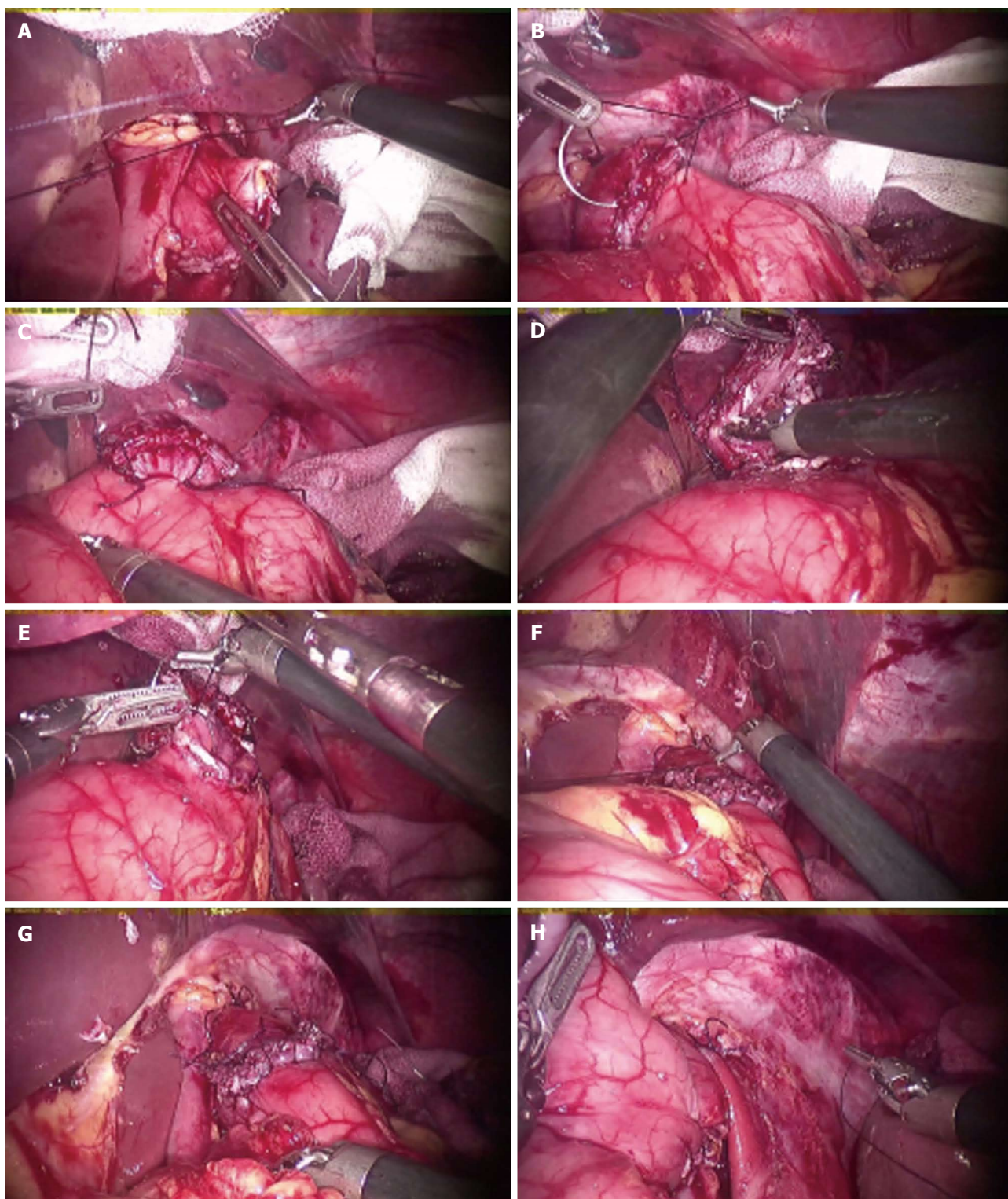


Figure 5 Proximal gastric resection with esophagogastrostomy. A: The terminal esophagus fully mobilized. Diaphragmatic crura are exposed and freed from the surrounding adipose and lymphatic tissue. The esophagus was stitched to the crura for better exposure; B-G: The remnant distal stomach was brought up just below the dissociated esophagus for end-to-end anastomosis; H: Complete anastomosis.

converted due to difficult exposure; 2 patients underwent extra-corporeal anastomosis by minilaparotomy to ensure adequate tumor margin. There were no cases of pancreatic or spleen injury during surgery.

Robot-assisted gastrectomy with total robot-sewn anastomosis were successfully performed in 104 cases,

including 66 males and 38 females with an average age of 58.2 ± 12.6 years (range: 40-76 years) and body mass index (BMI) of $22.12 \pm 4.64 \text{ kg/m}^2$ (range: 16-26 kg/m^2). Patient characteristics are presented in Table 1.

Fifty-four esophagojejunostomies with Roux-en-Y reconstruction for 54 total gastrectomies, 22 gastroduo-

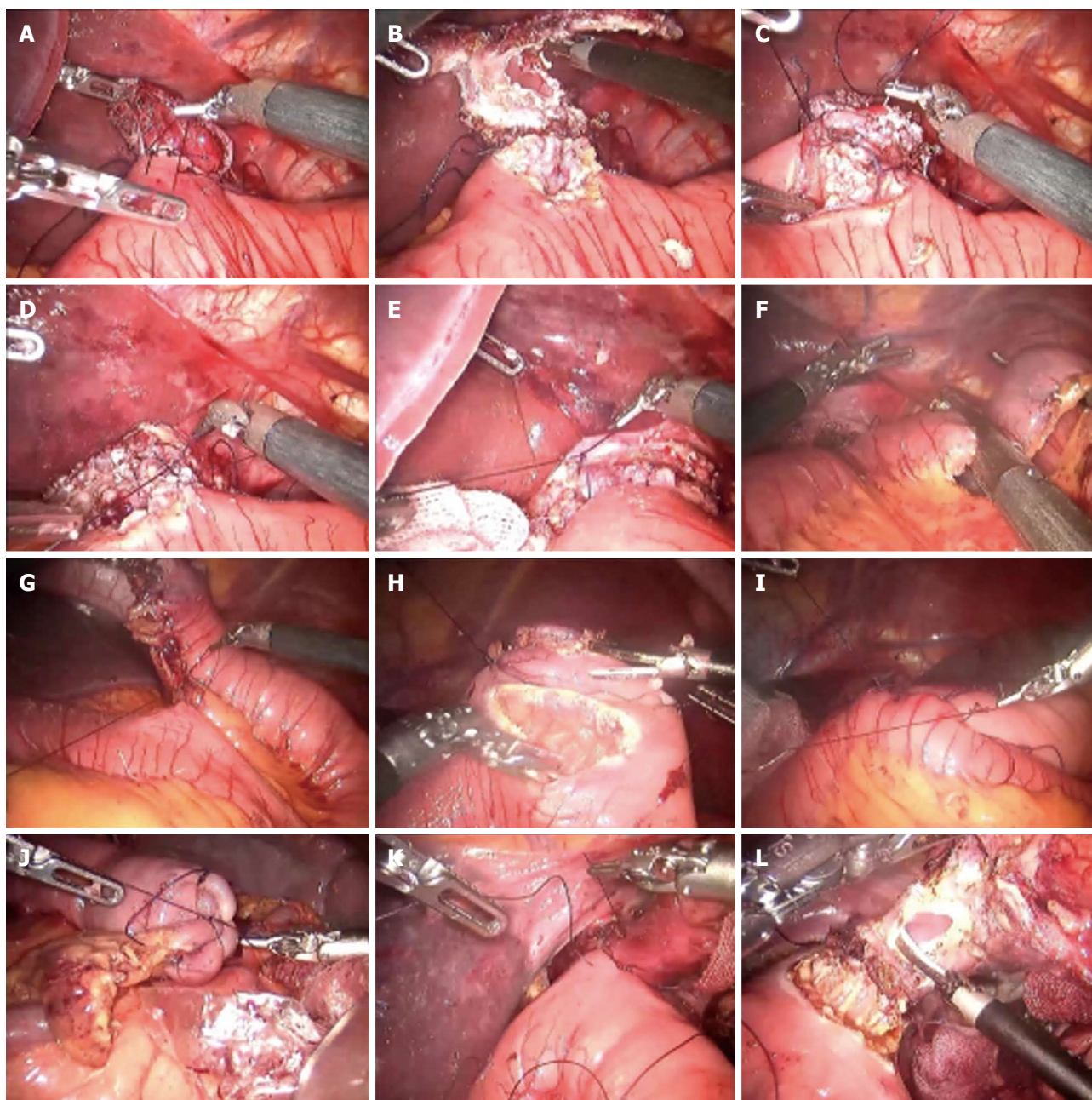


Figure 6 Total gastrectomy with esophagojejunostomy and Roux-en-Y reconstruction. A-E: The small bowel which is 15-20 cm away from the Treitz was brought up just below the dissociated esophagus for antecolic end-to-side anastomosis. Robotic anastomosis for esophagojejunostomy was performed in the same manner; F: The proximal jejunum 5 cm away from the esophagojejunal anastomotic stoma is transected by a 45-mm cartridge endostapler; G-I: The side-to-side jejunojunction and jejunal stump were achieved using the hand-sewn technique in the same manner; J: Jejunal stump was achieved using the hand-sewn technique in the same manner; K, L: Sometimes, the esophagus was not transected until the posterior wall suturing of the esophagojejunostomy was finished.

denostomies and 16 gastrojejunostomies for 38 distal subtotal gastrectomies, and 12 esophagogastromies for 12 proximal subtotal gastrectomies were successfully performed. The average operation time was 272.52 ± 53.91 min, and median reconstruction time was 45.8 ± 26.0 min (Table 2). The average amount of bleeding during surgery was approximately 80.78 ± 32.37 mL. From the pathologic findings, the average number of harvested lymph nodes was 23.1 ± 5.3 . No tumor specimens showed positive surgical margins. The final pathological staging was as follows: stage I, 26 cases; stage II, 32

cases; stage III, 46 cases.

The average time to first flatus and semi-liquid diet after surgery was 2.5 ± 0.7 and 4.1 ± 1.3 d, respectively. The average length of postoperative hospital stay was 6.2 d. Postoperative complications were observed in 12 (11.5%) patients and included anastomotic leakage in 1 (0.96%) patient, gastroplegia in 2 (1.9%) patients, prolonged ileus in 2 (1.9%) patients and poor wound healing in 2 (1.9%) patients. Postoperative complications are shown in Table 3. The anastomotic leak was mild and occurred in the duodenal stump. The patient recovered

Table 1 Patient characteristics

Characteristics (n = 104 cases)	
Age (yr, mean ± SD)	58.2 ± 12.6
Gender (male/female)	66:38
BMI (kg/m ² , mean ± SD)	22.12 ± 4.64
ASA status	
I	28
II	72
III	4
Comorbidity	
Diabetes	14
Valvular heart disease	6
Chronic atrial fibrillation	4
Hypertension	26
Occlusive vascular disease	4
Chronic anemia	18
Primary bronchiectasis	2

BMI: Body mass index; ASA: American Society of Anesthesiologists.

Table 2 Intraoperative data and early outcome (mean ± SD)

Type of gastrectomy and anastomosis	
Total esophagojejunostomy with Roux-en-Y reconstruction	54
Distal Gastroduodenostomy/gastrojejunostomy	38
Proximal Esophagogastrostomy	12
TNM staging	
I	26
II	32
III	46
Operative time (min)	
Overall	272.52 ± 53.91
Total gastrectomy	302.5 ± 20.28
Distal subtotal gastrectomy	266.54 ± 35.26
Proximal gastrectomy	264.82 ± 40.33
Construction time (min)	45.8 ± 26.0
Total number of retrieved lymph node	23.1 ± 5.3
Estimated blood loss (mL)	80.78 ± 32.37
Hospital stay after surgery (d)	6.2 ± 2.5

fully following treatment with continuous irrigation drainage for 12 d. One patient underwent re-operation on post-operative day 14 due to jejunal afferent loop obstruction and recovered 10 d later. Another patient who underwent distal gastrectomy with gastroduodenostomy was readmitted due to intra-abdominal infection after surgery. She was treated with abdominal puncture and drainage and recovered.

DISCUSSION

The first robotic cholecystectomy was performed by Cadière *et al*^[19]. Currently, robotic surgery is widely applied in most operations. Recent studies have shown that robotic gastrectomy is feasible for patients with gastric cancer^[20,21].

A recent trend in minimally invasive surgery for the treatment of gastric cancer has attempted to reduce the length of the skin incision. The fact that minilaparotomy

Table 3 Postoperative complications using the Clavien-Dindo classification

Complications	Grade 1	Grade 2	Grade 3a	Grade 3b	Grade 4	Grade 5
Anastomosis leakage	1					
Gastroplegia		2				
Prolonged ileus		2				
Alimentary tract obstruction				1		
Alimentary tract hemorrhage		1				
Poor incision healing			2			
Pulmonary infection		1			1	
Abdominal infection or abscess			1			
Intra-abdominal bleeding		1				
Total	1	7	3	1	1	

itself can cause traumatic stress to surgical patients led to the development of a totally laparoscopic technique in which all of the surgical procedures, including reconstruction, are performed intraabdominally under a laparoscopic field^[22]. Various methods have been established to facilitate intracorporeal anastomosis^[23-25]. A recent study in a large volume center showed that extracorporeal anastomosis can be changed to intracorporeal anastomosis using a stapling device. However, this laparoscopic method, especially in esophagojejunal anastomosis, presented many technical problems including exposure difficulty, impossible reinforced suturing, variation in the diameter of the esophagus and a weak point in double stapling^[26-28]. Due to the technical difficulties of laparoscopic anastomosis and concern regarding anastomotic complications using the stapling method^[29], many surgeons still prefer extracorporeal reconstruction. However, with the advance of robotic surgery, the da Vinci system has become a minimally invasive cutting edge surgical technique. Since articulating instruments of the robotic device may provide complete wrist dexterity, allowing fine control with precision when performing intracorporeal sutures, a robot-sewn anastomosis in robotic gastric cancer surgery could avoid minilaparotomy and additional laparoscopic techniques, and provide surgeons with a reduced risk of anastomotic complications similar to hand sewing^[22].

Robotic operations improved the time to completion and the quality of choledochojejunostomy compared with laparoscopy in an *ex vivo* bench model, especially for surgeons with less experience with minimally invasive surgery^[30]. Compared with standard laparoscopy, robotic assistance significantly improved intracorporeal suturing performance and the safety of novices in the operating room, thus significantly shortening the learning curve^[31]. Three dimensional vision allows significant improvements in performance times and error rates for both inexperienced residents and advanced laparoscopic surgeons^[32]. Hur *et al*^[33] reported 2 cases of successful esophagojejunostomy using the full robot-sewn technique after total gastrectomy with lymph node dissection. The study further confirmed that all types of hand-

sewn anastomoses in gastrectomy, which are performed in the deep and narrow space of the abdominal cavity, were technically feasible^[33]. Our study of 104 cases also demonstrated that full robotic hand-sewn anastomosis was technically feasible and safe.

Recent studies have demonstrated that the robotic approach does not provide an advantage over laparoscopy^[34-37]. Twenty of the initial robot-assisted gastrectomies had similar results to those for experienced laparoscopically-assisted gastrectomies in one report. Other studies have shown that patients who undergo a robot-assisted gastrectomy have a larger number of dissected lymph nodes and a smaller amount of bleeding during radical surgery for early gastric cancer than those who undergo a laparoscopically-assisted gastrectomy^[38,39], but not in terms of hospitalization time after surgery^[32]. Some scholars have indicated that one reason for the insufficient demonstration of this surgical system's advantages is that full robot-assisted reconstruction of the alimentary tract^[40] was not performed in these studies. Before this clinical trial, we performed more than 100 cases of robot-assisted gastrectomy with minilaparotomy for anastomosis^[41]. According to our experience, hospitalization time after robotic surgery with full intracorporeal anastomosis decreased by approximately 1 d compared to that with minilaparotomy for anastomosis. The rate of incision infection was sharply reduced in robotic surgery with full intracorporeal anastomosis. However, to achieve a definite result, a large number of robot-assisted gastrectomy cases and well-designed research are needed. As the number of robot-assisted gastrectomy cases increase, surgical outcomes may improve.

Although this trial showed many benefits in terms of clinical outcomes, limitations were still encountered. As a result of full robotic intracorporeal surgery, the tumor location may not be identified as easily as extra-corporeal anastomosis. Thus, preoperative examination with gastro-endoscopy and computed tomography is obligatory to determine the location of the tumor and the type of gastrectomy. As the specimen was extracted from the abdominal cavity through the extended intraumbilical port site, the stomach was opened to ensure the tumor margin was adequate. As in our study, 2 cases were diagnosed with early stage tumor in the middle part of stomach and the precise location of tumor was not palpable even with 3-D vision during surgery. Thus, these two cases were converted to extra-corporeal anastomosis *via* minilaparotomy to ensure adequate tumor margin.

The optimal method for full intracorporeal anastomosis remains to be established. It is probable that there is not one single optimal method. As we have shown, full robot hand-sewn anastomosis can be safely and rapidly performed by surgeons familiar with intracorporeal suturing and knot-tying techniques. This technique is feasible and can produce satisfying postoperative outcomes, and may be a minimally invasive technique in future gastrectomy surgery.

COMMENTS

Background

To achieve a minimally invasive method in gastrectomy surgery, a minimal gastroenteral anastomosis must be completed intracorporeally. Various modified procedures for reconstruction have been reported, but an optimal method has not been established due to technical difficulties. Robotic surgery has theoretical advantages such as increased degrees of freedom of instruments and a three-dimensional view. The aim of this study was to determine the feasibility and effectiveness of full robot-assisted total gastrectomy using intracorporeal robot hand-sewn anastomosis in the treatment of gastric cancer.

Research frontiers

Hand-sewn suturing is technically demanding, but with the advantages of robotic surgery it can be performed safely by trained surgeons. This technique is feasible and can produce satisfying postoperative outcomes. Its convenience and reliability in anastomosis for gastrectomy were confirmed in the study. This is the first large scale report on full robot-assisted gastrectomy with intracorporeal robot-sewn anastomosis.

Innovations and breakthroughs

The details of the surgical technique were well illustrated in this article. This technique is feasible and can produce satisfying postoperative outcomes, and may be a minimally invasive technique in future gastrectomy surgery.

Applications

Intracorporeal robot-sewn anastomosis can be widely used in robotic surgery centers. It may be a minimally invasive technique in future robotic gastrectomy surgery.

Terminology

Robotic surgery: Computer-assisted surgery and robotically-assisted surgery are terms used for technological developments which use robotic systems to aid surgical procedures. Robotically-assisted surgery was developed to overcome the limitations of minimally invasive surgery and to enhance the capabilities of surgeons performing open surgery. Minilaparotomy: A small abdominal incision for surgical procedures, such as liver biopsy, open transhepatic cholangiography, or alimentary anastomosis to ensure minimal traumatic stress.

Peer review

Authors present their prospective experience with full robotic-assisted gastrectomy. They performed 104 successful operations ranging from distal gastrectomy with intracorporeal gastroduodenotomies or gastrojejunostomies to total gastrectomy with esophagojejunostomy. The average surgical time was 272 min and blood loss was 81 cc. Patients averaged 6.2 d in hospital. The authors conclude that robotic gastrectomy with intracorporeal anastomosis is feasible and safe. Further case-control studies need to be conducted to investigate the advantage of intracorporeal robot's hand sewn anastomosis.

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HER2 in gastric cancer: Comparative analysis of three different antibodies using whole-tissue sections and tissue microarrays

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Abstract

AIM: To compare the performance of three commercially available anti-human epidermal growth factor re-

ceptor 2 (HER2) antibodies in whole-tissue sections and tissue microarrays (TMAs) of a series of gastric tumors.

METHODS: We present a comparative analysis of three anti-HER2 antibodies (HercepTest, 4B5 and SP3) using TMA and whole-tissue sections prepared from the same paraffin blocks of 199 gastric adenocarcinomas operated upon between January 2004 and December 2008 at a Brazilian cancer hospital. The data on the patients' age, sex, the anatomical location of the tumor and the Lauren's histological classification were collected from clinical and pathological records. The immunohistochemical (IHC) results were examined by two pathologists and the cases were classified as positive (3+), equivocal (2+) and negative (0 or 1+), according to the criteria of the IHC scoring system of gastric cancer. TMAs and whole-tissue sections were evaluated separately and independently. All cases yielding discordant IHC results and/or scored as 2+ were subjected to dual-color *in situ* hybridization in order to determine the final HER2 status. Besides determining the sensitivity and predictive value for HER2-positive status, we measured the accuracy of each antibody by calculating the area under the receiver operating characteristic (ROC) curve. The agreement between the results obtained using the TMAs and those obtained using the whole-tissue sections was assessed by means of Kappa coefficient.

RESULTS: Intratumoral heterogeneity of HER2 expression was observed with all antibodies. HER2-positive expression (3+) in the whole-tissue sections was observed in 23 cases (11.6%) using the 4B5 antibody, in 18 cases (9.1%) using the SP3 antibody and in 10 cases (5.1%) using the HercepTest antibody. In the TMAs, 11 positive cases (5.6%) were identified using SP3 antibody, 9 (4.6%) using the 4B5 antibody and 6 (3%) using the

HercepTest antibody. The sensitivity using whole-tissue sections and TMA, respectively, was 95.2% and 42.9% with 4B5, 90.5% and 66.7% with SP3 and 47.6% and 42.9% with HercepTest. The accuracy, calculated from the area under the ROC curve, using whole-tissue sections and TMA, respectively, was 0.91 and 0.79 by 4B5, 0.86 and 0.80 by SP3 and 0.73 and 0.71 by HercepTest. The concordance of the results obtained using whole-tissue sections and TMA was 97.4% (Kappa 0.75) using HercepTest, 85.6% (Kappa 0.56) using SP3 and 84.1% (Kappa 0.38) using 4B5.

CONCLUSION: The use of the 4B5 antibody on whole-tissue sections was the most accurate IHC method for evaluating HER2 expression in gastric adenocarcinoma.

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Key words: Gastric cancer; Human epidermal growth factor receptor 2; Immunohistochemistry; Whole-tissue sections; Tissue microarray; Trastuzumab

Core tip: This is the first study to compare the three widely used anti-human epidermal growth factor receptor 2 (HER2) antibodies 4B5, SP3 and HercepTest in tissue microarrays and whole-tissue sections prepared from paraffin blocks of a single series of gastric tumors. We aimed to find the best method to assess HER2 expression in gastric cancer, facilitating the choice of the antibody with the greatest ability to identify the most patients who could benefit from the use of trastuzumab. Besides, we demonstrated that HER2 expression in small samples of gastric cancer (such as tissue microarrays and biopsies) should be evaluated cautiously because these tumors exhibit intratumoral heterogeneity that may influence the results.

Abrahão-Machado LF, Jácome AAA, Wohnrath DR, Santos JS, Carneseca EC, Fregnani JHTG, Scapulatempo-Neto C. HER2 in gastric cancer: Comparative analysis of three different antibodies using whole-tissue sections and tissue microarrays. *World J Gastroenterol* 2013; 19(38): 6438-6446 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6438.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6438>

INTRODUCTION

The incidence of gastric cancer (GC) is gradually decreasing; however, it remains one of the leading causes of cancer-related death worldwide because the vast majority of GC patients are diagnosed with advanced disease^[1-4]. Despite the improvement in surgical techniques and the use of multimodal treatments, the prognosis for GC is generally poor and treatment continues to be a challenge for physicians^[1,4]. Recently, several oncogenes and tumor suppressor genes were studied in an attempt to clarify the process of gastric carcinogenesis, and specific monoclonal antibodies were developed as a potential form of adjuvant treatment for patients with advanced disease.

The HER2 (CerbB-2) or human epidermal growth factor receptor 2 (HER2) gene is a proto-oncogene located on chromosome 17q21 that encodes a transmembrane protein that is a member of the HER receptor family. These receptors possess tyrosine kinase activity and are typically involved in signal transduction pathways that lead to cell growth and differentiation^[5]. Amplification of the *HER2* gene and overexpression of its product have been identified in several tumors and have been widely studied in breast cancer^[6]. In GC, however, the reported frequency of HER2 overexpression ranges from 8.2% to 53.4%, and its clinical significance and prognostic value remain controversial, although HER2-positive tumors are usually associated with more aggressive biological behavior and tumor recurrence^[7-13]. A recent meta-analysis showed that in 7 of the 15 papers evaluated, HER2 positivity was correlated with a worse prognosis^[14].

New advances in molecular targeting therapy have identified HER2 as an important target for anti-cancer therapy of gastric tumors. The ToGa study recently indicated improved survival of patients with advanced GC who were treated with trastuzumab (a chimeric anti-HER2 targeted drug) combined with chemotherapy compared with those treated with chemotherapy alone^[15]. This randomized clinical trial achieved the longest median survival to date of patients with advanced gastric carcinomas. The mechanism by which trastuzumab acts is not completely understood, but the likely possibilities are that it prevents the dimerization of HER2 with other members of the HER family, activates the immune response by promoting antibody-dependent cell-mediated toxicity and induces endocytosis of HER2^[16,17]. Given the demonstration of its clinical benefits and its approval for use in systemic therapy by the Food and Drug Administration (FDA), trastuzumab is the new standard treatment option for patients with HER2-positive advanced GC. Therefore, it is crucial to determine the HER2 status of GCs to select patients who may benefit from this promising targeted therapy.

Several assays are available to determine HER2 status; however, many of them require fresh tissue, involve complicated procedures and are costly. The most commonly used method is immunohistochemistry (IHC), which is a low-cost technique that can be performed on small samples, even formalin-fixed and paraffin-embedded tissues. Fluorescent *in situ* hybridization (FISH) is considered the gold standard and can be used to analyze this type of sample. However, because of its higher cost and the need for a fluorescence microscope, as well as the high concordance between FISH and IHC reported in literature^[18-21], generally only equivocal cases are subjected to FISH. An alternative for equivocal cases is provided by the use of other *in situ* hybridization methods such as silver *in situ* hybridization (SISH), including dual-color *in situ* hybridization (DISH), which allows the use of an ordinary light microscope and has shown excellent correlation with results obtained using FISH^[18,22,23].

Although a widely used and FDA/CAP-approved IHC scoring system already exists for HER2 in breast

cancer, it was necessary to develop a suitable scoring system for gastric tumors, mainly because of morphological differences and the intratumoral heterogeneity of HER2 expression in GC^[8,9,11,18,24]. The system proposed by Hoffmann *et al.*^[9] for GC and incorporated as standard by CAP and FDA differentiates between surgical specimens and biopsies.

Currently, commercially available IHC antibodies include the HercepTest and A0485 (Dako, Glostrup, Denmark) rabbit polyclonal antibodies, the SP3 (Labvision; Thermo Fisher Scientific, Fremont, CA, United States) and 4B5 (Ventana Medical Systems, Tucson, AZ, United States) rabbit monoclonal antibodies and the CB11 mouse monoclonal antibody (Novocastra, Newcastle upon Tyne, England). Only the HercepTest, 4B5 and CB11 antibodies are approved by the FDA, although the international literature also shows high-quality of the SP3 antibody in samples of breast cancer^[20,25].

In the present study, HER2 expression in 199 GC was investigated by IHC on whole-tissue sections and tissue microarrays (TMA) using HercepTest, 4B5 and SP3. To date, no published results have compared these three antibodies. Moreover, this is the first study of GC to compare HER2 expression using both TMAs and whole-tissue sections prepared from samples of the same paraffin blocks, *i.e.*, the same tumors. All cases yielding divergent IHC results or results considered equivocal (2+) were subjected to DISH. We hypothesized that if the TMA samples were considered to be biopsies because they are small tissue samples, the reproducibility of the HER2 scoring system for GC could be tested using the two types of specimens.

Given that HER2 expression in the stomach is heterogeneous, the main purpose of our study was to compare the performance of three commercially available anti-HER2 antibodies. Furthermore, we aimed to determine the concordance of results obtained from whole-tissue sections and TMAs from the same tumors to evaluate the feasibility of TMA as an alternative method for assessing HER2 expression in GC.

MATERIALS AND METHODS

Patients

In the present study, we selected 199 cases of surgically resected primary gastric or gastro-esophageal adenocarcinomas. All the patients were operated upon between January 2004 and December 2008 at the Barretos Cancer Hospital. Clinical data were collected from medical charts and pathology reports, including sex and patient age as well as the anatomical location of the tumor and its Lauren histological classification.

TMA construction and IHC

Paraffin blocks containing representative samples of the tumors were selected by reviewing all of the hematoxylin and eosin (HE) stained slides. For the TMAs, two tissue cores with a diameter of 0.6 mm were extracted from

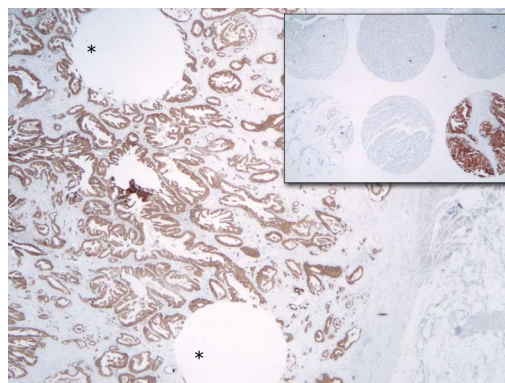


Figure 1 Photomicrograph of immunohistochemistry ($\times 100$) in a whole-tissue section. Asterisks indicate two round voids where the tissue microarray cores (inset) were extracted.

each tumor using the TMA arrayer MTA1 (Estigen, Tartu, Estonia). The tumor cores were sequentially placed in molds, embedded in paraffin and cooled to form the tissue array blocks. Each TMA also contained various non-gastric tissue samples as control tissues.

Sections of a thickness of 4 μm were obtained from the whole-tissue paraffin blocks and TMA blocks and used for IHC (Figure 1). The slides were stained using automatic staining devices: the Benchmark XT (Ventana Medical Systems, Tucson, AZ) for the 4B5 and SP3 antibodies and the Autostainer Link 48 (Dako, Glostrup, Denmark) for the HercepTest. After antigen retrieval processing for 60 min (at pH 8.4), 4B5 (prediluted form as provided by the manufacturer) and SP3 (diluted 1:100) were applied for a 32 min incubation period. Antibody visualization was enabled using the Ventana Ultraview DAB detection kit. The HercepTest was performed according to the manufacturer's guide provided with the kit, using the prediluted "ready-to-use" form for all of the steps and incubation periods preprogrammed in the stainer software. The kit also contained the visualization reagent. All the slides were subsequently counterstained with hematoxylin.

The criteria suggested by Hoffmann *et al.*^[9] were used to evaluate the expression of HER2. Sections of the surgical specimens were considered HER2-positive (3+) when strong complete or basolateral membranous staining was detected in $\geq 10\%$ of the neoplastic cells; equivocal (2+) when moderate/weak complete or basolateral membranous staining was detected in $\geq 10\%$ of the cells; 1+ (negative) when the staining was weak or detected in only one part of the membrane in $\geq 10\%$ of the cells and 0 (also negative) in cases in which there was no membranous staining or staining of $< 10\%$ of the tumor cells. The criteria for evaluating biopsies were applied to the TMAs, and the percentage above (10%) was replaced by a cellular group of at least 5 cells. Full-tissue sections or TMA cores with excessive tissue fragmentation, scant invasive tumor and excessive cytoplasmic or background staining were rejected, and IHC was repeated on more suitable samples.

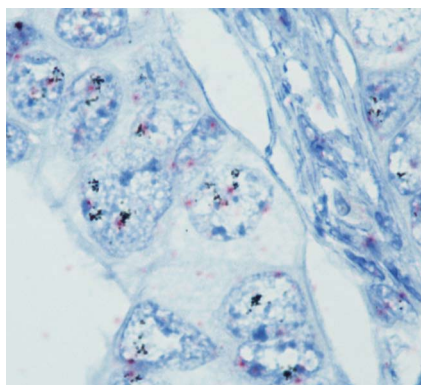


Figure 2 Dual-color *in situ* hybridization ($\times 1000$), human epidermal growth factor receptor 2 amplification. Black dots: Human epidermal growth factor receptor 2 gene; Pink dots: Chromosome 17.

In situ hybridization

DISH was performed in all cases that were scored 2+ in either of the samples stained with any of the antibodies, in accordance with the guidelines recommended by CAP and routine laboratory practice. In addition, all of the cases with discordant IHC results were tested by DISH. The tissue sections used for DISH were obtained from the whole-tissue paraffin blocks. HER2 DISH was performed using the Ventana Benchmark XT-machine (Ventana Medical System, Tucson, AZ) following its standardized protocol. DISH is dual-color *in situ* hybridization in which the *HER2* gene is labeled using silver to produce a black dot, and the centromere of chromosome 17 (Chr 17) is labeled with alkaline phosphatase to produce a pink dot. Therefore, the *HER2* gene and Chr 17 are both simultaneously stained on the same slide (Figure 2). The DISH slides were examined using an HE slide to assist with the tumor location and morphology within each section. At least 40 tumor cell nuclei were scored for the Chr 17 signal and HER2 signal in different areas of the tumor. Only nuclei displaying both signals were scored, and a HER2/Chr 17 ratio was obtained for each specimen. HER2 amplification was defined as a ratio of HER2/Chr 17 ≥ 2 . Chromosome 17 polysomy was defined as ≥ 3 Chr 17 signals per cell on average^[26].

Statistical analysis

The statistical analysis was conducted using SPSS version 19.0 software (SPSS Inc., Chicago, IL). The IHC results were compared with a final variable of positivity or negativity for HER2 protein expression. In equivocal cases or cases of nonconcordant results obtained with the three antibodies, *HER2* gene amplification was assayed by DISH, and the DISH results determined the definitive HER2 status. Therefore, the results considered final, *i.e.*, the gold standard for statistical analysis, were those that were identical for the three antibodies, in addition to the results of DISH. The area under the receiver operating characteristic (ROC) curve (AUC) for each test was used to measure the accuracy of antibody labeling. To verify the agreement between the results obtained using the TMAs and those obtained using the whole-tissue sec-

tions, we employed the Kappa coefficient^[27]. We defined $P < 0.05$ as statistically significant.

RESULTS

Patients and tumor characteristics

There were 123 male and 76 female patients, and their age ranged from 27 to 87 years (median: 60.7 years). The location of the tumor was in the antrum in 86 cases (43.2%), in the body in 26 cases (13%), in the fundus in 2 cases (1%), in the cardia in 38 cases (19%) and multicentric (in more than 2 regions) in 47 cases (23.6%). Fifty-five cases were histologically classified as the diffuse type (27.6%), 123 as the intestinal type (61.8%), 17 as the mixed type (8.5%) and four as not otherwise classified (2%).

Immunohistochemistry

HER2-positive expression (3+) in the whole-tissue sections was observed in 10 cases (5.1%) using the HerceptTest, in 23 cases (11.6%) using the 4B5 antibody and in 18 cases (9.1%) using the SP3 antibody. Using the TMAs, 6 HER2-positive cases (3%) were identified using the HerceptTest, 9 (4.6%) using the 4B5 antibody and 11 (5.6%) using SP3 antibody. The immunohistochemistry results are shown in Table 1.

The HerceptTest demonstrated the lowest number of positive cases in both the whole-tissue sections and the TMAs. The SP3 antibody yielded the highest number of equivocal (2+) cases for both types of samples. The frequency of a score of 2+ was higher among the whole-tissue sections than among TMAs, except when using the HerceptTest, which showed the opposite pattern.

The overall concordance between the results obtained using the TMAs and those obtained using the whole-tissue sections was 97.4% with the HerceptTest, 84.1% with the 4B5 antibody and 85.6% with the SP3 antibody. According to the values of the Kappa coefficient, HerceptTest provided substantial agreement between the TMAs and whole-tissue sections, the SP3 antibody provided moderate agreement and the 4B5 antibody provided fair agreement (Table 2).

Stronger membrane staining in positive cases was observed for the 4B5 antibody than for the other two antibodies (Figure 3). The diffuse cytoplasmic staining in the gastric foveolar epithelium and intestinal metaplasia that was observed when using 4B5 antibody was less pronounced when using the SP3 antibody and not observed when using the HerceptTest antibody. Heterogeneous HER2 expression within the tumors was observed with all antibodies (Figure 4). All the positive cases were classified as intestinal type. Nuclear staining with the 4B5 and SP3 antibodies was observed in some of the diffuse adenocarcinomas.

DISH and final HER2 status

Cases with divergent results and those considered equivocal by IHC (scored as 2+) were subjected to DISH; there

Table 1 Results of HER2 immunostaining using the three antibodies on whole-tissue sections and tissue microarrays *n* (%)

Score	Whole-tissue sections			TMAs		
	HercepTest	4B5	SP3	HercepTest	4B5	SP3
0	179 (90.9)	125 (63.1)	128 (65.2)	185 (93.5)	174 (88.8)	162 (82.6)
1+	7 (3.5)	30 (15.2)	17 (8.5)	3 (1.5)	10 (5.1)	8 (4.1)
2+	1 (0.5)	20 (10.1)	34 (17.2)	4 (2.0)	3 (1.5)	15 (7.7)
3+	10 (5.1)	23 (11.6)	18 (9.1)	6 (3.0)	9 (4.6)	11 (5.6)
Total	197 (100.0)	198 (100.0)	197 (100.0)	198 (100.0)	196 (100.0)	196 (100.0)

TMA: Tissue microarray.

Table 2 Concordance between the tissue microarrays and whole-tissue sections staining results using the HercepTest, 4B5 and SP3 antibodies

Antibody	Overall concordance	Kappa coefficient (95%CI)
HercepTest	97.40%	0.75 (0.54-0.96)
4B5	84.10%	0.38 (0.22-0.53)
SP3	85.60%	0.56 (0.43-0.70)

were 58 (29.1%) such cases, of which 14 cases (24.1%) exhibited *HER2* gene amplification and 44 cases (76.9%) did not exhibit *HER2* gene amplification. Chr 17 polysomy was present in 5 cases (8.6%), but it was not related to amplification in our study. Table 3 shows the *HER2* gene status in the cases that presented an IHC score of 2+.

A final positive *HER2* status (either by IHC or DISH) was obtained in 20 of the 199 cases tested (10%). The sensitivity using whole-tissue sections was 47.6% with HercepTest, 95.2% with 4B5 and 90.5% with SP3 in cases with an immunoscore of 2+/3+. The sensitivity using TMA was 42.9% with HercepTest, 57.1% with 4B5 and 66.7% with SP3 (Table 4).

Table 5 demonstrates the accuracy of each antibody. The 4B5 and SP3 antibodies gave similar AUC values in whole-tissue sections (Table 5), and both were significantly more accurate than HercepTest ($P = 0.002$ and 0.035 , respectively). Although the 4B5 and SP3 antibodies both gave greater AUC values than HercepTest in TMAs, the difference was not statistically significant. Based on the AUC of each antibody for both types of samples and the respective P values (Table 6), we determined that the use of the 4B5 antibody on whole-tissue sections was the most accurate method. SP3 staining of whole-tissue sections was also more accurate than the HercepTest using TMAs ($P = 0.013$).

Histological type and anatomical location

Of the 123 adenocarcinomas of the intestinal type, nine (7.3%) were given a final positive *HER2* status. None of the 76 cases of the other histological types was positive.

Of the 86 carcinomas located in the antrum, nine (10.4%) had a final positive *HER2* status. Eight (21%) of the 38 tumors situated in the cardia and three (6.4%) of the 47 multicentric tumors were positive.

DISCUSSION

With the demonstration of the benefits of trastuzumab therapy for advanced GC^[15], the clinical demand for *HER2* assessment is rapidly increasing. The use of trastuzumab in association with platinum and capecitabine or 5-FU for *HER2*-positive GC has shown the longest median survival in GC patients^[15]. Because IHC appears to be the easiest, least expensive and most widely used method, our goal was to compare three commercially available antibodies. Although the use of different clones can be problematic for GC, only two other reports in the literature have compared the performance of *HER2* antibodies^[18,28]. An ideal antibody test would be sufficiently sensitive to identify all possible treatment candidates and would have a low false-positive rate to minimize over-treatment.

Variability in performance among commercially available anti-*HER2* antibodies has been demonstrated in several studies, although most of these studies were performed in breast tumors^[29,30]. Cho *et al.*^[28] compared the HercepTest, A0485, 4B5 and CB11 antibodies in TMAs of gastric carcinomas, and they found that the sensitivity and specificity were 78.9% and 96% with HercepTest, respectively, 86.5% and 94.4% with the A0485 antibody, 76.3% and 95.6% with 4B5 and 60.5% and 98.4% with the CB11 antibody. Boers *et al.*^[18] tested the SP3 and 4B5 antibodies on biopsy specimens of gastro-esophageal adenocarcinomas, and they showed sensitivities of 77% and 96% as well as specificities of 100% and 98.4%, respectively. The latter result is consistent with the results we obtained when comparing the SP3 and 4B5 antibodies. In the present study, however, the difference in sensitivity among antibodies was much higher, as the sensitivity ranged from 47.6% to 95.2% when whole-tissue sections were analyzed and ranged from 42.9% to 66.7% when TMAs were analyzed. The sensitivity of HercepTest was far lower than that of the other two tests, which contributed to this difference. The specificity ranged from 81.2% to 99.4% for the whole-tissue sections and from 93.1% to 100% for the TMAs. For all the antibodies, lack of staining (score 0) was highly predictive of a negative/nonamplified case as confirmed by DISH, and positive staining (score 3+) was highly predictive of an amplified case as confirmed by DISH.

The 4B5 and SP3 antibodies exhibited similar perfor-

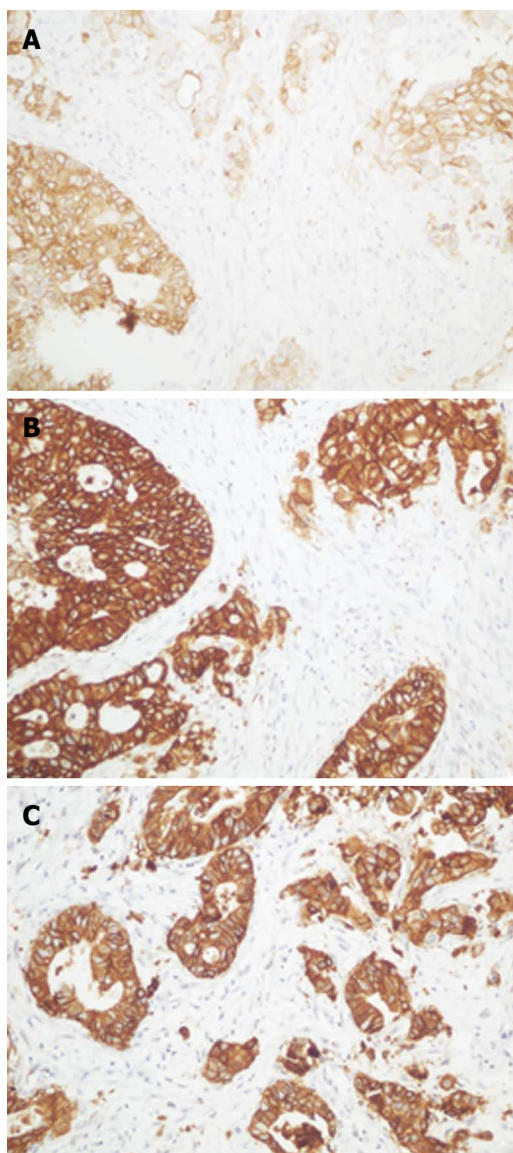


Figure 3 Comparison of positivity (3+) using the HercepTest (A), 4B5 (B) and SP3 (C) antibodies ($\times 200$).

mance, with high NPV values and AUC values that indicated higher accuracy, compared to HercepTest, although the difference was only statistically significant ($P < 0.05$) for whole-tissue sections. Even though HercepTest had high values for PPV and specificity, it presented the lowest sensitivity. Thus, this antibody provided the highest number of tumors with immunoscores of 0 or 1+ that were positive for HER2 amplification using DISH, which agrees with the report of Dekker *et al.*^[29] for breast tumors. Thus, in our view, HercepTest is not the best antibody to use as a first-line test to assess the HER2 status of GC because the ideal antibody should be highly sensitive, even though high sensitivity could increase the number of equivocal cases (2+) and the need for *in situ* hybridization tests. The 4B5 and SP3 antibodies were highly sensitive; therefore, these two antibodies appear to be more reasonable for first-line tests than HercepTest.

Another issue that we wanted to address was the use

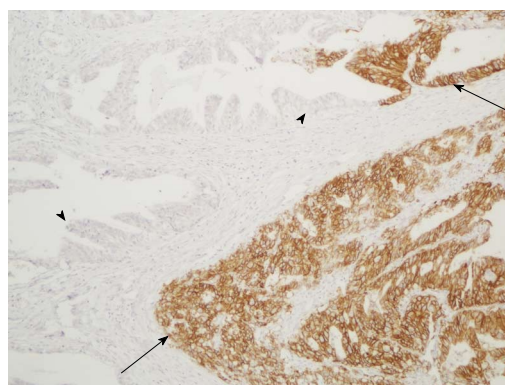


Figure 4 Representative image of the intratumoral heterogeneity of HER2 expression ($\times 100$). Arrows indicate areas with strong continuous membranous staining (score 3+) and arrowheads indicate negative areas (score 0).

of TMAs for assessment of HER2 and, by analogy, the reliability of testing endoscopic biopsies. The use of TMAs permits the inclusion of several different tumors in the same assay on a single slide. This cost-effective technique has become a standard procedure for many contemporary IHC studies. Dekker *et al.*^[29] found that TMAs were reliable for retesting large volumes of previously HER2-classified breast carcinomas. Similarly, Drev *et al.*^[31] observed a high concordance between whole-tissue sections and TMAs for breast tumors. Despite these favorable results for TMAs of breast cancers, HER2 assessment of gastric adenocarcinomas is more problematic. The obvious disadvantage of TMAs is that this preparation enables the analysis of only a limited sample of the tumor and for GCs, TMAs are even more unfavorable because of the generally observed heterogeneous expression of HER2 within these tumors.

Intratumoral heterogeneity can be defined as areas with different HER2 scores within the same tumor. This is the predominant pattern for GCs but not for breast tumors, and may thus cause sampling error when randomly sampled TMA cores of GCs are used^[8,9,11,24]. The difference in HER2 status between primary and metastatic tumor samples is still a matter of debate, and although the few studies in the scientific literature have demonstrated high concordance among the results obtained using these samples, some cases gave discordant results^[32,33], which suggests an effect of intratumoral heterogeneity.

Conspicuously heterogeneous HER2 staining on the whole-tissue sections and TMA core samples from most of the tumors was noted in our study. To minimize the discrepancy with results obtained with whole-tissue sections, we included two core samples from different areas of each tumor in the TMAs. Regardless, the TMA staining was much less sensitive than the staining of whole-tissue sections (mean values for the antibodies: 55.5% *vs* 77.7%, respectively). Although the HercepTest provided greater agreement between the TMAs and the whole-tissue sections, with a substantial Kappa value, its sensitivity was low for both types of sample, as mentioned above. The difference in HER2 expression detected in

Table 3 Human epidermalgrowth factor receptor 2 gene status assessed by dual-color *in situ* hybridization in cases with an immunohistochemistry score of 2+ with HercepTest, 4B5 and SP3 staining on whole-tissue sections and tissue microarrays *n* (%)

HER2 gene status	Whole-tissue sections			TMAs		
	HercepTest	4B5	SP3	HercepTest	4B5	SP3
Amplified	0 (0)	6 (30)	6 (17.6)	3 (75)	3 (100)	4 (26.6)
Not amplified	1 (100)	14 (70)	28 (82.4)	1 (25)	0 (0)	11 (73.4)
Total	1 (100)	20 (100)	34 (100)	4 (100)	3 (100)	15 (100)

TMA: Tissue microarray; HER2: Human epidermalgrowth factor receptor 2.

Table 4 Specificity, sensitivity, positive and negative predictive values and the area under the receiver operating characteristic curve of each antibody according to the final human epidermalgrowth factor receptor 2 status

		HercepTest (95%CI)	4B5 (95%CI)	SP3 (95%CI)
TMA	Sensitivity	42.9 (21.7-64.0)	57.1 (35.9-78.3)	66.7 (46.5-86.8)
	Specificity	99.4 (98.3-100.0)	100.0	93.1 (89.4-96.9)
	PPV	90.0 (71.4-100.0)	100.0	53.8 (34.7-73.0)
	NPV	93.6 (90.1-97.1)	95.1 (92.0-98.2)	95.9 (92.9-98.9)
	AUC	0.71 (0.60-0.782)	0.79 (0.68-0.89)	0.80 (0.69-0.90)
Whole-tissue sections	Sensitivity	47.6 (26.2-68.9)	95.2 (86.1-100.0)	90.5 (77.9-100.0)
	Specificity	99.4 (98.3-100.0)	87.0 (82.0-91.9)	81.2 (75.5-87.0)
	PPV	90.9 (73.9-100.0)	46.5 (31.6-61.4)	36.5 (23.4-49.6)
	NPV	94.1 (90.7-97.5)	99.3 (98.1-100.0)	98.6 (96.7-100.0)
	AUC	0.73 (0.63-0.84)	0.91 (0.86-0.96)	0.86 (0.78-0.93)

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the receiver operating characteristic curve; TMA: Tissue microarray.

Table 5 Accuracy of the three antibodies in the whole-tissue sections and the tissue microarrays

		HercepTest	4B5	SP3
Whole-tissue sections	AUC	0.73	0.91	0.78
	HercepTest	-	<i>P</i> = 0.002	<i>P</i> = 0.035
	4B5	<i>P</i> = 0.002	-	<i>P</i> = 0.265
	SP3	<i>P</i> = 0.035	<i>P</i> = 0.265	-
TMAs	AUC	0.71	0.79	0.8
	HercepTest	-	<i>P</i> = 0.058	<i>P</i> = 0.075
	4B5	<i>P</i> = 0.058	-	<i>P</i> = 0.714
	SP3	<i>P</i> = 0.075	<i>P</i> = 0.714	-

AUC: Area under the receiver operating characteristic curve; TMA: Tissue microarray.

our study between TMAs and whole-tissue sections was caused by the prominent heterogeneity of HER2 staining. The 4B5 antibody results in whole-tissue sections were significantly different from the results of the three antibodies in TMAs. Because 4B5 antibody staining of whole-tissue sections had the highest accuracy, which was much different from that obtained for TMAs, our results suggest that TMA staining is less accurate and lacks sufficient sensitivity to reliably assess the HER2 status in GC. Two tissue cores for the TMA were definitely not sufficient to prevent sampling error and minimize false results because of the intratumoral heterogeneity and the small amount of tissue in the cores. Therefore, studies in the literature that used TMA to test the HER2 status of GCs must be carefully analyzed, and it must be noted that this technique does not seem to reflect the real status of the HER2 gene in GCs.

Table 6 Comparison of the areas under the receiver operating characteristic curve according to the type of sample (whole-tissue section and tissue microarray) and the antibodies

		Whole-tissue sections			
		HercepTest	4B5	SP3	
TMAs	AUC	0.73	0.91	0.78	
	HercepTest	0.71	<i>P</i> = 0.572	<i>P</i> = 0.001	<i>P</i> = 0.013
	4B5	0.79	<i>P</i> = 0.289	<i>P</i> = 0.027	<i>P</i> = 0.201
	SP3	0.80	<i>P</i> = 0.265	<i>P</i> = 0.034	<i>P</i> = 0.244

AUC: Area under the receiver operating characteristic curve; TMA: Tissue microarray.

Endoscopic biopsies with few fragments, such as those used in the TMA, may underestimate the incidence of HER2-amplification, as Yang *et al.*^[24] demonstrated in a recent study in which large surgical specimens had higher rates of HER2 positivity than biopsy specimens. We believe that endoscopic biopsies are not optimal to identify the maximal number of patients who could be eligible for treatment. Therefore, to represent the tumor better and reduce misinterpretation, it is important to examine as many pieces of a biopsy as possible. We also suggest that all excisional specimens that had a previous HER2-negative result in a biopsy specimen should be retested to increase the chance of classifying the tumor as HER2-positive. Because the intratumoral heterogeneity of HER2 expression also seems to cause divergent results for primary and metastatic tumor samples^[32], is highly advisable to analyze the HER2 status of both primary and metastatic specimens when possible.

Thus, among the HercepTest, 4B5 and SP3 antibodies, HercepTest was the least sensitive and therefore had the lowest ability to identify a large number of patients eligible for trastuzumab treatment. According to our results, the most accurate IHC method to assess HER2 expression in GC is the use of the 4B5 antibody on whole-tissue sections. Intratumoral heterogeneity appears to be a major limitation for the use of TMA because TMA does not reflect the true HER2 status of many tumors. Because the number of cells that respond to a targeted therapy directly affects the tumor's responsiveness to treatment, there must be a great difference between cases that are diffusely positive and those that are only focally positive but still meet the criteria of positivity. Given the promising results from the use of trastuzumab and the particularities of HER2 expression in the stomach, further trials are needed to determine the clinical significance of the intratumoral heterogeneity and its impact on treatment outcome.

COMMENTS

Background

The vast majority of patients with gastric cancer are diagnosed with advanced disease and the prognosis is generally poor. Human epidermal growth factor receptor 2 (HER2)-positive tumors are usually associated with more aggressive biological behavior and recurrence. In view of the recently demonstrated clinical benefit of the anti-HER2 drug trastuzumab in the treatment of advanced gastric cancer, reliable HER2 testing is of key importance. HER2 status is usually determined by immunohistochemistry (IHC) and occasionally by *in situ* hybridization (ISH). However, little is known regarding the performance difference among the commercially available anti-HER2 immunohistochemical antibodies in gastric adenocarcinomas. This study compared three anti-HER2 antibodies (HercepTest, 4B5 and SP3) using two different arms: samples prepared in tissue microarray (TMA) device and whole-tissue sections counterpart prepared from paraffin blocks of a series of 199 gastric adenocarcinomas.

Research frontiers

HER2 is a member of the family of tyrosine kinase receptors. Overexpression of the HER2 receptor has been identified in various cancers and is most widely studied in breast cancer. Like in breast cancer, HER2-positive gastric tumors are correlated with worse prognosis. An important difference in HER2 immunoreactivity between breast cancer and gastric adenocarcinomas is the striking heterogeneity of HER2-positivity in the latter, which can affect the determination of HER2 status in small samples such as biopsies and TMA.

Innovations and breakthroughs

Many studies have compared anti-HER2 antibodies in breast cancer, however only few reports have done it in gastric cancer. Gastric adenocarcinomas exhibit intratumoral heterogeneity of HER2 expression and have a unique IHC scoring system. This is the first study to compare HercepTest, 4B5 and SP3 in a series of gastric tumors. Most contemporary studies use the cost-effective TMA technique for testing HER2 expression; however, given the high incidence of heterogeneous HER2-immunoreactivity and the risk of underestimating the incidence of HER2-amplification rate; we also aimed to compare the results obtained from TMA to those obtained from whole-tissue sections.

Applications

The study results indicate the best method to assess HER2 expression in gastric cancer, facilitating the choice of the antibody with the greatest ability to identify the most patients who could benefit from the use of trastuzumab. The results also demonstrated that HER2 expression in small samples of gastric cancer should be cautiously evaluated because the intratumoral heterogeneity may influence the results.

Terminology

According to the four-tiered IHC scoring system for gastric cancer, samples scored as 0 and 1+ are negative, 2+ as equivocal and 3+ as positive. Intratumoral heterogeneity is defined as areas with different HER2 scores within the

same tumor.

Peer review

This is a highly stringent study in which the authors examined HER2 immunostaining in a series of gastric cancers, using three different commercially available anti-HER2 antibodies in samples of TMA and whole-tissue sections. The sensitivity, predictive value for HER2 amplification and accuracy of each antibody were determined. The results are provoking and indicate the most accurate IHC method for HER2 evaluation, emphasizing the limitations of the TMA technique. The results of the study should encourage a more judicious assessment of the HER2 status in gastric cancers, especially in small tissue samples.

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Emergency admissions due to swallowed foreign bodies in adults

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Abstract

AIM: To study a retrospective analysis of patients who presented to the emergency departments (ED) with complaints related to foreign body ingestions.

METHODS: Patients older than 16 years of age who presented to the ED between January 1st and December 31st of 2010 with complaints related to swallowed foreign bodies were identified from electronic health records and patient charts.

RESULTS: A total of 100 patients presented with a complaint of foreign body ingestion during the study period. Overall, an X-ray was performed on 75 patients, and a fiberoptic evaluation was performed on 45 patients. A foreign body was detected in 46 (46%) patients. The diagnostic yield of the X-ray was 27 (36%)

out of 75 patients, while the diagnostic yield of the fiberoptic evaluations was 21 (47%) out of 45 patients. The detected foreign bodies were mostly located in the esophagus (17 out of 46 foreign bodies detected). When the types of ingested foreign bodies were evaluated, 52 (52%) patients reported ingesting food, and 19 (19%) patients reported swallowing pins. An X-ray was performed on 33 patients with accidental food ingestions but yielded a positive result in only two cases. In 12 out of 21 patients with accidental food ingestion who underwent fiberoptic evaluation, the foreign material was detected and removed.

CONCLUSION: Plain radiography is helpful in the localization of radiopaque swollen foreign bodies, while fiberoptic methods are useful as both diagnostic and therapeutic tools, regardless of radiopacity.

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Key words: Foreign body; Ingestion; Gastrointestinal tract; Endoscopy; Emergency

Core tip: The majority of foreign bodies swallowed by patients who present to the emergency departments cannot be detected using standard imaging studies and evaluation. Plain radiography is especially useful in the localization of radiopaque foreign bodies, while fiberoptic methods can be used as both diagnostic and therapeutic tools, regardless of the radiopacity of the foreign body ingested.

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INTRODUCTION

Visits related to gastrointestinal foreign bodies are relatively common causes of admission to emergency departments (ED)^[1,2]. The ingestion or insertion of a foreign body into the gastrointestinal (GI) tract can be a clinically serious condition with associated risks for morbidity and mortality^[2,3]. An estimated 1500 to 1600 patients die in the United States each year as a result of complications related to the ingestion or insertion of foreign bodies into the GI tract^[1,3-5]. Although this problem can be encountered in every age group, almost 80% of cases comprise patients in early childhood (18-48 mo), with a majority of cases resulting from swallowing coins, toys, crayons, or pen caps^[3,4]. The ingestion of foreign bodies is rarely seen in adults, is generally accidental and is commonly seen in the form of food (meat and bones) ingestion. Other risk groups for this type of injury include patients with psychiatric disorders, adults without teeth, prisoners and patients under the influence of substances that obscure judgment^[3,5-7]. The clinical presentation, symptoms and management of foreign bodies depend on their location within the GI tract. Depending on the size and shape, almost 80%-90% of such foreign bodies pass freely from the GI tract without any complication^[4,7,8].

The purpose of the present study was to conduct a retrospective analysis of patients who presented to our ED with complaints related to foreign body ingestions.

MATERIALS AND METHODS

Patients older than 16 years of age who presented to the Emergency Department between January 1st and December 31st of 2010 with complaints related to swallowed foreign bodies were analyzed retrospectively using the data obtained from electronic health records and patient charts. The patients' present complaints, demographic characteristics, previous medical history and medication use, physical exam findings, diagnostic studies performed, type and location of the foreign body, treatment provided, need for conservative or invasive/surgical treatment, complication rates, radiological findings and rate of survival/mortality were all recorded.

RESULTS

During the study period, we identified a total of 100 patients (42 male, 58 female; mean age 38 years, range 16-88 years) who were admitted with a complaint of foreign body ingestion. Of those, 65 (65%) localized their complaints to the pharynx, while 35 (35%) told us that they had ingested the foreign bodies. Among the list of complaints, 53 (53%) patients had difficulty swallowing; 33 (33%) had pain in the throat; 6 (6%) had difficulty breathing; 5 (5%) had abdominal pain; 4 (4%) had vomiting; 4 (4%) had bleeding from the mouth; 2 (2%) had a foreign body sensation in the throat; 2 (2%) had coughing; and 1 (1%) had chest pain. The incident was self-reported as

accidental in all patients. When facilitating factors were considered, 3 (3%) patients were undergoing dental interventions, and another 3 (3%) patients had dental plates. None of the study patients had any established diagnosis of psychiatric disease or history of substance abuse, alcohol or sedative use. Physical examination revealed oropharyngeal foreign bodies in 7 patients, epigastric tenderness in 1 patient, and rhonchus in 1 patient.

The diagnostic approaches to our patients are summarized in Figure 1. A foreign body was detected in 46 (46%) patients. The diagnostic yield of X-rays was 27 (36%) among the 75 patients evaluated by lateral neck, chest or abdominal X-ray. The foreign bodies were detected for 10 out of 51 patients using the chest X-ray, for 14 out of 29 patients using the abdominal X-ray and for 3 out of 52 patients using the lateral neck X-ray. The diagnostic yield was 21 (47%) out of 45 for all patients undergoing fiberoptic evaluations (Figure 1). The detected foreign bodies were mostly located in the esophagus (17 out of 46 foreign bodies detected) (Table 1). When the types of ingested foreign bodies were evaluated, 52 (52%) patients reported ingesting food, and 19 (19%) patients reported swallowing pins (Figure 2, Table 2). With respect to the types of ingested food, 20 were fish bones, 9 were bone fragments (Figure 3), and 23 were unknown food parts.

In 53 (53%) of the patients, a conservative approach for management was considered. Nineteen (19%) patients were followed with serial radiological examinations. In 21 patients, of whom 17 were undergoing upper GI endoscopy, 2 were undergoing laryngoscopy and 2 were undergoing bronchoscopy, the foreign body was removed by fiberoptic means. In total, 19 (19%) of the study patients were admitted for further evaluation and treatment. Out of all the patients, the clinical course was complicated by aspiration (food material) in two patients, by GI bleed (pin) in 1 patient and by mediastinitis (food material) secondary to perforation in 1 patient.

DISCUSSION

The medical history obtained from the patient is highly critical in the diagnosis of swollen GI foreign bodies. Therefore, the planning of the diagnostic work-up and the extent and urgency of a possible intervention are decided according to the information provided by the patient regarding the type of foreign body ingested, together with the clinical complaints and physical examination^[6,9-12]. Most GI foreign-body ingestions occur in pediatric patients aged between 6 mo and 6 years^[5,7,9]. GI foreign body exposure tends to be accidental in adults, with food particles and bones constituting the majority of the foreign bodies^[4,13]. The rest of the cases occur in the setting of facilitating factors, such as adults without teeth or with dental plates, prisoners and psychiatric patients^[4,6,9,14,15]. Our results were also similar among all of the patients evaluated in the study reporting accidental intake. Patients who suffer foreign body ingestion can present with a wide range of symptoms, which can vary

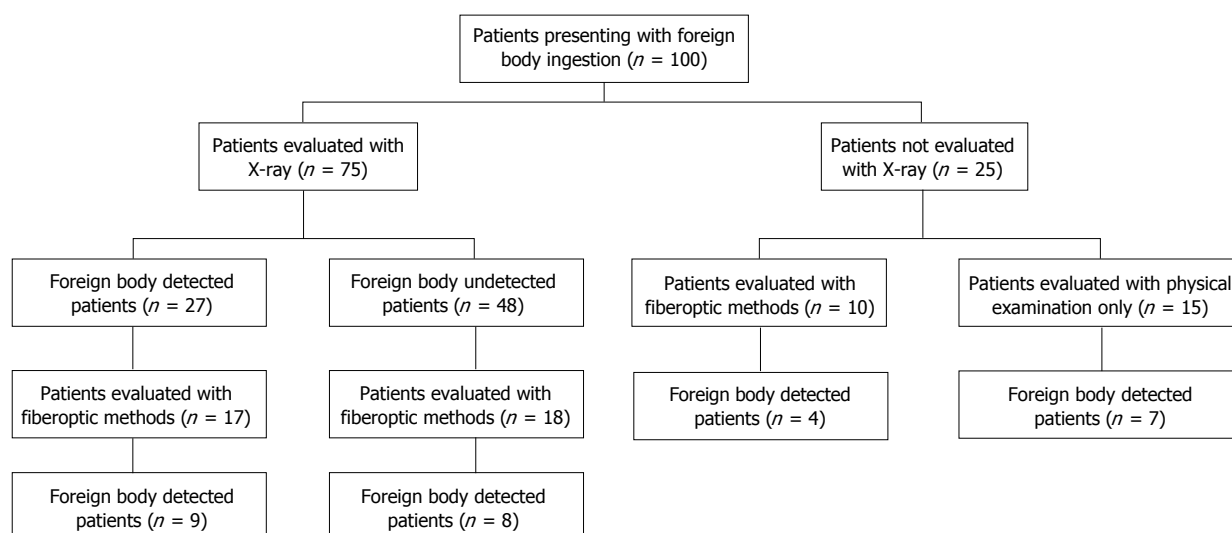


Figure 1 Diagnostic approach to patients presenting with foreign body ingestions.

Table 1 Location of the foreign bodies (n = 100)

Foreign body location	n (%)
Esophagus	17 (17)
Oropharynx	8 (8)
Small intestine	6 (6)
Stomach	6 (3)
Trachea	2 (2)
Larynx	3 (3)
Colon	3 (3)
Undetermined location	1 (1)
Undetected	54 (54)

based on the physical characteristics and the content that is absorbed in the GI tract. Diagnosis is based on the patient's history and complaints, which typically include the sudden onset of difficulty of swallowing during eating, chest pain, odynophagia or insufficiency in tolerating secretions. However, symptoms range from mild to life-threatening, including shortness of breath, abdominal pain, vomiting, hematemesis, foreign body sensation, coughing and chest pain^[3,5,9,11,16,17]. In agreement with the literature, the majority of patients in our cohort presented with difficulty in swallowing and foreign body sensations in the throat.

Different types of foreign bodies are observed in the GI tract based on the age group. During childhood, swallowed coins, small toys, crayons or batteries are observed, whereas during adulthood, food, bones and dental-related foreign bodies are more common^[4,6,7,9,13,16,17]. The types of foreign bodies may also differ by country. The high number of pin ingestions in our study group is thought to be related to the regional dress code, which results in women holding pins between their lips before attaching their headscarves. While certain conditions, such as parental attitudes and dietary habits, can provide clues for the types of foreign bodies that are ingested, prevention strategies are also dependent on various cultural, social,

religious and economic factors^[7,11,17-19].

The presentation, clinical findings, and management of foreign bodies are distinct and based upon the anatomical region where the foreign body is located^[4,9,11,17]. Determining the type and location of the foreign body in the ED changes the treatment approach^[5,9,16]. In our study, most of the foreign bodies were detected in the upper GI tract. The majority of the radiopaque foreign bodies in the GI tract can be detected using radiography. This simple modality provides crucial information, such as the number, size, location and direction of the foreign body, as well as the presence of sharp edges^[2,3,6,8]. However, the presence of fish bones, chicken bones, glass, wood and thin metals cannot be ruled out by plain radiographies^[2,3,6,11,13,20]. Neck, chest and abdominal radiographies are able to show perforations as well as metal objects and bones^[6,13]. In our study, we detected a foreign body with plain radiography in 27 (36%) out of 75 patients evaluated by X-rays; all of these foreign bodies were radiopaque. All of the 19 patients with radiopaque foreign bodies in whom an emergency intervention was not planned were admitted for serial radiographic evaluations to determine the passage of the foreign body. Serial radiographic studies can be used to determine the passage of the foreign body and the complications resulting from it. If perforation is suspected based on the clinical or radiological findings, neck, chest or abdominal computed tomography is then indicated^[13]. Computed tomography (CT) is especially useful when radiolucent materials cannot be detected with plain X-rays. A three-dimensional reconstruction with CT also increases the sensitivity of the detection modality^[2,6,21-23]. CT can also be useful in determining, treatment options and complications.

Foreign bodies in the GI tract are typically treated conservatively, based on the type of foreign body and the patient's clinical condition. Between 80% and 90% of foreign bodies pass through the GI tract freely, while 10% to 20% require an endoscopic intervention, and 1%



Figure 2 Plain abdominal X-ray showing a pin (white arrow) in the bowel in an adult. The pin passed spontaneously.



Figure 3 Lateral neck X-ray showing a bone fragment (white arrow). The fragment was removed by fiberoptic means.

Table 2 Types of foreign bodies swallowed

	Foreign body type	Total detected foreign bodies	Patients underwent X-ray	Foreign bodies detected with X-ray	Patients who underwent fiberoptic evaluation	Foreign bodies detected with endoscopy
	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>
Food	52	20	33	2	21	12
Pin	19	17	19	17	10	6
Toothpick	5	1	3	-	4	1
Dental instrument	5	2	5	2	2	-
Tooth filling	4	2	4	2	2	1
Nail	2	2	2	2	2	1
Water gel beads (pearl shape crystal soil)	1	-	1	-	-	-
Gelatin paper	1	-	-	-	1	-
Pen cap	1	-	-	-	1	-
Earring	1	-	1	-	1	-
Coin	1	1	1	1	-	-
Medication	2	-	1	-	-	-
Chewing gum	1	1	1	1	1	-
Unknown	5	-	4	-	-	-
Total	100	46	75	27	45	21

require surgery^[4,6-9,11,13,17,24,25]. Physicians should determine if and when an intervention is needed. The patient management strategies depend on a patient's age and clinical condition, the type and size of the foreign body, the presence of sharp edges, the anatomical location and the endoscopic capability of the treating unit^[2,3,5]. In general, foreign bodies larger than 2.5 cm in diameter cannot pass the pylorus, while objects longer than 6 cm cannot pass the duodenal curve. Therefore, these objects require endoscopic removal^[2,6,19,26]. Endoscopic intervention is also indicated if the patient's condition is not stable or if the foreign body is impacted or presents risks of further damage to the patient^[13,27]. An emergent endoscopic removal should be performed in patients with esophageal obstruction (*e.g.*, cannot swallow secretions), those with disc batteries in the esophagus and those who have swallowed pointed objects^[6,13]. However, endoscopic removal is contraindicated if the foreign body is above the upper esophageal sphincter or if there is clinical or radiological evidence of perforation. Objects containing illegal drugs must be removed with endoscopy, but this technique should be avoided in cases where ruptured cocaine pack-

ages are present in the GI tract^[6,13]. Objects located proximal to the upper esophageal sphincters are suggested to be removed by ear-nose-throat specialists^[6,13]. Emergent endoscopy should be performed to remove magnets if they are within the reach of the technique^[6]. Monitoring of the spontaneous passage of coins in asymptomatic patients is recommended. If there is no spontaneous passage, then removal within 24 h of ingestion is recommended^[6,9]. We conducted conservative monitoring in slightly over half of our patients (53 out of 100). The diagnostic yield was 21 (47%) out of 45 for all patients undergoing fiberoptic evaluations. Endoscopic treatment options for meat or other food impactions included food extraction and advancement of the bolus into the stomach^[6,9]. In our study, an X-ray was performed on 33 patients with accidental food ingestions but yielded a positive result in only two cases. By contrast, among 21 patients with accidental food ingestion who underwent fiberoptic evaluation, 12 patients were found to have a foreign material, and the material was removed. Food ingestion, a subjective feeling of foreign body sensation, and other properties of foreign bodies may have resulted

in the poor yield of standard imaging studies and endoscopic and physical examinations^[11,27].

An alternative radiological tool that was not systematically assessed in our study was ultrasonography, which is uncommonly used to diagnose GI foreign bodies in adults^[28]. However, there are reports in the literature where ultrasonography proves to be useful in the detection of abdominal foreign bodies^[9,29]. However, abdominal ultrasonography can be used as an initial imaging modality in the diagnosis of GI foreign bodies in pediatric patients^[30].

Conditions such as acute abdomen due to intestinal perforations are seen in nearly 1% of patients who have ingested foreign bodies^[8,31]. This condition can lead to severe complications and even death^[32]. The most common complication of foreign body ingestion is perforation^[33]; the ingestion of a sharp and pointed object is more likely to cause perforations^[9,33,34]. Approximately 30%-35% of such objects can penetrate the GI tract, requiring endoscopic management^[35]. In the presence of complications or in the case of unsuccessful endoscopic interventions, emergency surgery is preferred^[35,36]. The majority of foreign bodies pass through the GI tract freely, without any complication, and only a small percentage of these cases require intervention^[4,9].

In conclusion, a majority of the swallowed foreign bodies in patients presenting to the ED cannot be detected using standard imaging studies and evaluation. Plain radiography is especially useful in the localization of radiopaque foreign bodies, while fiberoptic methods can be used both as diagnostic and therapeutic tools, regardless of the radiopacity of the foreign body ingested. The goal of ED management is to refer patients with clinical significance to the appropriate departments for further evaluation and treatment. It is therefore important to evaluate the type and location of the foreign body and to identify complications that might develop as a result of this entity.

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COMMENTS

Background

Ingestions or insertions of foreign bodies are rarely seen in adults, are generally accidental and are commonly seen in the form of food ingestion (such as meat and bones). Nonetheless, foreign body ingestions into the gastrointestinal tract can lead to clinically serious conditions with significant morbidity and mortality. Approximately 80%-90% of the foreign bodies pass through the gastrointestinal tract freely, while 10%-20% require an endoscopic intervention, and 1% require surgery.

Research frontiers

The majority of swallowed foreign bodies in patients presenting to emergency departments cannot be detected using standard imaging tools. The research hotspot discussed here is that fiberoptic methods were implemented in both the diagnosis and treatment of gastrointestinal foreign bodies.

Innovations and breakthroughs

The most common approach for the detection of ingested foreign bodies in the gastrointestinal tract is physical examination combined with radiological studies. While radiological examinations are especially helpful in the detection of radiopaque foreign bodies, these studies are unyielding in a significant proportion of patients, depending on the timing of presentation and the nature of the ingested foreign body. Early evaluation with fiberoptic methods helps not only in the diagnosis and localization of the foreign body but also in its removal.

Applications

Dietary habits and various cultural factors can provide clues for the types of foreign bodies likely to be ingested. Fiberoptic methods can be used to diagnose and treat accidental foreign body ingestions in an emergency department setting.

Peer review

The present study is a retrospective analysis of 100 patients with complaints related to foreign body ingestions. The data suggest that plain radiography is especially useful in the localization of radiopaque foreign bodies, while fiberoptic methods can be used as both diagnostic and therapeutic tools, regardless of the radiopacity of the foreign body ingested. This study highlights interesting points regarding the clinical treatment of complaints related to foreign body ingestions.

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Nonalcoholic fatty liver disease is associated with coronary artery disease in Koreans

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RESULTS: Of the 134 patients who met the inclusion criteria, 82 (61.2%) had ultrasonographically diagnosed NAFLD. Among the 46 patients with CAD, 37 (80.4%) had evidence of a fatty liver. The two groups (A vs B-D) were significantly different in terms of age, total cholesterol, triglycerides, low-density lipoprotein levels and fatty liver. Coronary artery stenosis was strongly associated with fatty liver in a grade-dependent manner ($P = 0.025$). In binary logistic regression, NAFLD was a significant independent predictor of CAD ($P = 0.03$, OR = 1.685; 95%CI: 1.051-2.702). Among the candidate mediators, the serum adiponectin level showed a trend toward lowering based on CAD progression ($P = 0.071$).

CONCLUSION: NAFLD is an independent risk factor for CAD in a grade-dependent manner. Moreover, adiponectin might be related to the pathogenesis of NAFLD.

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Key words: Nonalcoholic fatty liver disease; Coronary artery disease; Coronary angiography; Adiponectin; Insulin resistance

Abstract

AIM: To investigate whether nonalcoholic fatty liver disease (NAFLD) affects coronary artery disease (CAD) and identify candidate mediators.

METHODS: Patients who underwent coronary angiography were consecutively recruited. The patients were classified into four groups by coronary artery stenosis: A, insignificant; B, one-vessel disease; C, two-vessel disease; and D, three-vessel disease. Abdominal ultrasonography was performed to determine the presence of a fatty liver and categorize by grade: 0, no evidence; 1, mild; 2, moderate; and 3, severe. We measured not only known CAD risk factors, but also serum insulin, HOMA-index, adiponectin, interleukin-6, tumor necrosis factor- α and high-sensitivity C-reactive protein levels.

Core tip: This article shows that angiographically proven coronary artery stenosis is strongly associated with nonalcoholic fatty liver disease (NAFLD) in a grade-dependent manner. Although many recent studies used coronary artery calcification score, carotid artery intima-media thickness, or carotid artery plaque measurements as surrogate markers for coronary artery disease (CAD), we evaluated the interaction between fatty liver and cardiovascular outcomes using coronary angiograms in a prospective case-controlled study. Because the pathogenesis of NAFLD and CAD is not fully elucidated, we attempted to identify mediators of these diseases and believe that adiponectin might be related to the development and progression of CAD in patients with NAFLD.

Choi DH, Lee SJ, Kang CD, Park MO, Choi DW, Kim TS, Lee W, Cho BR, Kim YH, Lee B, Ryu DR, Lee JW. Nonalcoholic fatty liver disease is associated with coronary artery disease in Koreans. *World J Gastroenterol* 2013; 19(38): 6453-6457 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6453.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6453>

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common disorder with an increasing prevalence of approximately 34% of the adult population in the United States^[1]. Patients with NAFLD can progress to more aggressive forms of nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis, end-stage liver disease, and eventually hepatocellular carcinoma^[2]. Because NAFLD is related to metabolic syndrome and obesity, many patients with NAFLD have coronary artery disease (CAD). Several studies have reported that NAFLD is a strong independent risk factor for CAD^[3,4]. However, these studies have some clinical application limitations because of the use of indirect modalities, such as coronary artery calcification or intima-media thickness despite coronary artery imaging. Authors of these studies suggested that the presence of CAD was indicated by coronary artery calcification or intima-media thickness despite conducting coronary artery imaging^[5,6]. Many NAFLD studies conducted in Western populations have found a relationship between NAFLD and CAD in relatively obese patients, which has not been found in Asian populations^[6,7]. Therefore, the relationship between NAFLD and CAD in relatively thin Asian people must be evaluated. This study was conducted to evaluate whether NAFLD independently affects angiographically proven CAD in Asians and, if so, which mediator is responsible for this association.

MATERIALS AND METHODS

Subjects and study design

From January 2009 to June 27, 2011, 184 adult patients who underwent elective coronary angiography (CAG) at Kangwon National University Hospital were consecutively recruited. Indications for CAG included Canadian Cardiovascular Society class III or IV angina upon medical treatment, high-risk findings upon noninvasive testing, acute coronary syndrome, or a chest pain evaluation according to the American College of Cardiology/American Heart Association recommendations^[8]. Standard selective CAG was performed by three experienced cardiologists and reviewed by another cardiologist. CAD was defined as the presence of at least a 50% stenosis in at least one major coronary artery. The patients were classified into four groups according to the number of major coronary arteries affected by CAD: A, insignificant coronary artery stenosis; B, one-vessel disease; C, two-vessel disease; and D, three-vessel disease.

We excluded patients with viral hepatitis (positive for

hepatitis B surface antigen and anti-hepatitis C virus), history of alcohol ingestion (> 30 g/d for men and > 20 g/d for women), history of drug use reported to cause steatosis (steroids, estrogens, tamoxifen, amiodarone, valproic acid, diltiazem, or methotrexate), improved steatosis (metformin, statins, or glitazones) within 3 mo of enrollment, or other history of chronic liver disease. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using antihypertensive medications. Diabetes was defined as fasting blood sugar ≥ 126 mg/dL or using glucose-lowering medications (oral agents or insulin). Of the 184 patients, we excluded 50 with at least one potential cause for chronic liver disease. Altogether, 134 patients were enrolled and underwent abdominal ultrasonography within 2 d after CAG by a single experienced physician to determine the presence of four fatty liver grades: 0, no evidence of fatty liver; 1, mild; 2, moderate; and 3, severe degree. The presence of a fatty liver was identified by characteristic echo patterns such as a diffuse increase in the echogenicity of the liver compared with that of the kidney according to conventional criteria^[9]. We measured not only known risk factors (*i.e.*, age, male gender, high low-density lipoprotein, low high-density lipoprotein, triglyceride, body mass index, diabetes, and hypertension) for CAD but also serum insulin, HOMA index, adiponectin, interleukin (IL)-6, tumor necrosis factor (TNF)- α and high-sensitivity C-reactive protein (hs-CRP) levels. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of Kangwon National University Hospital.

Statistical analysis

Clinical and biochemical variables were compared between the two groups (A *vs* B-D). Continuous variables were assessed with the unpaired Student's *t*-test, and nominal variables were compared with the chi-square test. Variables that were significantly different between the two groups were extracted and included as covariates in a binary logistic regression with CAD as the dependent and NAFLD as the independent variable. Correlations between CAD severity and NAFLD degree were analyzed using Pearson's correlation analysis. A *P*-value < 0.05 was considered significant. All analyses were conducted using the SPSS for Windows 12.0.1 statistical software (SPSS, Inc., Chicago, IL, United States).

RESULTS

A total of 134 (37 males and 97 females) patients met the inclusion criteria for the study. Table 1 demonstrates the demographic, clinical and laboratory data of the subjects without CAD (A) and those with CAD (B-D). The two groups were significantly different in terms of age, total cholesterol, triglycerides, low-density lipoprotein levels and presence of NAFLD. In addition, there tended to be more clinical features associated with metabolic syndrome in the CAD group, but the difference was not significant. In each

Table 1 Comparison of clinical characteristics and laboratory data between subjects with and without coronary artery disease

	Group A Insignificant stenosis (<i>n</i> = 88)	Group B-D Significant stenosis (<i>n</i> = 46)	<i>P</i> value
Age (yr)	62.5 ± 10.8	65.2 ± 9.2	0.010
Sex (male)	20 (22.7)	17 (37.0)	0.104
DM	11 (12.5)	10 (21.7)	0.211
HTN	49 (55.7)	33 (71.7)	0.093
Height (cm)	155.0 ± 7.4	156.4 ± 8.4	0.333
Weight (kg)	61.9 ± 8.2	62.5 ± 10.0	0.734
BMI (kg/m ²)	25.8 ± 3.3	25.6 ± 3.4	0.697
Waist circumference (cm)	86.8 ± 13.4	89.7 ± 6.9	0.169
Hip circumference (cm)	97.6 ± 13.8	98.7 ± 8.3	0.607
WHR	0.89 ± 0.9	0.91 ± 0.8	0.238
Total cholesterol (mg/dL)	177.1 ± 30.8	195.6 ± 39.1	0.009
HDL cholesterol (mg/dL)	41.2 ± 12.2	38.4 ± 12.1	0.227
Triglyceride (mg/dL)	134.9 ± 72.4	177.4 ± 94.4	0.012
Measured-LDL cholesterol (mg/dL)	102.3 ± 26.1	115.5 ± 33.3	0.015
Calculated-LDL cholesterol (mg/dL)	108.6 ± 28.3	121.7 ± 33.7	0.033
Creatinine (mg/dL)	0.8 ± 0.3	1.1 ± 0.4	0.068
Uric acid (mg/dL)	4.6 ± 1.4	4.9 ± 1.6	0.399
Hemoglobin (g/dL)	13.4 ± 1.8	13.0 ± 1.5	0.356
HbA1c (%)	5.7 ± 0.7	6.3 ± 1.2	0.072
Systolic BP (mmHg)	123.3 ± 16.6	125.6 ± 15.6	0.409
Diastolic BP (mmHg)	73.8 ± 10.7	75.9 ± 9.6	0.250
FBS (mg/dL)	104.2 ± 21.2	115.3 ± 37.3	0.082
Total bilirubin (mg/dL)	1.0 ± 0.5	1.1 ± 0.6	0.432
Albumin (g/dL)	3.9 ± 0.3	3.9 ± 0.4	0.465
AST (U/L)	34.1 ± 55.0	27.1 ± 11.0	0.394
ALT (U/L)	30.7 ± 53.4	22.8 ± 9.7	0.321
PT INR	0.9 ± 0.3	0.8 ± 0.1	0.182
HOMA-index	6.29 ± 9.16	5.99 ± 5.39	0.838
NAFLD	44 (51.2)	36 (78.3)	0.002

Data are expressed as mean ± SD or *n* (%). DM: Diabetes mellitus; HTN: Hypertension; BMI: Body mass index; WHR: Waist-hip ratio; HDL: High density lipoprotein; LDL: Low density lipoprotein; BP: Blood pressure; FBS: Fasting blood sugar; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PT: Prothrombin time; HOMA-index: Homeostatic model assessment of insulin resistance, fasting insulin (mU/L) × fasting glucose (mmol/L)/22.5; NAFLD: Nonalcoholic fatty liver disease.

group, women were predominant, and all subjects were post-menopausal except for one person in the CAD group.

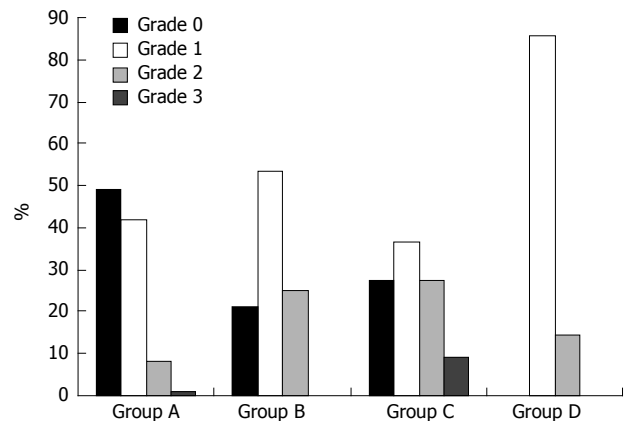
An analysis of the relationship between NAFLD and the presence of CAD is shown in Table 2. In addition to the significantly different variables between the two groups in Table 1, well-known established risk factors for CAD, such as age, gender, glucose, HbA1c and body mass index, were considered as covariates in conducting the multivariate analysis. In those models, as shown in Table 2, NAFLD was the significant independent predictor for CAD ($P = 0.03$, OR = 1.685; 95%CI: 1.051-2.702).

Next, we evaluated the correlation between the NAFLD degree and CAD severity. The proportion of patients with NAFLD increased from 51.1% in group A to 100% in group D. In group A, most of the fatty livers were grade 1. However, in the higher grade CAD group, the proportion of patients with more severe fatty livers was increased. No subject in group D (three-vessel

Table 2 Multivariate analysis of coronary artery disease with age, nonalcoholic fatty liver disease and metabolic risk factors

	OR (95%CI)	<i>P</i> value
NAFLD	1.685 (1.051-2.702)	0.030
Age	1.056 (1.010-1.104)	0.057
Total cholesterol (mg/dL)	1.012 (0.982-1.043)	0.427
TG (mg/dL)	1.004 (0.998-1.010)	0.873
Measured-LDL cholesterol (mg/dL)	1.003 (0.970-1.036)	0.225

NAFLD: Nonalcoholic fatty liver disease; TG: Triglycerides; LDL: Low density lipoprotein.

**Figure 1** Relationship between the grade of nonalcoholic fatty liver disease and severity of coronary artery disease.

disease) had a normal liver. Figure 1 shows that angiographically proven coronary artery stenosis was strongly associated with NAFLD in a grade-dependent manner by Pearson's correlation analysis ($P = 0.002$).

In addition, we measured the serum level of candidate mediators of metabolic syndrome, such as insulin, the HOMA index, IL-6, TNF- α , and hs-CRP (Table 3). In our results, none of the factors assessed were found to be related to CAD. However, serum adiponectin level demonstrated a trend toward lowering based on CAD progression ($P = 0.071$).

DISCUSSION

Our findings demonstrate that NAFLD is strongly associated with coronary artery stenosis in a grade-dependent manner. Our results also demonstrate that NAFLD is a significant predictor of CAD independent of traditional risk factors in Asians. Furthermore, we suggest that adiponectin might have a potential pathogenic role in the development and progression of CAD in patients with NAFLD.

Because NAFLD is a hepatic manifestation of metabolic syndrome, many studies have suggested that NAFLD results in increased cardiovascular risk and mortality^[7,10]. The risk for developing cardiovascular morbidity and mortality is thought to be higher than the risk for developing hepatic disease because of its slow progression. Therefore, many studies have investigated the association between NAFLD and cardiovascular diseases. As a result,

Table 3 Comparison of candidate mediators between subjects with and without coronary artery disease (mean \pm SD)

	Group A Insignificant stenosis (n = 88)	Group B-D Significant stenosis (n = 46)	P value
Adiponectin (μ g/mL)	8.40 \pm 5.97	6.95 \pm 5.85	0.071
IL-6 (pg/mL)	4.55 \pm 7.75	4.71 \pm 7.41	0.894
TNF- α (ng/mL)	4.00 \pm 3.95	4.85 \pm 4.73	0.273
hs-CRP(mg/dL)	0.45 \pm 1.70	0.74 \pm 1.18	0.366

IL: Interleukin; TNF: Tumor necrosis factor; hs-CRP: High sensitive C-reactive protein.

a number of studies have demonstrated that NAFLD is an independent risk factor for CAD^[4,5,11-13]. However, most studies used coronary artery calcification score, carotid artery intima-media thickness, carotid artery plaque measurements, or circulatory endothelial dysfunction as surrogate markers for CAD^[5,6,14]. Despite the fact that the coronary calcification score is a well-known marker for an increased risk of coronary events, the direct relationship between the presence of NAFLD and clinical CAD must be evaluated for use in the clinical setting^[3]. Recently, Wong *et al*^[15] evaluated the interaction between fatty liver and cardiovascular outcomes using coronary angiograms in a prospective cohort study and demonstrated that fatty liver is associated with CAD independently of other metabolic factors, which is consistent with our results. In contrast, our study was different from that study because we demonstrated that angiographically proven coronary artery stenosis was strongly associated with fatty liver in a grade-dependent manner.

Although the pathogenesis of NAFLD and CAD has not been fully elucidated, several explanations are present for the relationship between NAFLD and CAD. One widely accepted hypothesis implicates low-grade inflammatory conditions as key factors leading to hepatic steatosis and atherosclerosis^[16,17]. Moreover, oxidative stress is presumed to play a role in NASH pathogenesis. Many investigators have studied additional mechanisms that might be associated with NAFLD, which are supported by the levels of various biomarkers, such as reactive oxygen species, adipocytokines (leptin and adiponectin), CRP, and caspase-generated cytokeratin-18^[18-21]. In this study, we also tried to find candidate mediators of the mechanism of this relationship. We investigated several mediators, including adiponectin, IL-6, TNF- α , and hs-CRP. Among these candidate mediators, adiponectin may have been related to the development and progression of CAD in patients with NAFLD in our study. Adiponectin is the most abundant adipose-specific adipokine, and decreases hepatic insulin resistance and attenuates liver inflammation^[22]. Low levels of serum adiponectin might play an important role in the pathogenesis of clinical CAD and NAFLD. In contrast, NAFLD is also characterized by increased insulin resistance^[23]. We measured fasting serum insulin levels and calculated the HOMA index to confirm this relationship in our study. Because we included obese Asians, which in contrast with previous

Asian-Pacific NAFLD studies that included non-obese subjects, our study subjects had relatively high insulin resistance^[24]. However, fasting serum insulin levels and HOMA-IR were not different between our two groups (with/-without CAD and with/-without a fatty liver).

Some limitations of our study merit comment. First, our results were not based on a biopsy-proven NAFLD. There is no histology or staging of fibrosis by use of elastography to determine the liver fibrosis. We diagnosed NAFLD using hepatic ultrasonography. This technique does not identify fatty infiltration < 30% although it is a safe and confirmed reliable noninvasive method^[25]. This technique also has additional weak points, which are intra- and interobserver differences when making a diagnosis. However, to overcome these limitations, ultrasonography was performed by a single experienced physician to determine the presence of the four fatty liver grades. In addition, standard selective CAG was performed to diagnose and measure CAD severity by three experienced cardiologists in our study. To reduce interobserver variability for CAG, another cardiologist also reviewed all of the data. Second, this study was conducted at a single center in a rural area, which increased the chance for selection bias. Women were predominant in the included subject. A possible explanation for this gender imbalance is that men in this area had a high prevalence of alcohol intake and were excluded based on a history of alcohol ingestion.

Because NAFLD is considered a hepatic manifestation of metabolic syndrome, many studies have investigated the association between NAFLD and cardiovascular diseases. As a result, our study demonstrates that NAFLD is an independent risk factor for angiographically proven CAD in a grade-dependent manner. Because the pathogenesis of NAFLD and CAD are not fully elucidated, we also attempted to identify mediators and believe that adiponectin might be related to the development and progression of CAD in patients with NAFLD. Therefore, future large-scale studies are needed to elucidate the precise mechanism of this relationship.

COMMENTS

Background

Although recent many studies used coronary artery calcification score, carotid artery intima-media thickness, or carotid artery plaque measurements as surrogate markers for coronary artery disease (CAD), this study evaluated the interaction between fatty liver and cardiovascular outcomes using coronary angiograms in a prospective case-control study of Asians.

Research frontiers

The relationship between nonalcoholic fatty liver disease (NAFLD) and CAD in relatively thin Asian people must be evaluated. Moreover, because the pathogenesis of NAFLD and CAD are not fully elucidated, the authors attempted to identify candidate mediators.

Innovations and breakthroughs

This article show that angiographically proven coronary artery stenosis was strongly associated with NAFLD in a grade-dependent manner. In addition, the authors attempted to identify mediators and believe that adiponectin might be related to the development and progression of CAD in patients with NAFLD.

Applications

By understanding the association between NAFLD and CAD, patients with a se-

vere degree of fatty liver disease have to be concerned about CAD to improve their prognosis.

Peer review

This is a prospective single center study, which investigate the relationship between NAFLD and CAD and seeks candidate mediators. Future large-scale studies are needed to elucidate the precise mechanism of this relationship.

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Diet of patients after pouch surgery may affect pouch inflammation

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Abstract

AIM: To investigate the diet of pouch patients compared to healthy controls, and to correlate pouch patients' diet with disease behavior.

METHODS: Pouch patients were recruited and prospectively followed-up at the Comprehensive Pouch Clinic at the Tel Aviv Sourasky Medical Center. Pouch behavior was determined based on clinical, endoscopic and histological criteria. Healthy age- and sex-matched volunteers were selected from the "MABAT" Israeli

Nutrition and Public Health Governmental Study and served as the control group. All the participants completed a 106-item food frequency questionnaire categorized into food groups and nutritional values based on those used in the United States Department of Agriculture food pyramid and the Israeli food pyramid. Data on Dietary behavior, food avoidance, the use of nutritional supplements, physical activity, smoking habits, and body-mass index (BMI) were also obtained. Pouch patients who had familial adenomatous polyposis ($n = 3$), irritable pouch syndrome ($n = 4$), or patients whose pouch surgery took place less than one year previously ($n = 5$) were excluded from analysis.

RESULTS: The pouch patients ($n = 80$) consumed significantly more from the bakery products food group (1.2 ± 1.4 servings/d *vs* 0.6 ± 1.1 servings/d, $P < 0.05$) and as twice as many servings from the oils and fats (4.8 ± 3.4 servings/d *vs* 2.4 ± 2 servings/d, $P < 0.05$), and the nuts and seeds food group (0.3 ± 0.6 servings/d *vs* 0.1 ± 0.4 servings/d, $P < 0.05$) compared to the controls ($n = 80$). The pouch patients consumed significantly more total fat (97.6 ± 40.5 g/d *vs* 84.4 ± 39 g/d, $P < 0.05$) and fat components [monounsaturated fatty acids (38.4 ± 16.4 g/d *vs* 30 ± 14 g/d, $P < 0.001$), and saturated fatty acids (30 ± 15.5 g/d *vs* 28 ± 14.1 g/d, $P < 0.001$)] than the controls. In contrast, the pouch patients consumed significantly fewer carbohydrates (305.5 ± 141.4 g/d *vs* 369 ± 215.2 g/d, $P = 0.03$), sugars (124 ± 76.2 g/d *vs* 157.5 ± 90.4 g/d, $P = 0.01$), theobromine (77.8 ± 100 mg/d *vs* 236.6 ± 244.5 mg/d, $P < 0.001$), retinol (474.4 ± 337.1 μ g/d *vs* 832.4 ± 609.6 μ g/d, $P < 0.001$) and dietary fibers (26.2 ± 15.4 g/d *vs* 30.7 ± 14 g/d, $P = 0.05$) than the controls. Comparisons of the food consumption of the patients without ($n = 23$) and with pouchitis ($n = 45$) showed that the former consumed twice as many fruit servings as the latter (3.6 ± 4.1 servings/d *vs* 1.8 ± 1.7 servings/d, respectively, $P < 0.05$). In addition, the pouchitis patients consumed significantly fewer liposoluble antioxidants, such as cryptoxanthin (399 ± 485

$\mu\text{g/d}$ vs $890.1 \pm 1296.8 \mu\text{g/d}$, $P < 0.05$) and lycopene ($6533.1 \pm 6065.7 \mu\text{g/d}$ vs $10725.7 \pm 10065.9 \mu\text{g/d}$, $P < 0.05$), and less vitamin A ($893.3 \pm 516 \mu\text{g/d}$ vs $1237.5 \pm 728 \mu\text{g/d}$, $P < 0.05$) and vitamin C ($153.3 \pm 130 \text{ mg/d}$ vs $285.3 \pm 326.3 \text{ mg/d}$, $P < 0.05$) than the patients without pouchitis. The mean BMI of the pouchitis patients was significantly lower than the BMI of the patients with a normal pouch: 22.6 ± 3.2 vs 27 ± 4.9 ($P < 0.001$).

CONCLUSION: Decreased consumption of antioxidants by patients with pouchitis may expose them to the effects of inflammatory and oxidative stress and contribute to the development of pouchitis.

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Key words: Ulcerative colitis; Dietary reference intake; Body mass index; Ileal-pouch anal anastomosis; Pouch surgery; Food frequency questionnaire

Core tip: The diet of patients who had pouch surgery differed significantly from that of healthy individuals. Patients with pouchitis consumed significantly fewer fruit servings and antioxidants than patients with normal pouches, thus possibly exposing the former to inflammatory and oxidative stress. The body mass index of patients with pouchitis was significantly lower than patients with normal pouches, probably as a result of the continuous inflammatory burden.

Ianco O, Tulchinsky H, Lusthaus M, Ofer A, Santo E, Vaisman N, Dotan I. Diet of patients after pouch surgery may affect pouch inflammation. *World J Gastroenterol* 2013; 19(38): 6458-6464 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6458.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6458>

INTRODUCTION

Total proctocolectomy and the formation of a small bowel reservoir-ileal pouch anal anastomosis (IPAA, “pouch surgery”) is the surgery of choice for the treatment of severe, refractory or complicated ulcerative colitis (UC)^[1,2]. Pouch surgery has good short- and long-term outcomes and is associated with improved quality of life^[2]. Inflammation of the pouch (“pouchitis”) is the most common long-term complication, with a reported incidence of up to 60%^[3]. Nutrition is increasingly incorporated into the management of inflammatory bowel disease (IBD)^[4]. However, few studies have assessed the influence of nutrition on the health status of pouch patients. Rather most have focused on patients’ subjective feelings after consuming specific food products^[5]. Nevertheless, the potential contribution of nutrition to the development of inflammation in the pouch, as well as to IBD in general, remains unclear. Studies have shown that probiotic supplements, such as various strains of lactobacilli 3

(VSL#3), may prevent pouchitis after closure of the ileostomy, shorten the duration of the inflammation, and maintain remission^[6,7]. Several nutritional imbalances may also result from pouch surgery itself, including vitamin B12 and iron deficiency, fat malabsorption and electrolyte and trace element deficiencies^[8-11]. Such deficiencies may, in turn, cause or increase inflammation by mechanisms such as increased tissue oxidative stress^[10].

We hypothesized that diet modification and nutritional imbalance may occur after pouch surgery and that these could be associated with and predispose pouch patients to the development of pouchitis. We further assumed that such major changes might be related to the consumption of essential vitamins, minerals, antioxidants or fibers, which could thus potentially contribute to pouch inflammation^[11-15]. The purpose of the current investigation was thus to gather and analyze the detailed intake of food groups and nutrients as well as examine the nutritional and lifestyle habits of pouch patients, and test for correlations between these parameters and the occurrence of pouchitis.

MATERIALS AND METHODS

Study population

Pouch patients were recruited from the Comprehensive Pouch Clinic at the Tel Aviv Sourasky Medical Center (Tel Aviv, Israel), a tertiary referral center for IBD and the national referral center for pouch patients. Both an IBD-oriented gastroenterologist (Dotan I) and a colorectal surgeon (Tulchinsky H) examined all pouch patients. Healthy age- and sex-matched volunteers were selected from the “MABAT” Israeli Nutrition and Public Health Governmental Study cohort^[16]. Pouchitis was diagnosed by accepted clinical, endoscopic and histological criteria (the pouchitis disease activity index, PDAI^[17]). Pouch status was further defined as normal or pouchitis (recurrent acute pouchitis and chronic pouchitis) as previously described^[2]. Briefly, normal pouch status was defined as no clinical, endoscopic or histological criteria for pouchitis during the previous 2 years and no antibiotic or anti-inflammatory therapy of any type. Chronic pouchitis was defined by clinical, endoscopic and histological criteria that called for chronic administration of antibiotics or anti-inflammatory therapies for more than one month or when there were more than 5 flares of pouchitis within a year^[2,18,19]. Recurrent acute pouchitis was defined as ≤ 5 flares of pouchitis responding to a 2-wk course of antibiotics/year. The data on pouch patients who had familial adenomatous polyposis ($n = 3$), irritable pouch syndrome ($n = 4$), or patients who had had their pouch for less than one year ($n = 5$) were excluded.

Since there was no significant difference in the food and nutrient consumption between the patients with chronic pouchitis and those with recurrent acute pouchitis, they were combined into a single “pouchitis” group. The data for all of the enrolled pouch patients were compared to those of the healthy controls. Patients with a normal pouch status ($n = 23$) were further compared to

Table 1 Food group consumption in pouch patients and healthy controls (mean \pm SD)

Food group	Consumption healthy controls (<i>n</i> = 80, servings/d)	Consumption pouch patients (<i>n</i> = 80, servings/d)	Recommendation ¹	<i>P</i> value
Grains	6.1 \pm 4.0	6.9 \pm 4.01	6-11 ²	0.213
Bakery	0.6 \pm 1.1	1.2 \pm 1.4	6-11 ²	0.030
Potatoes	0.5 \pm 0.5	0.7 \pm 0.6	6-11 ²	0.063
Vegetables	3.9 \pm 2.7	3.6 \pm 2.7	3-5	0.49
Fruits	2.2 \pm 2.1	2.5 \pm 2.8	2-4	0.47
Dairy	3.7 \pm 2.6	4 \pm 3.3	2-3	0.52
Meat, fish and poultry	2.4 \pm 2.2	2.3 \pm 1.6	2-3 ³	0.945
Eggs	0.4 \pm 0.5	0.5 \pm 0.4	2-3 ³	0.206
Legumes	0.4 \pm 0.6	0.3 \pm 0.5	2-3 ³	0.094
Oils and fats	2.4 \pm 2.0	4.8 \pm 3.4	Limited	0.000
Nuts and seeds	0.1 \pm 0.4	0.3 \pm 0.6	Limited	0.012
Snacks and soft drinks	4.5 \pm 3.0	5.0 \pm 4.5	Limited	0.353
Water	6.0 \pm 3.9	6.0 \pm 3.5	-	0.913

¹Serving recommendations according to food pyramid (*n* = 20); ²6-11 servings are recommended for the grains, baked goods or potato categories; ³2-3 servings are recommended for the meat, fish and poultry, eggs, and legume categories.

patients with pouchitis (*n* = 45). All participants gave their informed consent. The study complied with the Helsinki Declaration and the ethical guidelines of our institution.

Questionnaires

All participants were prospectively interviewed using a food frequency questionnaire (FFQ). The 106 items on the FFQ were categorized into food groups according to those defined in the United States Department of Agriculture (USDA) food pyramid^[20] and the Israeli food pyramid^[21]. The questionnaire also included sub-food groups defined in the “MABAT” Israeli Nutrition and Public Health Governmental Study^[16]. The nutritional values of the food items were taken from the USDA FNDD, version 4.1^[22]. The nutritional values of several specific Israeli food items that do not appear in the USDA FNDD database were taken from the Israeli Ministry of Health food consumption and nutrients “TZAMERET” database, version 2^[23]. Pouch patients were also asked about their dietary behavior, food avoidance, and the use of nutritional supplements, as well as physical activity, smoking habits, and body-mass index (BMI). Assessment of the questionnaires was based on the recommended range of values established by the USDA FNDD^[22] and Israeli Health Ministry “TZAMERET”^[23] databases. The nutrient consumption of all participants was compared to the upper limits for daily nutrient recommendations for healthy males and females between the ages of 31-50 years as indicated in the USDA Dietary Reference Intake (DRI) 2010^[24].

Statistical analysis

Statistical analyses were conducted using SPSS software version 19.0 (SPSS Inc. Headquarters, S Wacker Drive, Chicago, IL, United States). A *P* value of < 0.05 was considered significant. Data are presented as mean \pm SD for continuous variables, and frequencies and percentages for categorical variables. Independent *t*-tests were used to compare pouch patients *vs* healthy controls for

food group and nutrient consumption. Fisher's exact test and independent *t*-tests were used to compare normal pouch patients to recurrent acute and chronic pouchitis patients for the categorical and continuous variables, respectively.

RESULTS

Diets of pouch patients vs controls

Eighty adult pouch patients were recruited and compared to 80 healthy adult volunteers. Subjects from both groups were matched for sex and age. Differences in their nutritional intake were first examined by comparing their consumption of servings of the main food groups. The major food groups were divided into subgroups based on the “MABAT” study distribution^[16]. The pouch patients consumed significantly more bakery, oils and fats, and nuts and seeds compared to the controls (Table 1). The consumption of other food groups was comparable. The total nutrient content of foods^[25] consumed by the patients and the control groups is shown in Table 2. The pouch patients' increased consumption of fat servings included significantly more total fat and fat components; *i.e.*, mono-unsaturated fatty acids and saturated fatty acids, than the controls. The pouch patients also consumed significantly higher amounts of several nutrients than the controls, *e.g.*, niacin, zinc, and vitamins C and D (Table 2). These higher levels were usually attributed to external supplements rather than to the diet itself. In contrast, the pouch patients consumed significantly fewer carbohydrates, sugars, theobromine, retinol and dietary fibers compared to the controls. Interestingly, neither the controls nor the pouch patients met DRI recommendations for dietary fiber intake (38 g/d^[24]).

Normal pouch vs pouchitis patients' diets

The pouch patients were divided into a normal pouch group (*n* = 23) and a pouchitis group (*n* = 45) (both recurrent acute and chronic). The demographic parameters of

Table 2 Consumption of nutrients in pouch patients and healthy controls (mean \pm SD)

Nutrient	Consumption healthy controls (<i>n</i> = 80)	%DRI	Consumption pouch patients (<i>n</i> = 80)	%DRI	<i>P</i> value
Energy (kcal)	2655.2 \pm 1313.7	-	2509.9 \pm 986.4	-	0.430
Proteins (g)	112.8 \pm 59.4	200%	113 \pm 42.5	201%	0.977
Total fat (g)	84.4 \pm 39	-	97.6 \pm 40.5	-	0.038
Carbohydrates (g)	369.0 \pm 215.2	284%	305.5 \pm 141.4	234%	0.029
Theobromine (mg)	236.6 \pm 244.5	-	77.8 \pm 100	-	< 0.001
Total sugars (g)	157.5 \pm 90.4	-	124 \pm 76.2	-	0.012
Total dietary fiber (g)	30.7 \pm 14.0	80%	26.2 \pm 15.4	69%	0.055
Zinc (mg)	13.3 \pm 6.5	121%	16.6 \pm 9.2	151%	0.01
Retinol (μ g)	832.4 \pm 609.6	-	474.4 \pm 337.1	-	< 0.001
Vitamin D (μ g)	7.9 \pm 5.7	158%	15.7 \pm 19.9	314%	0.001
Vitamin C (mg)	148.2 \pm 80.6	164%	210.3 \pm 225.4	233%	0.022
Niacin (mg)	34.4 \pm 20.0	212%	43.0 \pm 18.1	269%	0.005
Total monounsaturated fatty acids (g)	30 \pm 14	-	38.4 \pm 16.4	-	< 0.001
Total polyunsaturated fatty acids (g)	17.9 \pm 9.2	-	20.4 \pm 9.2	-	0.082
Total saturated fatty acids (g)	28.0 \pm 14.1	-	30 \pm 15.5	-	0.006
Total W3 fatty acids (g)	0.14 \pm 0.13	-	1.2 \pm 3.4	-	0.03
Total W6 fatty acids (g)	16.8 \pm 9.0	-	19.9 \pm 9.1	-	0.4

DRI: Dietary reference intakes.

Table 3 Demographic characteristics of pouch patient subgroups

	Normal pouch (<i>n</i> = 23)	Recurrent acute and chronic pouchitis (<i>n</i> = 45)	<i>P</i> value
Male/female	11/12	22/23	0.56
Age (yr)	53.2 \pm 13.7	43.0 \pm 14.9	< 0.001
Mean time since surgery (yr)	7.8 \pm 4.4	11.0 \pm 6.3	0.04
Operation stages (1/2/3)	4/16/3	4/34/7	0.4
Body mass index (kg/m ²)	27 \pm 4.9	22.6 \pm 3.2	< 0.001
Food avoidance	60.90%	73.30%	0.21
Probiotics consumption	30.40%	31.10%	0.6
Vitamins/supplement consumption	43.50%	66.70%	0.06
Smokers	4.30%	13.30%	0.24

the two groups are shown in Table 3. Comparison of the food consumption of the normal pouch patients to that of patients with pouchitis revealed significant differences in two main food groups; namely, fruits and vegetables (Table 4). Patients with a normal pouch consumed twice as many fruit servings as patients with pouchitis ($P < 0.01$) and tended to consume more vegetable servings than the pouchitis patients ($P < 0.01$). The consumption of other food groups was comparable regardless of pouch status. We hypothesized that these findings would be reflected in significantly less consumption of antioxidants. As predicted, pouchitis patients consumed significantly less liposoluble antioxidants, such as cryptoxanthin and lycopene, as well as less vitamins A and C than the normal pouch patients. Taken together, these data suggest that patients with pouchitis may be more exposed to oxidative stress as a result of their consumption of fewer fruits and vegetables. Interestingly, two-thirds of the patients with pouchitis supplemented their diet with vitamins and minerals, compared to 43.5% of the patients with a normal pouch ($P = 0.06$). Nevertheless, even after this supplementation, the total consumption of antioxidants was still significantly lower in the pouchitis group than in the normal pouch group. Seventy percent of all pouch

patients reported some type of food avoidance. The most frequently avoided foods were milk, citrus fruits, and spicy foods. Although up to 25% of all pouch patients avoided milk products, they met the recommended calcium intake level, mostly through supplements.

Only 26.2% ($n = 21$) of all pouch patients in the cohort used probiotics; 30.4% ($n = 7$) in the normal pouch group and 31.1% ($n = 14$) in the pouchitis group. Most of these were over-the-counter probiotics, and only 4 patients used the probiotic VSL#3.

BMI comparisons

Despite the comparable mean energy intake of patients with normal pouches and those with pouchitis, the mean BMI of both groups was significantly different, with the former having a significantly higher BMI than the latter. In terms of the normal BMI range for the healthy population (18.5-25 kg/m²)^[26], 15 patients (65%) in the normal pouch group fell into the overweight range compared to 35 patients (77%) the pouchitis group who were categorized in the normal or underweight range. This may suggest that inflammatory activity itself, rather than decreased caloric intake, plays a role in the significantly lower BMI of patients with pouchitis.

Table 4 Food group consumption in patients with recurrent acute and chronic pouchitis *vs* patients with a normal pouch (mean \pm SD)

Food group	Consumption normal pouch patients (<i>n</i> = 23, servings/d)	Consumption recurrent acute and chronic pouchitis patients (<i>n</i> = 45, servings/d)	Recommendation ¹	<i>P</i> value
Grains	7.0 \pm 3.5	7.3 \pm 4.5	6-11 ²	0.7
Bakery	1.0 \pm 1.4	1.2 \pm 1.3	6-11 ²	0.4
Potatoes	0.5 \pm 0.4	0.8 \pm 0.6	6-11 ²	0.15
Vegetables	4.5 \pm 3.0	3.3 \pm 2.1	3-5	0.06
Fruits	3.6 \pm 4.1	1.8 \pm 1.7	2-4	0.015
Dairy	4.3 \pm 3.0	3.7 \pm 3.0	2-3	0.43
Meat, fish and poultry	2.4 \pm 1.5	2.4 \pm 1.8	2-3 ³	0.99
Eggs	0.6 \pm 0.5	0.5 \pm 0.4	2-3 ³	0.37
Legumes	0.2 \pm 0.4	0.3 \pm 0.5	2-3 ³	0.28
Oils and fats	5.3 \pm 3.0	4.7 \pm 3.8	Limited	0.5
Nuts and seeds	0.4 \pm 0.8	2.3 \pm 0.4	Limited	0.55
Snacks and soft drinks	4.4 \pm 3.0	5.5 \pm 5.0	Limited	0.38
Water	6.0 \pm 3.9	6.0 \pm 3.5	-	0.93

¹Serving recommendations according to food pyramid (*n* = 20); ²6-11 servings are recommended for the grains, baked goods or potato categories; ³2-3 servings are recommended for the meat, fish and poultry, eggs, and legume categories.

Table 5 Consumption of nutrients in patients with recurrent acute and chronic pouchitis *vs* patients with a normal pouch (mean \pm SD)

Nutrient	Consumption normal pouch patients (<i>n</i> = 23)	%DRI	Consumption recurrent acute and chronic pouchitis patients (<i>n</i> = 45)	%DRI	<i>P</i> value
Energy (kcal)	2592.7	-	2538.2	-	0.836
Proteins (g)	117.9	210%	113.1	201%	0.667
Total fat (g)	98.2	-	99.5	-	0.882
Carbohydrates (g)	321.3	247%	307.3	236%	0.709
Vitamin A-RAE (μ g)	1237.5 \pm 728.0	137%	893.3 \pm 516.0	99%	0.027
Beta-carotene (μ g)	7180.5 \pm 7394.1	66%	4453 \pm 4960.6	41%	0.075
Cryptoxanthin (μ g)	890.1 \pm 1296.8	-	399 \pm 485	-	0.027
Lycopene (μ g)	10725.7 \pm 10065.9	-	6533.1 \pm 6065.7	-	0.036
Vitamin C (mg)	285.3 \pm 326.3	316%	153.35 \pm 130	170%	0.02

RAE: Retinol activity equivalents; DRI: Dietary reference intake.

DISCUSSION

Increased attention has been paid in recent years to the role of nutrition in the treatment of IBD patients^[4,27], and its putative contribution to inflammation continues to be a topic of considerable interest^[14,15]. UC patients undergoing pouch surgery are exposed not only to the consequences of total removal of the large bowel and reconstruction of an ileal reservoir, but also to the potential influence of nutrition on inflammatory processes. Thus it is surprising that there are no nutritional guidelines for these patients. Moreover, there is only sparse information on nutrition among pouch patients and its relationships to the development, treatment, and prevention of pouch inflammation. In this prospective cross-sectional study, we employed the FFQ to characterize pouch patients' dietary consumption to analyze correlations between diet and pouch inflammation. We hypothesized that nutrition could be significantly impaired in these patients, which would have possible implications for the inflammation of the pouch.

The results indicate major differences in the diet of pouch patients as compared to healthy individuals and, more importantly, between patients with normal pouches and those with pouchitis. In particular, pouch patients

consumed significantly higher servings of fats and oils compared to healthy controls, and patients with pouchitis consumed fewer fruit servings and antioxidants than patients with a normal pouch. These findings on fat and oil consumption may be crucial since USDA nutritional guidelines recommend that fats should be consumed sparingly^[20,28]. Sakamoto *et al.* for instance found that high consumption of fats and oils is associated with increased risk of CD^[12]. The same may apply to the development of pouchitis, which, similar to CD, is an inflammation of the small bowel in an IBD patient.

Our patients with normal pouches consumed twice as many servings from the fruit food group than the pouchitis patients (Table 4). They also tended to consume more servings from the vegetable food group. Low consumption of fruits and vegetables has been shown to be inversely related to inflammation, as reflected by higher CRP levels^[29]. El Muhtaseb *et al.*^[10] for instance showed that pouch patients have significantly lower plasma concentrations of liposoluble antioxidants such as beta carotene, and that they have increased oxidative stress in plasma compared to healthy controls. This may imply that the low consumption of antioxidants and vitamin C observed in the pouchitis patients here may contribute to their low serum levels. According to D'Odorico *et al.*^[30]

this may lead to further oxidative damage. When DRI consumption of dietary fibers is below the recommended level, several mechanisms may lead to a similar effect^[31]. Intestinal bacteria ferment soluble fibers, producing short chain fatty acids such as butyrate^[31,32] as well as lactic acid^[32]. A shortage in butyrate was shown to be associated with the development of pouchitis^[32]. Second, lactic acid decreases fecal pH^[31], which may contribute to protection from pouchitis^[31] by inhibiting the proteolytic activity of bacterial glycosidases^[33].

Taken together, these results on the low consumption of antioxidants, vitamins and dietary fibers by pouchitis patients support our hypothesis that these imbalances may both predispose and be associated with the development of pouchitis in pouch patients. Whether the consumption of more antioxidants and vitamins can prevent further intestinal inflammation or even reverse it is an open question reserved for future studies. Notably, probiotic supplements were consumed by 26.2% of our pouch patients, but the probiotic formula VSL#3 that has been reported to be beneficial for the prevention of pouchitis^[34] was consumed by only 5%. This low rate of use may change in the near future since 2011 VSL#3 has now been included in the Israeli MOH health basket as a supplement for patients with pouchitis^[35].

A major finding of the current work is the correlation between BMI and the inflammatory state. Patients with normal pouches had significantly higher BMI ratios than patients with pouchitis, even to the point of being in the “overweight” range^[26]. This finding is intriguing given that there was no difference in energy intake between the normal pouch and the chronic pouchitis patient groups. Thus, differences in BMI might be due to increased malabsorption^[3], increased energy expenditure^[3] or differences in microbiota composition, which may lead to differential utilization of nutrients^[36]. This correlation between BMI and pouch inflammatory state also suggests that the inflammation itself contributes to energy expenditure, as we reported elsewhere for CD patients^[3].

In conclusion, the results of this study revealed significant differences in the consumption of food groups and nutrients between healthy controls and pouch patients, and between patients with normal pouches and those with pouchitis. These differences correlated, in part, with pouchitis and affected the patients’ BMI levels. Further studies on the mechanistic effects of nutrition on pouch inflammation are needed to help provide guidelines for nutritional counseling and interventions to alleviate the symptoms of pouchitis and modify its course.

COMMENTS

Background

Total proctocolectomy and the formation of a small bowel reservoir-ileal pouch anal anastomosis (IPAA, “pouch surgery”) is the surgery of choice for the treatment of severe, refractory or complicated ulcerative colitis (UC). Inflammation of the pouch (“pouchitis”) is the most common long-term complication, with a reported incidence of up to 60%. Nutrition has been increasingly incorporated into the management of inflammatory bowel disease (IBD). However, few studies have assessed the influence of nutrition on the health of pouch patients.

Moreover, the potential contribution of nutrition to the development of inflammation in the pouch, as well as in IBD in general, remains under-researched.

Research frontiers

The characteristics of pouch patients’ nutrition were prospectively evaluated using a food frequency questionnaire. The questionnaire data were analyzed for correlations between pouch disease behavior, as determined by clinical, endoscopic and histological criteria. Most previous nutritional studies on pouch patients have focused on their subjective feelings after consuming specific food products rather than on the overall relationships between various food groups and nutrients and pouch disease behavior.

Innovations and breakthroughs

The dietary intake and nutrient composition of pouch patients was analyzed for relationships with pouch disease behavior. The key finding shows that the diet of patients with pouch surgery differed significantly from that of healthy individuals. Moreover, patients with pouchitis consume significantly fewer fruit servings and antioxidants compared to patients with normal pouches, possibly exposing the former to inflammatory and oxidative stress. The body mass index of patients with pouchitis was significantly lower than patients with normal pouches, probably as a result of the continuous inflammatory burden.

Applications

The findings suggest that the consumption of fruits and vegetables, as well as supplementation with specific vitamins, minerals and antioxidants may be beneficial for patients with pouchitis. Specific nutritional consultation for pouch patients is advisable.

Terminology

Pouch surgery: This is the surgery of choice for the treatment of severe, refractory or complicated UC. The large bowel and the rectum are removed (total proctocolectomy), and a reservoir (“pouch”) constructed of the normal small bowel is created and connected to the anus (IPAA). Pouchitis: Inflammation of the small bowel (that was originally normal, not inflamed) creating the pouch.

Peer review

This research compares the dietary and nutritional treatment of pouch patients and pouchitis. This manuscript is a meaningful and enlightening study in general because it reveals the dietary differences between pouch patients and controls as well as between patients with or without pouchitis, which can guide further investigations on this topic.

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Consumption of spicy foods and the prevalence of irritable bowel syndrome

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of spicy foods and prevalence of irritable bowel syndrome (IBS) among Iranian adults.

METHODS: In this cross-sectional study, data from 4763 Iranian adult participants were used. Consumption of spicy foods was estimated using a dietary habits questionnaire that included a question on spicy foods consumption: "how frequently do you use spicy foods (pepper, curry, ginger, cinnamon and turmeric) during a week?" Participants could respond to the question by choosing one of these choices: never, 1-3 times, 4-6 times, 7-9 times, or more than 10 times per week. A modified Persian version of the Rome III questionnaire was used to determine the prevalence of IBS.

RESULTS: IBS was prevalent in 21.7% (18.6% of men and 24.1% of women) of the study population. After controlling for potential confounders including dietary behaviors, those consuming spicy foods ≥ 10 times per week were 92% more likely to have IBS compared with those who never consumed spicy foods (OR = 1.92; 95%CI: 1.23-3.01, $P_{\text{trend}} < 0.01$). The association remained significant even after taking lactose intolerance into account (OR = 1.85; 95%CI: 1.18-2.90, $P_{\text{trend}} < 0.01$). Stratified analysis by gender revealed that the association between consumption of spicy foods and IBS was not significant in men; however, a significant association was found among women after taking potential cofounders, including meal regularity and lactose intolerance, into account. Women who consumed spicy foods ≥ 10 times per week were two times more likely to have IBS compared with those who never consumed spicy foods (OR = 2.03; 95%CI: 1.09-3.77, $P_{\text{trend}} = 0.02$).

CONCLUSION: Consumption of spicy foods is directly associated with IBS, particularly in women. Further, prospective studies are warranted to (1) examine this association in other populations; and (2) evaluate whether dietary interventions, for example a reduction

Abstract

AIM: To explore the association between consumption

in spice consumption, would improve IBS symptoms.

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Key words: Spice; Diet; Condiments; Red pepper; Irritable bowel syndrome; Functional gastrointestinal disorders

Core tip: The role of dietary habits, including consumption of spicy foods, in the development of functional gastrointestinal disorders remains controversial. In this cross-sectional study in a large sample of Iranian adults, we found that women with high consumption of spicy foods had a two-fold increased risk of developing irritable bowel syndrome compared with women who reported not to consume spicy foods. The results underline the need for further studies to characterize potential relationships between diet-related practices and the risk of functional gastrointestinal disorders, in order to design appropriate, and effective, diet-based interventions.

Esmailzadeh A, Keshteli AH, Hajishafiee M, Feizi A, Feinle-Bisset C, Adibi P. Consumption of spicy foods and the prevalence of irritable bowel syndrome. *World J Gastroenterol* 2013; 19(38): 6465-6471 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6465.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6465>

INTRODUCTION

Functional gastrointestinal disorders (FGIDs) are characterized by chronic and recurrent gastrointestinal (GI) symptoms with ambiguous pathophysiology^[1]. The most common FGID is irritable bowel syndrome (IBS), which is characterized by abdominal pain and changes in stool consistency and/or frequency^[2-5]. It has been estimated that 5%-10% of the adult population in Asian countries^[6] and 10%-20% of the population in developed countries^[7] are affected by IBS. In Iran, the prevalence of IBS has been reported to be 1.1%-25% based on different studies^[8]. Since there is no established medical therapy to alter the natural history of IBS in the longer term, the disorder represents a considerable financial burden to the health service, owing to medical consultations and consumption of other valuable resources^[9].

Diet appears to play an important role in the etiology of FGIDs^[10-12]. Dietary intake of carbohydrates and fatty foods along with caffeine, alcohol and spices have been linked to IBS^[13,14]. Consumption of other foods and nutrients has also been implicated in the induction of symptoms in IBS^[15-20]. Some studies have suggested that IBS symptoms might result from food sensitivities rather than altered diet composition^[10].

The consumption of spicy foods has received attention in relation to FGIDs^[21,22]. Earlier studies have shown that chili, with its pungent ingredient, capsaicin,

exacerbates abdominal pain and burning in IBS patients. In contrast, chronic consumption of chili has been found to result in an improvement in IBS-related symptoms^[11]. Six-week administration of four pills per day each containing 150 mg of red pepper powder was reported to be effective in improving the intensity of abdominal pain in IBS patients^[21]. Other studies have also reported the beneficial effects of spicy foods in the management of FGID symptoms^[22]. However, most previous studies have focused on chili and its ingredients, and no information is available on other spicy foods. Furthermore, earlier investigations have mostly used high doses of spices as a treatment, and limited data are available examining the habitual consumption of spicy foods and its relationship to the prevalence of IBS.

The traditional Iranian diet contains large amounts of spicy foods, including turmeric, saffron, and ginger, providing an opportunity to assess consumption of spicy foods in relation to health. In addition, few data exist about the association between diet and FGIDs, and available evidence has mostly been reported from small samples, thus, no data are available from large populations. The Study on the Epidemiology of Psychological, Alimentary Health and Nutrition (SEPAHAN), which has been performed in a large group of Iranian adults, provides a unique opportunity to investigate the epidemiological aspects of FGIDs and their relationship with different lifestyles, including nutritional factors^[23]. Here, we present the sub-study that aimed to explore the association between consumption of spicy foods and the prevalence of IBS among Iranian adults.

MATERIALS AND METHODS

Study population

This cross-sectional study was carried out within the framework of the SEPAHAN project. This project was conducted in two main phases in a large sample of Iranian adults working in 50 different healthcare centers across Isfahan province, Iran^[23]. In the first phase of SEPAHAN, questionnaires on demographic information, medical history, anthropometric measures, lifestyle and nutritional factors were sent to 10087 persons, and 8691 subjects returned the completed questionnaires (response rate: 86.16%). In the second phase, another set of questionnaires was sent out to obtain data on gastrointestinal health of participants. After linking data from both phases and considering missing data, 4763 people who provided complete information on diet and FGIDs were included in the current analysis. The Bioethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran, approved the study.

Assessment of spicy foods consumption

We used a dietary habits questionnaire that contained detailed questions about meal frequencies, regularity of meals and drinking fluids before and after meals, as well as a question relating to the consumption of spicy foods:

“how often do you use spicy foods (chili pepper, curry, ginger, cinnamon, and turmeric) during a week?” Participants could respond to this question by choosing one of the following choices: never, 1-3 times, 4-6 times, 7-9 times, or more than 10 times per week. Responses to this question were used as the main exposure variable in the current study.

Assessment of IBS

A modified Persian version of the Rome III questionnaire, as part of the main comprehensive questionnaire, was used for the identification of FGIDs, including IBS^[23]. During the face validation of the questionnaire, we found that most participants were unable to distinguish between the descriptors used in the original Rome III questionnaire (never, less than one day a month, one day a month, two to three days a month, one day a week, more than one day a week, every day). We, therefore, modified the rating scales to consist of only four descriptors (*i.e.*, never or rarely, sometimes, often, always)^[23]. Participants were also asked about the presence of each symptom in the previous three months. IBS was defined according to Rome III criteria as recurrent abdominal pain or discomfort at least sometimes in the previous 3 months associated with two or more of the following criteria: (1) improvement with defecation at least sometimes; (2) pain onset associated with a change in stool frequency; and (3) pain onset associated with a change in form (appearance) of stool at least sometimes.

Assessment of other variables

Standard questionnaires were distributed to collect information on age, gender and educational status. Weight, height and the presence of diabetes mellitus were evaluated by a self-administered questionnaire. Data on smoking were collected through self-reported responses to the questionnaire and participants were categorized as non-smokers, ex-smokers and current smokers. The use of dietary supplements (yes/no) and oral contraceptive pills (OCP) (yes/no) as well as patterns of tea consumption (never or less than 1 cup/mo, 1-3 cups/mo, 1-3 cups/wk, 4-6 cups/wk, 1 cup/d, 2-4 cups/d, 5-7 cups/d, 8-11 cups/d, or at least 12 cups/d) were also assessed by a pre-tested questionnaire. Fluid intake was evaluated through questions on the consumption of water, soft drinks, yogurt drink (“dough”) and other beverages, before, after or during meals, which participants could answer as never, sometimes, often, or always. Regularity of meals was also assessed and quantified as never, sometimes, often, or always having regular meals. Study subjects were also categorized in terms of dental status as fully dentate, lost 1-3 teeth, lost 4-5 teeth, or lost half or more teeth. Quality of chewing was also evaluated (How thoroughly do you chew food?), with responses including: not very well, well, or very well. We also asked participants to describe their feelings/symptoms after milk intake. Lactose intolerance was defined as the existence of abdominal pain, bloating, diarrhea or belching after milk consumption^[24].

Statistical analysis

We categorized participants based on their reported frequency of consumption of spicy foods *i.e.*, never, 1-3 times, 4-6 times, 7-9 times, or 10 times or more during a week. Comparison of continuous variables across different categories of spicy foods consumption was performed using one-way analysis of variance. Distribution of study participants in terms of categorical variables across different categories of intake of spicy foods was compared using χ^2 test. To assess the relationship between spicy foods consumption and IBS, logistic regression analysis was performed in different models. First, we adjusted for age (continuous) and gender (categorical). We further controlled for smoking (non-smoker, ex-smoker and current smoker), dietary supplement (yes/no) and OCP use (yes/no), self-reported diabetes (yes/no) and body mass index (continuous) in the second model. Additional adjustments were made for meal regularity (never, sometimes, often and always), quality of chewing foods (not very well, well and very well), intra-meal fluid intake (never, sometimes, often and always), dental status (fully dentate, lost 1-3 tooth, lost 4-5 tooth, lost half or more tooth) and pattern of tea consumption (never or < 1 cup/mo, 1-3 cups/mo, 1-3 cups/wk, 4-6 cups/wk, 1 cup/d, 2-4 cups/d, 5-7 cups/d, 8-11 cups/d or at least 12 cups/d). In the final model, a further adjustment was made for lactose intolerance (yes/no). In all analyses, the category of never consuming spicy foods was considered as the reference category. To assess the trend of odds ratios across increasing categories of spicy foods intake, we applied Mantel-Haenszel extension chi-square. A stratified analysis by gender was also performed to examine gender-specific associations. All analysis was performed using SPSS version 16 (SPSS Corp, Chicago, IL, United States). *P* values less than 0.05 were considered statistically significant.

RESULTS

IBS was prevalent among 21.7% (18.6% of men and 24.1% of women) of the study population. General characteristics of study participants across different categories of spicy food consumption are summarized in Table 1. Those consuming spicy foods ≥ 10 times/wk were younger, had lower weight and were more likely to be women, married and highly educated compared with those who never consumed spicy foods. High consumption of spicy foods was associated with a lower prevalence of smoking and high prevalence of dietary supplement consumption and OCP use. There was no significant difference in the prevalence of self-reported diabetes among different groups of spicy food intake.

The prevalence of IBS across different categories of spicy foods consumption in the entire study population and each gender is shown in Figure 1. Consumption of spicy foods was associated with an increased prevalence of IBS among women, so that those consuming spicy foods ≥ 10 times/wk were more likely to have IBS com-

Table 1 General characteristics of study participants across different categories of spicy foods consumption

	Consumption of spicy foods (times/wk)					¹ P value
	Never	1-3	4-6	7-9	≥ 10	
Age (yr)	38.0 ± 8.3	37.4 ± 8.4	36.5 ± 7.8	35.1 ± 7.3	33.9 ± 7.6	< 0.001
Weight (kg)	70.3 ± 15.2	70.7 ± 13.4	68.2 ± 12	66.9 ± 14.0	65.4 ± 11.7	< 0.001
BMI (kg/m ²)	25.1 ± 5.3	25.2 ± 5.0	24.9 ± 4.0	25.0 ± 5.0	24.5 ± 3.8	0.16
Female	38.10%	41.30%	63.50%	73.50%	76.50%	< 0.001
Marriage	78.70%	81.10%	82.70%	79.00%	81.60%	0.03
University degree	44.90%	50.50%	62.00%	65.50%	65.20%	< 0.001
Current smokers	6.30%	4.20%	4.00%	1.40%	1.60%	0.01
Supplement use	4.10%	6.10%	8.30%	9.10%	9.80%	< 0.001
OCP use	1.00%	1.60%	3.00%	4.70%	4.40%	< 0.001
Self-reported diabetes	1.70%	1.80%	1.90%	1.70%	1.40%	0.72

All values are mean ± SD; ¹Obtained from ANOVA or χ^2 test, as appropriate. BMI: Body mass index; OCP: Oral contraceptive pill.

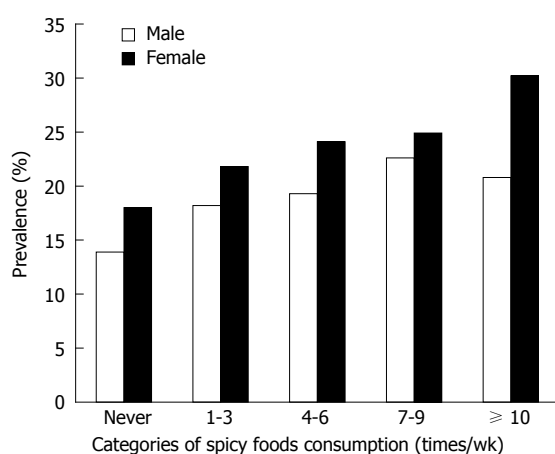


Figure 1 Prevalence of irritable bowel syndrome across different categories of spicy food consumption in men and women. Irritable bowel syndrome (IBS) was defined according to the Rome III criteria. Consumption of spicy foods was associated with an increased prevalence of IBS among women ($P < 0.05$). A trend for greater prevalence of IBS with consumption of spicy foods was also seen in men; however, a slight drop in its prevalence was evident in the top category.

pared with those who never consumed spicy foods (30.2% *vs* 18.0%, $P < 0.05$). A trend for greater prevalence of IBS with consumption of spicy foods was also seen in men; however, a slight drop in its prevalence was evident in the top category.

Multivariable adjusted odds ratio (OR) for IBS in the entire study population among different categories of spicy foods intake are illustrated in Table 2. Increased consumption of spicy foods was associated with a greater chance of having IBS in the crude model. After controlling for age and gender, those consuming spicy foods ≥ 10 times/wk were two times more likely to have IBS compared with those who never consumed spicy foods. The association remained significant even after taking other cofounders into account (OR = 1.92, 95%CI: 1.23-3.01). Further adjustment for dietary behaviors and lactose intolerance slightly attenuated the association, but it remained significant (OR = 1.85, 95%CI: 1.18-2.90).

Stratified analysis by gender revealed that the association between consumption of spicy foods and IBS

was not significant in men, even after controlling for cofounders (Table 3); however, a significant association was found among women after taking potential cofounders, including meal regularity and lactose intolerance, into account. Those who consumed spicy foods ≥ 10 times/wk were twice as likely to have IBS compared with those who never consumed spicy foods (OR = 2.03, 95%CI: 1.09-3.77, $P_{\text{trend}} = 0.02$).

DISCUSSION

We found that consumption of spicy foods was associated with increased prevalence of IBS among Iranian adults. This association remained significant even after adjustment for potential cofounders, including dietary behaviors. After stratified analysis by sex, the associations remained significant only in women. To the best of our knowledge, our study is among the first population-based studies that assessed habitual intake of spicy foods as a major exposure variable in relation to IBS.

Associations between the consumption of spicy foods and FGIDs, including IBS, have been examined previously^[11,13,14,21,22]. However, most studies have attempted to use spices to alleviate pain in these patients^[4,25,26], and limited data are available on the relationship between habitual consumption of spicy foods and symptoms of IBS^[11]. Furthermore, prior studies have mostly focused on pepper^[11,13,14,21,22], and effects of other spicy foods on IBS remain to be identified. In the current study, we found a significant, and direct, association between consumption of spicy foods and IBS. Our findings are in line with a population-based study in China, which showed a significant association between excessive intake of pepper and prevalence of IBS in adolescents^[14]. The link between spicy food consumption and IBS symptoms is also supported by an acute, meal-based study^[27], in which administration of a standard meal containing 2 g chili, either mixed into the meal or given separately in capsules, caused more abdominal pain and burning in IBS patients than in healthy participants. In contrast, in other studies, ingestion of 4 enteric-coated pills per day (each containing 150 mg of capsaicin-equivalent) for 6 wk, signifi-

Table 2 Multivariable-adjusted odds ratios and 95%CI for irritable bowel syndrome across different categories of spicy foods consumption¹

	Consumption of spicy foods (times/wk)					<i>P</i> _{trend}
	Never	1-3	4-6	7-9	≥ 10	
Crude	1.00	1.34 (0.95-1.88)	1.57 (1.12-2.21)	1.75 (1.21-2.52)	2.12 (1.45-3.10)	< 0.001
Model I	1.00	1.35 (0.92-1.97)	1.48 (1.00-2.18)	1.58 (1.04-2.39)	1.99 (1.30-3.06)	< 0.001
Model II	1.00	1.28 (0.87-1.88)	1.44 (0.98-2.13)	1.45 (0.95-2.20)	1.94 (1.26-2.98)	< 0.001
Model III	1.00	1.21 (0.81-1.80)	1.35 (0.90-2.01)	1.35 (0.87-2.07)	1.92 (1.23-3.01)	< 0.01
Model IV	1.00	1.20 (0.80-1.78)	1.33 (0.89-1.98)	1.32 (0.86-2.03)	1.85 (1.18-2.90)	< 0.01

¹Model I : Adjusted for age and gender; Model II : Adjusted for age, gender, body mass index (BMI), smoking, dietary supplements and oral contraceptive pill (OCP) use and self-reported diabetes; Model III : Adjusted for age, gender, BMI, smoking, dietary supplements and OCP use, self-reported diabetes, meal regularity, chewing quality, fluid intakes, pattern of tea consumption and dental status; Model IV: Adjusted for all variables in model III and lactose intolerance.

Table 3 Multivariable-adjusted odds ratios and 95%CI for irritable bowel syndrome across different categories of spicy foods consumption, stratified by gender

	Consumption of spicy foods (times/wk)					<i>P</i> _{trend}
	Never	1-3	4-6	7-9	≥ 10	
Men						
Crude	1.00	1.38 (0.88-2.17)	1.48 (0.92-2.37)	1.81 (1.03-3.16)	1.62 (0.85-3.08)	0.07
Model I	1.00	1.32 (0.79-2.22)	1.34 (0.78-2.31)	1.78 (0.95-3.32)	1.78 (0.87-3.60)	0.10
Model II	1.00	1.27 (0.75-2.14)	1.35 (0.78-2.33)	1.69 (0.89-3.19)	1.8 (0.88-3.67)	0.17
Model III	1.00	1.16 (0.67-2.01)	1.26 (0.71-2.24)	1.55 (0.79-3.04)	1.65 (0.78-3.48)	0.22
Model IV	1.00	1.18 (0.68-2.05)	1.27 (0.71-2.27)	1.55 (0.79-3.04)	1.64 (0.78-3.48)	0.29
Women						
Crude	1.00	1.26 (0.75-2.12)	1.44 (0.87-2.40)	1.5 (0.89-2.55)	1.96 (1.14-3.37)	< 0.01
Model I	1.00	1.38 (0.78-2.43)	1.58 (0.90-2.78)	1.57 (0.87-2.81)	2.14 (1.18-3.88)	< 0.01
Model II	1.00	1.32 (0.75-2.35)	1.53 (0.87-2.68)	1.43 (0.79-2.52)	2.05 (1.12-3.73)	< 0.01
Model III	1.00	1.3 (0.72-2.33)	1.45 (0.81-2.59)	1.38 (0.75-2.52)	2.13 (1.15-3.95)	0.01
Model IV	1.00	1.26 (0.70-2.27)	1.41 (0.79-2.52)	1.34 (0.73-2.44)	2.03 (1.09-3.77)	0.02

Model I : Adjusted for age and gender; Model II : Adjusted for age, gender, body mass index (BMI), smoking, dietary supplements and oral contraceptive pill (OCP) use and self-reported diabetes; Model III: Adjusted for age, gender, BMI, smoking, dietary supplements and OCP use, self-reported diabetes, meal regularity, chewing quality, fluid intakes, pattern of tea consumption and dental status; Model IV: Adjusted for all variables in model III and lactose intolerance.

cantly improved abdominal pain and bloating in IBS patients^[21]; ginger was found to be the most common type of complementary and alternative medicine used for IBS treatment^[4]; cinnamon administration has been found to reduce the number of IBS symptoms^[25]; and beneficial effects of turmeric on abdominal pain and discomfort in IBS patients have also been reported^[26]. While the causes for the discrepant study outcomes are not clear, there are a number of potential reasons. Different spices may have different modes of action; almost all studies that reported beneficial effects of individual spices used high doses in the form of supplements, and study designs and methodologies varied markedly between studies. It appears that the current study is the first observational study in an adult population, in which habitual consumption of spicy foods has been linked to IBS, although, due to the use of a single question to assess spicy food intake, it is not possible to distinguish between the potential effects of individual spices.

The mechanisms through which consumption of spicy foods might affect IBS are unknown. The effect of red pepper has been related to its pungent ingredient, capsaicin, which can modify gastrointestinal sensation

via transient potential vanilloid 1 (TRPV1) receptors^[11,27]. Increased TRPV1 receptors are associated with visceral hypersensitivity in the proximal gut and colon^[11,27]. It seems that capsaicin intake in IBS patients can lead to hypersensitivity, which in turn can result in TRPV1 up-regulation^[4,11,28]. However, few studies have postulated that intermittent and chronic ingestion of capsaicin or capsaicin containing chili can improve FGID symptoms by desensitization of TRPV1 receptors^[11]. This can be explained by the action of capsaicin, which when administered chronically, depletes nerve terminals of substance P, while acute application leads to maximal release of transmitters, resulting in pain. Further research is required to prove this hypothesis. The mechanisms of other spices remain to be identified.

This study has several strengths. Firstly, it is a large population-based study, which examined habitual intake of spicy foods, rather than the effects of high doses of spices. Earlier studies have mostly been performed in small sample sizes. Secondly, we performed rigorous statistical analyses, including adjustments for several potential contributing factors to IBS. Therefore, the associations we identified are independent of many factors,

including dietary behaviors. Nevertheless, the findings need to be interpreted in the light of some limitations. We used a pre-tested questionnaire for assessing dietary intakes of spicy foods; misclassification is a potential concern in our study as in any epidemiological studies. In addition, high consumption of spicy foods was associated with a complex pattern of lifestyles that may not have been accurately captured and controlled in our analysis, resulting in residual confounding. The significant direct association of spicy foods intake and IBS may be attributed to the other factors (*e.g.*, having irregular meals, not chewing foods very well, *etc.*) associated with higher intake of these foods. That said, the apparently 1 direct effect of spicy food consumption persisted in multivariate models accounting for known potential confounders. Furthermore, some intermediate factors might lead to changes in diet and may, therefore, confound the association between spicy food intake and IBS. In addition, the observed association may not apply to other sections of the Iranian population, including the young, elderly or those from different socio-economic backgrounds. However, participants in the current study were selected from different areas of Isfahan province with diverse socio-economic status and their dietary intakes covered a broad range of dietary habits. Given these characteristics, it is unlikely that this type of bias could explain the observed associations between spicy food intake and IBS.

In conclusion, we found evidence indicating that spicy food consumption was positively associated with IBS, particularly in women. Further studies, in particular of a prospective nature, are required to examine this association in more detail and to potentially develop novel dietary approaches to manage IBS and other FGIDs.

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COMMENTS

Background

The relationship between dietary factors and irritable bowel syndrome (IBS) remains yet to be clearly defined. Some research suggests that carbohydrate, protein, fiber, or water all may play a role. However, currently there are many controversial findings regarding the relationship between diet-related practices and different gastrointestinal disorders including IBS.

Research frontiers

While some studies have indicated that acute chili ingestion can aggravate abdominal pain and burning symptoms in functional gastrointestinal disorders, chronic ingestion of chili was found to improve functional dyspepsia and gastroesophageal reflux disease symptoms in small controlled studies. It is worth noting that most previous studies focused on one type of spice (*e.g.*, chili pepper), and there are few studies that have investigated the relationships between habitual intake of spicy foods and functional gastrointestinal disorders.

Innovations and breakthroughs

In a large cross-sectional study, information on habitual spicy food intake and symptoms related to IBS were gathered from 4763 adults using standard questionnaires. Individuals with a high intake of spicy foods (≥ 10 times/wk) had an

almost two-fold increased risk of having IBS compared with those who reported a lower intake of spicy foods. After taking into account different variables that might distort the association between spicy food intake and IBS, the relationship was significant only among women.

Applications

The findings of the current study, if confirmed in well-designed prospective studies, may assist with the design of novel dietary therapies that take into account, and modify, the dietary intake of spicy foods and, thus, may be useful in the management of IBS related symptoms.

Peer review

In this interesting manuscript, the authors explored the association between consumption of spicy foods and prevalence of IBS among Iranian adults. They performed a cross-sectional study from 4763 Iranian adult participants. Consumption of spicy foods was estimated using a dietary habits questionnaire, and the prevalence of IBS was estimated using a modified Persian version of the Rome III questionnaire. The study has concluded that consumption of spicy foods is directly associated with IBS, particularly in women. This article is interesting and the readers will get some beneficial information from this.

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Midterm outcome of stapled transanal rectal resection for obstructed defecation syndrome: A single-institution experience in China

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RESULTS: The median follow-up was 30 mo (range, 30-46 mo). Late postoperative complications occurred in 11 (14.7%) patients. Three of these patients required procedure-related reintervention (one diverticulectomy and two excision of staple granuloma). Although the recurrence rate was 10.7%, constipation scores (CSS, ODS score and SSS) significantly improved after STARR ($P < 0.0001$). Significant reduction in ODS symptoms was matched by an improvement in the PAC-QOL and VAS ($P < 0.0001$), and the satisfaction index was excellent in 25 (33.3%) patients, good in 23 (30.7%), fairly good in 14 (18.7%), and poor in 13 (17.3%). Nevertheless, the WS increased after STARR ($P = 0.0169$). Incontinence was present or deteriorated in 8 (10.7%) patients; 6 (8%) of whom were new onsets. Univariate analysis revealed that the occurrence of fecal incontinence (preoperative, postoperative or new-onset incontinence; $P = 0.028$, 0.000, and 0.007, respectively) was associated with the success of the operation.

Abstract

AIM: To assess midterm results of stapled transanal rectal resection (STARR) for obstructed defecation syndrome (ODS) and predictive factors for outcome.

METHODS: From May 2007 to May 2009, 75 female patients underwent STARR and were included in the present study. Preoperative and postoperative workup consisted of standardized interview and physical examination including proctoscopy, colonoscopy, anorectal manometry, and defecography. Clinical and functional results were assessed by standardized questionnaires for the assessment of constipation constipation scoring system (CSS), Longo's ODS score, and symptom severity score (SSS), incontinence Wexner incontinence score (WS), quality of life Patient Assessment of Constipation-Quality of Life Questionnaire (PAC-QOL), and patient satisfaction visual analog scale (VAS). Data were collected prospectively at baseline, 12 and 30 mo.

CONCLUSION: STARR is an acceptable procedure for the surgical correction of ODS. However, its impact on symptomatic recurrence and postoperative incontinence may be problematic.

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Key words: Colorectal surgery; Constipation; Stapled transanal rectal resection

Core tip: As a less-invasive surgical procedure, stapled transanal rectal resection (STARR) is becoming an important option in the treatment of obstructive defecation syndrome. However, its clinical and functional outcomes are still conflicting and controversial. The present study assessed the midterm results after STARR performed by the same team in our department to identify factors for predicting outcome. Our data provide evidence to attest the clinic benefits of this pro-

cedure, but its impact on symptomatic recurrence and postoperative incontinence may be problematic.

Zhang B, Ding JH, Zhao YJ, Zhang M, Yin SH, Feng YY, Zhao K. Midterm outcome of stapled transanal rectal resection for obstructed defecation syndrome: A single-institution experience in China. *World J Gastroenterol* 2013; 19(38): 6472-6478 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6472.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6472>

INTRODUCTION

Obstructed defecation syndrome (ODS) is defined as the normal desire to defecate but with an impaired ability to evacuate the rectum satisfactorily^[1]. The anatomical and physiological disturbances underlying ODS are complex and only partly understood, but rectocele and intussusception have been identified as the two most important organic causes of ODS^[2].

Although a variety of surgical approaches has been described in the literature for correction of ODS, most of these have high recurrence and complication rates. Stapled transanal rectal resection (STARR) was introduced in 2003 by Longo^[3] as a minimally invasive transanal operation for ODS associated with rectocele and intussusception. The novel procedure is carried out using double circular stapler devices to resect a full-thickness segment of rectal wall and subsequently to restore normal rectal anatomy. In contrast to traditional techniques, STARR addresses correction of both rectocele and intussusception.

Several multicenter trials have demonstrated that STARR significantly improves constipation with low morbidity and high comfort for patients^[4-8]. In addition, the procedure could even offer long-term clinical benefits^[9-11]. Nevertheless, worrisome complications and unsatisfactory functional results have been described^[12,13]. There are also reports of high rates of reintervention for both symptomatic recurrence and procedure-related complications after this surgery^[14,15]. As a consequence, although STARR is increasingly being accepted as an important option for surgical treatment of ODS, its clinical and functional outcomes are still conflicting and controversial.

We have shown previously that STARR can be performed safely and is effective for eligible patients with ODS secondary to rectocele and intussusceptions^[16,17]. The objective of this study was to assess midterm clinical and functional results and to identify factors for predicting outcome after STARR.

MATERIALS AND METHODS

Patients

From May 2007 to May 2009, a consecutive series of 86 female patients was treated with STARR for ODS in our Department of Colorectal Surgery at the Second Artillery General Hospital, Beijing, China. A total of 75 (87.2%)

patients completed the scheduled follow-up and formed the study population. All patients were prospectively included in a database. Study protocol was approved by the institutional ethics committee of our hospital. Written informed consent was obtained from all patients enrolled in the study. Preoperative assessment included symptom evaluation, clinical and gynecological examinations, and investigations with proctoscopy, colonoscopy, colonic transit time study, anorectal manometry, and defecography. Anorectal manometry was performed as previously described^[17]. Patients were carefully selected according to the inclusion and exclusion criteria for STARR proposed by the consensus recommendations^[18] and the decision-making algorithm^[2].

Surgical procedures

Polyethylene glycol electrolyte solutions were preoperatively prescribed for bowel preparation. Patients received routine broad-spectrum antibiotics immediately after anesthesia induction. Under spinal anesthesia, patients were placed in the lithotomy position with a catheter in the bladder. The STARR procedure was performed using the circular stapler (PPH-01; Ethicon Endo-Surgery, Inc., New Brunswick, NJ, United States) as described previously^[4]. Subsequent bleeding from the staple line was controlled with full-thickness 2-0 Vicryl stitches (Ethicon Endo-Surgery). All STARR procedures were conducted by the same surgical team.

Outcome measures

The severity of ODS was quantified by the validated constipation scoring system (CSS; range: 0-30 at increments of 1; no symptoms = 0)^[19]; Longo's ODS score (range: 0-40 at increments of 1; no symptoms = 0)^[16]; and symptom severity score (SSS; range: 0-36 at increments of 1; no symptoms = 0)^[7]. Patient's fecal incontinence was assessed by the Wexner incontinence score (WS; range: 0-36 at increments of 1; perfect continence = 0)^[20]. The validated Patient Assessment of Constipation-Quality of Life Questionnaire (PAC-QOL) was used to measure the quality of life in patients with ODS^[21]. The first three subscales of the self-reported questionnaire were used to assess the patient dissatisfaction index, with an overall score ranging from 0 to 96 (lower scores corresponding to better quality of life). The satisfaction subscale included four items with a global score ranging from 0 to 16 (high scores corresponding to better quality of life)^[22]. Moreover, the index of patient satisfaction was also measured by the visual analog scale (VAS) with scores from 0 to 10, and a higher score suggested an improvement in patient satisfaction with surgery.

Postoperative follow-up

The patients were followed up in our clinic at 3, 6, 12 and 30 mo postoperatively. At each visit, digital rectal examination was used to assess the anal sphincter, and proctoscopy or colonoscopy to evaluate the anastomosis and the presence or absence of local complications (stenosis,

Table 1 Univariable analysis of predictive factors correlated with therapy success after stapled transanal rectal resection

Factors	Total (n = 75)	Successful (n = 62)	Unsuccessful (n = 13)	P value
Mean age (yr) ¹	54.30	53.80	56.50	0.287
Multiparous/non-multiparous ²	31/44	24/38	7/6	0.314
Hysterectomy/no hysterectomy ³	10/65	7/55	3/10	0.364
Anorectal operation before STARR/no operation ²	36/39	29/33	7/6	0.643
Constipation scores ¹				
CSS score	15.57	15.60	15.46	0.569
ODS score	18.39	18.03	20.08	0.994
SSS score	13.69	13.55	14.38	0.537
Manometric parameters ¹				
Resting pressure (mmHg)	54.13	54.27	53.46	0.497
Squeeze pressure (mmHg)	109.0	109.5	106.7	0.726
First initial sensation (mL)	87.05	86.53	89.54	0.649
Maximum tolerable rectal volume (mL)	238.2	238.2	238.0	0.248
Defecographic parameters				
Rectocele (mm) ¹	35.12	34.62	37.46	0.220
Intussusception/no intussusception ³	65/10	56/6	9/4	0.064
Increased perineal descent/no perineal descent ³	21/54	15/47	6/7	0.171
Sigmoidocele/no sigmoidocele ³	9/66	7/55	2/11	0.650
Fecal incontinence ³				
Preoperative incontinence/no incontinence	2/73	0/62	2/11	0.028
Postoperative incontinence/no incontinence	8/67	2/60	6/7	0.000
New-onset incontinence/no incontinence	6/69	2/60	4/9	0.007

¹Unpaired *t* test; ²Pearson's χ^2 test; ³Fisher's exact test. STARR: Stapled transanal rectal resection; CSS: Constipation scoring system; SSS: Symptom severity score; ODS: Obstructed defecation syndrome.

granulomas or mucosal prolapse). We also recorded the occurrence of postoperative complications, which were considered to be early if they occurred within 1 mo after surgery and late if they occurred after this period. A complete clinical reassessment including anorectal manometry and defecography was performed at 12 mo after surgery. Functional results were further updated at 30 mo of follow-up using the same standardized questionnaires (CSS, ODS score, SSS, WS, PAC-QOL and VAS). The STARR procedure was considered successful at 30 mo when PAC-QOL (satisfaction index) scores were classified as excellent, good, or fairly good, defined as follows: 13-16 classified as excellent, 9-12 as good, 5-8 as fairly good, and 0-4 as poor.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 for Windows XP (SPSS Inc. Chicago, IL, United States). The variation of total scores of the CSS, ODS, SSS, WS, PAC-QoL and VAS were expressed as mean values with 95%CI. Data were compared between groups using the two-sample *t* test, paired *t* test, Pearson's χ^2 test, Fisher's exact test, and Wilcoxon signed-rank test, as indicated. *P* < 0.05 was considered statistically significant.

RESULTS

Preoperative data

Of the 75 female patients (mean age, 54.3 years; range, 29-75 years) included in this study, 60 (80%) had experienced vaginal delivery and 31 (41.3%) were multiparous. Sixty-four (61.3%) patients underwent previous anorec-

tal/gynecological surgery, including episiotomy (18 patients), hemorrhoidectomy (14 patients), fistulectomy (3 patients), sphincterotomy (1 patient), and hysterectomy (10 patients). Defecographic and manometric findings are detailed in Table 1.

Perioperative data

A staple-line dehiscence necessitating handsewn suturing was the only intraoperative complication that we observed. There were no major complications, rectovaginal fistula, pelvic sepsis, or deaths. The operative data, early postoperative complications, and short-term results were described in our previous studies^[16,17].

Late postoperative complications

A total of 12 late complications occurred in 11 patients, giving an overall morbidity rate of 14.7%. The most frequently reported complication was postoperative incontinence, which was present or deteriorated in eight (10.7%) patients. Although defecatory urgency vanished spontaneously in most patients within the first 3 mo postoperatively, one (1.3%) patient reported this complaint at the time of the latest interview. Two (2.7%) patients suffered from inflammatory granulomas on the staple line, which had to be removed because of chronic pain or bleeding. Additionally, there was one (1.3%) case of iatrogenic rectal diverticulum with impacted fecalith confirmed 34 mo after surgery. It presented as severe recurrence of obstructed defecation and was treated by transanal diverticulectomy^[23]. Thus, 3 (4%) patients required transanal reintervention for procedure-related complications after STARR.

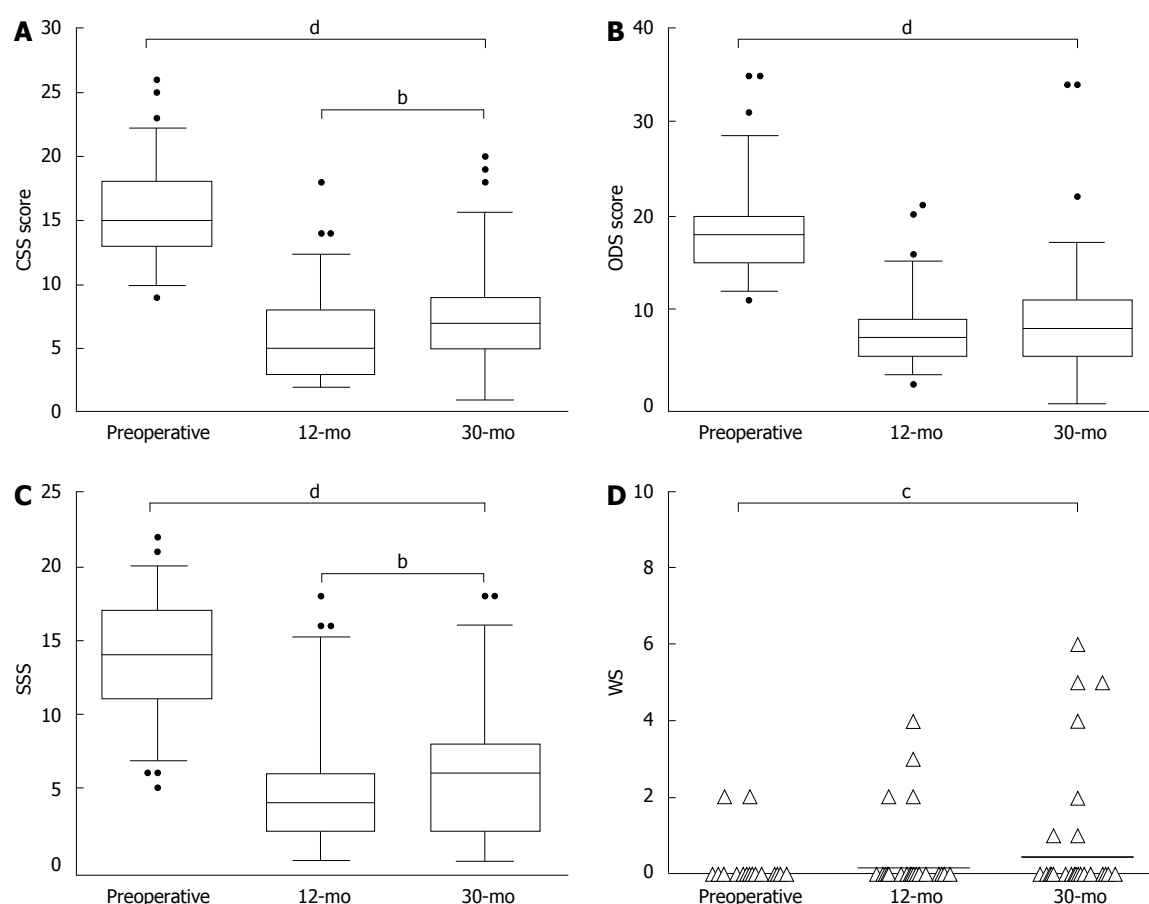


Figure 1 Preoperative and postoperative assessment in 75 patients with obstructed defecation syndrome undergoing stapled transanal rectal resection. A: CSS scores (paired *t* test, ^b*P* < 0.01, ^d*P* < 0.01); B: Longo's ODS scores (paired *t* test, ^b*P* < 0.01); C: SSS (paired *t* test, ^b*P* < 0.01, ^d*P* < 0.01); D: WS (paired *t* test, ^c*P* < 0.05). CSS: Constipation scoring system; ODS: Obstructed defecation syndrome; SSS: Symptom severity score; WS: Wexner incontinence scores.

Follow-up data

Changes in the constipation scores (CSS, ODS score and SSS) and the incontinence scores (WS) are presented in Figure 1. Globally, a significant reduction in the CSS, ODS score and SSS was observed at 12 mo as compared with baseline, and this reduction was maintained at 30 mo [CSS at baseline *vs* 30 mo: 15.57 (95%CI: 14.78-16.36) *vs* 7.07 (95%CI: 6.16-7.98); ODS score: 18.39 (95%CI: 17.27-19.51) *vs* 8.55 (95%CI: 7.12-9.97); SSS: 13.69 (95%CI: 12.74-14.64) *vs* 6.16 (95%CI: 5.12-7.20); *P* < 0.0001 in each group]. However, these scores started to increase slightly after 12 mo [CSS at 12 mo *vs* 30 mo: 5.99 (95%CI: 5.28-6.70) *vs* 7.07 (95%CI: 6.16-7.98); SSS: 4.59 (95%CI: 3.73-5.45) *vs* 6.16 (95%CI: 5.12-7.20), *P* < 0.01; ODS score: 7.49 (95%CI: 6.65-8.34) *vs* 8.55 (95%CI: 7.12-9.97), *P* = 0.07]. Overall, the symptoms of ODS had persisted or recurred in 8 (10.7%) patients with adequate follow-up. Two patients who had initial improvement presented with persistence of ODS symptoms 3 mo after surgery, and another 6 patients developed symptomatic recurrence after 12 mo.

Although the WS rose slightly after STARR, two cases of new-onset fecal incontinence and two of worsened incontinence were observed during 12 mo follow-up, there was no significant difference before and after surgery [WS

at baseline *vs* at 12 mo: 0.05 (95%CI: -0.02-0.13) *vs* 0.15 (95%CI: -0.003-0.30), *P* = 0.052]. However, another four patients had new-onset incontinence after 12 mo and the WS increased significantly at 30 mo follow-up [WS at baseline *vs* at 30 mo: 0.05 (95%CI: -0.02-0.13) *vs* 0.43 (95%CI: 0.09-0.76), *P* = 0.017]. On the whole, incontinence was present or deteriorated in 8 (10.7%) patients, 6 (8%) of whom had new onset.

As shown in Table 2, improvement in the constipation scores was matched by an overall improvement in quality of life as assessed by the PAC-QOL and VAS scores at both 12 and 30 mo follow-up [PAC-QOL (dissatisfaction index) at baseline *vs* 30 mo: 44.45 (95%CI: 41.15-47.76) *vs* 13.21 (95%CI: 10.36-16.07); PAC-QOL (satisfaction index): 0 *vs* 10.12 (95%CI: 9.21-11.03); VAS: 3.83 (95%CI: 3.54-4.11) *vs* 7.07 (95%CI: 6.69-7.46); *P* < 0.0001]. At the end of follow-up, the self-reported definitive outcome was reported as excellent by 25 (33.3%) patients, good by 23 (30.7%), fairly good by 14 (18.7%), and poor by 13 (17.3%). Symptomatic recurrence and postoperative incontinence were the main reasons for a poorer outcome.

Predictive factors for outcome

In accordance with the patient's assessment of the clini-

Table 2 Preoperative and postoperative scores of quality-of-life questionnaires and visual analog scale in 75 patients undergoing stapled transanal rectal resection

Items	Median		
	Preoperative	12 mo	30 mo
PAC-QoL (dissatisfaction index)	44	7 ^b	9 ^b
PAC-QoL (satisfaction index)	0	12 ^b	10 ^b
VAS satisfaction index	4	8 ^b	7 ^b

The Wilcoxon signed-rank test was used; All the comparisons *vs* the preoperative data were statistically significant; ^b $P < 0.01$. PAC-QoL: Postoperative scores of quality-of-life questionnaires; VAS: Visual analog scales.

cal outcome at 30 mo follow-up, 17 patient- and disease-related factors were used to compare 65 patients who acquired any improvement after STARR with 13 patients who considered an absence of success for further statistical analyses (Table 1). The result of the univariate analysis revealed that lack of improvement was more likely in patients with fecal incontinence (preoperative, postoperative or new-onset incontinence; $P = 0.028, 0.000$, and 0.007 , respectively). However, multiparous, hysterectomy, previous anorectal operation, CSS, ODS score, SSS, and defecographic or manometric findings were not correlated with the functional success of the operation.

DISCUSSION

Controversy exists in the literature regarding the results after STARR, therefore, this study aimed to evaluate the midterm results and predictive factors for outcome. We assessed a series of 75 patients before and 30 mo after STARR, in which late postoperative complications were seen in 14.7% and reintervention was required in 4%. Despite the recurrence rate of 10.7%, clinical and functional outcome scores (CSS, ODS, SSS, PAC-QOL, and VAS) significantly improved after surgery. Nevertheless, the significant reduction in ODS symptoms was not matched by impairment of the WS. The success of the STARR procedure was associated with the occurrence of fecal incontinence, which was present or deteriorated in 10.7% of patients after surgery.

Several studies have indicated the midterm efficacy of STARR in relieving ODS symptoms with high patient satisfaction rates^[4,5,24-27]. Similar clinic benefits were obtained in the present study; we were able to demonstrate that defecation difficulties were significantly improved after STARR. Improvement remained stable at 30 mo follow-up as compared with baseline, albeit the constipation scores started increase 12 mo after surgery. Meanwhile, the satisfaction index was reported as excellent in 25 (33.3%), good in 23 (30.7%), fairly good in 14 (18.7%), and poor in 13 (17.3%). Hence, our midterm follow-up suggests that early postoperative benefits were maintained. Other reports, however, showed that ODS symptoms may not improve or even deteriorate after STARR^[13,14]. The main reason for these conflicting observations may be the patient selection criteria. Inadequate

indications for this operation will necessarily result in poor outcome. The outcomes of an Italian multicenter study were worse in none-selected patients and improvement after STARR was noted in only 65% of the patients^[14]. In our series, all patients were carefully selected on the basis of the consensus recommendations and the decision-making algorithm^[2,18], but further observations should evaluate whether the midterm efficacy deteriorates with time.

Although STARR produced good midterm results, eight (10.7%) patients in our study presented with persistent or recurrent symptoms of ODS. In the literature, the incidence of midterm recurrences is between 4.3% and 17.1%^[5,8,14,28]. More recently, however, it has been shown that none of the patients who underwent STARR by the curved Contour Transtar stapler had recurrence of ODS symptoms during a 36-mo follow-up^[29]. This discrepancy may be attributed to the limited capacity of PPH-01 casing with risk of leaving residual disease, especially in patients with large rectocele and intussusception. It should also be stressed that rectocele and intussusception are only the emerging tip of the ODS iceberg syndrome; pelvic floor pathology caused by the “underwater rocks” or occult lesions are likely to persist and contribute to persistent or recurrent symptoms after surgery^[30].

Some series therefore have been designed to define predictive factors for outcomes after STARR. Gagliardi *et al*^[14] have suggested that the results were worse in patients with preoperative digitation, puborectalis dyssynergia, enterocele, larger rectocele, lower bowel frequency, and sense of incomplete evacuation. Contrary to this observation, a subsequent study showed that the number of pelvic floor changes was associated with the success of the operation^[11]. Another study demonstrated that factors for an unfavorable outcome after STARR included small rectal diameter, low sphincter pressure, and increased pelvic floor descent^[8]. In the present study, we only indicated that the occurrence of fecal incontinence, including preoperative, postoperative or new-onset incontinence, was associated with poorer midterm outcome. In addition, postoperative incontinence was one of the main reasons for patient dissatisfaction. No doubt more evidence is needed to clarify this issue.

Fecal incontinence after STARR is one of the main concerns of surgeons. Postoperative incontinence and urgency have been reported as being transient and disappeared within 6 mo^[4], but were still present after 30 mo in some of our patients. Incontinence may be caused by reduced rectal volume or by muscle stretching and transient sphincter dysfunction secondary to the 36-mm dilator^[4,31]. We did not systematically evaluate the anal sphincter using ultrasound, but there was no evidence of sphincter dysfunction according to our manometry results. Intriguingly, 6 (8%) patients in our study had new-onset incontinence after the STARR procedure. A possible explanation is that intussusception in the anal canal may function as a barrier with a subsequently beneficial effect on fecal continence. After its removal, fecal incontinence becomes

uncovered^[31]. Consequently, a careful patient selection with the awareness of occult incontinence is crucial. It is noteworthy that incontinence improves in some patients, which is attributed to improved internal sphincter function after STARR^[6,7,25,28]. Few patients with preoperative incontinence were enrolled, thus, it could not be assessed in our study.

In the current study, STARR was confirmed as a safe procedure for the treatment of ODS. Nevertheless, an unexpected major complication was observed in one patient who developed an iatrogenic rectal diverticulum after STARR. Concordant with previous findings^[12,32], the diverticulum was located along the lateral wall of the rectum, an area of weakness, where anterior and posterior suture lines cross over one another. Iatrogenic diverticulum may also occur as a consequence of technical failure in that the lateral part of the rectal wall remained outside the staple casing during the second resection, or an incomplete section of the mucosal band was retained after STARR^[32]. To the best of our knowledge, no patient has developed rectal diverticulum after Transtar for the surgical correction of ODS; therefore, this major complication may be the inherent drawbacks of the PPH-01 stapler that could be avoided by using the new device.

We conclude that STARR may be an acceptable procedure for the treatment of patients with ODS caused by rectocele and intussusception, but its impact on symptomatic recurrence and postoperative incontinence may be problematic. In this study, patients were strictly selected and systematically assessed prospectively. However, there were still some limitations such as the lack of a control group. Moreover, postoperative defecography or magnetic resonance imaging with longer follow-up is also crucial for providing more details on pelvic floor anatomy as well as physiology. Finally, this was a midterm follow-up study. Further studies are needed to assess long-term results and to optimize patient selection, which is required to enhance and maintain patient satisfaction after surgery.

COMMENTS

Background

Obstructed defecation syndrome (ODS) is a frequent but multifactorial disease that usually afflicts middle-aged women. Although a variety of surgical procedures has been proposed to correct ODS, no one has found unanimous consensus. Stapled transanal rectal resection (STARR) was recently introduced as a minimally invasive transanal procedure, the advantage of which is the simultaneous treatment of rectocele and rectal intussusception, both representing the main anatomical cause of ODS.

Research frontiers

In recent years, STARR is increasingly being accepted as an important option for surgical treatment of ODS. However, the clinical and functional outcomes after STARR are still conflicting and controversial. In the area of treatment of ODS by the STARR procedure, the research hotspots are how to optimize patient selection and to predict the functional outcome after surgery.

Innovations and breakthroughs

The authors assessed midterm results and predictive factors for outcome after STARR. Even though the recurrence rate was 10.7%, the clinical and functional outcome scores significantly improved after surgery. In addition, symptomatic recurrence and postoperative incontinence were the main reasons for a poorer

outcome.

Applications

The study results suggest that STARR may be an acceptable procedure for the treatment of ODS, but its impact on symptomatic recurrence and postoperative incontinence may be problematic.

Peer review

This study assessed the midterm outcome of STARR for ODS. This topic has been previously studied, and the results of several studies have been discordant. Nevertheless, the topic is interesting for the readers of the journal and suitable to be published.

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A new endoscopic ultrasonography image processing method to evaluate the prognosis for pancreatic cancer treated with interstitial brachytherapy

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Abstract

AIM: To develop a fuzzy classification method to score the texture features of pancreatic cancer in endoscopic ultrasonography (EUS) images and evaluate its utility in making prognosis judgments for patients with unresectable pancreatic cancer treated by EUS-guided interstitial brachytherapy.

METHODS: EUS images from our retrospective database were analyzed. The regions of interest were drawn, and texture features were extracted, selected, and scored with a fuzzy classification method using a C++ program. Then, patients with unresectable pancreatic cancer were enrolled to receive EUS-guided iodine 125 radioactive seed implantation. Their fuzzy

classification scores, tumor volumes, and carbohydrate antigen 199 (CA199) levels before and after the brachytherapy were recorded. The association between the changes in these parameters and overall survival was analyzed statistically.

RESULTS: EUS images of 153 patients with pancreatic cancer and 63 non-cancer patients were analyzed. A total of 25 consecutive patients were enrolled, and they tolerated the brachytherapy well without any complications. There was a correlation between the change in the fuzzy classification score and overall survival (Spearman test, $r = 0.616$, $P = 0.001$), whereas no correlation was found to be significant between the change in tumor volume ($P = 0.663$), CA199 level ($P = 0.659$), and overall survival. There were 15 patients with a decrease in their fuzzy classification score after brachytherapy, whereas the fuzzy classification score increased in another 10 patients. There was a significant difference in overall survival between the two groups (67 d vs 151 d, $P = 0.001$), but not in the change of tumor volume and CA199 level.

CONCLUSION: Using the fuzzy classification method to analyze EUS images of pancreatic cancer is feasible, and the method can be used to make prognosis judgments for patients with unresectable pancreatic cancer treated by interstitial brachytherapy.

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Key words: Digital image processing; Fuzzy classification; Endoscopic ultrasonography; Pancreatic cancer; Interstitial brachytherapy; Prognosis

Core tip: Digital image processing (DIP) of endoscopic ultrasonography (EUS) images has been proven to be useful in diagnosis of malignant tumor. Currently commonly used method of DIP is only to concludes the dif-

ferential diagnosis of solid tumors ("yes" or "no"), can not provide the numerical data describing the texture parameters in the EUS image. EUS-guided brachytherapy has been applied preliminarily in the study of advanced pancreatic cancer. However, prognosis judgment of these patients was still difficult. So we develop a fuzzy classification method to score texture features of pancreatic cancer in EUS images to supply more information and validated its utility in prognosis judgment of patients with unresectable pancreatic cancer treated by EUS-guided interstitial brachytherapy.

Xu W, Liu Y, Lu Z, Jin ZD, Hu YH, Yu JG, Li ZS. A new endoscopic ultrasonography image processing method to evaluate the prognosis for pancreatic cancer treated with interstitial brachytherapy. *World J Gastroenterol* 2013; 19(38): 6479-6484 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6479.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6479>

INTRODUCTION

The application of digital image processing (DIP) in endoscopic ultrasonography (EUS) images and other imaging scenarios has been proven to be a useful adjunct to endoscopic diagnoses and often comparable with specialists' interpretation in different pathologic settings^[1-3]. The texture parameters of EUS images are extracted and classified from the returned echoes to identify the tissue type present in the images. One effective approach is to use DIP based on a support vector machine (SVM), which is a computer algorithm that learns by example to assign labels to objects^[4,5]. The SVM technique, as a subfield of digital signal processing, has been applied to a series of pathologically proven diseases^[6-12].

The typical method of SVM, which is only able to provide a differential diagnosis for solid tumors ("yes" or "no"), cannot provide numerical data describing the texture parameters in the EUS image. In this study, a new DIP method based on fuzzy classification is applied to obtain the feature value of texture parameters in EUS images of pancreatic cancer and observe the change of texture parameters to evaluate its utility in making prognosis judgments for patients with unresectable pancreatic cancer after EUS-guided interstitial brachytherapy.

MATERIALS AND METHODS

The whole study protocol was approved by the Institutional Review Board and Ethics Committees of the Second Military Medical University. All patients had provided their written informed consent before the study. DIP of EUS images using the fuzzy classification method was retrospective, whereas its application in the prognosis evaluation was prospective.

Principle of fuzzy classification

Given that the unidentified object u had p classes, which

meant there were p cases that such an object could be classified to, a number of features were extracted from the object u , and the sum of these features had a membership degree A to every class. Therefore, the membership degree of the unidentified object u to each class was $A_1(u), A_2(u), \dots, A_p(u)$. It is generally assumed that the larger the membership degree's value of a certain class is, the greater the feature value of the objects belonging to this class will be.

Given that the j th feature extracted from the unidentified object u was u_j , its membership degree to the j th feature of class i was:

$$A_{ij}(u) = (1 + (u_j - a_{ij})^2 / \sigma_{ij}^2)^{-1} \quad (1)$$

where a_{ij} was the j th feature's mean value for the training data belonging to class i , and σ_{ij} was the variance.

Thus, every feature of the unidentified object u could obtain a membership degree to class i . In addition, a corresponding weight α_j was also assigned to it. Therefore, the membership degree of belonging to class i should be:

$$A_i(u) = \left| \sum_{j=1}^m (\alpha_j \times A_{ij}(u)) \right| \quad (2)$$

The weights could be optimized by taking advantage of the training data.

In terms of the application object in this study, there were two classes: pancreatic cancer and non-pancreatic cancer. For an unidentified case, its membership degree to the two categories A_1 and A_2 was computed, and then the feature value was obtained according to the following normalized evaluation function: $Eval = [A_1 / (A_1 + A_2)] \times 100\%$. The object is more likely to be a cancer as the feature value gets closer to 100%, and vice versa. Thus, the fuzzy classification of pancreatic cancer was achieved.

Processing of the fuzzy classification method

The analysis database of EUS images was compiled from data collected between March 2005 and December 2007, which was described in a previous study of our group^[14]. All EUS procedures were performed with an Olympus GF-UM2000 at 7.5 MHz. Regions of interest (ROIs) of all EUS images were manually outlined by endoscopic specialists who were blind to the final diagnosis. Texture features were extracted from every ROI and analyzed using a C++ program. Texture features generally referred to the spatial arrangement and interconnection of the basic elements of images^[15]. The sequential forward search algorithm was applied to select the features after extracting the feature. Then, a few optimum feature combinations were obtained^[16,17]. Finally, real time was taken into account, and 22 features falling into three categories were selected. First, the mean feature value of the image was extracted. It was a first-order statistical feature. Second, the gray level co-occurrence matrix (GLCM) features were selected, which were based on the second-order joint feature distribution matrix of the images proposed by Haralick *et al*^[18]. GLCMs for four directions (0°, 45°, 90°, and 135°) were constructed. For each matrix, five features were extracted, which were energy, entropy, moment of inertia, correlation, and local stationary. Finally,

the fractal feature was obtained. Recently, the fractal dimension feature has been widely used in pattern recognition and texture analysis. In this study, the second-order multi-fractal dimension feature was used, and the differential box-counting approach was applied to calculate the fractal dimension^[19,20]. The previously described fuzzy classification method assessed all the features contained in the ROI of the EUS image and estimated a score between 0 and 100. Given that two states existed - cancer and a normal pancreas - 0 represented all the features of a “normal pancreas” that were contained in the ROI with no “cancer” features, whereas 100 represented all the features of “cancer” with no “normal pancreas” features.

Application of the fuzzy classification method

Written informed consent for EUS-guided interstitial brachytherapy (EUS-guided iodine 125 radioactive seed implantation) was required from all included patients. Patient eligibility criteria included histologically confirmed unresectable pancreatic adenocarcinoma. To be included in the study, patients had to have a Karnofsky performance status score ≥ 60 and be expected to survive for more than 2 mo after diagnosis; in addition, they had to exhibit adequate bone-marrow function (absolute neutrophil count $\geq 1.5 \times 10^9$ cells/L, platelet count $\geq 100 \times 10^9$ /L, and hemoglobin ≥ 100 g/L), kidney function (serum creatinine ≤ 132.6 μ mol/L), and a prothrombin time within 3 s of the control. Exclusion criteria included the inability to give informed consent. Abdominal pain and other accompanying diseases had to be controlled in all patients before inclusion in the study. While receiving implantation treatment, the patients received other necessary treatments such as chemotherapy or biological therapy. The procedure used for radioactive seed implantation was the same one detailed in our previous description^[21].

All patients received repeated EUS before and after brachytherapy. All images were reviewed by endoscopists who were blinded to the prognosis. A total of 10 EUS images, 5 images each before and after brachytherapy, were chosen for each patient. The boundary of the ROI was manually delineated, and all the feature values within the ROIs were averaged together. By setting the appropriate range for the estimated scores, the influences of necrotic tissue and radioactive seeds on the calculation results were avoided. The fuzzy classification method calculated two scores for every patient.

All patients were evaluated by weekly physical examinations, complete blood counts, and chemistry profiles. The serum level of carbohydrate antigen 199 (CA199) was measured every 3 wk after the therapy. Standard WHO response criteria were used to define the best anti-tumor effects, toxicities, complications, and adverse events^[22]. Tumor assessment by the same endoscopic expert with an EUS scan was required every 3 mo. The largest and smallest diameters were recommended to be measured by EUS, and the tumor volume was estimated according the following formula: $V = 1/2 ab^2$, where a and b are the largest and smallest tumor diameters, re-

spectively, and V is the tumor volume. Overall survival (OS) was calculated from the day of treatment until the date of death.

Statistical analysis

The descriptive results of continuous variables were presented as the median (interquartile range, IQR). The relationships between the change of fuzzy classification score, tumor volume, CA199 level, and overall survival were assessed using the Spearman rank correlation test. The patients were divided into two groups according to an increase or decrease in the fuzzy classification score. The overall survival rates of the two groups were compared using the log-rank test. The inter-group comparison of the change of tumor volume and CA199 level was conducted by a Mann-Whitney U test. The results were considered statistically significant at $P < 0.05$. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS version 18.0).

RESULTS

Database of EUS images

Between March 2005 and December 2007, 153 patients with pancreatic cancer and 63 non-cancer patients with a normal pancreas (20 patients) or chronic pancreatitis (43 patients) were included in the analysis database. All EUS images of these patients were analyzed. The ROIs were drawn, and texture features were extracted and selected.

Characteristics of the included patients

From April 2007 to March 2009, a total of 25 consecutive patients were enrolled. There were fourteen men and eleven women, with a median age of 67 years (range 54-80 years) and a median KPS score of 80 (range 60-90). Five patients were in stage III, and twenty were in stage IV. The average number of seeds (0.5 mCi per seed) implanted was 14.6 per patient (range 5-30 per patient). All patients tolerated the brachytherapy well without any complications throughout the study.

Change of the fuzzy classification score

A total of 250 EUS images from the 25 patients were analyzed using the fuzzy classification method, and every patient was scored twice. There was a correlation between the change in the fuzzy classification score and overall survival ($r = 0.616$, $P = 0.001$), whereas no correlation was found to be significant between the change of tumor volume ($P = 0.663$), CA199 level ($P = 0.659$), and overall survival. There were 15 patients with a decrease in the fuzzy classification score after the brachytherapy, whereas the fuzzy classification score increased in other 10 patients (Table 1). There was a significant difference in the overall survival between the two groups (67 d *vs* 151 d, $P = 0.001$, Figure 1). There was no significant difference in the change of tumor volume ($P = 0.345$) and CA199 level ($P = 0.371$) between the two groups (Table 1).

Table 1 The change in the fuzzy classification result and clinical parameters after brachytherapy in 25 unresectable pancreatic cancer patients

No.	FCS before brachytherapy	FCS after brachytherapy	FCS ¹	Tumor volume ¹	CA199 ^{1,2}	Survival time (d)
1	22.30	80.40	-260.50%	-93%	-53%	58
2	16.30	33.52	-105.60%	14%	69%	69
3	70.60	88.61	-25.50%	42%	75%	50
4	50.30	60.24	-19.80%	53%	0%	80
5	52.60	56.80	-8.00%	2%	0%	53
6	60.30	63.50	-5.30%	0%	8%	67
7	87.52	90.31	-3.20%	0%	66%	198
8	88.50	90.20	-1.90%	-320%	17%	54
9	83.10	83.68	-0.70%	0%	NA	103
10	90.80	91.20	-0.40%	12%	0%	125
11	88.20	87.20	1.10%	-15%	NA	200
12	90.20	88.93	1.40%	-171%	0%	143
13	91.30	88.70	2.80%	0%	0%	138
14	92.43	89.20	3.50%	48%	0%	221
15	95.10	88.20	7.30%	-1%	6%	312
16	89.21	80.12	10.20%	-104%	-30%	108
17	93.70	81.10	13.40%	71%	85%	61
18	87.52	75.21	14.10%	44%	-265%	182
19	92.20	61.23	33.60%	96%	0%	122
20	82.30	47.60	42.20%	78%	-3%	200
21	78.56	44.00	44.00%	-66%	-1%	150
22	75.90	35.62	53.10%	42%	13%	104
23	51.20	18.30	64.30%	40%	-358%	151
24	90.10	22.70	74.80%	61%	96%	194
25	89.30	18.64	79.10%	4%	95%	378
Groups						
Increase (<i>n</i> = 10) (interquartile range)			-6.7% (43.9%)	1% (44%)	8% (68%)	67 (49) ³
Decrease (<i>n</i> = 15) (interquartile range)			14.1% (49.6%)	40% (76%)	0% (41%)	151 (78) ³

¹The parameter change was calculated by (pre-post)/pre; ²When the levels of carbohydrate antigen 199 (CA199) were larger than 1000 μmol/L before and after treatment, 0% meant no improvement; ³*P* = 0.001. FCS: Fuzzy classification score; NA: Not available.

DISCUSSION

The analysis of texture features is the core of DIP of digital images. Texture features are helpful for classifying lesions on sonography, and the potential of sonographic texture analysis to improve tumor diagnosis has already been demonstrated^[23-27]. However, only a few reports exist about the application of DIP techniques to EUS. For the diagnosis of pancreatic cancer, research using DIP and pattern recognition remains rare. Two recent studies successfully used neural network analysis of EUS images to differentiate pancreatic cancer from chronic pancreatitis^[1,3]. Das *et al.*^[3] reported high sensitivity (93%) and specificity (92%), with excellent positive predictive values (87%) and negative predictive values (96%). An SVM model was evaluated as a potential method to differentiate between malignant and benign lesions with excellent accuracy rates^[28]. Its performance characteristics in differentiating pancreatic cancer from benign lesions or normal tissue of the pancreas are closely rivaled by those of EUS-FNA.

In our study, the feature extraction and selection based on fuzzy classification was applied to EUS images of pancreatic cancer patients. All the work was carried out by the developed C++ program. According to the fuzzy algorithm, the classification result was not just “yes” or “no”, but a score from 0 to 100^[2,13,29]. Compared with the SVM method^[13], the fuzzy classification method

proposed in our study could additionally give the precise numerical difference between a cancer case and a non-cancer case.

EUS-guided brachytherapy has been applied preliminarily in the study of advanced pancreatic cancer^[30]. Two clinical series showed that pancreatic cancer could be treated safely with EUS-guided brachytherapy with pain control^[31,32]. The number of patients enrolled in these two series was 22 and 100, respectively, with stage III or IV pancreatic cancer in a majority of cases. The estimated median overall survival in the two studies was 9.0 and 7.0 mo. The brachytherapy’s effect on overall survival was uncertain because of the lack of a control. Meanwhile, making prognosis judgments for these patients is still difficult. Given that brachytherapy aims to destroy the tumor closely, if it were effective, the EUS images of pancreatic cancer ought to change to be more similar to those from a normal pancreas, and the fuzzy classification score of EUS images after the brachytherapy ought to show a decrease. Thus, the change of the fuzzy classification score most likely reflected the treatment effect to some extent and the prognosis after brachytherapy. Our study results validated this hypothesis. First, the change of the fuzzy classification score was significantly correlated with overall survival, which meant the more the score decreased, the longer the patient survived. Second, 15 of 25 patients (60%) had a decreased fuzzy classification score after the

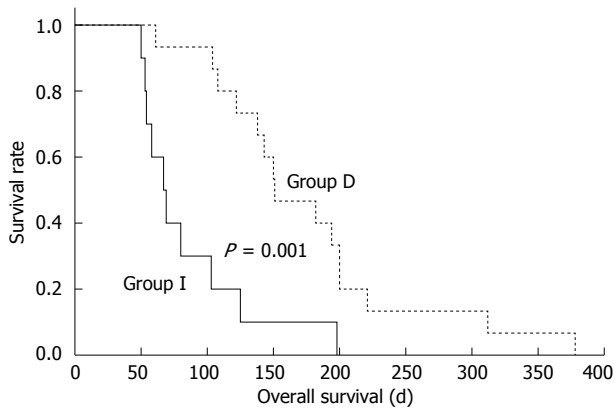


Figure 1 Survival curve of two groups of patients with pancreatic cancer treated with endoscopic ultrasonography-guided interstitial brachytherapy. Group I ($n = 10$): Patients whose fuzzy classification score increased after the brachytherapy. Group D ($n = 15$): Patients whose fuzzy classification score decreased after the brachytherapy.

brachytherapy. The median overall survival was nearly 5 mo. As a control, the fuzzy classification results increased in 10 patients after treatment, and the median overall survival was only approximately 2 mo. The log-rank test indicated a significant difference between these two groups.

The tumor volume is an important candidate for making prognosis evaluations for pancreatic cancer. In our study setting, the metal package of radioactive seeds made it difficult to measure the tumor volume by computed tomography. Thus, the EUS scan was a more suitable and convenient way to measure the volume. Meanwhile, as a diagnostic marker, CA199 is also another candidate for prognosis evaluation^[3]. However, our results found no association between the change of tumor or CA199 and the overall survival, which meant they were not a suitable prognosis marker in the patient population.

There were some limitations in our study. The new method can distinguish pancreatic cancer from chronic pancreatitis or a normal pancreas, but it cannot differentiate different cancer types. The probable approach to overcome this problem is to train multiple, 1-*vs*-all classifiers^[19]. Furthermore, enlarging the sample size and selecting new effective features are future possibilities for further study to improve the practicability of the technique.

In conclusion, the fuzzy classification method to score texture features of pancreatic cancer in EUS images is feasible and can be used as an effective tool to judge the prognosis of patients with unresectable pancreatic cancer treated by interstitial brachytherapy.

COMMENTS

Background

The application of digital image processing (DIP) in endoscopic ultrasonography (EUS) images and other imaging scenarios has been proven to be a useful adjunct to endoscopic diagnoses and is often comparable with specialists' interpretations in different pathologic settings. The typical method of support vector machine, which is only able to provide a differential diagnosis for solid tumors

("yes" or "no"), cannot provide numerical data describing the texture parameters in the EUS image. Thus, authors applied a new DIP method based on fuzzy classification to quantify the images and supply more information about the status of pancreatic cancer.

Research frontiers

The prognosis for pancreatic cancer is poor, and the effects of all currently available therapies are poor. EUS-guided brachytherapy has been applied preliminarily in the study of advanced pancreatic cancer as a potential new therapy. However, making prognosis judgments for these patients after brachytherapy is still difficult. EUS has become a useful tool to monitor cancer lesions. DIP of the change in EUS images after brachytherapy may be useful for making prognosis judgements.

Innovations and breakthroughs

Making prognosis judgments for patients with unresectable pancreatic cancer after EUS-guided interstitial brachytherapy is difficult. Authors developed a new DIP method based on fuzzy classification to analyze EUS images of pancreatic cancer, and they validated its utility in making prognosis judgements.

Applications

The new DIP method based on using fuzzy classification to analyze EUS images supplies more information than other DIP methods and has a significant potential to assist in clinical decision making in terms of diagnosis, prognosis, and diseases monitoring, especially for solid tumors.

Terminology

Fuzzy classification is the process of grouping elements into a fuzzy set whose membership function is defined by the truth value of a fuzzy propositional function.

Peer review

This study provided a new method to evaluate the effect of EUS-guided interstitial brachytherapy on unresectable pancreatic cancer. Through digital image processing of EUS images, the current study indicates that using the fuzzy classification method to score the texture features of pancreatic cancer in EUS images is useful for making prognosis judgments for patients with unresectable pancreatic cancer treated by interstitial brachytherapy.

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Ectopic liver: Different manifestations, one solution

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Abstract

Developmental abnormalities are rare in the liver. This study presents two case reports of ectopic liver. The first case was a 31-year-old male with clinical indication for laparoscopic appendectomy. Laparoscopy identified a perforated appendix and an unknown tumorous lesion in the ligamentum hepato umbilicalis. The patient underwent a laparoscopic appendectomy, intraoperative lavage of the peritoneal cavity, and extirpation of the lesion in the ligamentum hepato umbilicalis. Histopathological examination of the excised tumor revealed that it comprised liver tissue with fibrinous changes. The tumor was completely separate from the liver with no connection. It was classified as an ectopic liver. No further therapy was required. The second case was a 59-year-old male with a tumor on the upper pole of the spleen, incidentally diagnosed in an ultrasound examination. The biopsy raised suspicion of hepatocellular carcinoma. A positron emission tomography-computed

tomography examination revealed accumulation of F-18 fluorodeoxyglucose only in the tumor. The patient underwent a splenectomy with a resection and reconstruction of diaphragm. After the hepatocellular carcinoma was confirmed, adjuvant therapy (sorafenib) was initialized. The operations and postoperative recoveries were uncomplicated in both cases. Despite the low incidence of ectopic liver and rare complications, it is necessary to maintain awareness of this possibility. The potential malignancy risk for ectopic liver tissue is the basis for radical surgical removal. Therapy for hepatocellular carcinoma in an ectopic liver follows the same guidelines as those followed for treating the "mother" liver.

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Key words: Ectopic; Liver; Hepatocellular carcinoma; Diagnostic; Treatment

Core tip: Ectopic liver presents a rare clinical finding resulting from liver tissue migration to various organs during embryogenesis. Although the condition is typically asymptomatic, it can lead to different clinical manifestations such as intraabdominal bleeding or hepatocarcinogenesis. The potential malignancy risk is the basis for radical surgical removal; which represents the only correct solution. Therapy for hepatocellular carcinoma in an ectopic liver follows the same guidelines (National Comprehensive Cancer Network Guidelines) as those followed for treating the "mother" liver. Despite the low incidence of ectopic liver and rare complications, it is necessary to maintain an awareness of this possibility.

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INTRODUCTION

The liver is the largest abdominal organ. It occupies a substantial portion of the upper abdominal cavity. Abnormalities in the position or number of liver parts are considered rare developmental anomalies. They are typically asymptomatic, and incidental detection, though extremely rare, may occur during an operation or autopsy. The incidence of ectopic liver is 0.24%-0.56%, according to data described in laparoscopic or autopsy studies^[1-3], but this estimate seems high. Most authors distinguish two types of ectopic liver. The first is an accessory liver lobe connected to the liver, and the second is a truly ectopic liver. Collan classified four types. The first is the ectopic liver, which is not connected to the mother liver, but is typically attached to the gallbladder or intra-abdominal ligaments. The second is a microscopic ectopic liver, which is occasionally found in the gallbladder wall. The third is a large accessory liver lobe, attached to the mother liver by a stalk (pedunculated liver). The fourth is a small, accessory liver lobe attached to the mother liver^[4]. Here, we presented two manifestations of ectopic livers.

CASE REPORT

Case 1

A 31-year-old male patient was admitted with an 8-h history of pain in the right lower abdominal quadrant with a gradual onset. The patient reported nausea, but no vomiting, normal bowel function, and normal miction. He was subfebrile, but no infection was observed. His medical history included pollinosis. He took no regular medication and had no previous surgeries.

The clinical examination showed right lower quadrant abdominal pain with tenderness. The bowel sounds were diminished. The patient was hemodynamically stable without any signs of sepsis (temperature 37.5 °C, noninvasive blood pressure 120/80 mmHg, heart rate 76 beats/min, respiratory rate 14 breaths/min). The white blood count was 14800 cells/mL and C-reactive protein was 12.3 mg/L. Other biochemical results were normal and the urinalysis revealed no pathological findings. An abdominal ultrasound showed a small amount of pericaecal fluid. No other abnormal findings were identified by ultrasound in other parts of the abdomen or pelvis.

The signs and symptoms suggested appendicitis; therefore, we performed an acute laparoscopic appendectomy. First, antibiotic therapy was introduced. Initially, the routine diagnostic laparoscopy revealed perforated appendicitis with circumscribed peritonitis. Incidentally, a small oval tumor (3 cm × 2 cm × 2 cm) was found in the ligamentum hepato umbilicalis next to the liver (Figure 1). No other pathological signs were observed. The appendectomy was performed, followed by an intraoperative lavage of the peritoneal cavity. The tumor was excised. The operation and the postoperative recovery were uncomplicated. The patient was discharged on the third postoperative day. The histology of the appendix revealed an ulcerophlegmonous appendix. The histopath-

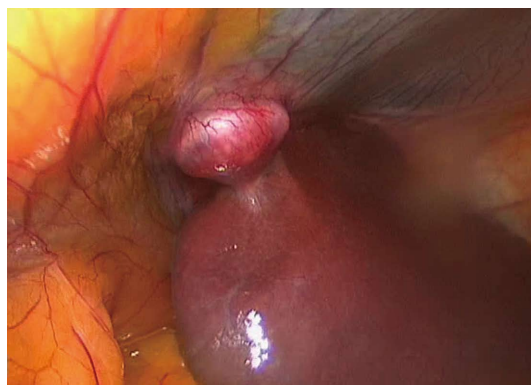


Figure 1 Small oval tumor (3 cm × 2 cm × 2 cm) was found in the ligamentum hepatoumbilicalis next to the liver.

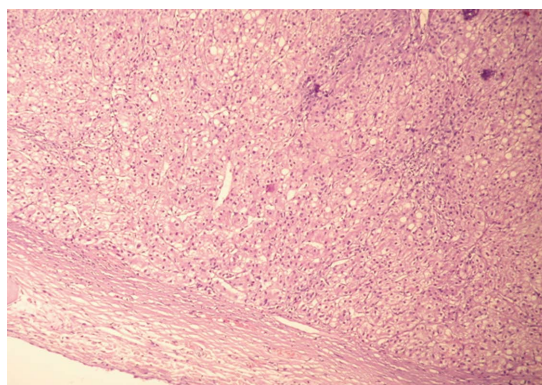


Figure 2 Histopathology of the tumor from the ligamentum hepatoumbilicalis (liver tissue with moderate steatosis and a thick fibrotic capsule).

ological examination of the tumor from the ligamentum hepato umbilicalis revealed liver tissue with moderate steatosis and a thick fibrotic capsule (Figure 2). The specimen examination showed that the tumor was completely separate, with no connection to the liver. It was classified as the first type of ectopic liver according to the Collan classification. No further therapy was required.

Case 2

During an ultrasound examination, a 59-year-old male was incidentally diagnosed with a tumor (10 cm × 8 cm × 6 cm) on the upper pole of the spleen. The finding was confirmed with a computed tomography (CT) scan (Figure 3), and a biopsy was performed. A histological examination of the biopsy specimen, including immunohistochemistry, raised the suspicion of a metastatic hepatocellular carcinoma, but bile production was not caught and renal carcinoma could not be reliably ruled out. To detect the primary tumor location, we performed a positron emission tomography-computed tomography examination. This showed a localized accumulation of F-18 fluorodeoxyglucose only in the suspicious tumor. There was no other pathological finding in the abdominal or thoracic cavities. The medical history included toxic-nutritive hepatopathy (alcoholic liver disease) and chronic gastritis. The biochemical results were within normal

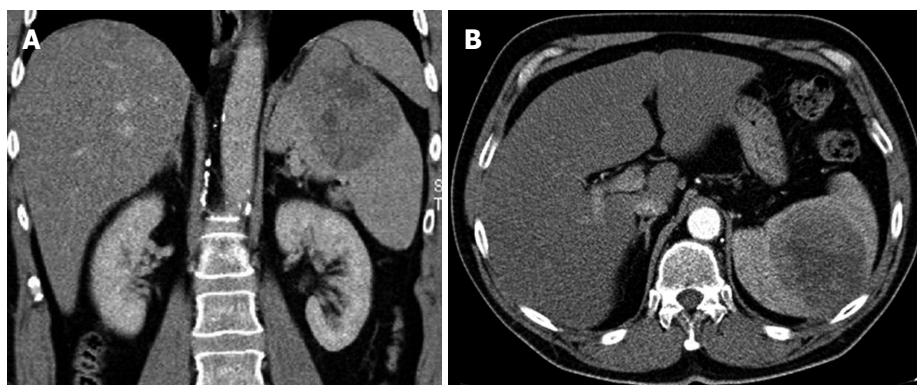


Figure 3 Tumor (10 cm × 8 cm × 6 cm) on the upper pole of the spleen. A: Computed tomography scan, arterial phase with a coronal reconstruction; B: Computed tomography scan, arterial phase - axial orientation.

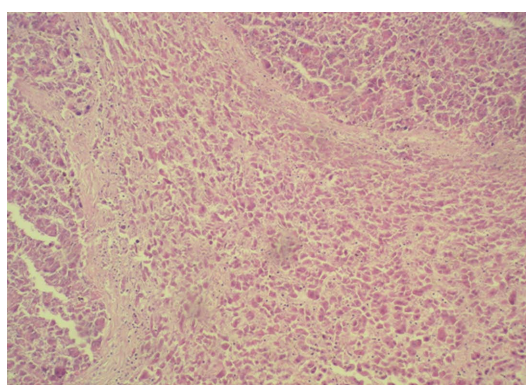


Figure 4 Histopathology of the hepatocellular carcinoma found in an ectopic liver in the spleen.

limits, and oncomarkers (carcinoembryonic antigen, alpha-fetoprotein, CA 19-9) were negative. A perioperative examination confirmed that the tumor was located on the upper pole of the spleen and was connected to diaphragm, but did not invade other surrounding tissues. It was classified as the first type of ectopic liver according to the Collan classification. No other pathology was found in the abdomen. A splenectomy was performed, with partial diaphragm resection and reconstruction. The postoperative recovery was uneventful. A definitive histological examination, including immunohistochemistry, confirmed a hepatocellular carcinoma (HCC) in the spleen tissue (Figure 4). Two small additional tumor sites (satellite tumors) were found in addition to the main lesion. Several investigations showed that the orthotopic liver tissue was negative for HCC, but a histological verification (a biopsy of the mother liver tissue) was not performed. Due to the high risk of tumor recurrence (additional tumor sites were found), we initialized a targeted adjuvant therapy with sorafenib. The American Association for the Study of Liver Diseases (AASLD) practice guidelines on the management of hepatocellular carcinoma do not recommend the routine use of adjuvant therapy with sorafenib (recurrence rates reduction was not reliably proven)^[5], nevertheless, there are data available that indicate that sorafenib was effective for treating patients with advanced HCC^[6,7].

DISCUSSION

In development, the hepatic diverticulum comprises the liver and biliary tree, and it appears late in the third week or early in the fourth week of gestation. The foregut endoderm of the hepatic diverticulum develops into the liver parenchyma (hepatocytes) and the epithelial lining of the biliary tract. The hepatic diverticulum divides to form a small ventral portion, the future gall bladder, and a larger cranial portion, the liver primordium. Developmental errors are relatively rare in the liver. Other errors in foregut development are more frequently observed, like errors in pancreas or duodenum formation^[8]. Liver tissue can migrate to various organs during embryogenesis. Sites of ectopic liver include the gallbladder, spleen, retroperitoneum, pancreas, adrenal gland, portal vein, diaphragm, thorax, gastric serosa, testes, and umbilical vein^[9]. Most authors distinguish ectopic and accessory liver formations, based on whether there is a connection to the mother liver. The Collan Classification mentioned above is not widely used. In many cases, it is difficult to make a clear distinction between ectopic liver and accessory liver. The precise incidence of ectopic liver or accessory liver is unknown. Examination of several studies indicated that the incidence is approximately 0.24%-0.56%. Watanabe's series of 1060 patients revealed an incidence of 0.47% for ectopic liver and 0.09% for accessory liver. These numbers could be over-estimated, because histological verification was not performed in all cases. Ectopic and accessory liver are typically asymptomatic, but occasionally they cause unexpected problems, like intra-abdominal bleeding or hepatocarcinogenesis. The first clinical sign of ectopic or accessory liver could be an acute complication that leads to acute surgery and diagnosis of ectopic liver. Various clinical symptoms, like recurrent abdominal pain and impaired liver function could be caused by ectopic or accessory liver, but in the majority of cases, an ectopic/accessory liver remains undetected.

In some cases, the ectopic or accessory liver may undergo torsion, infarction, rupture, or other disorders. Torsion and subsequent infarction of an accessory liver lobe has been described in children and adults^[10-14]. Ladurner

presented a very interesting case of a patient with hepatic ischemia caused by complete vascular occlusion due to a twisted accessory liver lobe. In that case, the accessory liver lobe produced serious, life-threatening problems, and an orthotopic liver transplantation was performed^[15]. Ito reported a small omphalocele that involved an accessory liver lobe embedded in the cranial portion of the amniotic sac. In that case, the pedicle of liver tissue was markedly elongated^[16].

An ectopic or accessory liver can lead to benign or malignant diseases. Benign cases in the literature report hemangiomas, adenomas, or focal nodular hyperplasia associated with an ectopic or accessory liver^[17-19]. Benign lesions seem to be less frequent; however, the higher frequency of malignancies could be based on the fact that many benign lesions remain undiagnosed because they are asymptomatic. Moreover, due to their abnormal locations, asymptomatic lesions may be misdiagnosed in the absence of histology.

The ectopic liver has been associated with malignancies more often than with benign lesions. Many authors have pointed out that ectopic liver tissue is more predisposed to malignancy than normal liver tissue. Ectopic livers have completely functional architecture, but may be metabolically handicapped; this may facilitate carcinogenesis. Ectopic liver tissue also has increased neoplastic potential compared to orthotopic liver tissue. This may have given rise to the hypothesis that ectopic livers are particularly predisposed to the development of hepatocellular cancer. A high incidence of hepatocellular cancer in ectopic livers was described in Japan^[20]. In most cases, a malignant tumor was found in the ectopic liver, but not in the mother liver. Ectopic or accessory livers with cancer may be amenable to surgical resection. Many case reports have described surgical treatments. Some authors suggested that the outcome after resection to remove hepatocellular cancer was superior when it involved an ectopic or accessory liver, compared to when it involved the mother liver. However, long-term follow-up data are poor.

Many anatomical locations have been described for ectopic livers with cancer^[20-23]. The favorable outcome after resection of ectopic livers could depend on the specific anatomical location^[24,25]. Shigemori described a case of ectopic hepatocellular carcinoma in the jejunum^[26]. Cardona *et al.*^[27] reported a case of a primary, well-differentiated hepatocellular carcinoma arising from ectopic liver tissue in the pancreas. Leone presented interesting data regarding three cases of hepatocellular carcinomas that arose in ectopic livers. The clinical presentations were very interesting; one patient reported dull epigastric pain; the second reported abrupt onset with signs and symptoms of acute abdomen caused by intra-abdominal bleeding; and the third presented with an unexplained, progressive increase in alpha-fetoprotein serum levels^[28]. Seo *et al.*^[29] reported a case of hepatocellular carcinoma that arose from hepatic parenchyma located in the left subphrenic space in the upper portion of the gastrosplenic

ligament. The preoperative diagnosis was a nonspecific stomach mass, with suspicion of gastrointestinal stromal tumor. An operation was performed laparoscopically. Takavasu reported another case with high serum alpha-fetoprotein combined with a suspicion of a submucosal stomach tumor^[30]. The two latter cases diagnosed the ectopic liver postoperatively, after histological examination.

It is important to consider an ectopic/accessory liver when evaluating perihepatic lesions. It is common to misdiagnose an ectopic liver as a malignant tumor. Statatus suggested that the diagnosis should be based on a biopsy of ectopic liver or a NMR with liver specific contrast^[31]. However, all investigative imaging methods are limited for diagnosing an ectopic/accessory liver, due to its limited volume. Despite the low incidence and rare complications of ectopic/accessory liver, it is necessary to maintain an awareness of this possibility. Because this entity presents with a broad spectrum of clinical symptomatology, it is rarely diagnosed; thus, most discoveries of ectopic and accessory liver are incidental. An elevation in serum alpha-fetoprotein and lack of focus in liver CT image may be the first signs of malignant transformation in an ectopic liver. The suspicion of hepatocellular carcinoma in an ectopic liver is substantial reason for radical surgical removal of an ectopic liver found incidentally. When hepatocellular carcinoma is definitely, histologically confirmed in an ectopic liver, it should be treated with the same approaches used for treating carcinoma in the mother liver (National Comprehensive Cancer Network Guidelines).

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A case of a duodenal duplication cyst presenting as melena

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Key words: Gastrointestinal hemorrhage; Duodenum; Duplication

Core tip: Duodenal duplication cysts are rare congenital anomalies that have been seldom reported in adults. Most cases of duodenal duplication have been associated with pancreatitis or jaundice and few have been reported as a cause of gastrointestinal hemorrhage. We submit a case of duodenal duplication cyst causing gastrointestinal hemorrhage. In rare cases, duodenal duplication cysts may cause gastrointestinal bleeding and must be included in the differential diagnosis.

Ko SY, Ko SH, Ha S, Kim MS, Shin HM, Baeg MK. A case of a duodenal duplication cyst presenting as melena. *World J Gastroenterol* 2013; 19(38): 6490-6493 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6490.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6490>

Abstract

Duodenal duplication cysts are benign rare congenital anomalies reported mainly in the pediatric population, but seldom in adults. Symptoms depend on the type and location and can present as abdominal pain, distension, dysphagia or dyspepsia. They have been reported to be responsible for duodenal obstruction, pancreatitis and, in rare cases, gastrointestinal bleeding. We present a case of a duodenal duplication cyst in a 43-year-old man presenting as melena. Initial gastroduodenoscopy and colonoscopy did not reveal any bleeding focus. However, the patient began passing melena after 3 d, with an acute decrease in hemoglobin levels. Subsequent studies revealed a duplication cyst in the second portion of the duodenum which was surgically resected. Histology revealed a duodenal duplication cyst consisting of intestinal mucosa. There was no further bleeding and the patient recovered completely. In rare cases, duodenal duplication cysts might cause gastrointestinal bleeding and should be included in the differential diagnosis.

INTRODUCTION

Duplication cysts are rare congenital anomalies of the gastrointestinal (GI) tract. Duodenal duplication cysts are extremely rare, representing only 2%-12% of GI tract duplications^[1]. Most duplication cysts are detected in children and fewer than 30% of all duplications are diagnosed in adults^[2]. They are difficult to diagnose, as the presenting symptoms are nonspecific and are closely related to the type, size and location of the lesion^[1]. We report a rare case of duodenal duplication with duodenoduodenal intussusception presenting as GI bleeding.

CASE REPORT

A 43-year-old man was admitted to the emergency department complaining of melena. He had complained of recurrent burning, nonradiating upper abdominal pain,

usually lasting for several minutes, irregular in nature and relieved by taking food since childhood. Ten years previously, he had been diagnosed with a gastric ulcer and had taken medications for 1 year.

His vital signs upon admission were a blood pressure of 120/80 mmHg and a heart rate of 105 beats/min. Initial laboratory studies revealed a hemoglobin level of 9.7 g/dL (normal range, 14-18 g/dL), hematocrit 28.8% (normal range, 42.0%-52.0%) and mean red corpuscular volume 88.4 fL (normal range, 80-94 fL). A nasogastric tube was inserted, but no blood was aspirated.

An initial gastroduodenoscopy showed no evidence of active bleeding or obvious focus. A colonoscopic examination was unremarkable, except for the presence of internal hemorrhoids. After a blood transfusion of 220 mL packed red blood cells, his hemoglobin level rose to 10.3 g/dL and there was no clinical evidence of further bleeding.

On the third day of hospitalization, the patient passed about 200 mL of melena and his hemoglobin levels fell to 7.6 g/dL. Gastroduodenoscopy revealed fresh blood oozing without a definite bleeding focus in the second portion of the duodenum. As there was no obvious bleeding focus, a high-resolution computed tomography (CT) scan of the abdomen was done. It revealed a circumferential cystic lesion in the proximal duodenum arising from a duodenoduodenal intussusception (Figure 1). As the patient did not have any symptoms except for melena and physical examinations did not reveal any palpable sausage-shaped abdominal mass suggestive of intussusception, an upper GI series was done. This demonstrated a large elongated sac-like 10 cm long mass in the second and proximal third portion of the duodenum (Figure 2). The junction of the first and second portions of the duodenum, where the mass began, was narrowed locally and the second portion of the duodenum was enlarged. This feature suggested a large elongated communicating duplication cyst (about 10 cm long) in the second and proximal third portion of the duodenum with duodenoduodenal intussusception.

As rare cases of GI cysts with malignancies have been reported in the literature, bleeding from a cancer could not be ruled out. Because of the recurrent bleeding symptoms and the possibility of a cancer being present, we chose surgical resection. This revealed a mass attached to the second and third portion of the duodenum. After duodenotomy, the cyst was opened and blood clots were found in its cavity. Subtotal resection of the cyst was done followed by primary closure of the duodenum. A macroscopic examination showed an irregular, flat mass measuring 4 cm × 4 cm × 0.5 cm, covered with intestinal mucosa and with hemorrhagic contents (Figure 3). Microscopy revealed showed the cyst wall to be composed of two mucosal layers sharing a common muscle layer (Figure 3). The patient had an uneventful recovery and was discharged 12 d later. Follow-up visits at our outpatient clinic have revealed no sign of further GI bleeding.

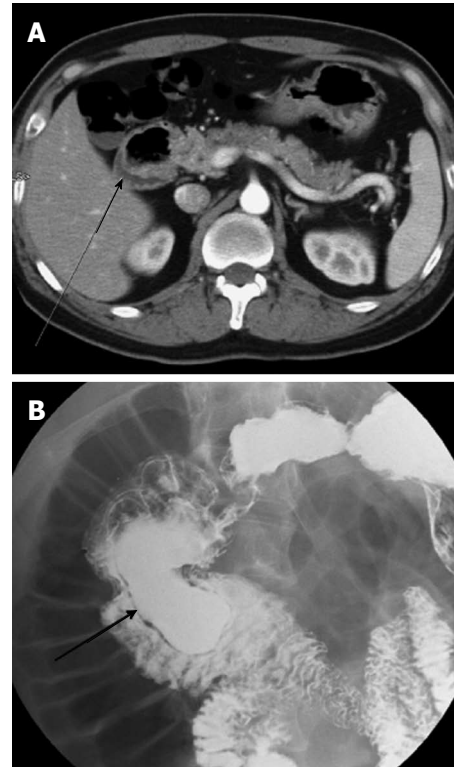


Figure 1 Computed tomography image. A: A high resolution computed tomography image of the abdomen reveals a circumferential cystic lesion (arrow) in the vicinity of the second part of the duodenum; B: Upper gastrointestinal series showing an large elongated sac-like mass (about 10 cm in length, arrow) arising from the second and proximal third portion of the duodenum.

DISCUSSION

GI duplication cysts are rare congenital anomalies formed during the embryonic development of the alimentary tract. They are defined by a smooth muscle coat, an intimate attachment to the native GI tract and a GI mucosal lining^[3]. They can occur anywhere along the GI tract, with varying types, shapes and sizes. GI duplication cysts are most commonly found in the distal ileum, followed by the esophagus, colon and jejunum^[4].

Duodenal duplication cysts are among the rarest of all intestinal duplications. Most are located in the second or third portion of the duodenum. The cysts are usually filled with clear fluid, but might contain gallstones, bile or pancreatic fluid depending on communication with the biliary or pancreatic systems^[5]. Most are diagnosed in childhood, with diverse, nonspecific clinical presentations^[6]. The most common symptoms reported are abdominal pain and nausea/vomiting. The most common complication is pancreatitis, reported in up to 53% of patients. Other manifestations such as intussusceptions, infection or weight loss have also been reported^[1]. Though rare, GI bleeding can result from peptic ulceration of the ectopic gastric mucosa within the cyst and be a cause of unexplained GI bleeding^[5]. The bleeding can be painless, brisk and life threatening, as shown in our patient. The bleeding in our case was made more interesting by the

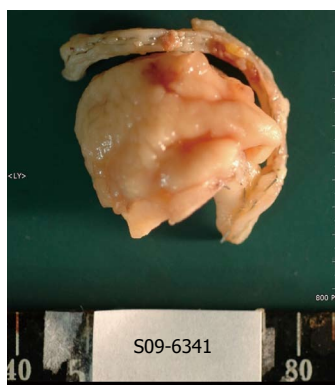


Figure 2 Gross specimen of the excised duodenal duplication cyst.

fact that histology did not reveal any ectopic gastric mucosa within the cyst, contrary to previous reports^[1,7,8].

The diagnosis of duodenal duplication cysts is difficult. The site of origin, such as an intraluminal or intramural position, and the presence or absence of luminal communication has to be taken into account. If the cyst is intraluminal, differentiation must be made between a pedunculated neoplastic lesion or a duodenal diverticulum. In the case of an intramural mass, a distended duodenal duplication has to be differentiated from other cystic masses belonging to the duodenal-choledochal-pancreatic area such as cystic dystrophy of the duodenal wall, choledochal cyst, pseudocysts, cystic tumors of the pancreas, cystic lymphangiomas, or mesenteric cysts. An empty duodenal duplication shows a solid structure and has to be differentiated from neoplastic or inflammatory duodenal or pancreatic lesions^[9].

The preoperative diagnosis of duodenal duplications is often inaccurate. Diagnosis is usually done using imaging modalities such as ultrasonography or CT scans. On ultrasonography, duodenal duplication is seen as having an echogenic inner mucosa surrounded by a hypoechoic outer muscular layer^[4]. CT scans often reveal a cystic mass associated with the alimentary tract and is more useful in demonstrating the precise anatomical relationship between the cyst and surrounding structures^[4]. Magnetic resonance images as well as endoscopic ultrasonography allow us to suspect duplications and are useful in evaluating upper GI tract masses^[3,9]. On contrast CT series of the GI, duodenal duplication cysts can present as smooth submucosal or extrinsic masses, or as oval filling defects in the duodenum, as can be seen in our case. In case of bleeding, the most sensitive tool is gastroduodenoscopy, which can locate the bleeding duplication in the duodenum^[8]. However, all modalities allow us only to suspect the presence of an abnormal lesion, and diagnostic confirmation is possible only after resection.

Treatment of duodenal duplication has classically involved surgical resection. However, cases of endoscopic treatment have been reported, especially in cases where the duplication is in close proximity to the adjacent structures such as the major duodenal papillae^[10]. In our case,

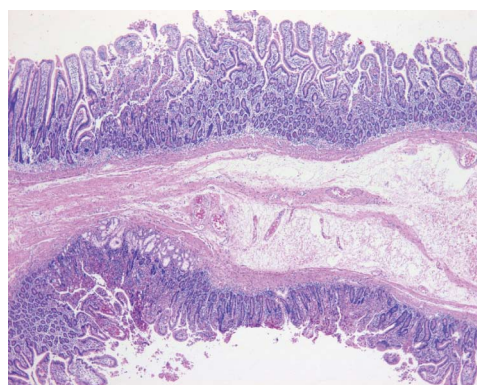


Figure 3 Histology of the duplication cyst showing two mucosal layers sharing a submucosal layer with muscular layer. Some of the mucosa are comprised of duodenal mucosa and the majority are jejunal mucosa (hematoxylin and eosin stain, $\times 40$).

endoscopic treatment was not possible because the duplication was not visible by endoscopy, leading to the need for surgical excision. Treatment of asymptomatic cases remains controversial. However, as neoplasms have been reported within duodenal duplication cysts, surgical resection must be considered^[1].

In conclusion, duodenal duplication cysts are rare congenital anomalies that are seldom reported in adults. Clinical presentations are diverse, but there are a few reports of GI bleeding. Here we report a case of a duodenal duplication cyst with overt GI bleeding and suggest that such cysts should be considered in cases of undiagnosed GI bleeding.

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E-Editor Zhang DN



Simultaneous intrahepatic and subgaleal hemorrhage in antiphospholipid syndrome following anticoagulation therapy

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Abstract

Warfarin is a widely used anticoagulant. Interindividual differences in drug response, a narrow therapeutic range and the risk of bleeding render warfarin difficult to use clinically. An 18-year-old woman with antiphospholipid syndrome received long-term warfarin therapy for a recurrent deep vein thrombosis. Six years later, she developed right flank pain. We diagnosed intrahepatic and subgaleal hemorrhages secondary to anticoagulation therapy. After stopping oral anticoagulation, a follow-up computed tomography showed improvement in the hemorrhage. After restarting warfarin because of a recurrent thrombosis, the intrahepatic hemorrhage recurred. We decided to start clopidogrel and hydroxychloroquine instead of warfarin. The patient has not developed further recurrent thrombotic or bleeding

episodes. Intrahepatic hemorrhage is a very rare complication of warfarin, and our patient experienced intrahepatic and subgaleal hemorrhage although she did not have any risk factors for bleeding or instability of the international normalized ratio control.

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Key words: Warfarin; Liver; Subgaleal; Hemorrhage; Antiphospholipid syndrome

Core tip: An 18-year-old woman with antiphospholipid syndrome received long-term warfarin therapy for a recurrent deep vein thrombosis. Six years later, she was diagnosed with intrahepatic and subgaleal hemorrhage and received clopidogrel and hydroxychloroquine in place of warfarin. She has not developed further recurrent thrombotic or bleeding episodes. Intrahepatic hemorrhage is a very rare complication of warfarin, and our patient experienced intrahepatic and subgaleal hemorrhages even though she did not have any risk factors for bleeding or an elevated international normalized ratio.

Park IC, Baek YH, Han SY, Lee SW, Chung WT, Lee SW, Kang SH, Cho DS. Simultaneous intrahepatic and subgaleal hemorrhage in antiphospholipid syndrome following anticoagulation therapy. *World J Gastroenterol* 2013; 19(38): 6494-6499
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INTRODUCTION

Antiphospholipid syndrome (APS) is characterized by

venous or arterial thrombosis or pregnancy morbidity, in the presence of antiphospholipid antibodies (aPL). A venous thrombosis is the most common presenting complication of APS^[1]. Long-term anticoagulation therapy is recommended for the secondary prevention of thromboembolism.

Warfarin is a widely used anticoagulant prescribed for patients with venous thrombosis, pulmonary embolism, chronic atrial fibrillation, and prosthetic heart valves. Interindividual differences in drug responses, a narrow therapeutic range and the risk of bleeding render warfarin difficult to use clinically. During treatment with oral anticoagulants, the risk of severe bleeding has been estimated to be 0.6%-1.0% per treatment year^[2,3]. In a retrospective study of hemorrhagic complications in 184 patients, gastrointestinal bleeding was the most common complication, and liver or splenic hemorrhage was identified in only 3 patients^[4]. Only a few cases of liver hematomas have been reported in the literature following anticoagulant therapy^[5]. We report a woman with primary APS who presented with a simultaneous intraparenchymal hemorrhagic complication in the liver and a subgaleal hematoma with anticoagulation treatment, without any risk factors of bleeding or an elevated international normalized ratio (INR). This type of condition has not been previously described.

CASE REPORT

An 18-year-old woman visited Dong-A University Hospital with swelling and tenderness of the left lower leg in March 2004. She had no past history of serious illness. Doppler ultrasonography (US) of her lower legs showed deep vein thromboses (DVT) in the left popliteal vein and the right common femoral vein. She received anticoagulation treatment with warfarin for 2 years. After 2 years, a follow-up US showed nearly complete improvement of the DVT, and anticoagulation therapy was stopped.

She returned four months later, in November 2006, with edema and tenderness of the right lower leg. US of her lower legs showed a chronic DVT in the right popliteal vein. We reexamined her for other conditions leading to a hypercoagulable state. The clinical lab values were as follows: white blood cell count (WBC) 4570/mm³, segmental neutrophils 29%, hemoglobin (Hb) 11.9 g/dL, platelets (PLT) 91000/mm³, prothrombin time (PT) 13.9 s, INR 1.23 and activated partial thromboplastin time (aPTT) 31.3 s. Tests for antinuclear antibodies were positive (at 1:40 dilution), and the particular types of immunofluorescence pattern were negative. An anti-double-stranded DNA antibody enzyme-linked immunoabsorbent assay test was moderately positive (326.7 WHO unit/mL). Anti-Smith antibody and anti-ribonucleoprotein antibody test were negative. Immunoglobulin (Ig)G anti- β_2 -glycoprotein I (anti- β_2 GPI) was positive (98.8 GPL), and IgM anti- β_2 -GPI was positive (95.5 GPL). IgG anticardiolipin antibodies (aCL) were highly positive (95.6 GPL),

and IgM aCL was positive (37.9 MPL). Lupus anticoagulant testing (diluted russell viper venom test, DRVVT) was positive (185.5 s). The patient was diagnosed with primary APS according to the revised Sapporo criteria for APS diagnosis^[6,7], and oral anticoagulation treatment was initiated.

After forty months of anticoagulation therapy, the patient was admitted in April 2010 with epigastric pain, headache, fever and chills. On physical examination, she had epigastric tenderness and body temperature of 38.2 °C. The laboratory findings were: WBC 17111/mm³, segmental neutrophils 82.9%, Hb 9.7 g/dL, PLT 136000/mm³, C-reactive protein (CRP) 8.38 mg/dL, alkaline phosphatase 211 I/U, PT INR 1.82 and aPTT 31.4 s. An abdominal computed tomography (CT) showed multiple low attenuated lesions without peripheral enhancement and perilesional edema of the liver on contrast-enhanced image (Figure 1). A brain magnetic resonance imaging (MRI) showed a crescentic high signal in the posterior parietal scalp on the T2 weighted image, a low signal on the T1 weighted image and mild enhancement on the contrast-enhanced scan images (Figure 2). We suspected intraparenchymal hemorrhage in the liver and a subgaleal hematoma in the posterior parietal scalp because of bleeding complications from anticoagulation. We stopped the warfarin treatment and administered antibiotics because there was the potential for an infected intrahepatic hemorrhage. After 2 wk of supportive care, a follow-up US on day 16 showed resolution of a previous hepatic lesion. We decided to restart warfarin because of her recurrent thrombotic episodes.

She was readmitted in June 2010 with right flank pain. On physical examination, she had abdominal tenderness in the right upper quadrant and her body temperature was normal. The WBC was 13250/mm³, the segmental neutrophils were 80.8%, the Hb was 10.2 g/dL and the PLT were 88000/mm³. The CRP was 7.93 mg/dL. The PT INR was 1.53, and the aPTT was 29.8 s. Liver and kidney function tests were unremarkable. An abdominal CT showed a low attenuated lesion in the S2/4 and S5 segments of the liver on the contrast-enhanced image (Figure 3A and B). We diagnosed the patient with intraparenchymal hemorrhage of the liver from the anticoagulation therapy and stopped warfarin treatment. Follow-up CT scanning on day 17 showed nearly complete improvement of intraparenchymal hemorrhage in the S2/4 and S5 segments of the liver (Figure 3C and D). After 1 mo, a follow-up US showed the previous intrahepatic lesions were completely resolved. We decided to initiate clopidogrel and hydroxychloroquine instead of warfarin. Subsequently, the patient has not developed recurrent thrombotic episodes or bleeding complications.

DISCUSSION

APS is characterized by the occurrence of venous or arterial thromboses or by specific pregnancy morbidity in the presence of laboratory evidence of aPL. According

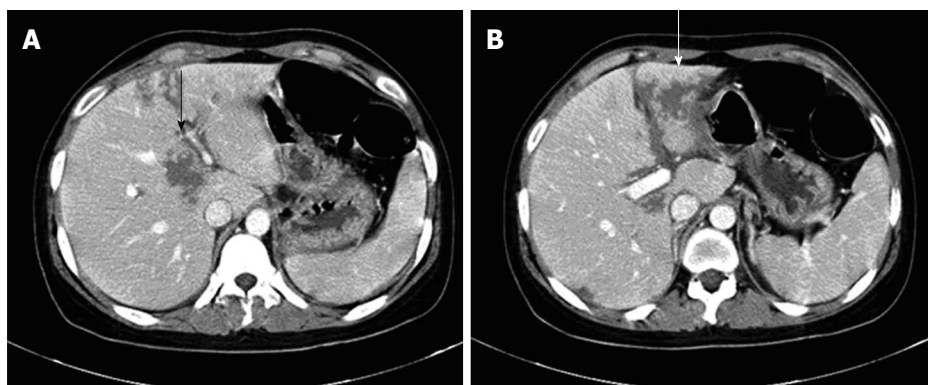


Figure 1 Intraparenchymal hemorrhage in the liver (arrows). An abdominal computed tomography showed multiple low attenuated lesions without peripheral enhancement (A) and perilesional edema of the liver on the contrast-enhanced image (B).

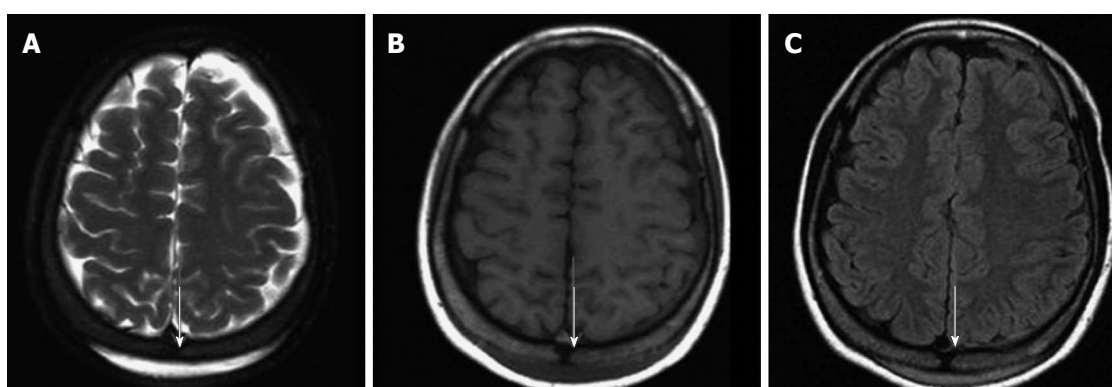


Figure 2 Subgaleal hematoma at the posterior parietal scalp (arrows). A brain magnetic resonance imaging showed a crescentic high signal in the posterior parietal scalp on the T2 weighted image (A), a low signal on the T1 weighted image (B) and mild enhancement on the contrast-enhanced image (C).

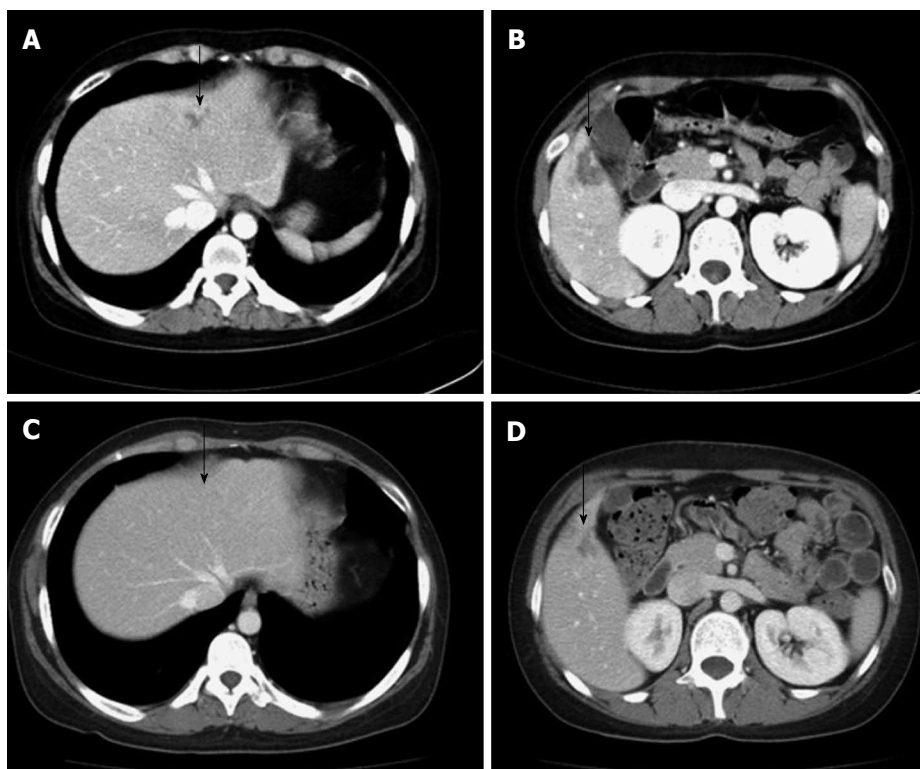


Figure 3 Recurrent and nearly complete resolution intraparenchymal hemorrhage in the liver (arrows). A, B: An abdominal computed tomography showed a low attenuated lesion (arrow) in the S2/4 (A) and S5 segments (B) of the liver on the contrast-enhanced image; C, D: An abdominal computed tomography showed resolution of the parenchymal hemorrhage in S2/4 (C) and the healing process in S5 (D) of the liver.

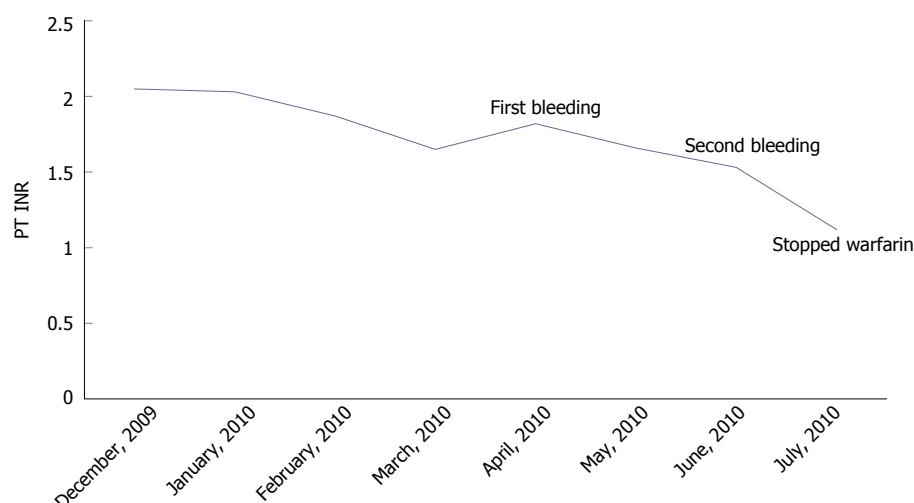


Figure 4 Extended follow-up data for the prothrombin time international normalized ratio. PT INR: Prothrombin time international normalized ratio.

to the revised Sapporo criteria^[6,7], definite APS is considered if at least one of the clinical criteria and at least one of the laboratory criteria are satisfied. APS occurs either as a primary or secondary condition in the setting of an underlying disease, usually systemic lupus erythematosus. In the largest prospective cohort study of patients with APS, venous thromboembolism was the most common presenting complication, including DVT (31.7%), pulmonary embolism (9.0%), and superficial thrombophlebitis (9.1%)^[11]. Warfarin is the standard of care for the chronic management of patients with APS who are not pregnant.

Warfarin is an oral anticoagulant used in a variety of clinical settings. The anticoagulant effect of warfarin is mediated through inhibition of the vitamin K-dependent gamma-carboxylation of coagulation factors II, VII, and X. Warfarin is among the top 10 drugs with the largest number of serious adverse event reports submitted to the United States Food and Drug Administration, because it has a high incidence of adverse effects as well as variable interactions with diet or medications through a mechanism of altered platelet function, altered vitamin K synthesis in the gastrointestinal tract and interference with vitamin K metabolism^[8-11]. In a prospective observational study, life-threatening bleeding occurred in 32 of 1999 patients. The gastrointestinal tract was the most common site, with bleeding occurring in 21 (66%) of the 32 patients^[12]. In another study of hemorrhagic complications in 184 patients, gastrointestinal bleeding was the most common complication and unusual hemoperitoneum and/or soft tissue bleeding were reported^[4]. Liver hemorrhage following anticoagulation therapy has been rarely reported in the literature^[5,13-15]. It is sometimes difficult to distinguish hepatic hemorrhage from a liver abscess. Liver abscesses appear as low attenuated lesions with peripheral enhancement or perilesional edema^[16,17]. We diagnosed a patient with an intrahepatic hemorrhage because these characteristics were not observed in our patient. We discussed the possibility of a microaneurysm in the liver and brain with radiology specialists who said that it was very unlikely.

There are many risk factors associated with bleeding complications following the use of vitamin K antagonist. These risk factor have been associated with a significantly increased risk of bleeding in one or more multivariate analyses: old age, diabetes mellitus, the presence of malignancy, hypertension, acute or chronic alcoholism, liver disease, severe chronic kidney disease, elevated creatinine, anemia, the presence of bleeding lesions or episodes, a bleeding disorder, concomitant use of aspirin, nonsteroidal anti-inflammatory drugs, antiplatelet agents, antibiotics, amiodarone, statins or fibrates, and instability of the INR (INR > 3.0, pre-treatment INR > 1.2)^[18,19].

In previously reported spontaneous liver hemorrhages, Erichsen *et al.*^[14] addressed the severe drug interaction between warfarin and trimethoprim-sulfamethoxazole. Dizadji *et al.*^[13] and Roberts *et al.*^[15] showed the two risk factors of old age and high INR at the time of admission. Behranwala *et al.*^[5] suggested multiple risk factors, including; old age, hypertension, instability of INR control, and drug interactions with statins and omeprazole. Our patient had a simultaneous intraparenchymal hemorrhage in the liver and subgaleal hematoma despite the absence of risk factors for bleeding and a therapeutic range of warfarin with a PT INR of 1.53 (Figure 4). This type of case has not been previously reported.

One study suggested risk factors that could be used in estimating the probability of major bleeding in outpatients treated with warfarin. The risk factors include the following; age ≥ 65 years, history of stroke, history of gastrointestinal bleeding, and one or more the following (recent myocardial infarction, hematocrit < 30%, serum creatinine > 1.5 mg/dL, and diabetes mellitus^[20]). The cumulative incidence of major bleeding at 48 mo in the low (no risk factor), intermediate (1 to 2), and high (3 or more) risk groups was 3%, 12% and 53% respectively. Our patient belonged to the low risk group with a cumulative incidence of only 3%. Some recent reports showed that APS could be correlated to a transient hemorrhagic event without anticoagulation therapy^[21,22]. Lupus anticoagulant-hypoprothrombinemia syndrome is a rare clinical

entity that can occur in association with systemic lupus erythematosus. It is characterized by prolongation of the coagulation test that is not corrected by normal fresh plasma because of non-neutralizing antibodies against Factor II. Our patient did not have this abnormal laboratory finding.

There are very few randomized studies comparing the treatment options for patients with an elevated INR and/or bleeding complications following the use of warfarin. If significant or life-threatening bleeding occurs, rapid reversal of excessive anticoagulation should be undertaken at any degree of anticoagulation^[23-26]. Warfarin should be stopped, and 10 mg of vitamin K should be administered by a slow intravenous infusion, supplemented by fresh frozen plasma for less urgent situations. For more urgent situations, recombinant human factor VIIa or prothrombin complex concentrate may be used. If needed, angiography may help with embolization in cases of severe major bleeding. In the majority of cases, bleeding is improved by stopping warfarin and conservative care. Restarting of warfarin is not recommended, and changing the drug used for maintaining long-term anticoagulation is suggested^[5].

In studies of the bleeding risk during antithrombotic therapy, the odds ratio is generally lower for clopidogrel than warfarin, and we decided to start clopidogrel instead of warfarin^[27,28]. We added hydroxychloroquine because limited data showed that it might be useful for thrombosis in patients with APS although there were no randomized controlled trials^[29,30]. After starting clopidogrel and hydroxychloroquine, our patients did not experience additional bleeding complications or recurrent thromboses.

Our patient had a simultaneous intrahepatic hemorrhage and a subgaleal hematoma despite the absence of risk factors and a therapeutic range of warfarin. This is the first case with simultaneous bleeding in the liver and brain in an APS patient treated with warfarin.

Patients with intrahepatic bleeding might experience right upper quadrant or epigastric pain and pain that radiates into the shoulder or flank. Fever might develop when an infection accompanies the intrahepatic hemorrhage. If a patient experience pain and/or fever during anticoagulation therapy, we should consider the possibility of abdominal bleeding such as an intrahepatic hemorrhage in any patient receiving oral anticoagulants, even though this circumstance remains unlikely. CT or US should be adopted for early diagnosis.

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Ileal duplication mimicking intestinal intussusception: A congenital condition rarely reported in adult

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Core tip: We reported a case of duplication of the alimentary tract involving the ileum, which mimicked intestinal intussusception, Meckel's diverticulum (MD) and Crohn's disease, in a young man. Computed tomography enterography identified the intussusception in the right lower quadrant, and the disease was positive on Tc-99m pertechnetate scintigraphy. Exploratory laparotomy and surgical pathology showed ileal duplication cyst with complicating ectopic gastric mucosa. Differential diagnosis of ileal duplication cyst was made, especially from MD.

Abstract

Intestinal duplication is an uncommon congenital condition in young adults. A 25-year-old man complained of chronic, intermittent abdominal pain for 3 years following previous appendectomy for the treatment of suspected appendicitis. Abdominal discomfort and pain, suggestive of intestinal obstruction, recurred after operation. A tubular mass was palpable in the right lower quadrant. Computed tomography enterography scan identified suspicious intestinal intussusception, while Tc-99m pertechnetate scintigraphy revealed a cluster of strip-like abnormal radioactivity in the right lower quadrant. On exploratory laparotomy, a tubular-shaped ileal duplication cyst was found arising from the mesenteric margin of the native ileal segment located 15 cm proximal to the ileocecal valve. Ileectomy was performed along with the removal of the duplication disease, and the end-to-end anastomosis was done to restore the gastrointestinal tract continuity. Pathological examination showed ileal duplication with ectopic gastric mucosa. The patient experienced an eventless postoperative recovery and remained asymptomatic within 2 years of postoperative follow-up.

Li BL, Huang X, Zheng CJ, Zhou JL, Zhao YP. Ileal duplication mimicking intestinal intussusception: A congenital condition rarely reported in adult. *World J Gastroenterol* 2013; 19(38): 6500-6504 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i38/6500.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i38.6500>

INTRODUCTION

Gastrointestinal tract duplication is a rare congenital gastrointestinal malformation reported in 1733 for the first time, and termed as duplication of the alimentary tract (DAT) by Fiorani *et al*^[1]. The exact etiology of DAT remains unknown, while multiple theories have been postulated, including "abortive twinning theory", "persistent embryonic diverticula theory", "split notochord theory", "intrauterine vascular accident theory" and more popular "aberrant luminal recanalization theory". More than 80% of DAT cases are diagnosed in children under 2 years but rarely in adults^[2,3]. DAT can arise from any segment of the gastrointestinal tract from the mouth to the anus, but involves the ileum in most cases (44%)^[4]. Clinical manifestations of DAT are highly variable, especially in adults, de-

pending on the type, size, location, and mucosal lining of the duplication. An intestinal DAT may be asymptomatic, while chief complaints consist mainly of belly pain, abdominal mass, intestinal obstruction, and hematochezia^[5]. Ileal duplication occasionally becomes symptomatic until adulthood and requires subsequent medical intervention. However, ileal duplication cannot be overlooked in adult patients due to its serious complications, such as refractory bleeding, gastrointestinal perforation, and possible malignant transformation if with complicating gastric mucosa heterotopia^[5]. Ileal duplication is also a diagnostic challenge in adults as it is almost impossible in the clinical setting to differentiate it from other common gastrointestinal malformations or inflammatory diseases, such as Meckel's diverticulum (MD), noncomplicating intestinal intussusception, and Crohn's disease^[6]. We report a case of ileal duplication mimicking intestinal intussusception in a young man in his mid 20s, which was previously misdiagnosed as appendicitis, Crohn's disease or MD.

CASE REPORT

A 25-year-old Han Chinese man complained of chronic abdominal pain and weight loss for 3 years. In previous hospitalization, contrast gastrointestinal radiography showed multiple mucosal filling defects in the terminal segment of the ileum, while abdominal contrast-enhanced computed tomography (CT) scan revealed localized dilation, effusion, thickening, and edema of the intestinal wall in the right lower quadrant with concomitant enlargement of multiple mesenteric lymph nodes. Ileal Crohn's disease was initially suspected but subsequently excluded due to the fact that colonoscopy only identified mild chronic inflammation. Moreover, the abdominal symptoms could not be alleviated by the medication with oral salicylazo-sulphapyridine but omeprazole. Open appendectomy was performed as indicated by a serious attack of right lower quadrant pain, and postoperative pathology showed mild simple appendicitis. However, intermittent abdominal pain free of hematochezia or melena, suggestive of intestinal obstruction, still recurred following appendectomy. The patient was referred to our department in November 2010 due to the consistently worsening abdominal symptoms in the absence of any clinically evident predisposing factors.

Physical examination revealed a 10 cm × 3 cm, tubular-shaped, soft, tender, mobile mass located in the right lower quadrant. Routine hematology and clinical chemical tests showed no clinically significant abnormalities. Repeated abdominal contrast-enhanced CT scan revealed a suspicious intestinal intussusception located in the right lower quadrant (Figure 1). Repeated contrast gastrointestinal radiography and colonoscopy identified clinically insignificant results. Tc-99m pertechnetate scintigraphy also showed a cluster of strip-like abnormal radioactivity in the right lower quadrant (Figure 2). Explorative laparotomy was indicated for suspicious MD in this patient with a history of previous abdominal surgery.

Intraoperative exploration showed no clinically sig-

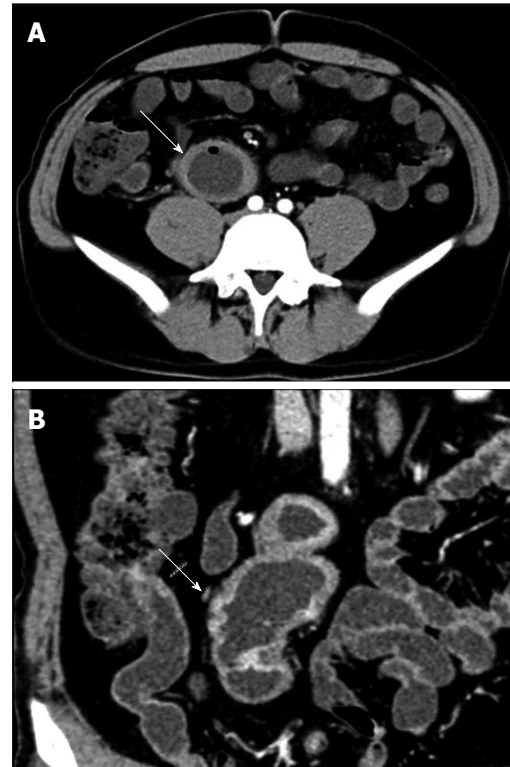


Figure 1 Computed tomography enterography scan. A: Transverse view showed suspicious ileal intussusception (white arrow) in the right lower quadrant; B: Coronal view revealed a similar result (white arrow).

nificant abnormalities, except for moderate intestinal adhesions, in the peritoneal cavity. However, a duplicating, tubular-shaped intestinal segment, 15.5 cm in length and 4 cm in diameter (Figure 3A), was found arising from the mesenteric margin of the native ileal segment located 15 cm proximal to the ileocecal valve (Figure 3B). The distal part of the ileal duplication cyst had a 2-cm, completely patent orifice into the native ileal lumen, while the proximal part ended in a blind pouch (Figure 3C). The ileal duplication cyst was easily resected along with a 7.5 cm native ileal segment, and an end-to-end ileal anastomosis was performed to restore the gastrointestinal continuity. The resection specimen showed no signs of inflammation, infection, ulceration, hemorrhage, obstruction or malignant transformation (Figure 4A). Additionally, gross pathology (Figure 4B) and histology (Figure 4C) showed that the duplication cyst was lined with ileal mucus glands and heterotopic gastric mucosae. Therefore, this disease was diagnosed as ileal duplication cyst with complicating gastric mucosa heterotopia. The patient experienced an eventless postoperative recovery, and he was discharged from hospital on postoperative day 7. The patient was followed up at the outpatient clinic and remained asymptomatic throughout a two-year follow-up period until the time of drafting this manuscript.

DISCUSSION

DAT was firstly reported by Fitz^[7] and subsequently defined by Ladd *et al*^[8] as a spherical- or tubular-shaped

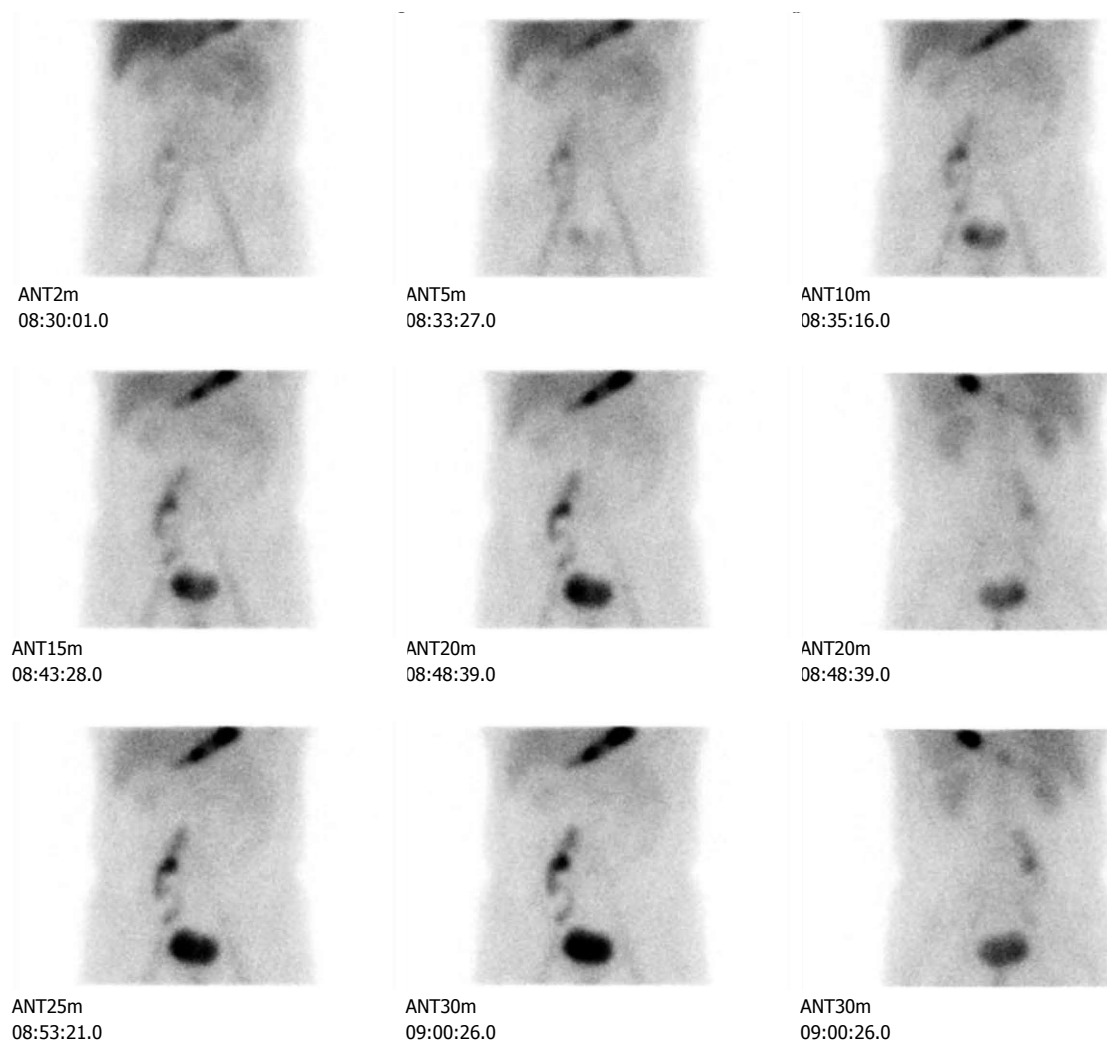


Figure 2 Tc-99m pertechnetate scintigraphy. A cluster of stripy abnormal radio-activity was located in the right lower quadrant.

anomaly that was attached or adherent to and shared the identical phenotypic characteristics with the normal alimentary tract. Although DAT is known as a rare congenital malformation (1/10000 live births), this anomaly can occur anywhere along the gastrointestinal tract, with the ileum being the most frequently affected segment^[2,6], either on the mesenteric margin or the contralateral side^[9,10]. Daudet *et al*^[11] reviewed 764 DAT cases, the majority of which occurred at infancy but rarely at adulthood, with a male dominance.

Ileal duplication normally exhibits highly variable and nonspecific clinical manifestations in adults. The most frequent complaints consist mainly of symptoms suggestive of gastrointestinal bleeding and intestinal obstruction^[5,6,12,13]; and abdominal pain and palpable abdominal mass are also reported by approximately 50% of patients^[13]. Furthermore, gastrointestinal bleeding and refractory abdominal pain may be underlain with heterotopic gastric mucosae lining the duplication cyst. Therefore, antacids can be effective in relieving abdominal pain as shown in this patient.

Multiple diagnostic tools are reported to be useful in the investigation of DAT, including contrast-enhanced

gastrointestinal ultrasonography and radiography, abdominal CT scan, and gastrointestinal endoscopy^[14,15]. Moreover, Tc-99m pertechnetate scintigraphy is recommended as the first-line option of choice for the workup of DAT^[16]. The positive result depends mainly on the abnormal enrichment of radionuclides accumulated by the heterotopic gastric mucosae. Ileal DAT mainly needs to be differentiated from MD. In pathogenesis, MD is a true congenital diverticulum deriving from the remnant of the omphalomesenteric duct during the development of the terminal ileum, while DAT can occur anywhere along the gastrointestinal tract but most frequently in the ileum; MD is normally located on the contralateral side of the mesenteric margin, while ileal duplication cyst occurs either on the mesenteric margin or the contralateral side. MD is often complicated with ectopic gastric mucosa; therefore, abdominal pain responsive to antacids is more frequently present in MD patients than in ileal DAT patients. Furthermore, MD is known to often cause a series of complications, such as diverticulitis, gastrointestinal bleeding or perforation, and intestinal obstruction, whereas these complications are relatively less common in ileal DAT. However, it is almost impossible to distinguish DAT from

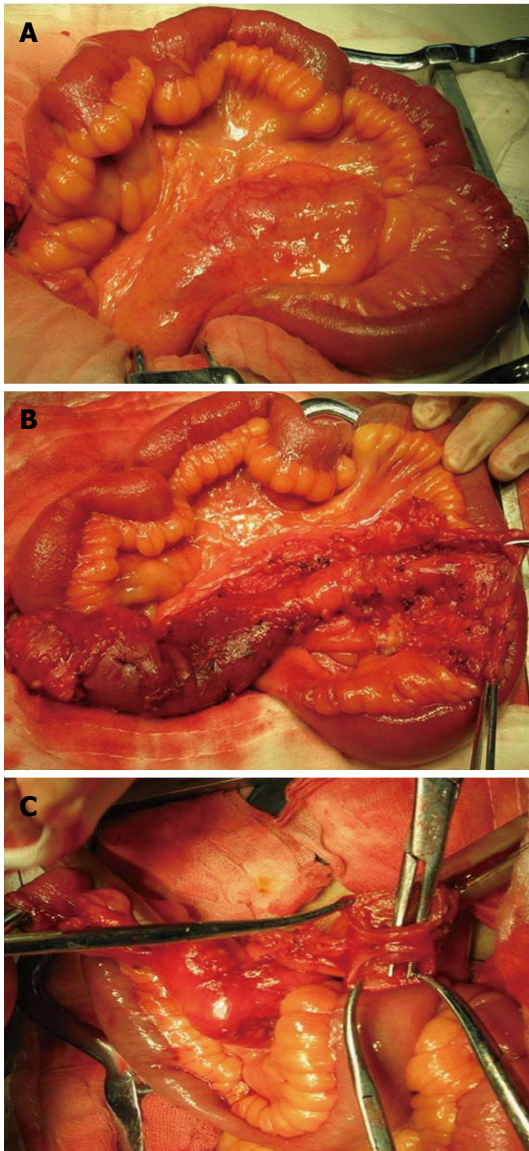


Figure 3 Exploratory laparotomy findings. A: A 25-cm duplicating, tubular small intestinal segment was found arising from the ileal mesenteric margin; B: This duplication cyst was intimately attached to the native ileal segment located 15 cm proximal to ileocecal valve; C: this cyst had a blind end proximally and a completely patent orifice into the native ileal lumen distally.

MD prior to operation if the ileum is involved. A previous study conducted in Japan reported that only 11.2% of ileal duplication cases could be correctly diagnosed before operation, 18.2% misdiagnosed as ileal intussusception, 15.1% as ileal mass, 14.4% as ileus, and 26.7% as abdominal pain of unknown cause^[17]. Therefore, it will easily lead to the misdiagnosis of ileal DAT as MD. As MD is the most frequent gastrointestinal malformation, a suspected diagnosis of MD was made and indicated for exploratory laparotomy in our case. Appendicitis also needs to be excluded as the primary complaint was right lower quadrant pain in this patient, especially if complicating infection occurred in the duplication cyst. Unfortunately, the ileal duplication, which located 15 cm proximal to the ileocecal valve, was missed in previous appendectomy. The possibility of ileal duplication should be excluded for a patient

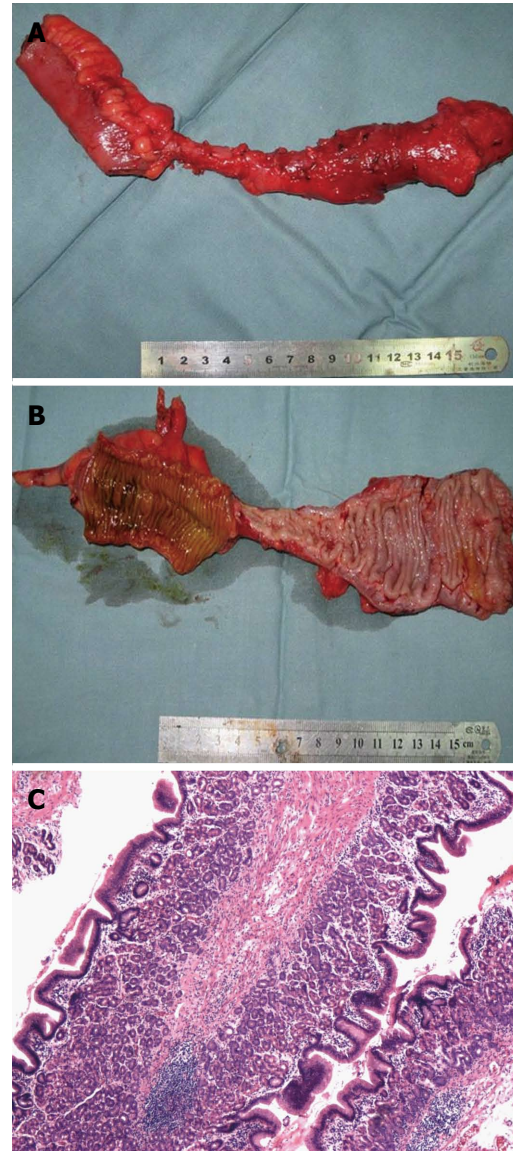


Figure 4 Gross and histological pathology of the resection specimen. A: The resection specimen showed no signs of inflammation, infection, ulceration, hemorrhage, obstruction, or malignant transformation; B: The mucosal layer of the duplication cyst was lined with both small intestinal and gastric mucosae; C: Histology revealed that the duplication cyst was lined with ileal mucus glands and heterotopic gastric mucosae (hematoxylin-eosin, $\times 100$).

diagnosed with suspicious MD or appendicitis but exhibiting gastrointestinal bleeding and/or intestinal obstruction. Use of laparoscopy may be helpful in identifying any suspicious ileal diseases when a diagnosis of MD or appendicitis is doubtful. Double-balloon enteroscopy may be another effective investigational technique for the diagnosis of ileal DAT if an additional ileal luminal orifice is visualized^[4,18,19].

Symptomatic treatment, such as acid-suppressing medications, may be effective in some cases if the symptoms are primarily associated with ectopic gastric mucosae. Like the possibility of adenocarcinoma in MD with complicating ectopic gastric mucosa, malignant transformation of ileal duplication cyst with complicating gastric mucosa heterotopia is also a major concern in adult patients as

epithelial instability is seen in long-standing duplication cysts. A historic review published by Johnson and his colleagues^[5] reported that three out of 13 (23.1%) adult ileal DAT patients had ileal cancer, including adenocarcinoma in two patients and squamous cell carcinoma in one patient. Thus, radical resection of the duplication cyst along with the affected native intestinal segment remains the mainstay modality of definitive treatment^[5,20].

In conclusion, our report described ileal DAT, a rare congenital gastrointestinal malformation uncommonly seen in adults. This rare condition exhibits no specific manifestations although CT enterography scan and Tc-99m pertechnetate scintigraphy may identify some characteristic appearance of intussusception and MD. Surgical resection is thought to be the most effective treatment modality. Use of laparoscopy will allow a direct visualization and concomitant resection of possible ileal DAT.

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Plastic tube-assisted gastroscopic removal of embedded esophageal metal stents: A case report

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Abstract

A patient with stent embedding after placement of an esophageal stent for an esophagobronchial fistula was treated with an ST-E plastic tube inserted into the esophagus to the upper end of the stent using gastroscopy. The gastroscopy was guided into the esophagus through the ST-E tube, and an alligator forceps was inserted into the esophagus through the ST-E tube alongside the gastroscopy. Under gastroscopy, the stent wire was grasped with the forceps and pulled into the ST-E tube. When resistance was met during withdrawal, the gastroscopy was guided further to the esophageal section where the stent was embedded. Biopsy forceps were guided through a biopsy hole in the gastroscopy to the embedded stent to remove silicone membranes and connection threads linking the Z-shaped wire mesh. While the lower section of the Z-shaped stent was fixed by the biopsy forceps, the alligator forceps were used to pull the upper section of the metal wire until the Z-shaped metal loops elongated. The wire mesh of the stent was then removed in stages through

the ST-E tube. Care was taken to avoid bleeding and perforation. Under the assistance of an ST-E plastic tube, an embedded esophageal metal stent was successfully removed with no bleeding or perforation. The patient experienced an uneventful recovery after surgery. Plastic tube-assisted gastroscopic removal of embedded metal stents can be minimally invasive, safe, and effective.

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Key words: Esophagus; Stents; Gastroscopy; Complication

Core tip: A patient presented with a disordered stent structure as a result of failure of repeated attempts at gastroscopic removal. An ST-E tube was inserted into the esophagus. An alligator forceps was inserted into the ST-E tube alongside a gastroscopy. The stent wire was gripped and pulled up into the tube. Biopsy forceps were inserted into the lower section of the stent through the biopsy hole to fix the stent, while the alligator forceps continued to be used to pull up the stent wire until the Z-shaped metal loops became elongated stripes. All the stent wire was removed through the ST-E tube.

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INTRODUCTION

The use of fully covered self-expandable esophageal metal stents has favorable results in treating a variety of

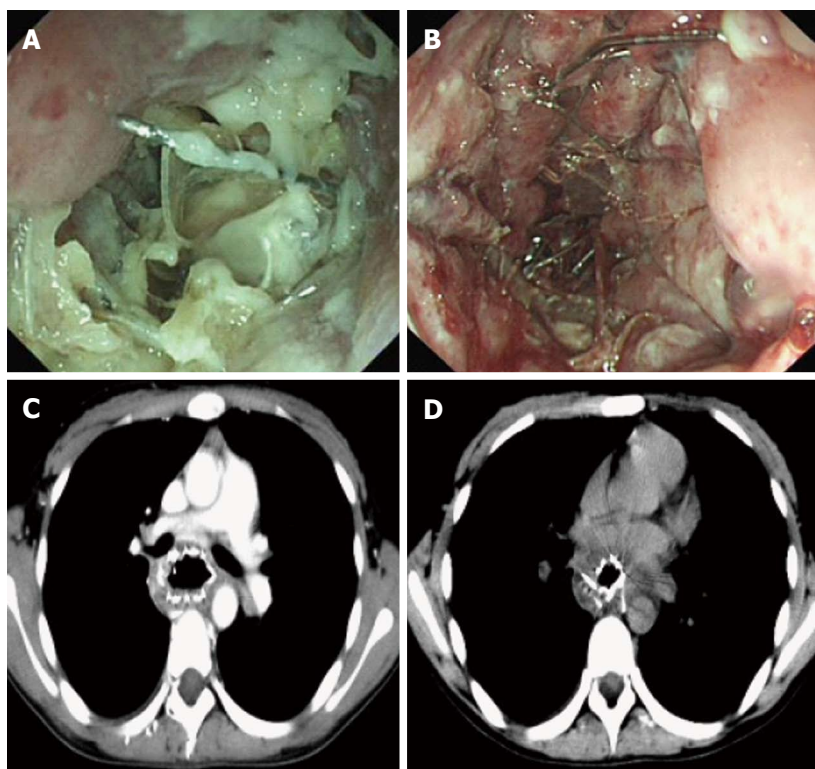


Figure 1 Disordered stent structure as a result of failure of repeated attempts at gastroscopic removal of the stent. A-D: Enhanced chest computed tomography scan performed showed the stent was situated in the middle section of the esophagus after esophageal stent placement, the wall of the middle and inferior segments of the esophagus thickened noticeably, part of the stent was embedded into the esophageal wall, borders between the stent and surrounding fat were blurred, and the upper end of the stent pressed against the trachea carina.

benign and malignant esophageal strictures and esophageal fistulae^[1-6]. However, stent placement over a prolonged period can result in hyperplastic tissue overgrowth on both ends of the stent, leading to in-stent restenosis^[7-9]. Therefore, stents should be removed after an appropriate period after treatment of benign esophageal strictures and fistulae^[10-12]. Notable tissue hyperplasia can occur at both stent ends, causing difficulty in stent removal and sometimes requiring surgical treatment. This procedure has a high risk of trauma^[13,14]. Therefore, a minimally invasive, low-risk method is needed for removal of embedded esophageal stents. Here, we report our experience with a novel approach to gastroscopic removal of an embedded esophageal stent.

CASE REPORT

The patient was a 15-year-old girl who had experienced coughing after drinking since early March 2012. The imaging of iodinated contrast-enhanced radiological examination showed an esophagobronchial fistula arising from the left bronchus to the middle portion of the esophagus. Under gastroscopy on May 30, 2012, a fully covered Z-shaped metal stent measuring 2 cm × 6 cm was placed within the esophagus to cover the fistula opening. After the procedure, the coughing after drinking disappeared. However, the patient developed esophageal obstruction. Gastroscopy on August 13, 2012 showed tissue hyperplasia on both stent ends, luminal stenosis, and embedding of both stent ends in the hyperplastic tissue.

Attempts to remove the stent under endoscopy failed and led to a disorganized stent structure. Enhanced chest computed tomography scan showed the wall of

the middle and inferior segment of the esophagus were noticeably thickened. Part of the stent was embedded in the esophageal wall, the boundary between the stent and surrounding fat was blurred, and the upper end of the stent was pressed against the trachea carina (Figure 1). After consultation with thoracic surgeons, we decided to perform gastroscopic removal on August 31, 2012.

Under the guidance of gastroscopy, an ST-E plastic tube (3 cm × 40 cm) was inserted into the esophagus to the upper end of the stent. A gastroscope was guided into the esophagus through the ST-E tube, and an alligator forceps was inserted into the esophagus through the ST-E tube alongside the gastroscope. Under gastroscopy, the stent wire was gripped with the forceps and pulled into the ST-E tube. When resistance was met during withdrawal, the gastroscope was guided further to the esophageal section where the stent was embedded. Biopsy forceps were sent through a biopsy hole in the gastroscopy and inserted near the embedded stent to remove the silicone membranes and connection threads linking the Z-shaped stent wire. Next, while the lower section of the Z-shaped stent was fixed by the biopsy forceps, the alligator forceps were used to pull the upper section of the metal wire until the Z-shaped metal loops elongated. The wire mesh of the stent was then removed in stages through the ST-E tube (Figure 2).

DISCUSSION

Benign esophagobronchial fistulae are rare and often result from trauma, esophageal spontaneous rupture, tuberculosis, and Crohn's disease^[2,4,5]. Treatment is usually difficult and surgical interventions involve a high risk of

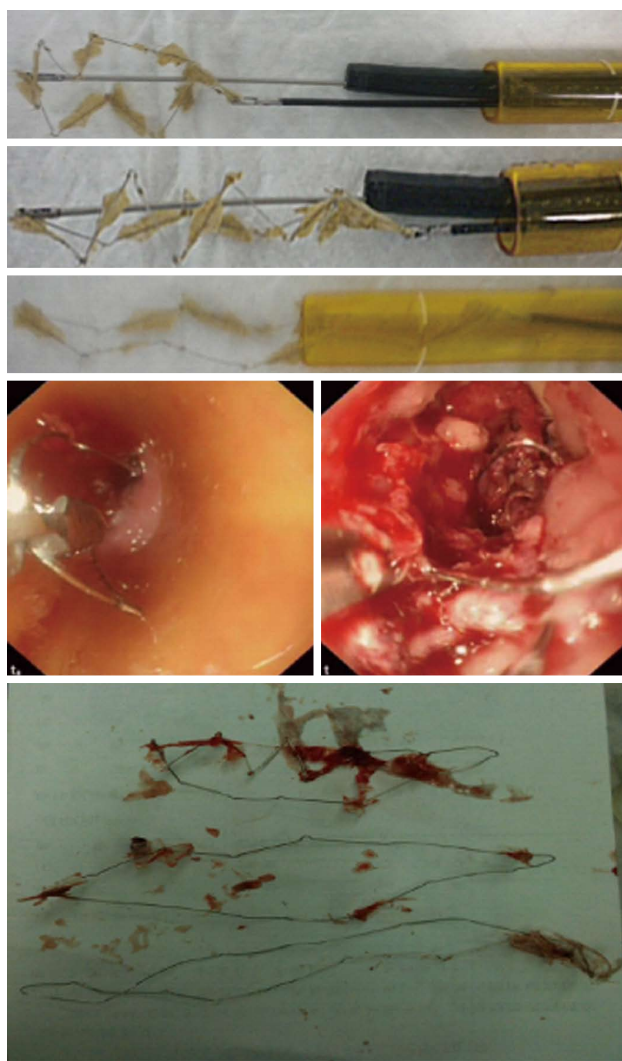


Figure 2 An alligator forceps was inserted into an ST-E tube alongside a gastroscop and, under the guidance of gastroscopy, the stent wire was gripped and pulled up into the ST-E tube. Biopsy forceps were inserted into the lower section of the Z-shaped stent through a biopsy hole in the gastroscop to fix the stent; in the meantime, the alligator forceps continued to be used to pull up the stent wire until the Z-shaped metal loops became elongated strips. All the stent wire was removed in stages through the ST-E tube.

trauma. Placement of fully covered self-expanding metal stents has become a viable treatment option. However, stent placement over a prolonged period can result in hyperplastic tissue overgrowth on both ends of the stent, leading to in-stent re-stenosis. Removing embedded stents is difficult. One study attempted to remove a stent by placing a secondary stent within the primary stent^[13]. However, in this case, the stent structure was compromised and became disordered during attempts to remove the stent under endoscopy, with the result that sharp parts of the stent were entering the esophageal wall. Thus, placement of another stent might have caused esophageal perforation and damage to surrounding organs.

In our view, two goals must be achieved to successfully remove a structurally disordered Z-shaped metal stent embedded in the esophageal wall under

gastroscopy. First, the esophageal entrance and throat must be protected from scratching by the stent wire during removal; second, each of the Z-shaped stent loops needs to be pulled outward until it is elongated. Gastroscopic procedures conducted through a ST-E tube meet these requirements, as the plastic tube fully protects the upper portion of the esophagus and throat from scratching by the stent wire. A two-handed operation is possible with an ST-E tube: with one hand, an alligator forceps is inserted into the esophagus through the ST-E tube alongside the gastroscop. Under the guidance of gastroscopy, the stent wire is gripped and pulled outward into the ST-E tube. If resistance is met during pulling, the gastroscop can be sent further to the esophageal section where the stent is embedded and biopsy forceps guided with the second hand through a biopsy hole in the gastroscop to insert into the embedded stent to remove silicone membranes and connection threads linking the Z-shaped stent. While the lower section of the Z-shaped stent is fixed by the biopsy forceps, the alligator forceps can be used to pull the upper section of the metal wire until the Z-shaped metal loops are elongated, to enable the wire mesh of the stent to be removed through the ST-E tube. The wire mesh of the stent is removed in stages by repeating the above procedures.

In our view, plastic tube-assisted gastroscopy is a minimally invasive, safe, and effective method for removal of esophageal embedded metal stents.

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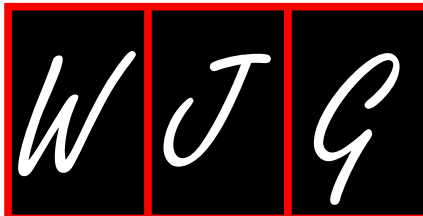
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- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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- 5 **Vallancien G**, Emberton M, Harving N, van Moorseelaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and

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- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; **(401)**: 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

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- 9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

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Electronic journal (list all authors)

- 15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

Patent (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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