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World Journal of Gastroenterology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
<http://www.wjgnet.com>

PUBLISHER

Baishideng Publishing Group Co., Limited
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Stool therapy may become a preferred treatment of recurrent *Clostridium difficile*?

Dinesh Vyas, Heidi E L'Esperance, Arpita Vyas

Dinesh Vyas, Heidi E L'Esperance, Department of Surgery, College of Human Medicine, Michigan State University, East Lansing, MI 48912, United States

Arpita Vyas, Department of Pediatrics, College of Human Medicine, Michigan State University, East Lansing, MI 48912, United States

Author contributions: Vyas D and L'Esperance HE collected the material and wrote the manuscript; Vyas D and Vyas A discussed the topic; Vyas D supervised the publication of this commentary.

Correspondence to: Dinesh Vyas, MD, MS, FICS, Department of Surgery, College of Human Medicine, Michigan State University, 1200 East Michigan Avenue, Suite 655, East Lansing, MI 48912, United States. vyasd@msu.edu

Telephone: +1-517-2672460 Fax: +1-517-2672488

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Core tip: Recurrent *Clostridium difficile* has been a challenge for patients, clinicians and hospital alike. Drug therapy for this epidemic is still not very effective. A more traditional method of fecal transplant has been discussed in this article, but it has been an uphill task to execute. We are discussing this first randomized control study, showing overarching benefits of stool transplant over traditional drug treatment. More studies needed with similar results, before making a strong recommendation in favor of it.

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Abstract

Fecal enemas were first reported to successfully treat life threatening enterocolitis in 1958, but fecal therapy to treat *Clostridium difficile* (*C. difficile*) infection has remained esoteric and not well investigated until recently. In the past few years, systematic reviews of case series and case reports of fecal microbiota transplant for recurrent *C. difficile* infection have become available and validate use of fecal transplant for *C. difficile* enterocolitis. Methods of fecal transplant reported in the literature include: nasogastric tube, gastroscopy, duodenal tube, colonoscopy, rectal tube, and fecal enemas administered at home; no method has been shown to be superior. A recent randomized study published in *New England Journal of Medicine* found fecal transplant to be superior to oral vancomycin alone in treatment of recurrent *C. difficile* enterocolitis. The significance of this trial cannot be underestimated as it lends credibility to the idea of intentionally using microbes to combat disease, providing an alternative to the older paradigm of disease eradication through use of antimicrobials.

COMMENTARY ON HOT TOPICS

Recurrent *Clostridium difficile* (*C. difficile*) is a growing epidemic with high rate of recurrence despite use of antibiotics. Fecal therapy to treat enterocolitis has been discussed in the literature since the late 1950's; despite anecdotal evidence suggesting its safety and efficacy, fecal therapy has remained an esoteric treatment. We read the recent article by van Nood *et al*^[1] describing the results of the European randomized study investigating fecal therapy *vs* oral vancomycin for treatment of recurrent *C. difficile* infection with great interest. This is first randomized study investigating and validating use of fecal transplant for treatment of recurrent *C. difficile* infection.

C. difficile infection is identified as the cause of 25%-55.4% cases of antibiotic-associated diarrhea^[2,3],

costs over \$1 billion dollars annually in the United States to treat^[4], and is a growing epidemic with twice as many cases reported in 2003 as in 1996 in part due to emergence of the more virulent, fluoroquinolone-resistant NAP1/BI/027 strain^[4,5]. *C. difficile* infection has a risk of initial recurrence rate following treatment with antibiotics of 20%-35%^[6,7]; risk of recurrence is increased by use of antibiotics for other infections, being female, having initial infection in the spring, and having number of previous *C. difficile* infection recurrences^[8]. Up to 65% of patients with recurrent disease ultimately develop pattern of recurrent *C. difficile* infection^[6,8]. Patients with recurrent *C. difficile* infection are at risk of developing antibiotic resistance, and complications from *C. difficile* infection including: colitis, pseudomembranous colitis, toxic megacolon, and death^[7,9]. Current therapies to treat recurrent *C. difficile* infection include tapered or pulsed dose oral vancomycin or metronidazole; these therapies are associated with high recurrence rates making it important that an effective treatment option for recurrent *C. difficile* infection become available^[6].

Regardless of the method, fecal transplant for recurrent *C. difficile* infection appears to resolve symptoms in 83%-96% patients with most patients having durable response following single treatment^[2,5,6,9]. Of patients requiring retreatment, 87.5% of patients experience symptom resolution^[6]. Fecal transplant for pseudomembranous colitis appears promising with 72%-88% patients reporting improvement in symptoms^[9]. Additionally, a case series found fecal transplant to be effective at treating the more pathogenic NAP1/BI/027 *C. difficile* strain in 89% patients^[10]. Fecal transplant was associated with few and infrequent adverse events related to the procedure in all available case series and reports^[2,6].

However promising the data from the systematic reviews of the case studies and case reports, it is not a substitute for data from prospective randomized controlled clinical trial data, as case reports and series are subject to bias from retrospective review of the data, subject selection and possible underreporting of adverse events. Results from a 3 arm randomized controlled clinical trial was recently reported by van Nood *et al*^[11] in the *New England Journal of Medicine* comparing fecal transplant to vancomycin $\pm$ bowel lavage. More than 50% of patients enrolled in the trial had experienced multiple episodes of recurrent *C. difficile* infection and had been previously exposed to tapered vancomycin. The trial was stopped after an interim analysis showing superiority of fecal transplant arm to the other arms; 94% patients on the fecal transplant arm experienced symptom resolution - 81% (13/16) following initial infusion, and 66% (2/3) having symptom resolution following second infusion from another donor, *vs* 31% (4/13) patients with symptom resolution on the vancomycin alone arm and 23% (3/13) patients with symptom resolution on the vancomycin and bowel lavage. Adverse events from this trial confirm fecal transplant to be well tolerated with most common events experienced to be diarrhea (94% patients), abdominal cramping (31%),

and belching (19%) immediately following fecal transplant and resolving within 3 h; and constipation (19%) as major adverse event reported during follow-up^[11]. This study is far from perfect as it enrolled a small number of participants (16 on fecal transplant arm, 13 each on vancomycin and vancomycin and bowel lavage arms), was not blinded, patients on vancomycin $\pm$ bowel lavage arms frequently crossed-over following recurrence of *C. difficile* infection (non-protocol directed) and received fecal transplant, and although it enrolled primarily elderly patients it excluded many patients at higher risk of recurrent *C. difficile* infection including: patients with prolonged immunodeficiency, critically ill intensive care unit patients, and patients requiring antibiotics to treat another infection. Despite the studies limitations, it appears to favor fecal transplant for treatment of recurrent *C. difficile* infection with results similar to previous systematic reviews of case reports and case series available in the literature.

Data from the randomized trial by van Nood *et al*^[11] provides further evidence that the efficacy of fecal transplant is not likely due to bowel preparation as it included a vancomycin and bowel lavage arm, but appears to be due to reconstitution of microbes in the gastrointestinal tract. As noted by observations the early 1980's, *C. difficile* growth can inhibit growth of certain strains of *Peptococcus*, *Peptostreptococcus*, and *Bacteroides* and its growth can also be inhibited by certain strains of *Staphylococcus*, *Pseudomonas*, *Bacteroides* and *Lactobacillus*, and recurrent *C. difficile* infection is likely due to germination of spores before balance of large bowel flora restored^[11], or reinfection with a new strain of *C. difficile* due to the lack of protective bacteria in the colon^[5]. Fecal transplant likely works by repopulating normal gut flora and preventing colonization with pathogenic *C. difficile* bacteria^[5].

Fecal microbiota transplant has had empiric evidence demonstrating effectiveness and safety in treating recurrent *C. difficile* infection and pseudomembranous colitis enduring for over 50 years and is relatively less cost than other treatment options, so why has it remained an esoteric treatment for these disorders and not been investigated in randomized controlled trials until recently? Some possibilities include concerns of transmitting infections from donors to recipients *via* fecal material, no clear fecal transplant protocol as several methods have been described in the literature, and the idea of transplanting fecal material from one individual to another is aesthetically unappealing^[9,11,12]. It should be noted that patients are reportedly receptive to the idea of fecal transplant following frustration at repeated antibiotic failure^[13] and high out of pocket medical expenses to treat recurrent *C. difficile* infection^[14].

Given the growing epidemic of *C. difficile* infection, cost and complications of treating recurrent disease, increasing antibiotic resistance, and growing body of evidence to support fecal microbiota transplant as a cost-effective and widely available therapy to treat recurrent *C. difficile* infection it is important that further research on fecal transplant be performed to identify methods and

indications for its use. The significance of the randomized study by van Nood *et al*^[1] cannot be underestimated as it lends credibility to the idea of intentionally using microbes to combat disease, providing an alternative to the older paradigm of disease eradication through use of antimicrobials.

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Strategies to rescue steatotic livers before transplantation in clinical and experimental studies

Qiang Liu, Maria-Louisa Izamis, Hongzhi Xu, Tim Berendsen, Martin Yarmush, Korkut Uygun

Qiang Liu, Maria-Louisa Izamis, Hongzhi Xu, Tim Berendsen, Martin Yarmush, Korkut Uygun, Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School, and Shriners Burns Hospital, Boston, MA 02114, United States

Martin Yarmush, Department of Biomedical Engineering, Rutgers University, Piscataway, NJ 08854, United States

Author contributions: Liu Q, Izamis ML, Xu H, Berendsen T, Yarmush M and Uygun K contributed to collect literatures and write the paper.

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Correspondence to: Martin Yarmush, MD, PhD, Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School, 51 Blossom Street, Boston, MA 02114, United States. ireis@sbi.org

Telephone: +1-617-3174882 Fax: +1-617-5739471

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receptor *e.g.*, peroxisomal proliferator-activated receptor, or anti-inflammation through suppressing cytokines *e.g.*, tumor necrosis factor- α , or antioxidant therapies to alleviate oxidative stress. This similarity of molecular mechanisms implies possible future attempts to reinforce each approach by repeating the same treatment approach at several stages of procurement and preservation, as well as utilizing these alternative approaches in tandem.

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Key words: Liver transplantation; Steatosis; Donor liver; Clinical; Experimental

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Abstract

The shortage of donor livers has led to an increased use of organs from expanded criteria donors. Included are livers with steatosis, a metabolic abnormality that increases the likelihood of graft complications post-transplantation. After a brief introduction on the etiology, pathophysiology, categories and experimental models of hepatic steatosis, we herein review the methods to rescue steatotic donor livers before transplantation applied in clinical and experimental studies. The methods span the spectrum of encouraging donor weight loss, employing drug therapy, heat shock preconditioning, ischemia preconditioning and selective anesthesia on donors, and the treatment on isolated grafts during preservation. These methods work at different stages of transplantation process, although share similar molecular mechanisms including lipid metabolism stimulation through enzymes or nuclear

INTRODUCTION

Liver transplantation (LTx) is a successful therapy for end-stage liver disease, but it is severely restricted by the donor organ shortage^[1-4]. In an effort to increase the size of the donor pool, livers from expanded criteria donors (ECD), including steatotic livers, are increasingly being used^[3]. The current contribution of steatotic livers is marginal however, since the majority has an increased risk of ischemia-reperfusion injury (IRI) after LTx^[3]. Here we review the clinical and experimental attempts to minimize this risk by modifying the quality of livers at different stages, on donors and isolated liver grafts during preservation, before LTx. We begin by briefly introducing the etiology, pathophysiology, categories and experimental models of hepatic steatosis, provide a summary of the techniques applied on donors and liver grafts, and con-

clude by providing future perspectives for these experimental approaches.

ETIOLOGY OF HEPATIC STEATOSIS

Livers are defined as steatotic or fatty when they have excessive (above 5% of wet liver weight) accumulation of lipids, mainly triglycerides. Steatosis occurs when lipid ingestion and synthesis exceed export and consumption in livers^[5]. Based on the patient's alcohol consumption^[5,6] fatty liver disease is classified as either alcoholic or non-alcoholic in origin (AFLD and NAFLD). Alcohol can decrease fatty acid oxidation and lipoprotein excretion, and increases the esterification of fatty acid to triglycerides *via* alpha-glycerophosphate^[6]. Several factors contribute to NAFLD including dietary lipid overload; insulin resistance, which results in abnormal lipid metabolism; and ingestion of drugs or toxins such as carbon tetrachloride, which induces a decrease in apoprotein synthesis and lipid export^[5-8].

PATHOPHYSIOLOGY OF HEPATIC STEATOSIS

A complicated pathophysiology has been exposed although the exact pathways have not been completely elucidated. Briefly, a “two-hit” theory is the current consensus, with the first “hit” being the initial abnormal fat accumulation and the second “hit” being the consequent inflammation (“steatohepatitis”) leading to fibrosis and cirrhosis^[6]. In the first “hit”, excessive fat accumulates in vacuoles within hepatocytes, increasing cell volume and narrowing sinusoidal lumens^[5,9]. This impairs microcirculation and decreases nutrient, oxygen and waste exchange. Excessive non-esterified fatty acids in hepatocytes inhibit β -oxidation thereby reduce acetyl-coenzyme A production^[10]. Mitochondria uncoupling protein-2 is upregulated and associated with a dysfunction of adenosine triphosphate (ATP) synthesis^[11-13]. Fat-induced hyperactivity of cytochrome P-450 enzymes increases the production of reactive oxygen species (ROS)^[6,14]. ROS in turn lead to lipid peroxidation, phospholipid depletion and membrane dysfunction^[6,14,15] as well as the release of inflammatory cytokines such as tumor necrosis factor (TNF)- α ^[14,16,17]. Inflammation occurs gradually and marks a significant downturn in disease progression as the second “hit”^[18,19]. Alcohol can exacerbate oxidative injury and Kupffer cell activation^[20], though alcoholic and non-alcoholic steatohepatitis (ASH and NASH) are thought to progress similarly^[18].

Steatotic livers have reduced tolerance to ischemia due to low ATP stores, and are thus prone to early onset of acidosis and cellular edema during standard liver preservation method, static cold storage (SCS)^[21]. Edema significantly impairs hepatic microcirculation further than the preceded impairment induced by excessive fat. Moreover, steatosis-induced inflammation is not addressed in the present liver SCS preservation solutions (Table 1).

Upon reperfusion, a complex inflammatory response involving Kupffer cells, lymphocytes, neutrophils, numerous cytokines^[22] and nuclear factor kappa-B (NF κ B)^[23], is inevitably worse in steatotic livers compared to non-steatotic livers. Oxidative stress is also exacerbated *e.g.*, through xanthine oxidase^[24,25]. The microcirculation is deteriorated further due to adherence of platelets in the sinusoids^[22]. Strategies to minimize fat content and ameliorate the inflammatory and oxidative injury of steatotic livers are essential for improving these organs for transplantation.

CATEGORIES OF HEPATIC STEATOSIS

Besides AFLD and NAFLD in etiology, steatosis is also classified as “macro-” or “micro-” in histology based on the size and number of the fat vacuoles and on the location of the nucleus in the hepatocytes^[5,26]. Macrosteatosis has a single fat vacuole larger than the nucleus filling the majority of the cell and pushing the nucleus to the periphery. Microsteatosis has many small fat vacuoles surrounding the nucleus in the central zone of the hepatocytes, and has more LTx success than macrosteatosis^[3,27]. Steatosis can also be classified based on the proportion of hepatocytes affected, being mild (< 30%), moderate (30%-60%), or severe (> 60%), with incremental risk of graft dysfunction after LTx^[5,26].

CLINICALLY APPLIED STRATEGIES

Approaches to improve steatotic livers before LTx have been tested in a handful of pilot clinical studies on living donors or donors after brain death (DBD) (Table 2). The interventions focused on reducing excessive fat (the first “hit”) through limiting lipid intake and increasing lipolysis, or stimulating factors likely to be protective against inflammation and oxidative stress (the second “hit”) of steatohepatitis. Living donors, though a minority in western countries^[1,2], are used extensively in Asia^[28] and are theoretically amenable to therapies before procurement after ethical concern is taken into account^[29]. DBD livers, which comprise the majority of donor organs for LTx in western countries, could be treated between brain death declaration and organ procurement since circulation is maintained until procurement. Livers from donors after cardiac death (DCD) are seldom utilized when they have steatosis because they experience a period of warm ischemia (WI) before procurement^[3] and thus were normally considered as unacceptable with two defects (steatosis and WI). Currently there are no attempts to rescue steatotic DCD livers.

Physical exercise and dietary intervention

Physical exercise and dietary restriction are general therapies for NAFLD patients, independent of whether or not they are organ donors^[30,31]. But this treatment normally needs several months and might be risky to increase the mortality of recipients during the waiting time for treat-

Table 1 Intrinsic composition of preservation solutions in this review

		UW	HTK	Celsior	IGL-1	UW-gluconate	Kreb-Henseleit	MEM
Electrolyte (mmol/L)	Na	25	15	100	125	125	143	143.4
	K	120	9	15	30	25	5.9	5.4
	Mg	5	4	13	5	5	1.2	0.8
	Ca		0.0015	0.25		0.5	1.25	1.4
	Cl	20	32	42		1	125.2	124
Buffer (mmol/L)	SO ₄	5			5		1.2	0.8
	Phosphate	25			25	15	1.2	1
	Bicarbonate					25	25	26.2
	HEPES					10	20	
	Histidine		198	30				0.27
Antioxidant (mmol/L)	Glutathione	3		3	3	3		
	Allopurinol	1			1	1		
	Mannitol		38	60				
	NAC						5	
	Vit C							0.25
Metabolic Substrates (mmol/L)	Glucose					10	5	5.5
	Adenosine	5			5	5		
	Adenine					5		
	Ribose					5		
	Tryptophan		2					
Impermeants (mmol/L)	Ketoglutarate		1					
	Glutamate			20				
	Amino acid							0.7
	Lactobionate	100		80	100			
	Gluconate					95		
Colloid (g/L)	Raffinose	30			30	30		
	HES	50						
Other intrinsic compounds	PEG				1	50		
	Insulin	100 U						
	Dexamethason	8 mg						
	Penicillin	40 U						
Osmolarity	Phenol-red (mmol/L)							0.03
		340	300	363	330	360	320	310

UW: University of Wisconsin; HTK: Histidine-tryptophan-ketoglutarate; IGL-1: Institut Georges Lopez-1; MEM: Minimum essential cell culture medium; HEPES: N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid; NAC: N-acetylcysteine; HES: Hydroxyethyl starch; PEG: Polyethylene glycol.

ing donors^[32]. An intensive protocol might be a solution, which was already reported to successfully reduce macrosteatosis on obese human living donors in 2-8 wk, through exercise burning 600 kcal/d, a protein-rich diet of 1000 kcal/d, and bezafibrate, an anti-hyperlipidaemia drug, at 400 mg/d^[33]. However, supplementary glucose to donors a few hours before living donor liver transplantation was recommended to supply additional energy reserves, since fasting before procurement can induce glycogen depletion, decrease glycolytic ATP generation, and compromise graft transplantability^[34].

The lack of omega-3 polyunsaturated fatty acids (PUFA) has been recognized in the development of NAFLD because they can activate peroxisomal proliferator-activated receptor (PPAR)- α , suppress sterol regulatory element-binding protein-1, improve microcirculation, and reduce Kupffer cell activity and inflammation^[35-43]. The mechanism on microcirculation might work through reducing TXA₂ synthesis after manipulating the composition of hepatic lipid (omega-3: omega-6 PUFA ratio)^[43]. Based on experimental success on rodents^[35-43], omega-3 PUFA was shown to be effective on NAFLD clinically after treatment at 1-2 g/d for 6-12 mo^[44-46]. This has not been applied specifically on living donors but is expected

to be a safe and promising approach.

Pharmacological preconditioning

Many drugs are being used clinically to treat NAFLD by decreasing lipid intake^[47-49], stimulating lipid metabolism^[50-53], or improving insulin sensitivity^[54-57]. Ursodeoxycholic acid, a natural bile acid, was used as a non-specific hepato-protector to treat NAFLD in a pilot clinical study^[58], but afterward was revealed to be controversial^[59]. Pentoxifylline was used against NASH and ASH in patients^[60,61] due to the effect of reducing TNF- α by inhibiting phosphodiesterase^[62] and lessening oxidative stress by increasing glutathione^[63]. But to date, only bezafibrate was used to treat human living donors for LTx^[33]. This drug works through activating PPAR- α and β/δ to stimulate lipid metabolism and decrease fat content in livers^[64,65]. While there are other candidate drugs that could potentially be taken by living donors, concerns of significant side effects are limiting their use^[66,67].

Ischemic preconditioning

Though extended ischemia is deleterious to organs, it has been recognized since the 1980s that a short period of ischemia with subsequent reperfusion triggers natural de-

Table 2 Overview of the clinical and experimental strategies in this review

	Clinically applied	Experimentally applied
Donors	Dietary Pharmacological Ischemic (except remote ischemic) Anesthetic	Pharmacological Heat shock
Liver grafts		SCS preservation New solution Pharmacological additives Additional oxygen (VSOP) MP preservation MP solely MP + pharmacological additives Flushing Pharmacological additives

SCS: Simple cold storage; MP: Machine perfusion; VSOP: Venous systemic oxygen persufflation.

fense mechanisms against future ischemic insults and protects the organ against IRI^[68]. Ischemic preconditioning (IP) was first observed in kidneys and hearts^[69,70], and then employed for clinical liver resections and transplantation^[68]. It can be applied intermittently^[71], or as a single short period (5-10 min) of ischemia followed by 10-15 min reperfusion before cold flush during liver procurement^[72,73]. Franchello *et al.*^[73] have used the technique clinically on marginal DBD livers including steatotic livers, and observed a reduction of hepatocyte swelling and enzyme release in recipients after LTx.

IP is protective because ATP consumption during the short ischemic period increases endogenous adenosine and nitric oxide^[74]. Adenosine protects sinusoidal endothelial cells through adenosine A2 receptor^[75]. Cyclic adenosine monophosphate (cAMP) worked as the second messenger, but whether increasing or blocking cAMP would be beneficial was still controversial^[75,76]. Nitric oxide is a vasodilator, and further it attenuates the release of TNF- α , decreases the injurious interleukin (IL)-1 β and increases the anti-inflammatory IL-10^[77]. Another effect of the intermittent ATP consumption is to increase the level of adenosine monophosphate (AMP), which stimulates AMP-activated protein kinase (AMPK). AMPK can regulate an energy-conserving state, decrease inflammation through inhibiting NF κ B, and induce the synthesis of nitric oxide as well^[78-80]. Overall, the advantages of IP were an improved microcirculation^[81,82], mitochondrial permeability transition and mitochondrial function^[83], cytochrome oxidase C activity and tissue oxygenation^[82,84], and the reduction of oxidative stress such as the xanthine accumulation and xanthine oxidase activity^[85,86].

Interestingly, IP can work remotely, *e.g.*, liver IP decreased lung IRI^[25,87] and limb IP decreased liver IRI^[88-90]. This possibly works through some protective agents, *e.g.*, heme oxygenase-1, endothelial nitric oxide synthase, and nuclear protein High Mobility Group-Box 1^[88-90]. But foreseeable ethical concerns exist with the logistics of implementing this technique in human donors.

Anesthesia selection

During clinical liver resection, Beck-Schimmer *et al.*^[91] observed the superiority of volatile anesthesia using sevoflurane in the prevention of hepatic injury after reperfusion compared to intravenous anesthesia with propofol. The mechanism was suggested to be the increased synthesis of nitric oxide with sevoflurane, which alleviates some of the effects of IRI as discussed above. Moreover they observed that patients with steatosis did benefit more^[91]. This method could be easily applied during the procurement of steatotic livers from both DBD and living donors.

EXPERIMENTAL STRATEGIES

Induction of steatosis in animal models

Steatosis in animal models are established with or without alcohol and classified also as micro- *vs* macro- or as mild, moderate, *vs* severe. The timeline to develop the different classifications largely depends on strain and method used (Table 3).

A common rodent NAFLD model induces cerebral deficiency of the leptin receptor through a genetic mutation that causes the animals to become obese through overeating^[92-98]. The methods inducing lipid overload with high fat or cholesterol diets are also quite common. A high fat diet (50% dextrose, 18% casein, 25% lipids, 4% minerals, 1% cholesterol, 0.5% sodium cholate, 0.2% choline, and 1% vitamins) for 7 d induces severe steatosis in rats^[99]. A high-cholesterol (2%) diet for 12 wk in rats^[82] and 8 wk in rabbits results in moderate steatosis^[84]. A cafeteria diet (65% of fat) for 4-15 wk was also used to create NAFLD on rats^[100,101]. Recent studies suggested it reflects human metabolic syndrome better than high-fat diet^[100,101]. A choline/methionine-deficient diet (CMDD) was another rather common method to develop steatosis, for 4-6 wk or short as 7 d in rodents^[5,27,102,103]. Choline is essential for the formation of phosphatidyl-choline and very low density lipoprotein needed for lipid export, while methionine is a good source of methyl groups for the endogenous synthesis of choline. This model could be criticized for not being clinically accurate because NAFLD patients due to CMDD are rather unrealistic. The most rapid induction of NAFLD in rats was with a high-starch, fat-free diet [saccharose (40%), starch (40%), casein (16%), and a mineral and vitamin mix (4%)] administered for 2 d after fasting for 2 d, which can lead to mild to moderate steatosis^[104-106]. While none of these models are unanimously agreed to be ideal in replicating clinical NAFLD, the high fat or cholesterol diet model was the most widely used to mimic steatosis in humans.

There are fewer large animal NAFLD models, which usually combine more than one method described above to achieve steatosis. Takahashi *et al.*^[107] established a dog model using a diet rich in fat and deficient in choline, which produced moderate to severe macrosteatosis after 8-12 wk feeding. Lee *et al.*^[108] used a high fat and high cholesterol diet (20% kcal from fructose, 46% kcal from fat,

Table 3 Animal models of hepatic steatosis for liver transplantation

Disease	Approaches	Description	Animals	Treatment time
NAFLD	Genetic	Cerebral leptin receptor deficiency	Rodent	
	Dietary	High fat	Rodent	7 d
		High cholesterol	Rodent, rabbits	8-12 wk
		Cafeteria diet	Rodent	4-15 wk
		Choline/methionine-deficient	Rodent	7 d-6 wk
		High starch and fat free after fasting	Rodent	4 d
		High fat and choline deficiency	Dog	8-12 wk
		High fat and high cholesterol, plus choline deficiency or not	Miniature pig	24 wk
		High fat and carbohydrate with streptozotocin for a diabetic state	Landrace pig	5 wk
		Ethanol in liquid diet, intragastric infusion or gavage	Rodent	20 h-9 wk
AFLD	Dietary	Ethanol and high fat diet	Rodent	6 wk
		Ethanol and deficient folate diet	Micropig	12 wk

NAFLD: Non-alcoholic fatty liver disease; AFLD: Alcoholic fatty liver disease.

2% cholesterol and 0.7% cholate by weight) for 24 wk to develop microsteatosis on miniature pigs. When using lowered choline content simultaneously, severe steatosis with fibrosis was observed with increased TNF- α and oxidative stress. A recent study on Landrace pigs used a diet rich in fat (20% in volume) for 5 wk together with intravenous streptozotocin (125 mg/kg) to induce a diabetic state in the last 2 wk; but this treatment led only to mild steatosis^[109].

In rodent AFLD models, ethanol was provided in a liquid diet^[110-114] or *via* intragastric infusion^[115,116]. Different degrees of alcohol exposure have been reported: 5%-8% in the concentration of liquid diet; 35%-40% of total energy consumption; 8-16 g/kg per day; or 150-300 mg/dL of blood ethanol^[110-116]. Normally several days are needed for the animals to adapt to the alcoholic diet, and an additional 4-9 wk to observe steatosis. Acute responses to ethanol have also been reported as early as 20 h after feeding 6 g/kg ethanol by gavage on rats^[117]. Combined ethanol with high-fat diet to develop steatosis on rats was also reported^[118]. Large animal AFLD models are uncommon though micropigs have been fed a diet with ethanol and a deficiency of folate, as a substrate for methionine synthase, with some efficacy^[119,120].

Experimental strategies applied to donors

Pharmacological preconditioning: Reduction of oxidative and inflammatory activity with heme oxygenase-1, a microsomal enzyme^[121-123], was used intravenously or intraperitoneally on AFLD and NAFLD rats 24 h before liver procurement. It decreased macrophage infiltration, improved portal venous blood flow, bile production, and survival rate after LTx^[121-123]. Bortezomib, a NF κ B inhibitor, was used intravenously on obese donor rats and reduced IRI after LTx^[23]. N-acetylcysteine, a precursor of glutathione, was injected through the mesenteric vein of CMDD rats 15 min before liver procurement and showed a protective effect on IRI in an isolated reperfusion system^[124]. The subcutaneous injection of IL-6 for 10 d was observed to be protective against IRI after *in situ* partial ischemia-reperfusion on NAFLD and AFLD mice^[92]. The mechanism might be the prevention of cell

death and the reduction of TNF- α ^[125], in addition to stimulating PPAR- α , β -oxidation of fatty acids, and the export of triglycerides and cholesterol^[92,125]. Theaflavin, a polyphenol substance extracted from black tea, was tested on CMDD mice and observed to have antioxidant, anti-inflammatory, and anti-apoptotic effect^[126]. A multi-drug approach was reported by von Heesen *et al.*^[127] including N-acetylcysteine as an antioxidant, pentoxifylline for anti-inflammation, glycine to stabilize Kupffer cells, deferoxamine as an iron chelator to reduce ROS, and erythropoietin, melatonin and simvastatin to protect against IRI. In the treated rats they observed no inflammatory response with significantly reduced parenchymal dysfunction and injury compared to the untreated rats.

Heat shock preconditioning: An intriguing experimental method to improve the quality of steatotic donor livers has been to induce protective heat shock proteins (HSPs) endogenously by exposure to heat. Termed "heat shock preconditioning" and applied at 3-48 h before organ procurement by exposing anesthetized donor animals to warm (42 °C) bath water for 10-15 min^[128-132], obese and CMDD rats showed an increased expression of HSP-32 (heme oxygenase-1), -72 and -90^[128,129]. These HSPs can decrease TNF- α production^[129], improve microcirculation through producing carbon monoxide, and inhibit platelet aggregation^[62,64]. Our group has also reported the inhibition of CD4+ T lymphocytes in CMDD rats after LTx with heat shock preconditioning^[130]. Other factors might be involved in the treatment since studies on normal and WI rat livers showed IL-6, inter-cellular adhesion molecule-1, and some neutrophil chemo-attractants were also impacted^[131,132].

Strategies applied on liver grafts during ex vivo preservation

Obviously, strategies to improve steatotic liver quality during preservation are more desirable than those on donors, as they have no effect on the donor's other organs, and are practical when it's not possible to work on the donor. The clinical standard for liver graft preservation has been SCS with University of Wisconsin (UW)

solution for more than 20 years^[3,21]. In the past decade, histidine-tryptophan-ketoglutarate (HTK) solution^[133-135] and Celsior solution^[136,137] were recognized as having similar efficacy and safety as UW solution; Institut Georges Lopez-1 (IGL-1) was reported to be comparable to UW for healthy human livers^[138], and better for steatotic rat livers^[139]. Besides the arising new solutions, many adaptations have been suggested through enriching the intrinsic composition of the solutions with additives, or replacing SCS by machine perfusion (MP) preservation in experimental studies to rescue steatotic livers.

Pharmacological additives during SCS preservation:

Liver preservation solutions normally comprise electrolytes, pH buffers, antioxidants, metabolic substrates, impermeants with or without colloid, insulin, dexamethasone, and antibiotics (Table 1)^[139-143]. The additives for improving steatotic liver preservation were intended to ameliorate metabolism or suppress oxidative injury and inflammation. They were reported to be effective during SCS, despite the reduced metabolic rate of liver grafts during hypothermic preservation.

Addition of IL-6 into UW solution for donor liver flushing and SCS was tested by Sun *et al.*^[144], leading to improvement in microcirculation and reduced IRI after LTx of NAFLD and AFLD rats. Arnault *et al.*^[99] added pentoxifylline into UW solution and also observed a benefit to the microcirculation, but the exact mechanism is yet to be identified.

Tolba *et al.*^[106] added L-carnitine into HTK solution for SCS preservation of steatotic rat livers and observed a reduction of IRI in an isolated reperfusion system. L-carnitine is a nonessential amino acid but is essential for transporting fatty acids through the inner mitochondrial membrane and for β -oxidation^[106]. It has also been reported to function as an antioxidant and to stabilize the membrane fluidity and stability *in vitro* and *in vivo*^[145].

Ben Mosbah *et al.*^[146] added carvedilol, a cardiologic drug to block α - and β -adrenergic receptor, into UW solution for SCS of obese rat livers, and reduced oxidative stress and mitochondrial damage after isolated reperfusion. The mechanism might be an enhanced release of nitric oxide that facilitates vasodilatation and ROS scavenging^[147]. AMPK activators, trimetazidine and aminoimidazole-4-carboxamide ribonucleoside (AICAR) were also tested as UW additives on obese rat livers by this group. Increased bile production, decreased enzyme release and vascular resistance, and reduced oxidative stress after isolated reperfusion were observed. It was noted that combination of trimetazidine and AICAR was not necessary^[95].

Zaouali *et al.*^[148] tested the use of epidermal growth factor and insulin-like growth factor-I as UW additives and observed that each additive resulted in the improvement of fatty rat liver function after LTx. The mechanisms are suggested to be upregulation of Akt, a cytoprotector^[149], and the subsequent over-expression of PPAR- γ . They also tested melatonin as additive in IGL

solution and reported its protective role through generating nitric oxide and decreasing oxidative stress and inflammation^[150].

Venous systemic oxygen persufflation during SCS preservation:

In 1990s, Minor *et al.*^[151] developed a new method, called venous systemic oxygen persufflation (VSOP) to supply gaseous oxygen to livers during SCS preservation. The oxygen was introduced into hepatic vasculature *via* the suprahepatic vena cava and allowed to exit *via* several small pin pricks on the liver capsule made using an acupuncture needle. This technique was employed on steatotic rat livers for 24 h, and resulted in improved preservation of mitochondria and sinusoidal endothelial linings, less Kupffer cell activation and reduced hepatocellular enzyme release compared to SCS preservation^[105]. Recently, by assessing the enzyme release, energy storage, bile production, and cell death during isolated reperfusion, it was demonstrated that application of VSOP for 90 min may rescue steatotic livers after extended (18 h) SCS preservation^[152].

MP preservation: MP is an alternative preservation method to SCS^[153], which can be further categorized based on the temperature employed^[154]. Hypothermic (4 °C) machine perfusion (HMP) preservation has proven to be superior to SCS for human kidneys^[155], and feasible for normal human livers^[156]. Normothermic (32 °C-37 °C) and sub-normothermic (20 °C-30 °C) machine perfusion (NMP and subNMP) preservation have been reported in experimental studies on livers, but mostly on their advantages for DCD models^[143,157-162]. MP preservation of steatotic livers is limited, but also reported to be beneficial on preserving energy content and liver function experimentally^[109,143,163,164]. The advantages of MP preservation result from continuously supplying nutrients, removing waste products, and maintaining microcirculation^[154]. Because MP, especially NMP, provides a physiologically-relevant environment to the isolated donor organ, the quality of liver grafts can be manipulated more efficiently than those simply stored in an ice-box during SCS. Another advantage of MP is the considerable convenience for non-invasively evaluating liver viability, a key issue when ECD livers are used^[153].

Bessems *et al.*^[163] employed HMP preservation with UW-gluconate solution on steatotic rat livers for 24 h and alleviated IRI compared to SCS. Vairetti *et al.*^[143] preserved steatotic rat livers by subNMP (20 °C) with Krebs-Henseleit solution for 6 h and obtained similar results. The longest preservation of steatotic livers was the NMP preservation for 48 h in a pig model by Jamieson *et al.*^[109], who employed blood containing additional insulin and vasodilators as perfusate, and observed a mild reduction of steatosis from 28% to 15%. This NMP setting provided the most physiological environment to liver grafts and lead to an activated function of the isolated organs with sufficient oxygen and nutritional support. This is expected to be the best preservation method in spite of the

highest logistic restriction.

Recently our group has combined NMP and pharmacological preconditioning for decreasing steatosis^[96]. A “defatting cocktail” was developed with 6 compounds to activate nuclear receptors such as PPARs, pregnane X receptor, and constitutive androstane receptor, to exert an insulin mimetic effect and to stimulate intracellular cAMP. This cocktail was added into Minimum Essential cell culture medium as a perfusate to stimulate lipid metabolism of obese rat liver grafts preserved at NMP. A significant decrease (50%) in steatosis was observed after 3 h NMP^[96].

Other experimental approaches

The solution used for SCS preservation needs to be flushed out of the donor organ prior to implantation to remove possible air bubbles, and preservative components such as high potassium, which are deleterious to the recipient. This provides an opportunity for treatment of ischemic injury, although it has been very little explored. In one study, polyphenols, an antioxidant extracted from *Camellia sinensis* (green tea), was added to the flushing solution and improved the hepatic injury and survival rate after LTx in a steatotic rat model^[117].

Another option is applying preconditioning at several phases. While it appears little explored, in one study Ye *et al.*^[118] injected glutathione intraperitoneally in rats with hepatic steatosis 2 d before procurement and preserved the livers by SCS with VSOP and additional glutathione. Remarkable improvement on survival rate, liver function, and oxidative stress in liver tissues after transplantation was observed.

SUMMARY AND FUTURE PERSPECTIVES

Based on the understanding on pathophysiology, current strategies to rescue steatotic donor livers work through ameliorating the abnormal lipid metabolism (the first “hit”), and the oxidative stress and inflammation (the second “hit”). Each approach employs various methods at different phases of organ recovery and preservation but generally targets similar molecular mechanisms. Notably, it may be possible to attack the same mechanism but reinforce the effects by applying the treatment at multiple points of organ recovery and preservation process, thereby producing a stacking effect. The use of glutathione on donors and in SCS solution by Ye *et al.*^[118] is a good example in this direction that demonstrates the therapeutic effects may stack. Stacking other medications, *e.g.*, IL-6 and pentoxifylline could also be promising as these could be given to the donors prior to recovery or readily added into preservation solutions to treat liver grafts. Whether the stacking approach works for different targets in lipid metabolism, oxidative stress or inflammation remain to be elucidated.

Similar to stacking the same/similar pharmacological agents at different stages, it is a reasonable idea to attack the two “hits” simultaneously by stacking medica-

tions targeting different pathways. Development of such combinations is usually shunned because it exponentially increases the complexity of development and clinical testing, but given that the disease itself is a very complex phenomenon spanning multiple pathways, it may be unavoidable, and a single silver bullet to treat steatosis simply nonexistent.

An intriguing alternative in development is the efforts to use MP for liver preservation. Especially in near normothermic conditions, MP provides a combined opportunity to improve energy storage, maintain microcirculation, and support pharmacological approaches to decrease fat content and treat IRI. While machine perfusion by definition is more complex than simple storage on ice, it is a very promising approach available in the near future and could be the ultimate solution to rescue steatotic as well as ischemic livers.

Both steatotic livers and DCD livers are highly susceptible to IRI. Therefore, potentially, a method to rescue DCD livers could be also applicable to steatotic livers even with the fat content intact. For instance, MP preservation was able to rescue DCD livers and steatotic livers^[143,157-164]. Similarly, perfluorocarbon as an artificial oxygen carrier to improve SCS preservation of DCD livers^[165] could also be tested on steatotic livers. If successful, we would then secure both DCD and fatty livers for transplantation, which would boost the organ availability dramatically and resolve donor liver shortage for a decade or more.

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Clinical utility of anti-*p53* auto-antibody: Systematic review and focus on colorectal cancer

Aravind Suppiah, John Greenman

Aravind Suppiah, Postgraduate Medical Institute, Biomedical and Environmental Sciences, University of Hull, Cottingham HU6 7RX, United Kingdom

Aravind Suppiah, Castle Hill Hospital, Cottingham HU16 5JQ, United Kingdom

John Greenman, School of Biological, Biomedical and Environmental Sciences, University of Hull, Cottingham HU6 7RX, United Kingdom

Author contributions: Suppiah A drafted, wrote and submitted the manuscript; Greenman J revised the manuscript, and provided final approval.

Correspondence to: Aravind Suppiah, MD, FRCS, Castle Hill Hospital, Castle Road, Cottingham HU16 5JQ, United Kingdom. aravind.suppiah@hotmail.com

Telephone: +44-79-20486056 Fax: +44-14-82622335

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Abstract

Mutation of the *p53* gene is a key event in the carcinogenesis of many different types of tumours. These can occur throughout the length of the *p53* gene. Anti-*p53* auto-antibodies are commonly produced in response to these *p53* mutations. This review firstly describes the various mechanisms of *p53* dysfunction and their association with subsequent carcinogenesis. Following this, the mechanisms of induction of anti-*p53* auto-antibody production are shown, with various hypotheses for the discrepancies between the presence of *p53* mutation and the presence/absence of anti-*p53* auto-antibodies. A systematic review was performed with a descriptive summary of key findings of each anti-*p53* auto-antibody study in all cancers published in the last 30 years. Using this, the cumulative frequency of anti-*p53* auto-antibody in each cancer type is calculated and then compared with the incidence of *p53* mutation in each cancer to provide the largest sample calculation and correlation between mutation and anti-*p53* auto-antibody published to date. Finally, the review focuses on

the data of anti-*p53* auto-antibody in colorectal cancer studies, and discusses future strategies including the potentially promising role using anti-*p53* auto-antibody presence in screening and surveillance.

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Key words: *p53* gene; *p53* mutation; Anti-*p53* auto-antibody; Cancer; Colorectal cancer

Core tip: Anti-*p53* auto-antibodies are commonly produced in response to *p53* mutations. Anti-*p53* auto-antibody titres generally increase with tumour load, but not all patients who are initially sero-negative develop an auto-antibody response despite disease progression and metastases. Conversely, sero-positive patients do not lose their anti-*p53* auto-antibodies despite the cancer being completely excised. In general, cancers with the highest *p53* mutation rate, *e.g.*, oesophageal and ovarian, demonstrate the highest anti-*p53* auto-antibody rates; conversely, melanoma and testicular carcinoma with the lowest mutation rate have the lowest serum auto-antibody levels. Measurement of anti-*p53* auto-antibodies may be useful in screening or monitoring for tumour recurrence.

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INTRODUCTION

The *p53* gene is located on the distal band of the short arm of chromosome 17p13^[1,2]. It consists of approximately 20000 base pairs spread over 11 exons^[2-5]. *p53* was initially discovered in 1979 as a protein binding to a viral

oncogene, Simian Vacuolating 40 (SV40) large T-antigen, and hence was thought to be an oncogene itself^[6-8]. It has since been established that it has a critical role as a tumour-suppressor gene^[9-11]. *p53* inactivation predisposes cells to malignant transformation in rodent models and in human clinical diseases such as Li-Fraumeni syndrome; the latter being characterized by germline mutations of *p53*^[12-14]. The tumour suppressive role of *p53* is so crucial that it is referred to as “the guardian of the genome”^[15,16]. It is the most common mutation found in cancers and is present in half of all solid tumours thus emphasising its importance in protecting cells from carcinogenesis^[3]. The frequency of mutation varies in individual cancers ranging from 5%-12% in cervical and haemopoietic malignancies to 40%-50% in colorectal and ovarian cancer^[1,5]. Additionally, the remaining cancers with no detectable *p53* mutation are still thought to have dysfunctional *p53* caused by mechanisms other than mutation^[9,17-20]. The most recent advances in colorectal cancer (CRC) treatment have been in the field of immunology with the use of antibodies against potent growth factors including epidermal growth factor receptor (EGFR) and vascular endothelial growth factors (VEGF)^[21,22]. As such, *p53*, with its diverse immuno-regulatory role maintains a vital role in future of management of cancer and benign diseases. This review begins with the description of the normal *p53* gene function and mechanisms of *p53* inactivation in cancer, followed by a systematic review of the association between the anti-*p53* auto-antibody response and underlying *p53* mutations, and finally a clinical focus on the current evidence and potential future role of anti-*p53* auto-antibody in colorectal cancer.

***p53* GENE AND GENE FUNCTION**

p53 acts as a tumour suppressor by preventing propagation of defective cells. It is up-regulated by various upstream factors in response to cellular stress or damage such as DNA damage, hypoxia, telomere shortening and oncogenic stimulation or radiation^[2,11]. Activated *p53* modifies downstream gene expression and co-factor transcription, which in conjunction with *p53*, lead to growth arrest (*e.g.*, via p21^{WAF1}) or apoptosis (*e.g.*, *p53*-upregulated modulator of apoptosis, PUMA)^[19,23,24].

The *p53* gene encodes for a 393 amino-acid, 53 kDa, phospho-protein which is divided into 3 domains-an amino (-NH₂) terminal region (approximately amino acids 1-100), a central “core” domain (amino acids 100-300) and a terminal carboxyl (-COOH) region (amino acids 320-360)^[25-27]. Almost all mutations are harboured in the central “core” which contains the DNA-binding regions. Thus *p53* dysfunction is most likely caused by mutations that alter DNA binding behaviour. However, most anti-*p53* auto-antibodies do not recognise central core mutations but rather recognise epitopes in the 2 terminal regions. An interesting observation is that these terminal regions which contain the least mutations are also found on the wild-type and the mutant *p53* protein^[25,27,28]. This suggests anti-*p53* is not only produced in response to

mutation but also elevated levels of normal *p53*. This is discussed later (see Anti-*p53* Auto-antibody).

Wild-type *p53* protein expression is intra-nuclear with a half-life of 5-30 min and is subject to complex regulation^[29]. The most important regulator is thought to be Murine Double Minute 2 (Mdm2)^[29,30]. Mdm2 is an ubiquitin-dependant E3 ligase which targets wild-type *p53* protein for nuclear and cytoplasmic proteasome-mediated degradation^[31,32]. When up-regulated *p53* binds to the Mdm2 promoter leading to increased levels of mdm-2 transcription; the *Mdm2* gene product then inhibits *p53* thus creating a negative feedback loop. This feedback process is complex and regulated by multitude of factors. Mdm-2 in itself is subject to modifications mainly self-degradation by (1) auto-ubiquitination^[33,34]; (2) small Ubiquitin-like Modifier (SUMO)-ylation^[35]; (3) acetylation^[36]; (4) post-translational upstream kinases (*e.g.*, ATM kinase phosphorylation of Mdm2 and Mdm-X)^[37-39]; (5) Mdm-2 in conjunction with Mdm-X (also known as Mdm-4) can form a Mdm2-MdmX-*p53* complex which represses *p53* activity; and (6) Mdm-X can furthermore act independently of Mdm-2 and repress *p53*-bound chromatin, without Mdm2, most likely by direct binding. The combinations of these mechanisms, regulate *p53* accumulation in response to various cellular stresses (Figure 1).

p53 increases in response to cellular stress caused by a variety of insults including DNA damage, oncogene activation, ribosomal stress and hypoxia^[11] by several mechanisms: (1) increased transcription; (2) increased intra-nuclear accumulation of active *p53*; (3) increased extra-nuclear export of Mdm-2^[40,41]; (4) down-regulation of Mdm2-Mdmx which usually represses chromatin-bound-*p53*^[24]; (5) various downstream post-translational modifications of both *p53* and its regulators *e.g.*, Mdm2^[17]; and (6) raised cytosolic *p53*^[42-45].

Active *p53* has tumour suppressive activity by causing cell cycle arrest, apoptosis and autophagy. Cell cycle arrest initially provides additional time for the cell to repair damaged DNA. However, cells unable to repair damage are directed towards apoptosis by shifts in the balance between pro-arrest, pro-autophagy and pro-apoptotic factors severe cellular damage, the cell pushed directly towards apoptosis by the relative increase in pro-apoptotic markers relative to cell-cycle arrest promoters^[19].

Autophagy is an evolutionary catabolic process of mass lysosomal self-degradation of cytosol/proteins/organelles which are sequestered into a double membrane vesicle which is then fused with lysosomes for bulk degradation^[42,43]. *p53* plays a dual role in activating and/or inhibiting autophagy by transactivating numerous autophagy regulators including mammalian target of rapamycin (mTOR)^[46], activated protein kinase (AMPK) and tuberous sclerosis protein (TSC2)^[43,47]. *p53* is also able to influence the decision between apoptosis and autophagy by selectively activating pro-autophagy proteins such as AMPK, death-associated protein kinase 1 (DAPK-1) and damage-regulated autophagy modulator (DRAM)^[48]. Alternatively, *p53* also promotes apoptosis by activating pro-apoptotic markers such as B-cell Lymphoma 2

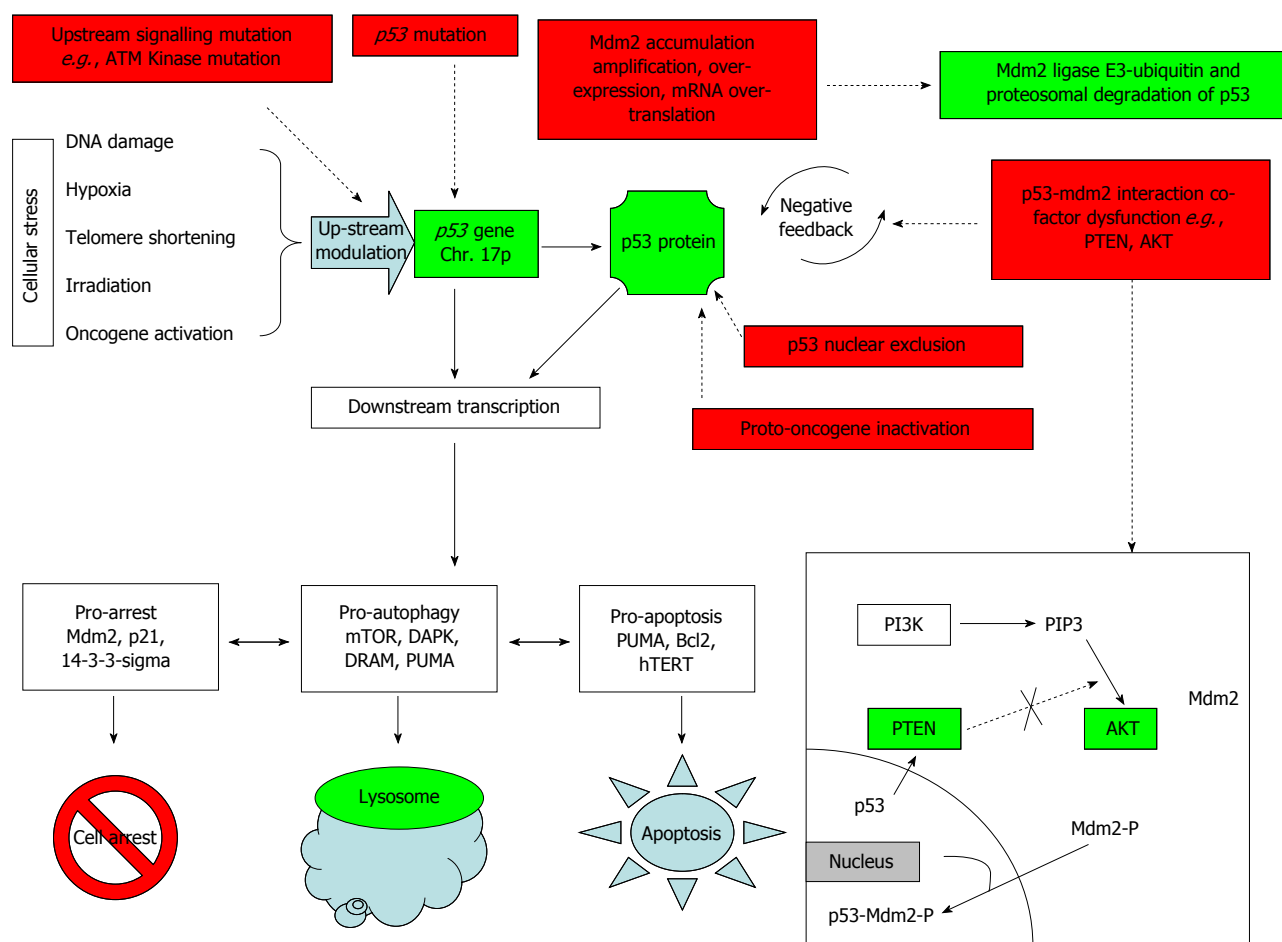


Figure 1 Normal *p53* function highlighted in green boxes and blue figures as described. Red boxes show mechanisms for inactivation; Normal *p53* function highlighted in green and blue figures. PI3K: Phosphatidylinositol-3-kinase; PTEN: Phosphatase and tensin homolog; AKT: Protein kinase B; ATM: Ataxia telangiectasia mutated kinase; mTOR: Mammalian target of rapamycin; DAPK: Death-associated protein kinase; DRAM: Damage-regulated autophagy modulator; PUMA: p53-upregulated modulator of apoptosis; hTERT: Human telomerase reverse transcriptase.

(Bcl-2), Bcl-2 associated death protein (BAD), Bcl-2 associated X-protein (BAX), *p53*-upregulated modulator of apoptosis (PUMA)^[49] and autophagy inhibitors such as TP-53 induced glycolysis and apoptosis regulator proteins (TIGAR)^[42].

Disruption of *p53* gene transcription function and subsequent production of an inactive mutant *p53* protein allows cells to escape the cellular arrest/apoptosis controls. This allows unregulated propagation of abnormal cells and a predisposition to malignant transformation. It is important to be aware that *in vivo* *p53* behaviour can be different from *in vitro* response. This could be due to different stress types^[50,51], cell types^[52] and immune responses^[24,53,54].

MECHANISMS OF *p53* INACTIVATION

The most common cause of *p53* inactivation is mutation which most frequently occurs within the *p53* core, and furthermore 70% occur at “hot spots”—amino acids 132-142, 151-159, 172-179, 237-249 and 272-286^[26,55]. The International Agency for Research on Cancer (IARC) TP53 database similarly reports the most frequent *p53* mutations at codons 175, 245, 248, 249, 273 and 282;

which is further corroborated by the UMD-TP53 mutation database, another international database that spans over 25 years^[1,3,56]. The most common type of mutation is mis-sense (73%) followed by frame shift (9%), non-sense (8%), silent (4%) and others (6%)^[1,3,57].

p53 is also inactivated by mechanisms other than *p53* gene mutation and are described below (Figure 1).

Proto-oncogene activation

Proto-oncogenes are normal proteins that become oncogenic with relatively minor modifications^[6]. These proto-oncogenes are usually important cell cycle regulators (e.g., p14^{ARF})^[58]. Human papilloma virus (HPV) 16/18 E6 protein which causes cervical cancer is able to inactivate *p53* without mutation. This explains the relatively low incidence of *p53* mutation in cervical cancer^[59,60]. The SV40 large-T-antigen is another viral oncogene which is able to inactivate wild-type *p53*^[61].

Mdm2 over-expression

Mdm2 is a negative regulator of *p53* and reduces the cell's ability to trigger the pro-arrest/apoptotic pathway in the event of cellular damage^[62,63]. Mdm2 over-expression can occur by gene amplification, gene over-expression or

mRNA over-transcription^[20,56]. Mdm2 over-expression is classically observed in soft tissue sarcomas^[64,65]. Interestingly, instead of a decrease in p53 expression, the levels of both Mdm2 and p53 expression are increased. This suggests Mdm2 may have an additional p53-independent oncogenic mechanism (in addition to p53 suppression by negative feedback) which can promote tumour growth.

Dysfunction of regulators of the p53-Mdm2 loop

Mutations of in the p53-Mdm-2 feedback loop such as AKT Kinase, Phosphatidylinositol-3-kinase (PI3K), Phosphatase and Tensin Homolog (PTEN) and Ataxia Telangiectasia Mutated (ATM)-Kinase can inappropriately influence levels of p53 (see detailed description below). p53 disruption has also been associated with inactivation of other tumour suppressors *e.g.*, BRCA1, Bcl-2, transforming growth factor (TGF)- β . AKT-kinase not only influences p53 levels but forms an apoptotic pathway with mTOR, an autophagy marker, in the PI3K/AKT/mTOR pathway demonstrating the complex interplay between p53 and the relative levels of its regulators in deciding cell fate^[19,42,43]: (1) AKT-kinase phosphorylates Mdm2 and induces migration of phosphorylated-Mdm2 into the nucleus where it inactivates p53. AKT over-expression has been shown to occur in cancer cells^[66,67]; (2) PTEN is tumour-suppressive and activated in response to stress leading to p53 up-regulation. Wild-type PTEN inhibits AKT-kinase phosphorylation of Mdm-2 and thus, intra-nuclear Mdm2 migration which suppresses p53 activity^[29,68,69]. In contrast mutated PTEN is unable to inhibit AKT-kinase which leads to continuous Mdm2- phosphorylation and Mdm-2 intra-nuclear migration leading to reduced p53 tumour suppressive ability^[69,70]; and (3) Cell stress (*e.g.*, irradiation) activates factors up-stream of p53 such as ATM kinase and checkpoint Kinase-2^[54,63]. Mutated ATM-kinase is unable to activate p53 in response to radiation-induced stress.

Nuclear exclusion and cytoplasmic p53

Extrusion of p53 into cytoplasm has been observed in certain tumours such as breast^[71], colon^[72], neuroblastoma^[73] and malignant melanoma^[74]. Nuclear extrusion prevents p53 from performing its intra-nuclear interactions.

Gain of oncogenic function

Mutant p53 has an impaired ability to regulate cell cycle which is referred to as “loss of function”^[3,6,75]. In addition to this, mutated p53 can also exhibit conformational changes which result in acquisition of new pro-oncogenic abilities; this is known as “gain of function”. Such functions include increased transcription of tumour-promoting factors such as MYC and VEGF^[76] and disruption of protective pro-apoptotic factors such as p73^[7,77].

ANTI-p53 AUTO-ANTIBODY

An anti-p53 auto-antibody response was first reported by Crawford *et al*^[78] in 1982 in 9% (14/155) of patients with breast cancer. Further interest in this anti-p53 auto-anti-

body response declined due to the lack of accurate quantification methods and no observable clinical relevance. Research into the auto-antibody was invigorated in the 1990s when the critical role of p53 gene in carcinogenesis was recognized. The exact cause of induction of anti-p53 auto-antibody production is unknown but is thought to be associated with the presence of p53 mutation and p53 protein over-expression.

An anti-p53 auto-antibody is not normally produced wild-type p53 protein induces tolerance of the host^[32,79]. In abnormal cells, mutant p53 protein is stabilised as discussed above which cause relatively high intra-nuclear p53 protein accumulation which then escapes into the cytoplasm. The resulting high cytoplasmic p53 levels increase the likelihood of p53 protein being degraded by proteasomes and presented on cell surfaces to be recognised by T-cells in a MHC I response^[16]. Auto-antibodies recognise epitopes on the terminal regions of the protein, and hence auto-antibody production can theoretically be triggered by either the wild-type or the mutant p53, provided sufficiently high levels of these immunodominant epitopes are present at the cell surface^[80]. Another probable antigen presentation mechanism is where cancer cells containing high cytoplasmic concentrations of p53 undergo necrosis and release p53 into the blood and lymphatic system where appropriate B-cells can interact. These antigens are also captured by Antigen Presenting Cells (APC) in their normal scavenging role and are presented in association with MHC class II response causing a Th2-like cell response^[16] (Figure 2).

p53 mutation alone is insufficient to trigger anti-p53 auto-antibody production as evidenced by several observations. Firstly, only 20%-50% of patients with detectable p53 mutations produce detectable auto-antibodies^[81,82]. This is attributed to the type of mutation, *e.g.*, mis-sense mutations are associated with higher auto-antibody production compared with other mutations^[5,56,57,83]. This is probably because mis-sense mutations are more likely to produce a stable mutant p53 protein which is more likely to accumulate to sufficient levels to increase the likelihood of antigen presentation. Other mutations such as non-sense, frameshift and deletions often lead to truncated mRNA and unstable protein sequences which are less likely to accumulate, and thus less likely to induce auto-antibody production^[84]. Secondly, anti-p53 auto-antibodies most frequently recognise terminal epitopes but not the central domain with the majority of mutations^[25,27,28,81]. Thirdly, large SV40 T-antigen stabilises p53 protein leading to accumulation of the wild-type protein which also induces auto-antibody production. Together these observations suggest that humoral response is triggered by elevated p53 protein levels *per se* (mutated and/or wild-type) rather than specifically directed at a mutated sequence.

Discrepancy between anti-p53 auto-antibody and p53 mutation

There are discrepancies between the presence of p53 mutation, p53 protein product expression and anti-p53

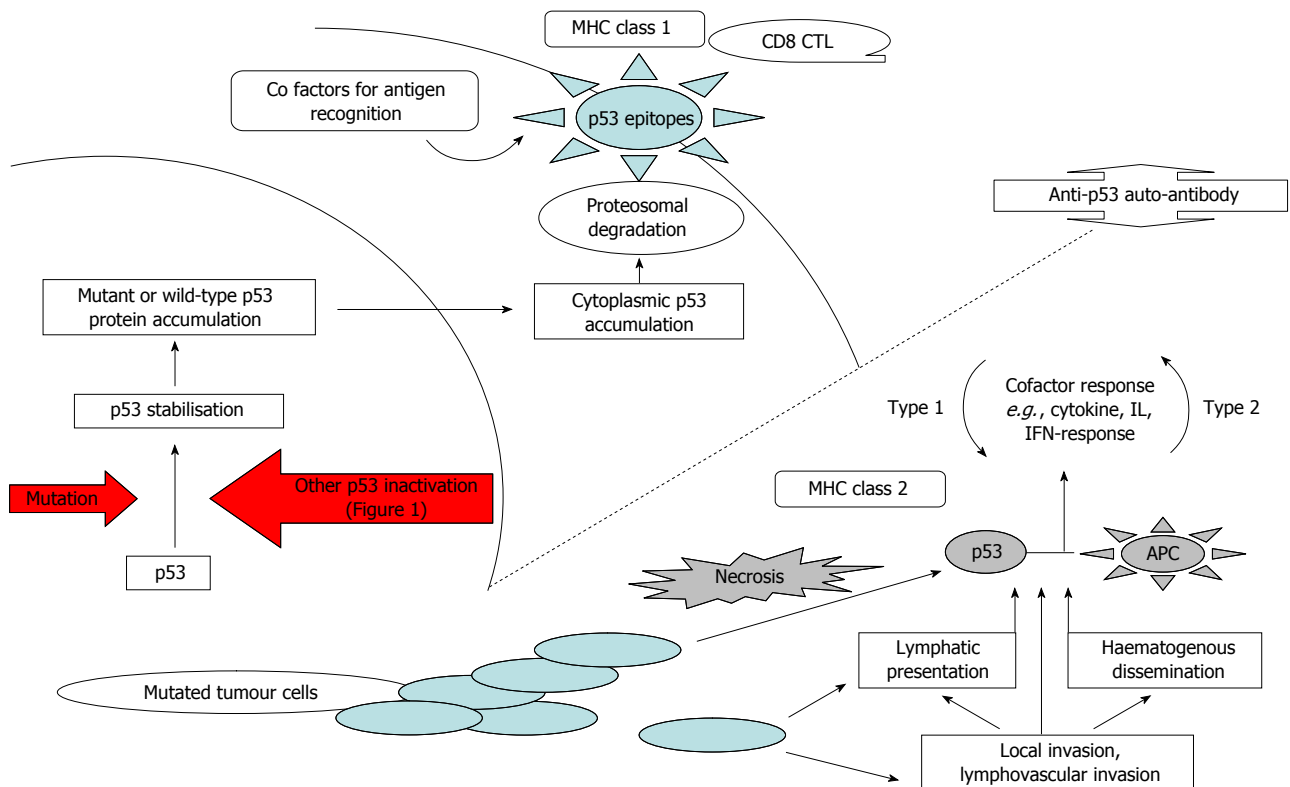


Figure 2 Proposed mechanisms of anti-*p53* auto-antibody induction. MHC: Major histocompatibility complex; CD8: Cluster of 8 differentiation; CTL: Cytotoxic T-cell; IL: Interleukin; APC: Antigen presenting cell.

auto-antibody production. This is largely attributed to the methodological differences of detection. Initial gene screening studies reported that most *p53* mutations were localised to exons 5-8 and to a lesser extent 4, 9, 10. Subsequent studies then only tended to screen these regions leading to substantial screening bias. It is now known that at least 10% of *p53* mutations occur outside these areas^[84,85]. Another source of methodological difference is *p53* protein detection which is inherently subject to tissue sampling and biopsy errors. Older studies (pre-1999) had different immuno-histochemical, fixation, paraffinization, antigen and antibody retrieval and observer scoring techniques. Finally, the antibody used to detect the mutant *p53* protein affects sensitivity of *p53* protein detection in IHC and also the detection of anti-*p53* auto-antibody in ELISA as described below.

Historically, the auto-antibody was initially detected using immunoblots or in-house enzyme linked immunosorbent assay (ELISA). These ELISA used different cut-off values leading to a vast range of reported frequencies of anti-*p53* auto-antibody within individual cancers in many older studies. Although standardised commercial ELISA are now widely available leading to an increase in anti-*p53* ELISA studies (2000 onward), auto-antibody detection can still vary depending on different manufacturers' product^[86]. Most importantly, these ELISAs only measure an antibody response against those *p53* epitopes, which are expressed by the recombinant proteins used as the coating antigen. This may account for the reason that there are minor variations in commercial ELISA studies in different populations but when the same ELISA is

used in the same population, inter and intra-coefficient of variations of 0.3%-2.7% are extremely reliable^[82].

Finally, the differences in individual's immune systems cannot be ignored. The humoral response is dependent on an individual's unique MHC presentation as shown by several observations. Firstly, patients with similar cancers containing the same *p53* mutations do not necessarily mount the same immune response^[81]. Secondly, whilst anti-*p53* auto-antibody titres increase in response to tumour load, all patients who are initially sero-negative do not develop an auto-antibody response despite disease progression and metastases. Conversely, patients who are sero-positive at diagnosis do not sero-convert to a negative anti-*p53* auto-antibody status even after the cancer is completely excised. It seems that once the patient's immune system has been primed, there is sufficient *p53* antigen available to maintain a long-term anti-*p53* humoral response^[28,87,88].

MATERIALS AND METHODS: SYSTEMATIC REVIEW

Literature searches were performed using Medline and PubMed up to January 2012. Keywords used were "*p53*", "anti-*p53*", "antibody", "auto-antibody", "cancer" and combinations. No language or time restrictions were applied. All abstracts were reviewed and the relevant articles retrieved. The results of all published anti-*p53* auto-antibody cancer studies were accumulated and compiled in Table 1 with relevant key findings. The anti-*p53* auto-

Table 1 Cumulative reported frequencies of anti-*p53* auto-antibody (anti-*p53*) in controls and individual cancers *n* (%)

Group	Ref.	Anti- <i>p53</i> positive	Summary of study and tumour type
Healthy/Benign	Park <i>et al</i> ^[107]	4/79 (5)	Comparative study with lung cancer
	Wu <i>et al</i> ^[133]	9/879 (1)	Case-control study of anti- <i>p53</i> in various cancers
	Kulić <i>et al</i> ^[134]	1/20 (5)	Comparative study with breast carcinoma
	Suppiah <i>et al</i> ^[130]	0/28 (0)	Comparative study with colorectal carcinoma
	Cai <i>et al</i> ^[125]	0/30 (0)	Comparative study with oesophageal carcinoma
	Atta <i>et al</i> ^[135]	5/29 (17.2); 13/26 (50) ¹	Comparative study with hepatocellular carcinoma
	Mattioni <i>et al</i> ^[136]	0/64 (0)	Comparative study with gastric carcinoma
	Akere <i>et al</i> ^[137]	4/45 (8.9)	Comparative study with hepatocellular carcinoma
	Müller <i>et al</i> ^[123]	0/57 (0); 0/379 (0) ²	Single study of anti- <i>p53</i> in various cancers
	Chang <i>et al</i> ^[85]	0/40 (0)	Comparative study with colorectal carcinoma
	Fonseca <i>et al</i> ^[96]	0/15 (0)	Comparative study with glioma
	Shimada <i>et al</i> ^[82]	10/205 (6.3); 13/189 (7) ³	Multi-institutional study of anti- <i>p53</i> in various cancers
	Neri <i>et al</i> ^[138]	0/51 (0)	Comparative study with lung carcinoma
	Numa <i>et al</i> ^[139]	0/9 (0)	Comparative study with uterine, ovarian, cervical carcinoma
	Mack <i>et al</i> ^[140]	1/46 (2.2)	Comparative study with SCLC
	Chow <i>et al</i> ^[141]	1/28 (3.6)	Comparative study with head and neck carcinoma
	Moch <i>et al</i> ^[142]	2/130 (1.5)	Comparative study with skin carcinoma (SCC/BCC)
	Hofele <i>et al</i> ^[143]	0/80 (0)	Comparative study with oral SCC
	Hagiwara <i>et al</i> ^[144]	0/13 (0)	Comparative study with oesophageal carcinoma
	Ralhan <i>et al</i> ^[145]	4/50 (8)	Comparative study with lung carcinoma
	Bielicki <i>et al</i> ^[111]	0/28 (0)	Comparative study with colorectal carcinoma
	Soussi ^[90]	35/2404 (1.5)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Total	102/4924 (2.1)	
Oesophageal	Blanchard <i>et al</i> ^[146]	24/97 (28)	Correlates with decreased overall and disease free survival
	Wu <i>et al</i> ^[133]	4/29 (13.8)	Case-control study of anti- <i>p53</i> in various cancers
	Cai <i>et al</i> ^[125]	18/46 (39.1)	Correlates with advanced histological grade, stage, lymph node metastases and decreased tumour response following radiotherapy
	Müller <i>et al</i> ^[123]	10/50 (20)	No correlation with stage or prognosis
	Bergström <i>et al</i> ^[147]	31/42 (73.8)	No correlation with clinico-pathological parameters, tumour size or survival
	Shimada <i>et al</i> ^[82]	90/301 (29.9)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Kozłowski <i>et al</i> ^[148]	20/75 (26.6)	No correlation with stage, lymph node metastases or size.
	Shimada <i>et al</i> ^[99]	14/35 (40)	Correlates with tumour p53 protein expression but not clinico-pathological parameters
	Hagiwara <i>et al</i> ^[144]	13/46 (28)	Correlates with increased stage and tumour p53 protein expression but not prognosis
	Ralhan <i>et al</i> ^[145]	36/60 (60)	Correlates with tumour p53 protein expression and missense mutations but not clinico-pathological parameters.
	Soussi ^[90]	85/274 (31)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Total	345/1055 (32.7)	
Head/Neck ¹⁶	Wu <i>et al</i> ^[133]	1/20 (5.0)	Case-control study of anti- <i>p53</i> in various cancers
	Shimada <i>et al</i> ^[82]	10/31 (32.3)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Chow <i>et al</i> ^[141]	23/75 (31)	Correlates with nodal metastases but not prognosis
	Total	34/126 (27.0)	
Oral	Wu <i>et al</i> ^[133]	5/15 (33.3)	Case-control study of anti- <i>p53</i> in various cancers
	Hofele <i>et al</i> ^[143]	19/102 (18.6) ⁴ ; 12/24 (50) ⁵	Correlates with poor prognosis
	Castelli <i>et al</i> ^[149]	3/61 (18.7); 9/13 (69.2) ³	Serum anti- <i>p53</i> is useful as a screening tool in pre-malignant lesions
	Soussi ^[90]	309/1062 (29.1)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
Ovary	Total	348/1219 (28.5)	
	Wu <i>et al</i> ^[133]	5/12 (41.6)	Case-control study of anti- <i>p53</i> in various cancers
	Qiu <i>et al</i> ^[150]	36/92 (39.1)	Correlates with p53 expression, not clinico-pathological parameters
	Shimada <i>et al</i> ^[82]	2/27 (7.4)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Numa <i>et al</i> ^[139]	8/30 (27)	Correlates with p53 tumour expression and poor prognosis
	Abendstein <i>et al</i> ^[151]	28/113 (25); 21/113 (19) ⁶	Correlation between serum and ascitic anti- <i>p53</i> . No correlation with stage or grade. Anti- <i>p53</i> in ascites associated with poor prognosis
	Soussi ^[90]	140/635 (22)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Total	219/909 (24.1)	
Colorectal (detailed results in Table 2)	Wu <i>et al</i> ^[133]	11/66 (16.7)	Case-control study of anti- <i>p53</i> in various cancers
	Suppiah <i>et al</i> ^[130]	20/92 (21.7)	No correlation with stage or prognosis
	Nozoe <i>et al</i> ^[97]	17/36 (47.2)	Correlates with advanced lymph node status and stage
	Müller <i>et al</i> ^[123]	63/197 (32) ⁷ ; 7/46 (15.2) ⁸	No correlation with stage or prognosis
	Chang <i>et al</i> ^[85]	47/167 (28.1)	p53 mutation, not anti- <i>p53</i> , correlates with poor prognosis
	Lechpammer <i>et al</i> ^[88]	40/220 (18.2)	? Correlation with stage or prognosis in Dukes' A/B1
	Shimada <i>et al</i> ^[82]	46/192 (23.9)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Forslund <i>et al</i> ^[84]	24/88 (27.3)	Correlates with p53 mutation
	Tang <i>et al</i> ^[89]	130/998 (13)	Correlates with advanced lymph node involvement but not prognosis
	Broll <i>et al</i> ^[152]	20/130 (15.4)	No correlation with stage or prognosis
	Takeda <i>et al</i> ^[98]	17/27 (63)	95% negative sero-conversion within 3 wk post-surgery
	Shiota <i>et al</i> ^[112]	18/71 (25.4)	Correlates with advanced stage and poor prognosis

HCC	Bielicki <i>et al</i> ^[111]	30/145 (20.7)	? Correlation with Dukes' A →B
	Soussi ^[90]	307/1244 (24.7)	Literature review of anti-p53 in various cancers (1979-1999)
	Total	797/3719 (21.4)	
	Wu <i>et al</i> ^[133]	15/93 (16.1)	Case-control study of anti-p53 in various cancers
	Atta <i>et al</i> ^[135]	28/41 (68.3)	Correlates with advanced stage and shorter survival.
	Akere <i>et al</i> ^[137]	5/41 (12.2)	Correlates with increased Okuda stage
	Müller <i>et al</i> ^[123]	19/80 (23.8)	Non-significant trend towards poor prognosis
	Charuruks <i>et al</i> ^[153]	26/141 (18.4)	Correlates with stage but not tumour p53 protein expression
	Tangkijvanich <i>et al</i> ^[154]	16/121 (13.2) ¹⁷	Preliminary report of Charuruks <i>et al</i> (2001). No correlation with severity, stage or prognosis. Survival too short for survival analysis (3 mo <i>vs</i> 4 mo)
	Sitruk <i>et al</i> ^[155]	19/159 (12)	Correlates with multinodular, infiltrative tumour but not survival
Bladder	Soussi ^[90]	82/387 (1.2)	Literature review of anti-p53 in various cancers (1979-1999)
	Total	210/1063 (19.8)	
	Wu <i>et al</i> ^[133]	0/11 (0)	Case-control study of anti-p53 in various cancers
	Müller <i>et al</i> ^[123]	3/24 (12.5)	No correlation with prognosis
	Watanabe <i>et al</i> ^[156]	17/63 (27) ⁹	Correlates with higher grade, stage, lymph node metastases and tumour p53 protein expression, but not prognosis
	Gumus <i>et al</i> ^[157]	14/80 (17.5)	Correlates with tumour p53 protein expression and poor prognosis.
	Gumus <i>et al</i> ^[158]	25/76 (33)	Negative sero-conversion post-treatment (35%, 8/23) associated with good prognosis.
	Shimada <i>et al</i> ^[82]	4/33 (12.1)	Multi-institutional study of anti-p53 in various cancers
	Morita <i>et al</i> ^[159]	12/100 (12)	Correlates with stage, and p53 protein expression but not prognosis
	Wunderlich <i>et al</i> ^[160]	4/32 (12.5)	Correlates with tumour protein p53 expression but not stage.
Lung	Soussi ^[90]	8/29 (27.6)	Literature review of anti-p53 in various cancers (1979-1999)
	Total	70/385 (18.2)	
	Park <i>et al</i> ^[107]	28/82 (34.1)	Sensitivity study with other markers for lung cancer
	Wu <i>et al</i> ^[133]	13/95 (13.7)	Case-control study of anti-p53 in various cancers
	Bergqvist <i>et al</i> ^[161]	14/84 (16.6)	No correlation with tumour volume. Correlates with survival in adenocarcinoma, but not SCC
	Bergqvist <i>et al</i> ^[162]	12/58 (20.7)	No correlation with tumour volume or lymph node metastases
	Neri <i>et al</i> ^[138]	2/30 (6.7) ¹⁰ ; 8/48(16.7) ¹¹	No correlation with stage, histology or prognosis. Non-significant increased survival in LC but not MM
	Cioffi <i>et al</i> ^[163]	35/109 (32.1)	Low sensitivity, but high specificity (100%) and accuracy (69%). Only 14% agreement with other tumour markers (CEA/TPA, CYFRA21-1, NSE.)
	Zalcman <i>et al</i> ^[126]	20/97 (20.6)	Correlates with poor prognosis in limited stage SCLC, but not all SCLC
	Mack <i>et al</i> ^[140]	4/35 (11.1) ¹² ; NSCLC 13/99 (13.3) ¹³	Correlates with stage and prognosis in NSCLC but not SCLC
Cervix	Shimada <i>et al</i> ^[82]	18/125 (14.4)	Multi-institutional study of anti-p53 in various cancers
	Soussi ^[90]	219/1282 (17.1)	Literature review of anti-p53 in various cancers (1979-1999)
	Total	373/2049 (18.2)	
	Shimada <i>et al</i> ^[82]	10/53 (18.9)	Multi-institutional study of anti-p53 in various cancers
	Numa <i>et al</i> ^[139]	12/86 (14)	No correlation with tumour p53 protein expression or prognosis
	Total	22/139 (15.8)	
	Wu <i>et al</i> ^[133]	7/43 (16.3)	Case-control study of anti-p53 in various cancers
	Qiu <i>et al</i> ^[150]	19/61 (31.1)	Correlates with tumour size but not prognosis.
	Mattioni <i>et al</i> ^[136]	17/111 (15.3)	Correlates with tumour p53 protein expression, prognosis and survival
	Lawniczak <i>et al</i> ^[164]	16/71 (22.5)	Correlates with tumour type and age, but not stage or prognosis
Gastric	Müller <i>et al</i> ^[123]	14/122 (11.5)	No correlation with prognosis
	Shimada <i>et al</i> ^[82]	13/123 (10.6)	Multi-institutional study of anti-p53 in various cancers
	Nakajima <i>et al</i> ^[165]	13/81 (16)	Correlates with lymph node metastases but not stage or prognosis
	Maehara <i>et al</i> ^[166]	23/120 (19.2)	Correlates with increased stage and tumour p53 protein expression but not prognosis
	Soussi <i>et al</i> ^[90]	105/727 (14.1)	Literature review of anti-p53 in various cancers (1979-1999)
	Total	227/1459 (15.6)	
	Nozoe <i>et al</i> ^[167]	15/42 (35)	Correlates with grade 3 and triple negative cancer
	Wu <i>et al</i> ^[133]	9/25 (16)	Case-control study of anti-p53 in various cancers
	Kulić <i>et al</i> ^[134]	21/61 (35)	Correlates with decreased 5 year survival
	Müller <i>et al</i> ^[123]	17/50 (34)	Non-significant trend towards poor prognosis
Breast	Gao <i>et al</i> ^[168]	31/144 (21.5)	Correlates with stage, lymph node metastases, ER negative, c-erb-2 and tumour p53 protein expression
	Shimada <i>et al</i> ^[82]	13/71 (18.3)	Multi-institutional study of anti-p53 in various cancers
	Volkman <i>et al</i> ^[169]	18/165 (10.9)	Poor concordance between recombinant/native p53 ELISA, immunoblot and immunofluorescence
	Metcalfe <i>et al</i> ^[87]	155/1006 (15.4)	No correlation with stage and prognosis
	Soussi ^[90]	296/2006 (14.8)	Literature review of anti-p53 in various cancers (1979-1999)
	Total	539/3467 (15.5)	
	Wu <i>et al</i> ^[133]	1/13 (7.7)	Case-control study of anti-p53 in various cancers
	Shimada <i>et al</i> ^[82]	5/22 (22.7)	Multi-institutional study of anti-p53 in various cancers
	Numa <i>et al</i> ^[139]	5/41 (12)	No correlation with tumour p53 expression/prognosis (see Cervix, Ovary)
	Total	11/79 (13.9)	
Pancreas	Wu <i>et al</i> ^[133]	0/17 (0)	Case-control study of anti-p53 in various cancers

	Müller <i>et al.</i> ^[123]	5/22 (22.7)	Increase sensitivity in conjunction with CA19-9. No correlation with prognosis.
	Shimada <i>et al.</i> ^[82]	3/28 (10.7)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Ohshio <i>et al.</i> ^[170]	19/82 (23.2)	No correlation with tumour <i>p53</i> expression or prognosis
	Soussi ^[90]	60/650 (9.2)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Total	87/799 (10.9)	
Lymphoma	Messmer <i>et al.</i> ^[171]	19/120 (15.8)	Associated with 17p deletions
	Wu <i>et al.</i> ^[133]	0/18 (0)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Soussi ^[90]	19/248 (14.3)	Case-control study of anti- <i>p53</i> in various cancers
	Total	38/386 (9.8)	
Biliary tract ¹⁶	Wu <i>et al.</i> ^[133]	1/8 (6.3)	Correlates with tumour <i>p53</i> protein expression but not stage
	Limpaiboon <i>et al.</i> ^[172]	6/49 (12.2)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Shimada <i>et al.</i> ^[82]	1/6 (16.7)	Correlates with tumour <i>p53</i> mutation
	Tangkijvanich <i>et al.</i> ^[173]	6/82 (7.3)	
	Total	14/145 (9.7)	
Haematological	Wu <i>et al.</i> ^[133]	8/33 (25)	Case-control study of anti- <i>p53</i> in various cancers
	Shimada <i>et al.</i> ^[82]	32/364 (6.3) ¹⁴	Multi-institutional study of anti- <i>p53</i> in various cancers
	Soussi ^[90]	14/428 (3.3) ¹⁵	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Total	54/825 (6.5)	
Glioma	Wu <i>et al.</i> ^[133]	1/24 (4.2)	Case-control study of anti- <i>p53</i> in various cancers
	Fonseca <i>et al.</i> ^[65]	5/24 (20.8)	No correlation with <i>p53</i> protein but increased in patients < 16 years
	Shimada <i>et al.</i> ^[82]	2/31 (6.5)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Soussi ^[90]	6/144 (4.2)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Total	14/223 (6.3)	
Prostate	Wu <i>et al.</i> ^[133]	1/8 (12.5)	Case-control study of anti- <i>p53</i> in various cancers
	Shimada <i>et al.</i> ^[82]	4/23 (17.4)	Multi-institutional study of anti- <i>p53</i> in various cancers
	Soussi ^[90]	4/148 (2.7)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
	Total	9/179 (5.0)	
Skin	Moch <i>et al.</i> ^[142]	3/105 (2.9)	No difference between controls and patients. Increased in aggressive SCC (8%) vs slow-growing BCC (1.5%)
Testicular	Soussi ^[90]	0/144 (0)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
Melanoma	Soussi ^[90]	0/58 (0)	Literature review of anti- <i>p53</i> in various cancers (1979-1999)
Total		3419/18595 (18.4)	All cancers (1979-2012)

Cancer types are listed in order of decreasing anti-*p53* auto-antibody frequency. The reported studies within each cancer type are listed in reverse chronology. Squamous cell carcinoma (SCC); basal cell carcinoma (BCC), hepatocellular carcinoma (HCC), carcino-embryonic antigen (CEA). Tissue polypeptide antigen (TPA), CYFRA21-1, Neurone-specific enolase (NSE), Oestrogen receptor (ER), c-erb-2. ¹Cirrhosis; ²Benign disease; ³Oral pre-malignant lesions-excluded from calculation; ⁴Primary carcinoma; ⁵Secondary/recurrent carcinoma; ⁶Ascitic titre, not included in calculation of serum titres; ⁷Colon; ⁸Rectum; ⁹Upper renal tract tumours, excluded from anti-*p53* titres in bladder carcinoma; ¹⁰Malignant mesothelioma (MM); ¹¹Lung carcinoma (LC); ¹²Small cell lung carcinoma (SCLC); ¹³Non-small cell lung carcinoma (NSCLC); ¹⁴Myeloma; ¹⁵Leukaemia; ¹⁶Tumour type not specified; ¹⁷Excluded as is preliminary report of the same cohort (duplicate) reported in Charuruks *et al.*

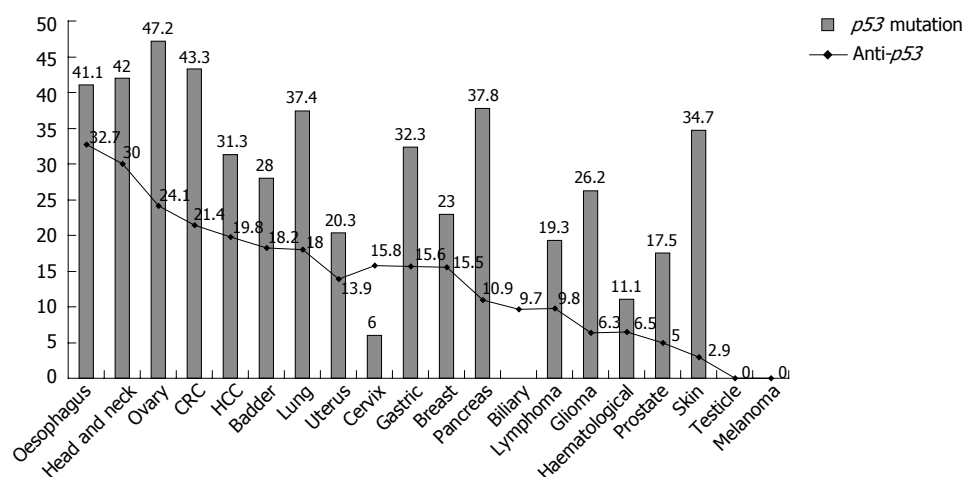


Figure 3 Percent *p53* mutations (International Agency for Research on Cancer, 2008) and percent anti-*p53* auto-antibody incidence calculated in this review. $r^2 = 0.45$, Correlation = 0.59. CRC: Colorectal cancer; HCC: Hepatocellular carcinoma.

antibody frequency from all published studies in each cancer type was calculated in this review. This calculated anti-*p53* auto-antibody frequency was then correlated with reported *p53* mutation rates to determine the associated between anti-*p53* auto-antibody presence and muta-

tion in each cancer (Figure 3).

Methodological quality of anti-*p53* and CRC publications

All published studies on anti-*p53* auto-antibody (1979-2012) were retrospective or cross-sectional case

control series with relatively small sample size (27-220 subjects tested) with a heterogeneous mix of cancer stages. The largest single study was published by Tang *et al.*^[89] that included a cohort of 998 CRC patients with anti-*p53* present in only small numbers ($n = 130$) for stage-specific analysis. An earlier non-systematic review by Soussi in 2000 recruited large numbers from various anti-*p53* studies but study quality was limited by different cancers at various stages and different auto-antibody detection methods^[90]. The primary outcome was not stated in most studies, and none was powered appropriately for survival outcomes.

ANTI-*p53* AUTO-ANTIBODY IN ALL CANCER TYPES

The reported frequency of anti-*p53* auto-antibody in individual cancer studies vary significantly due to small sample sizes, stage bias (usually a greater proportion of advanced stage tumours were included and different detection methods used). Anti-*p53* auto-antibody is usually measured in patients' sera but has also been measured in ascitic fluid of patients with ovarian cancer^[68], saliva of patients with oral cancer^[91] and in pleural effusions (12.5%) associated with lung, colon and pancreatic cancer^[92]. In a landmark review, Soussi compiled results of 80 anti-*p53* auto-antibody studies in 18 cancer types over a 20 year (1979-1999) period^[90]. The mean serum sero-positivity across all cancer types was 16.9% (1600/9489 patients, range 0%-31%) compared with 1.45% (35/2404) in controls thus demonstrating remarkable specificity (98%) but poor sensitivity. The specificity would be even higher as half the false positive subjects (17 out of 35) were from a single study reporting an extra-ordinarily high sero-positivity (24%, 17/70)^[93] (Table 1). When this study was excluded anti-*p53* auto-antibody specificity is near 100% for any cancer, which is confirmed by most recent reports.

Relationship between anti-*p53* auto-antibody and *p53* mutation

The 30-year cumulative sera anti-*p53* auto-antibody frequencies in individual cancers were calculated in this review to provide the most comprehensive anti-*p53* auto-antibody frequency in each cancer to date (Table 1). The auto-antibody frequencies are plotted (point) against the *p53* mutation rate (bars) as reported by the IARC TP53 Mutation Database to ascertain a relationship between anti-*p53* auto-antibody and *p53* mutation rates in each cancer.

The graph shows moderate correlation ($r^2 = 0.45$, correlation 0.59) between anti-*p53* auto-antibody and *p53* mutation (Figure 3). In general, cancers with the highest *p53* mutation rate such as oesophageal, head and neck, and colorectal demonstrate highest anti-*p53* auto-antibody rates^[82,90]. Conversely, melanoma and testicular carcinoma with the lowest mutation rate have the lowest serum anti-*p53* auto-antibody rates ($< 1\%$)^[90]. The two

exceptions are gliomas and skin cancers which have moderate *p53* mutation rate and low anti-*p53* auto-antibody rate (Figure 3). Proposed reasons for the low anti-*p53* auto-antibody production are poor brain antigenicity, poor *p53* antigen-presentation across the blood-brain barrier, and use of immuno-suppressive steroids (dexamethasone) in the majority of glioma cases^[90,94,95]. Similar arguments about poor antigen presentation across an epithelial barrier are made for the majority superficial skin cancer. In summary, anti-*p53* auto-antibody has up to 35% sensitivity, depending on cancer type, nearly 100% specific for any malignancy but varies in individual cancer types; and demonstrates moderate correlation with *p53* mutation rate of each cancer.

ANTI-*p53* AUTO-ANTIBODY AND COLORECTAL CANCER VARIATIONS

CRC has the second highest anti-*p53* auto-antibody sero-positivity rates due in part to the high frequency of *p53* mutation. Pre-1999, eight studies used an "in-house" developed ELISA, 1 used Western blotting (WB), 1 used immuno-precipitation (IP) and another used all 3 detection methods (ELISA, WB, IP)^[90]. Despite these methodological differences, most studies, 10 out of 11, reported a sero-positivity rate between 12.5% and 32%. The only study to report a discrepant and much higher sero-positivity rate (68%) used WB thus demonstrating the potential bias caused by non-standardised detection methodology^[96]. New standardised commercial ELISA kits have since been developed with less variation in sero-positivity (13%-27%) with intra- and inter-assay coefficient of variation of 1.85%-2.37% and 0.3%-3.32% respectively (MESACUP anti-*p53* Test; Medical and Biological Laboratories, MBL, Nagoya, Japan)^[82].

The mean sero-positivity from ELISA-only CRC studies calculated in this review was 19.9% (479/2409) with individual studies reporting of 13%-27% (Table 2). Only two studies reported inconsistently high rates of 47% and 63% in patients, and also in controls (2.6%), which suggests a lower cut-off value was used^[97,98]. The same authors then reported an unusually high (40%) sero-positivity in superficial oesophageal carcinoma in another series, compared with 20%-30% in the majority of other studies. These studies used the same ELISA (Pharmacell, France)^[99]. Interestingly, when the same authors later used a different ELISA (anti-*p53* EIA Kit II, MESACUP) in a similar population, they reported a much lower sero-positivity of 30% (oesophageal cancer) and 24% (CRC) which was more consistent with other published ELISA studies^[82]. This highlights potential methodological biases with anti-*p53* auto-antibody quantification even with commercially standardised ELISA kits.

Anti-*p53* auto-antibody in diagnosis and screening

Cancer screening is used when early detection and intervention can lead to improved outcome for example CRC where 5 year survival in Dukes' A is 95%-100% com-

Table 2 Anti-*p53* auto-antibody in all published colorectal cancer studies and key findings *n* (%)

Ref.	Method and manufacturer	Samples	Follow-up	Key findings
Suppiah <i>et al</i> ^[130]	ELISA (<i>p53</i> ELISAPLUS, Calbiochem, Darmstadt, Germany)	20/92 (21.7); 0/20 (0) ¹ 0/8 (0) ²	Median 97 mo	No correlation with tumour stage, differentiation or location. Multivariate analysis show only Stage (Dukes' and TNM) to be independent prognostic factors
Nozoe <i>et al</i> ^[97]	ELISA (Pharmacell, France)	17/36 (47.2)	Not stated	Anti- <i>p53</i> -ab (+) associated with greater lymphatic invasion (94.1%; 16/17 <i>vs</i> 68.4%; 13/19), nodal involvement (70/6%; 12/17 <i>vs</i> 17.6%; 3/17) and advanced stage (<i>P</i> = 0.02). Anti- <i>p53</i> frequency higher in <i>p53</i> protein expressing tumours (74%; 14/19 <i>vs</i> 18%; 3/17). Only 3 patients with Dukes' A CRC, all sero-negative
Muller <i>et al</i> ^[123]	Immunoblot	Colon 63/197 (32); Rectum 7/46 (15.2); 0/57 (0) ¹ 0/379 (0) ²	CRC patients enrolled into trial with 5 year follow-up	No correlation with clinico-pathological parameters or prognosis. Trend toward higher anti- <i>p53</i> sero-positivity in N2/3 disease, poor differentiation and metastases. There were no patients with Dukes' A in this study. Anti- <i>p53</i> independent of CEA and CA19-9 with 16% information gain. This is the only study to report negative to positive sero-conversion (3.6%, 11/303)
Chang <i>et al</i> ^[85]	ELISA (<i>p53</i> -AK, Dianova, Hamburg, Germany)	47/167 (28.1); 0/40 (0) ¹	Median 36.3 mo (4-58)	Anti- <i>p53</i> correlates with <i>p53</i> mutation (43% <i>vs</i> 18%) but not tumour <i>p53</i> expression, clinico-pathological features or prognosis. <i>p53</i> mutations, advanced stage and pre-operative CEA > 5 ng/mL were independent prognostic factors (in that order). <i>p53</i> mutation strongly associated with advanced stage and poor differentiation
Lechpammer <i>et al</i> ^[88]	ELISA (ELISAPLUS Oncogene Research Products, Cambridge, United States)	40/220 (18.2); 0/42 (0) ¹	40 patients up to 20 wk; 8 patients up to 48 wk	Anti- <i>p53</i> had higher tumour <i>p53</i> expression (70% <i>vs</i> 52%). Anti- <i>p53</i> frequency shows highest increase in Dukes' A (0%, 0/28) → Dukes' B: (24%, 21/87) but no increase in progression to Dukes' C (18%, 19/105). No correlation with overall tumour grade or metastases. Anti- <i>p53</i> reflects tumour load following surgery, during chemotherapy and with disease recurrence
Shimada <i>et al</i> ^[82]	ELISA (Anti- <i>p53</i> EIA Kit II, MESACUP anti- <i>p53</i> Test; MBL; Nagoya, Japan)	46/192 (23.9); 10/205 (4.9) ¹ ; 13/189 (6.9) ²	Not reported	Validation study for MESACUP ELISA using prevalence of anti- <i>p53</i> in various cancers. Good intra- and inter-assay coefficient of variation of 1.85-2.37% and 0.3-3.2% respectively. Demonstrates stability of anti- <i>p53</i> titres at room temperature for 7 d and following 10 freeze-thaw cycles. No comment on correlation with clinico-pathological parameters or prognosis
Forslund <i>et al</i> ^[84]	ELISA (Dianova, Hamburg, Germany)	24/88 (27)	Not reported	Cross-sectional study on relationship between <i>p53</i> mutations and anti- <i>p53</i> presence. Frequency of <i>p53</i> mutation higher in anti- <i>p53</i> sero-positive group (92%, 22/64 <i>vs</i> 34%, 22/64) Correlation with clinico-pathological and survival parameters not reported
Tang <i>et al</i> ^[89]	ELISA (Calbiochem-Novabiochem, Darmstadt, Germany)	130/998 (13); 2/211 (1) ³	Not reported	Anti- <i>p53</i> sero-positivity increases in progression from N2→N3 (2.9%-10.6%); but not N0→N1 (11.7%-12.3%), N1→N2 (12.3%-10.6%) or M0→M1 (12%-17%). No correlation with CEA, overall TNM stage or metastases. Anti- <i>p53</i> associated with shorter survival in uni- but not multi-variate analysis. Largest study on anti- <i>p53</i> in CRC
Broll <i>et al</i> ^[152]	ELISA (<i>p53</i> -autoantikörper ELISA, Dianova, Hamburg, Germany)	20/130 (15); 0/44 (0) ¹	Median 25.5 mo	Anti- <i>p53</i> positive predictive value of 100%, but accuracy 37% and negative predictive value 29% due to poor sensitivity (15%). Anti- <i>p53</i> correlated with <i>p53</i> expression (<i>P</i> < 0.05), but not TNM stage, grade or location (exact numbers not shown). Approximately 70% of series Stage I / II CRC
Takeda <i>et al</i> ^[98]	Anti- <i>p53</i> EIA (PharmaCell, Paris, France)	17/27 (63); 1/38 (2.6) ³	Up to 2 years Median not reported	Anti- <i>p53</i> correlates with <i>p53</i> protein expression and independent of CEA and CA-19-9. Sero-conversion in 94% (16/17) within 3 wk of endoscopic resection. No correlation with clinico-pathological parameters or prognosis/recurrence as all patients had early superficial CRC (23 mucosal, 4 submucosal invasion). This study reports exceptionally high anti- <i>p53</i> , especially considering very early CRC
Takeda <i>et al</i> ^[174]	ELISA (anti- <i>p53</i> -EIA kit, Pharmacell, Paris, France)	40 patients with anti- <i>p53</i> ab from previous studies	Up to 29 mo	No correlation between post-operative anti- <i>p53</i> sero-positivity and histological (depth, lymphatic or venous invasion) or clinico-pathological features of lymph node or liver metastases. High (96%; 27/28) sero-conversion in patients with complete tumour resection. No sero-conversion in patients with residual disease.
Shiota <i>et al</i> ^[112]	ELISA (GIF, Munster, Germany)	18/71 (25); 1/18 (6) ³	Not stated, median survival 56 mo anti <i>p53</i> ab negative	Anti- <i>p53</i> correlates with TNM stage (Stage I -IIIb: 9%, 4/45 <i>vs</i> IV: 56%, 14/25), Dukes' stage (A-C: 9%, 4/45 <i>vs</i> D: 56%, 14/25), CEA, CA19-9 and tumour <i>p53</i> protein expression. Anti- <i>p53</i> associated with shorted survival (56 mo <i>vs</i> 20 mo) and is weak poor prognostic indicator. Anti- <i>p53</i> prognostic significance secondary to other factors, including weak factors <i>e.g.</i> , CEA and CA19-9. Only small number of Stage I -IIIb patients

Bielicki <i>et al</i> ^[111]	ELISA (Dianova, Hamburg, Germany)	30/145 (21); 0/20 (0) ² ; 0/8 (0) ³	Not stated. Cross sectional study	No correlation with Dukes' Stage (A/B: 22%, 16/73 vs C/D 19% 14/72), size, location, CEA. Highest increase in anti- <i>p53</i> frequency from Dukes' A (0%, 0/6) to Dukes B1 (28%, 5/18) but no further difference in progression to Dukes' C (19%, 7/36). Only 6 Dukes' A patients in study, all sero-negative
Soussi ^[90]	ELISA/WB/IP	307/1244 (24.7)	ELISA/ WB/IP	Review combining all studies with different methodologies from 1979-1999. Range of sero-positivity (12.5%-68% in 11 studies)
Total (1999-2009)		479/2409 (19.9)		All modern studies (1999 onwards) using commercial ELISA only, with one exception using Immunoblot (Muller <i>et al</i> , 2006)
Review Total (1979-2009)		786/3653 (21.5)		All studies on anti- <i>p53</i> in CRC (1979-2009)

Studies prior to 1999 used different methodology and not included (see above). Enzyme-linked immunosorbent assay (ELISA); Western blotting (WB); Immunoprecipitation (IP) ¹healthy, ²benign disease, ³adenoma. The study by Muller *et al* was included despite using immunoblot technique as it was a recent study with relatively large sample size. CRC: Colorectal cancer; CEA: Carcino-embryonic antigen.

pared with 5% in Dukes' D. Colonoscopy is the current gold-standard diagnostic tool but is painful, expensive and is associated with life-threatening complications such as colonic perforation (0.01%-0.3%) and haemorrhage (0.6%)^[100,101]. The United Kingdom Flexible Sigmoidoscopy Screening Trial has provided evidence that one-off screening flexible sigmoidoscopy between age 55 and 64 was beneficial in reducing CRC incidence by 23%-33% and reducing mortality by 31%-43%^[102,103]. Similar mortality reduction has been reproduced in other screening trials such as Norwegian Colorectal Cancer Prevention (NORCCAP)^[104]. A recent meta-analysis similarly confirmed benefits of screening (endoscopy or stool-based screening) over an unscreened population in increasing detection and prognosis^[105].

There are intuitive benefits of screening with serum anti-*p53* auto-antibody compared to CT, barium enemas and colonoscopy. The titre is not subject to tumour sampling error, is quicker, cheaper, easier and less traumatic, thus making it more repeatable in the general population. The auto-antibody titre itself is remarkably stable, showing no significant change when stored at room temperature for up to 7 d, or when stored at -80 °C for 3 years^[81]. Repeated freeze-thaw cycles (up to 10 cycles) have minimal or no effect on serum levels as immunoglobulins are generally robust proteins^[82]. Also, anti-*p53* auto-antibody appears to be independent of other conventional CRC tumour markers such as carcino-embryonic antigen (CEA) which means it could detect CRC in CEA-negative patients. The combined advantages of serum testing and the characteristics of anti-*p53* auto-antibody (serum stability, 95%-100% specificity, independent of current tumour markers), makes anti-*p53* auto-antibody a potentially valuable screening modality.

The role of *p53* in screening is promising its specificity for cancer, but this enthusiasm is tempered by a low sensitivity (20%-30%). It would thus be required as part of a panel of tumour markers. This panel could then be used to guide more invasive investigations such as colonoscopy. Combined serum immuno-testing for 6 markers (CEA, anti-*p53* auto-antibody, CYFRA 21-1, osteopontin, separase and ferritin) has been reported to have comparable sensitivity (> 80%) to faecal immuno-testing^[106]. A similar tumour marker panel using CYFRA-21, CEA and anti-*p53* has been used in lung cancer also with 80%

sensitivity^[107]. Combined biomolecular and endoscopic strategies^[108] are being investigated, and in conjunction with other new diagnostic non-invasive modalities (*e.g.*, CT-colonography)^[109] may be able to further broaden the screening programmes for CRC and other cancers in the general population.

Anti-*p53* auto-antibody and clinico-pathological parameters of CRC

p53 mutation is usually a late event in the adenoma-carcinoma sequence and hence anti-*p53* auto-antibody is unlikely to be present in early pre-invasive lesions where *p53* mutations have not occurred^[110]. The largest study reports 1% (2/211) sero-positivity in adenomas which increased to 6% in carcinoma *in-situ*^[89]. This 1% could be due to undetected microfoci of invasive cancer within adenoma or changes that predate microscopic detection. The increase prevalence of anti-*p53* auto-antibody to 6% in carcinoma *in situ* can be expected in these tumours which are at the end of the adenoma-carcinoma sequence with greater proportion of *p53* mutation. This would then suggest that anti-*p53* auto-antibody should increase with further growth (CRC stage) but this is not seen. Almost all studies reported no association between anti-*p53* auto-antibody and CRC stage (Tables 1 and 2). This was reported in the largest cross-sectional series, and confirmed by other long-term follow-up studies^[89] (Table 2). Only a handful of studies have suggested an association between anti-*p53* and T-stage^[88,111], selected nodal disease^[89] and metastases^[112].

Tumour depth

Two studies reported increased anti-*p53* in progression from Dukes' A to B, but not with progression from Dukes' B to C^[88,111]. Lechpammer *et al*^[88] reported 0% (0/28) anti-*p53* in Dukes' A which increased significantly to 9.6% (21/87) in Dukes' B, but did not increase further with Dukes C (8.6%, 19/105). Bielicki *et al*^[111] similarly reported increased anti-*p53* auto-antibody from Dukes' A (0%) to Dukes' B (28% Dukes' B1, 22% Dukes' B2); but no increase in progression to Dukes C (19%). This suggests auto-antibody production is stimulated by early (Tis to T2) local invasion such as microvascular basement membrane invasion leading to antigen presentation; but not further progression. Further studies are required to

Table 3 Prevalence of anti-*p53* auto-antibody and carcino-embryonic antigen in studies reporting the presence of both tumour markers in colorectal cancer *n* (%)

	CEA	Anti- <i>p53</i>
Tang <i>et al</i> ^[89]	408/943 (43.3)	130/998 (13.0)
Shibata <i>et al</i> ^[96]	23/47 (48.9)	32/47 (68.0)
Bielicki <i>et al</i> ^[111]	46/148 (31.1)	29/148 (19.6)
Hammel <i>et al</i> ^[175]	20/54 (37.0)	14/54 (25.9)
Overall	497/1192 (41.7)	204/1247 (16.4)

CEA: Carcino-embryonic antigen.

understand the precise series of events in anti-*p53* auto-antibody production.

Nodal involvement

Anti-*p53* auto-antibody is produced in part due to response to *p53*-antigen presentation. Thus nodal involvement should also increase anti-*p53* auto-antibody production by increasing probabilities of antigen presentation to the humoral system. However, there is no correlation between anti-*p53* auto-antibody and nodal involvement in any of the studies (Table 2). Tang *et al*^[89] suggested increased anti-*p53* with “advanced” nodal disease (N3: > 10 regional nodes or systemic nodal metastases) compared to N0-2 CRC in selected analysis. We re-classified the data into node “positive” and “negative” disease and found no difference in sero-positivity of 12% *vs* 14% with nodal involvement (calculation not shown).

Metastases

It would be expected that haematogenous cancer cell dissemination should invoke a further immune response but there has been no association between anti-*p53* auto-antibody status and metastatic disease except one study^[112]. In this study, anti-*p53* auto-antibody had extremely high prevalence (56%, 14/25) in Stage IV disease, and unusually low prevalence in Stage I-III (9%, 4/45) leading to strong anti-*p53* bias towards Stage IV disease. This is the only study to report anti-*p53* auto-antibody association with stage and adverse prognosis which is discussed later (anti-*p53* auto-antibody in prognosis in CRC).

Summary of anti-*p53* auto-antibody and CRC Stage

Anti-*p53* auto-antibody production is initially most likely to be produced in the final stages the adenoma-carcinoma sequence (in keeping with *p53* mutation being a relatively late event. It is likely that anti-*p53* auto-antibody production is no longer dependant on antigen-presentation, but rather now dependant on immune-recognition by (1) tumour factors *e.g.*, *p53* mutation type and conformation, presence of co-factors; and (2) patients’ immune-specific factors such as MHC expression required for recognition. This response is not sufficiently consistent to justify a separate clinico-pathological parameter of its own. In the future, anti-*p53* auto-antibody may have some benefit in refining CRC stage if there is influence on prognosis or treatment, similar to k-RAS status in anti-EGFR and

anti-VEGF therapy for CRC and liver metastases, or oestrogen- or progesterone-status in breast cancer.

Anti-*p53* auto-antibody and carcino-embryonic antigen

CEA is the most common serum tumour marker used in CRC. It is a 180 kDa serum glycoprotein which is present at low levels in normal cells but over-expressed in adenocarcinoma, especially of the colon, rectum, breast and lung^[113]. Pre-operative CEA presence has been associated with aggressive CRC and poor prognosis^[114,115]. CEA has also been used as an adjunct in CRC screening, monitoring for disease recurrence following resection, or as part of tumour marker panel for metastases of unknown primary origin. CEA has high specificity (80%) with false elevations in smokers, inflammatory diseases, cirrhosis, obstructive jaundice, gastric ulcers, emphysema, diabetes and collagen vascular diseases^[116-118].

CEA in isolation is not recommended for screening or detection of recurrence due to its variable sensitivity (30%-80%)^[114,119]. CEA sensitivity can be modulated by changing the cut-off values for “positivity” but sensitivity has still remained low despite variations in the cut-off value used^[120,121]. Despite this, The American Society of Surgical Oncology (ASCO) guidelines suggest serial CEA measurements every 3 mo in Stage II/III CRC for at least 3 years following diagnosis, and during treatment of metastatic disease^[122].

Tumour markers used in conjunction with CEA could increase the efficacy of CRC screening in selected populations. Such tumour markers should be independent of CEA as to detect the CEA-negative CRC population and thus increase sensitivity of the tumour marker panel. The majority of studies have shown that anti-*p53* auto-antibody is independent of CEA (Table 3). The two studies which report a positive correlation had results inconsistent with other studies, with the first study having an unusually strong association between anti-*p53* auto-antibody and Stage IV disease (as discussed earlier)^[112] and the second reporting the highest anti-*p53* auto-antibody frequency (68%) and used WB, not ELISA^[96]. Methodological difference and sample bias are most likely responsible for the results observed.

In this review, information is compiled from all studies reporting CEA and anti-*p53* in Table 4. This shows that when used in isolation, anti-*p53* auto-antibody can detect CRC in 17% and 42% respectively. If both tumour markers are used, the sensitivity increases to 51% (as both markers are absent in 48.9%). This results in information/sensitivity gain of +9% (compared with CEA alone); and +34% (compared with anti-*p53* auto-antibody alone). The only other study to report “information gain” using anti-*p53* auto-antibody in CRC confirmed reported mean increased sensitivity of 16% with individual increased sensitivity from 55% to 71% in colon cancer and 78% to 83% in rectal cancer^[123]. This report is consistent with our calculation using data from all other published anti-*p53* auto-antibody and CEA studies in CRC (Tables 3 and 4).

Table 4 Combined carcino-embryonic antigen and anti-*p53* auto-antibody rates from all studies reporting the presence of both markers (*n* = 1192) *n* (%)

	CEA normal	CEA elevated
Anti- <i>p53</i> ab present	112 (9.4)	90 (7.6)
Anti- <i>p53</i> ab absent	584 (48.9)	406 (34.1)

CEA: Carcino-embryonic antigen.

The clinical utility of this “information gain” requires examination. CRC has low prevalence in the general population and thus pick-up rates would remain low despite the use of both tumour markers. Both markers also have preponderance towards later stage CRC (as opposed to Stage I) which reduces impact of earlier detection and thus screening efficacy. It is thus likely that these 2 markers alone are insufficient and additional markers would be required, i.e. panel of 6 tumour markers was used to form a panel with sensitivity similar to faecal occult testing in population screening^[108]. In post-operative surveillance, small studies have demonstrated overall 4 tumour markers (CEA, TPA, CA19-9, CA72.4) panel sensitivity of 81% compared with 9%-45% using individual markers^[124]. Hence, the optimal strategy would be to use other markers in addition anti-*p53* and CEA to select patients for investigations. The cost-effectiveness of these immunological-targeted strategies for general population screening, high risk population screening or post-operative surveillance requires further evaluation.

Anti-*p53* Auto-antibody and monitoring for recurrence or metastases

Anti-*p53* auto-antibody may have its most promising role in post-operative monitoring for disease recurrence or distant metastases. Several, but not all, studies have demonstrated that anti-*p53* reflects tumour load, with increasing serum titres corresponding with disease recurrence/progression and decreased titres following surgery/chemotherapy^[81,88,120]. Lechpammer *et al.*^[88] produced the most convincing series demonstrating clear decreases with post-surgery and during chemotherapy. More importantly, increases, especially during chemotherapy, predated clinical diagnosis of recurrence. Smaller subset analysis in other studies has also demonstrated fluctuations in serum titres with disease load. Similar fluctuations with resection and radiotherapy have been reported in oral, oesophageal, lung, ovarian and breast cancer^[125-127].

In almost all cases, the anti-*p53* auto-antibody persists but at a much lower level. Only one study has reported complete absence of the anti-*p53* auto-antibody in a series of patients with superficial (mucosal and submucosal) CRC treated with endoscopic resection^[98]. This may be because the early stage CRC had a smaller mutant *p53* load which may not have adequately stimulated the humoral system to produce a prolonged immune response following CRC removal. The other studies had more advanced CRC where there would have been prolonged antigen exposure to the humoral system^[81,88,98,128].

This cost efficacy of serial anti-*p53* auto-antibody for surveillance must be considered in the light of only 20%-30% prevalence at presentation and subsequent sero-conversion (Table 5). Assuming 1% future sero-conversion and 3-monthly serum measurements for 3 years as per ASCO recommendations, this would result in 20-30 initial positives at diagnosis; and an additional 1 positive over the subsequent 3 years. This results in an initial yield 20-30 positives followed by only 1 positive out of 960 samples over next 3 years (remaining 80 patients × 4 samples per year × 3 years). We then consider this 1 positive sero-conversions out of 960 samples may not alter treatment as serum measurements may predate clinical evidence of disease, and treatment cannot be offered based anti-*p53* auto-antibody titres alone.

An alternative more cost-effective strategy of screening would be to screen all patients for anti-*p53* auto-antibody at diagnosis with further serial measurements only in patients sero-positive at diagnosis. Post-operative patients with rising titres could be selected for expedited investigations and thus increase diagnostic yield, compared to current blanket strategy of routine investigations for surveillance at fixed time intervals. Preliminary studies in small groups using tumour marker panel (CEA, TPA, CA19-9 and CA72.4) demonstrated 81% sensitivity for recurrence with mean lead times of 5.3 mo prior to radiological confirmation of recurrence^[124]. A cost efficacy study would be required to ascertain the ability of anti-*p53* auto-antibody as part of a tumour marker panel to guide post-operative surveillance, improve resource allocation and prolong survival.

Anti-*p53* auto-antibody and prognosis in CRC

p53 mutations have been associated with poor prognosis, possibly in part due to chemo-resistance against *p53*-dependant chemotherapy (e.g., 5-fluorouracil) but reports of its prognostic significance are inconsistent^[90,129]. As the anti-*p53* auto-antibody response has been associated with *p53* mutations and serum testing is easier than DNA sequencing, studies have focused on using anti-*p53* auto-antibody to predict prognosis. The majority of studies report that anti-*p53* auto-antibody response has no independent prognostic value. This was confirmed in the study with the longest follow-up which reported CRC stage, but not anti-*p53* auto-antibody, to be an independent prognostic marker in multivariate analysis^[130], and also by the study with the second longest follow-up but larger sample size^[85] (Table 6).

Four studies report an adverse prognostic significance but in 3 of these, the prognostic significance was in selective univariate analysis where anti-*p53* was associated with advanced stage, and prognostic significance was lost when stage was incorporated in multivariate analysis^[112,131,132]. The fourth, and only study, to report anti-*p53* auto-antibody as an independent prognostic indicator in multivariate analysis strongly associated anti-*p53* auto-antibody with Dukes' D to an extent that median survival of anti-*p53* positive patients was extremely low (20 mo) compared to other studies reporting median survival up

Table 5 Anti-*p53* auto-antibody and sero-conversion in colorectal cancer

Ref.	Patients, method	Follow-up	Findings
Müller <i>et al</i> ^[123]	303 patients, 197 colon, 46 rectal	Median 6 mo	All cancers: 3.6% (11/303) sero(-)→(+); 3.6% (11/303) sero(+)->(-); Total 7.2% (22/303) sero-conversion. Colon cancer: 3% (4/137) sero(-)→(+); 3.6% (5/137) sero(+)->(-); Total 6.6% (9/137) sero-conversion. Rectal cancer: 6.5% (2/31) sero(-)→(+); 3.2% (2/31) sero(+)->(-); Total 12.9% (4/31) sero-conversion
Lechpammer <i>et al</i> ^[88]	Immunoblot 32, ELISA (Oncogene, Research Products, Cambridge, United States)	Up to 20 wk; 8 patients-48 wk	Non-significant decrease at 4 wk (pre-first cycle chemo) and significant decrease at 12 wk post-surgery Significant decreases during chemotherapy and 2 patients with anti- <i>p53</i> increase at 12 wk (during chemotherapy) developed recurrence 8 patients with extended follow-up: 7/8 had decreased anti- <i>p53</i> with no recurrence. 1/8 anti- <i>p53</i> decrease post-surgery/chemotherapy but increased at 12 wk corresponding with liver metastases. Anti- <i>p53</i> fluctuates in response to tumour load but does not disappear. Anti- <i>p53</i> levels reflects tumour load even during chemotherapy
Takeda <i>et al</i> ^[174]	30 CUR A, 5 CUR B, 5 CUR C, anti- <i>p53</i> EIA, Pharmacell	Median 26 mo (13-144)	CUR A (<i>n</i> = 30): 28/30 sero(+)->(-) in 6 mo; 2 no sero-conversion: 1 recurrence CUR B (<i>n</i> = 5): 2 sero(+)->(-) no recurrence. 3 no sero-conversion, 2 had metastases CUR C: No sero-conversion Correlation between post-operative negative conversion and operative curability
Takeda <i>et al</i> ^[98]	17 mucosal/submucosal, ELISA (anti- <i>p53</i> EIA, Pharmacell, France)	Up to 2 years	94%, 16/17 sero(+)->(-) within 3 wk post-surgery No recurrences as early stage tumours and hence not able to comment on anti- <i>p53</i> and recurrence rates
Polge <i>et al</i> ^[128]	10, ELISA (Dianova, Hamburg, Germany)	Up to 6 mo	8 followed-up: 5/8 remained sero(+) post-operatively. All developed metastases 3/8 decreased anti- <i>p53</i> titres. No metastases or recurrence. Anti- <i>p53</i> titres decreased within 1 mo of surgery/chemotherapy but no sero-conversion to anti- <i>p53</i> (-)
Angelopoulou <i>et al</i> ^[81]	6, "In house" immunofluorometric assay	Up to 17 mo	Anti <i>p53</i> decreases with surgery/chemotherapy but persists at low levels Anti- <i>p53</i> increases with recurrence Anti- <i>p53</i> reflects tumour load more sensitively than CEA (<i>n</i> = 5) and in non-CEA producing tumour (<i>n</i> = 1)
Hammel <i>et al</i> ^[175]	12, "In house" ELISA	Up to 20 mo	Anti- <i>p53</i> in 5/8 patients decrease by > 25% within 1 mo. At 1 year, 3 with normal anti- <i>p53</i> levels and 3 with substantial decrease in anti- <i>p53</i> remain disease-free 2 patients with post-operative increased anti- <i>p53</i> : 1 developed recurrence and 1 developed metastases Anti- <i>p53</i> decreased again following surgery in both patients. CEA and CA19-9 were normal in both cases

Sero(-): Sero-negative; Sero(+): Sero-positive; CUR A: No residual tumour macroscopically; CUR B: No residual tumour but not as evaluable as CUR A; CUR C: Definite residual tumour; CEA: Carcino-embryonic antigen.

to 60 mo and 5-year survival > 50%^[89,123,130]. Remarkably, anti-*p53* auto-antibody prognostic significance was even weaker than CA19-9, a pancreatic tumour marker considered unsuitable for pancreatic cancer screening by ASCO^[112,122]. The results of this study are hard to credit. As such, anti-*p53* auto-antibody has no independent prognostic value.

CONCLUSION

The anti-*p53* auto-antibody response is the end-point of a complex multi-factorial humoral response to the accumulation of *p53* protein which is a product mainly of *p53* gene mutation, but also mutation of *p53* regulators and non-mutative pathways. Anti-*p53* auto-antibody has

low (13%-32%) sensitivity in CRC but is nearly 100% specific for malignancy. The auto-antibody frequency may increase with early local invasion or late nodal progression but is not sufficiently consistent to form a separate stage classification. There may be a promising future role of anti-*p53* auto-antibody in screening and monitoring for disease recurrence. The characteristics of the immunoglobulin and the benefits of serum testing provide a promising role in guiding the radiological and endoscopic screening of high risk populations in conjunction with other current tumour markers. The most promising future focus of anti-*p53* auto-antibody lies in being part of a bio-molecular panel of tumour markers to guide endoscopic and radiological screening in general population and high-risk population screening; and in post-operative

Table 6 Anti-p53 auto-antibody and prognosis in colorectal cancer

Ref.	n (%)	Follow-up	Findings
Suppiah <i>et al</i> ^[130]	20/92 (21.7)	Median 97 mo	No difference in overall survival (62 mo <i>vs</i> 60 mo) or disease-free survival (73 mo <i>vs</i> 82 mo)
Müller <i>et al</i> ^[123]	70/243 (28.8)	5-year trial protocol	No survival difference with anti-p53 in CRC and other cancers. Trend towards decreased survival in anti-p53 positive patients with HCC and breast carcinoma
Tang <i>et al</i> ^[89]	130/998 (13)	Recruitment 1995-2000	Anti-p53 associated with decreased survival in univariate analysis but not multivariate analysis. Anti-p53 associated with advanced nodal disease (Stage N2→N3) and metastases (M1)
Chang <i>et al</i> ^[85]	147/167 (28)	Median 36.3 mo (22-85)	p53 mutation associated with poor differentiation and advanced stage. Multivariate analysis shows p53 mutation most significant survival predictor, followed by CRC stage. No prognostic significance of p53 protein expression or anti-p53
Shiota <i>et al</i> ^[112]	18/71 (25)	Not stated	Anti-p53 associated with shorter overall survival (20 mo <i>vs</i> 56 mo) but highly significant association with metastases (M1). Cox regression showed prognostic significance with liver metastases, TNM stage, Dukes stage, Ca19-9 and anti-p53 (in that order)
Kressner <i>et al</i> ^[131]	59/184 (32.1)	Median 6 years	Anti-p53 associated with decreased survival in univariate, but not multivariate analysis. Anti-p53 is independent prognostic indicator in Dukes' A-C with curative surgery (<i>i.e.</i> , when metastases excluded)
Houbiers <i>et al</i> ^[132]	65/255 (25.5)	36 mo	Anti-p53 associated with reduced overall (75% <i>vs</i> 88%) and disease-free survival (56% <i>vs</i> 64%) at 3 years in subgroup analysis of Dukes' A and B1. No difference in overall survival (61% <i>vs</i> 68%) or disease-free survival (51% <i>vs</i> 58%) when all stages included

CRC: Colorectal cancer; HCC: Hepatocellular carcinoma.

cancer surveillance to guide earlier detection of cancer and cancer-recurrence; and finally with more significant impact on cost-efficacy and survival.

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Update on small intestinal stem cells

Valentina Tesori, Maria Ausiliatrice Puglisi, Wanda Lattanzi, Giovanni Battista Gasbarrini, Antonio Gasbarrini

Valentina Tesori, Department of Internal Medicine and Gastroenterology, Università Cattolica del Sacro Cuore, Rome, Largo A. Gemelli 8, 00197 Rome, Italy

Valentina Tesori, Maria Ausiliatrice Puglisi, Antonio Gasbarrini, Department of Internal Medicine, Gemelli Hospital, 00168 Rome, Italy

Wanda Lattanzi, Institute of Anatomy and Cell Biology, Università Cattolica del Sacro Cuore, 00168 Rome, Italy

Giovanni Battista Gasbarrini, Medical Research Foundation ONLUS, Galleria Falcone Borsellino 2, 40123 Bologna, Italy

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Correspondence to: Valentina Tesori, PhD, Department of Internal Medicine, Università Cattolica del Sacro Cuore, Rome, Largo A. Gemelli 8, 00197 Rome, Italy. valentina.tesori@libero.it

Telephone: +39-6-30156018 Fax: +39-6-30157249

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Abstract

Among somatic stem cells, those residing in the intestine represent a fascinating and poorly explored research field. Particularly, somatic stem cells reside in the small intestine at the level of the crypt base, in a constant balance between self-renewal and differentiation. Aim of the present review is to delve into the mechanisms that regulate the delicate equilibrium through which intestinal stem cells orchestrate intestinal architecture. To this aim, special focus will be addressed to identify the integrating signals from the surrounding niche, supporting a model whereby distinct cell populations facilitate homeostatic vs injury-induced regeneration.

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Key words: Intestinal stem cells; Organoids; Intestinal

regeneration; Lgr5; Niche

Core tip: Among somatic stem cells, those residing in the intestine represent a fascinating and poorly explored research field. Aim of the present review is to delve into the mechanisms that regulate the delicate equilibrium through which intestinal stem cells orchestrate intestinal architecture, integrating signals from the surrounding niche and supports a model whereby distinct cell populations facilitate homeostatic vs injury-induced regeneration.

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INTRODUCTION

In an adult organism, stem cells are characterized by their ability to generate multiple differentiated cell types while maintaining their capacity for long-term self-renewal^[1,2]. These are generally known as “adult” or “somatic” stem cells, including all stem cells residing in adult organs, regardless of the age of the individual. These include mesenchymal stem cells^[3-7], residing in the connective stroma of most organs, and haematopoietic stem cells^[8,9] among the best known and characterized, that are already being tested in clinical trials^[10-14].

The amazing renewal capacity of the intestinal epithelium^[1] has made this organ an attractive site to study stem-cell regulation. The intestinal tract is anatomically subdivided into the small intestine and large intestine. The inner mucosal surface, composed by an absorptive and secretory epithelium, is folded into repeated units comprising finger-like invaginations (called crypts of Lieberkühn) associated with numerous protrusions (villi), which increase the surface area, allowing efficient absorption of nutrients from the bowel lumen^[2].

In normal homeostasis, the specialized differentiated cell types that orchestrate the uptake of nutrients into the body, are routinely and rapidly turned over. In fact, the intestinal epithelium is the most rapidly self-renewing tissue in the human body, with a 3-5 d turnover rate^[2]. It is widely accepted that this complex process is regulated, *via* a highly regulated process of self-renewal, by a population of multipotent stem cells, residing within the bottom of the crypt namely the intestinal stem cells (ISCs)^[15-19].

The number and location of these cells are still debated. Clonal analysis has demonstrated the existence of multiple stem cells in each crypt^[20], with an estimated number in the 4-6 cells per crypt range^[21]. ISCs have the properties of self-renewing and generating rapidly dividing transit-amplifying (TA) daughter cells, *via* asymmetric cell division^[22]. TA cells undergo rapid cell division and migrate upwards into the villus. During their migration, TA cells start differentiating and finally localize at the surface of the villus epithelium as either mature absorptive enterocytes, which represent the main cell type, or mucous secreting goblet cells, or hormone-producing enteroendocrine cells^[22]. Upon completing their life cycle, TA die and are discarded into the lumen^[23,24].

A distinct cell type, the Paneth cell, evades this upward migration program, completing the differentiation at the base of the crypt, where it start producing lysozyme, maintaining the sterile environment of the crypt, and regulating the stem cell compartment^[25-27].

Converging evidence suggests the existence of two distinct populations of intestinal stem cells: one that remains quiescent for a long time and one that actively proliferate^[28]. The actively dividing ISCs provide to the baseline regeneration, whereas quiescent stem cells represent a reserve subpopulation that copes to injuries. These two subpopulations are located in adjacent sites within the crypt and are probably maintained by specific signals from the surrounding niche. Nonetheless, the precise identity of the ISCs is still a matter of debate. Two alternative models are currently proposed in the literature: the label-retaining cells (LRC) + 4 model, which identifies the quiescent stem cells, and the crypt base columnar (CBC) cells model, which identifies the actively cycling stem cells.

According to the LRC+4 model, the ISCs should be located specifically at the +4 position from the bottom of the intestinal crypt region, precisely at the origin of the migratory epithelial cell column^[29]. This prediction was supported by Potten *et al.*^[30], who showed that cells most commonly found in this position, are characteristically label-retaining and extremely sensitive to X- and γ -radiation, two features ascribed to stem cells. Furthermore, the expression of Bmi1, a gene thought to be involved in stem cell maintenance, was shown to be elevated in the +4 cells^[31]. Alternatively, the CBC cell model is based on a series of electron microscopy studies on the crypts of the small intestine, showing slender, immature, cycling cells interspersed between Paneth cells at positions 1-4,

hence termed crypt base columnar cells. To support the hypothesis of CBC as the ISCs, mutagenesis studies demonstrated that 90% of the crypts, that contained a mixed population of mutant cells of different epithelial lineages, also contained mutant CBC cells, indicating the CBC cells as the common source of these different lineages^[32]. Further studies, based on targeted lineage tracing strategies, have definitively identified the CBC as the intestinal stem cells, and have revealed the strategy by which the balance between proliferation and differentiation is maintained^[33]. Taken together, these studies suggested that ISCs operate within a complex and dynamic environment, in which stochastic cell loss is compensated by the proliferation of neighboring stem cells.

INTESTINAL STEM CELLS MARKERS

The crypt stem cells responsible for the renewal capacity of the intestinal epithelium, represent a minority of the whole intestinal population, therefore, their identification is extremely troublesome^[14]. Indeed, until relatively recently, ISCs could be identified only by indirect measurements. The recent discovery of specific ISC markers has allowed their isolation and paved the way towards a clearer understanding of their biology and role in tissue homeostasis, repair, and cancer^[14].

Among the various ISCs markers, the best characterized one is the leucine rich-repeat containing G-protein coupled receptor (Lgr5), a Wnt-target gene that expressed by the cycling crypt base columnar cells, interspersed between Paneth cells^[22]. *Lgr5* encodes an orphan G-protein-coupled receptor, characterized by a large leucine-rich extracellular domain^[34].

Barker and co-workers demonstrated that CBC cells are capable of long-term maintenance and support the epithelium self-renewal, using the lineage tracing technique (*i.e.*, introducing permanent genetic marker into candidate stem cell genes *in situ*, thus allowing the visualization of the modified stem cells and their progeny over time)^[34]. One of the major advantages of *in vivo* lineage tracing, compared to transplantation-based methods, is the lack of a physical manipulation of the candidate stem cell, so that ISCs are studied in their physiological niche^[34]. In particular, the visualization and isolation of putative ISCs was obtained by targeting the *Lgr5/Gpr49* gene locus by knock-in of a dual expression cassette encoding enhanced green fluorescent protein (EGFP) and CreERT2. This resulted in the tamoxifen-induced expression of EGFP in the Lgr5+ fraction, which allowed the *in vivo* lineage tracing when combined with inducible reporter strains such as R26RLacZ^[34]. This study hence showed that Lgr5 expression was confined to CBCs, and that these cells give rise to the variety of epithelial cells present in crypts, proving that CBCs function as ISCs as well^[34].

In addition, it has been demonstrated that Lgr5+ cells form self-renewing epithelial organoids in *ex vivo* culture assays, resembling the *in vivo* structure and composition

of crypt/villus epithelial units. It is also worth noticing that cells expressing low, if any, *Lgr5* were unable to produce organoids^[35]. Gene expression analysis of purified *Lgr5*+ stem cells, indicated that they express additional specific markers, such as *Olfm4* and the Achaete scutellike 2 (*Ascl2*)^[36]. *Ascl2* is a basic helix-loop-helix transcription factor^[19]; its expression in the intestinal epithelium is regulated by the Wnt pathway and is restricted to *Lgr5*+ stem cells. *Ascl2* deletion results in the complete loss of *Lgr5*+ ISC, whereas transgenic *Ascl2* expression induces crypt hyperplasia^[36].

Moreover, several lines of evidence have demonstrated the existence of another putative intestinal stem cells marker, the Polycomb family member *Bmi1*^[31]. The Polycomb family plays a key role in the development and in the regulation of progenitor self-renewal in several tissues, including the nervous system^[37], the retina^[38] and hematopoietic organs^[39]. *In vivo* lineage tracing technique, showed that *Bmi1*+ cells are mainly located at the +4 position in the crypts of the small intestine, contributing to the long-term maintenance of all its epithelial cell types^[31]. In addition, the selective ablation of the *Bmi1*+ population led to a disorganization of the intestinal mucosa, resulting in the absence of the crypts^[31]. Unexpectedly, *Bmi1* transgenic expression was restricted only to a minority of the crypts in the proximal small intestine, while being completely absent in the distal tract^[31]. This could be possibly due to the existence of *Bmi1*-negative ISC populations in other regions of the intestine, or, perhaps, to an inaccurate reporting of endogenous *Bmi1* expression, as a result of the variegated transgene activity, frequently observed in the intestine. Interestingly, microarray analysis showed that sorted *Lgr5*+ cells express *Bmi1*, raising the question whether the two markers really characterize independent stem cells populations. Yan *et al.*^[40] clarified this issue by demonstrating that *Bmi1* and *Lgr5* mark two functionally distinct ISCs *in vivo*. *Lgr5* identifies actively cycling ISCs that are sensitive to Wnt modulation, involved in homeostatic regeneration and markedly ablated by irradiation, *i.e.*, the CBC cells. Conversely, *Bmi1* is expressed by quiescent ISCs insensitive to Wnt modulations, that contribute partly to homeostatic regeneration, and are resistant to radiation injury, namely the LRC stem cells^[40].

Another interesting molecule in the scenario of ISCs putative markers, is the CD133/Prominin 1(Prom1), originally discovered as novel glycoprotein expressed on neural^[41] and hematopoietic stem cells^[42,43]. More recently, CD133 has been described as marker of epithelial stem/progenitor cells in human kidney tubules^[44] and in the prostate^[45]. In the study by Zhu *et al.*^[46], a knock-in allele was used that integrated a CreERT2-IRES-nLacZ cassette at the first ATG codon of Prom1 (Prom1C-L). This allowed demonstrating a wide expression pattern for Prom1 in the colon; on the other hand, the expression in the small intestine, appeared to be restricted to the crypt base, overlapping with the *Lgr5*+ CBC cells^[46]. The Prom1+ cells were self-renewing, multipotent adult

stem cells. By contrast with these findings, Snippert *et al.*^[47] reported that Prom 1 expression occurred in *Lgr5*+ stem cells as well as in their TA progenitors. A possible explanation for this discrepancy may reside in the different sensitivity of detection methods used in the two studies.

The RNA-binding protein Musashi 1 (*Msi1*), a regulator of asymmetric cell division^[48], is also involved in stem cell maintenance^[49,50]. Particularly, in neural stem cells *Msi1* is able to maintain stemness properties through Notch pathway activation^[51]. Independent immunohistochemical and *in situ* hybridization analyses, demonstrated that, *Msi1* is expressed in the CBC cells immediately above the Paneth cells^[52-54].

Moreover, *Msi1* overexpression in the intestine increases both Wnt and Notch pathways, and induces the upregulation of *Lgr5* and *Bmi1*^[55]. Interestingly, although *Msi1* is expressed in putative ISCs, in knockout mice lacking this marker, no defects in the development of the intestine are detected^[56]. Taken together, these observations demonstrated that *Msi1* is not a specific ISCs marker, but is expressed in both ISCs and in their early progeny^[57].

To sum up, different markers point to distinct stem cells within the crypt: the marker *Lgr5* points to the crypt base columnar cells located in between the Paneth cells at the crypt bottom^[22], whereas the markers *Bmi1* identify the +4 position in the crypt, just above the Paneth cells^[31]. The existence and interdependency of these different types of ISCs remain a matter of debate.

STEM CELL NICHE: HOMEOSTASIS AND MORPHOGENESIS SIGNALS

A key role in the dynamics of ISCs is ascribed to the niche, a complex and dynamic setting, that adapts in response to environmental stimuli and provides the cells essential signals, including the morphogenetic pathways, such as Wnt, Notch, bone morphogenetic proteins (BMPs) and Hedgehog^[21,58-63]. The microenvironment of the niche surrounding ISCs features extracellular matrix, neural cells, lymphocytes, macrophages, endothelial cells, fibroblasts, smooth muscle cells, and myofibroblasts, that generate signals able to regulate stem cells properties and behavior^[64-67].

A wide range of evidence indicates that the Wnt pathway has a crucial role in intestinal proliferation and ISC maintenance^[68-74].

The Wnt pathway molecules are evolutionary conserved intracellular signaling molecules which regulate cellular fate in the crypt-villus axis in normal gut epithelium, and are implicated in stem cells self-renewal^[75]. Indeed, loss of Wnt signaling *in vivo* effectively blocks cell proliferation in the intestinal crypts, destroying the epithelium^[76]. Moreover, when the Wnt secretion inhibitor (IWP1) was added to organoids, the LacZ signal derived from *Lgr5*+ cells, was completely lost, and the proliferation was inhibited; this inhibition could be overcome administering exogenous Wnt3A^[76]. Recent evidences have

also demonstrated that Paneth cells residing next to ISCs are crucial for their maintenance and serve as the stem cell niche^[77]. Paneth cells are known to secrete a variety of bactericidal products, such as cryptidins/defensins and lysozyme, epidermal growth factor (EGF), transforming growth factor β (TGF- β), and represent the main sources of Wnt3a. Indeed, the ablation of Paneth cells, decreases the number of ISCs in the crypt^[78] confirming that an active Wnt signal is crucial for ISC maintenance^[79].

A Wnt signaling gradient exists along the crypt-villus axis. When cells migrate away from the Wnt source at the base of the crypt, they progressively lose their proliferative capacity and differentiate. The activity of the Wnt pathway, in conjunction with other pathways such as Notch and bone morphogenetic protein (BMP), is vital for the proper organization of the colic epithelium.

In the small intestine, Notch activity determines lineage differentiation between enterocytes and secretory cell differentiation; indeed, Notch inhibition results in an increase of goblet cells, while its activation results in goblet cells depletion^[78]. Recent data support the idea that Notch promotes proliferation when Wnt activity is high, while induces enterocyte differentiation when Wnt activity decreases at the top of the crypt^[29]. Given that Notch receptors are membrane-bound, it would appear that only the neighboring Paneth cells can maintain active Notch signaling in Lgr5 stem cells.

BMP belongs to a family of ligands which comprises BMP and TGF- β family members, acting through the SMAD intracellular signaling cascade^[80-82].

In the intestine, BMP2 and BMP3, are expressed by mesenchymal cells and are able to arrest proliferation at the crypt-villus edge, rather than promoting differentiation^[29].

In fact, both mice lacking the BMP receptor (Bmpr1a), and mice overexpressing the BMP inhibitor noggin, present hyperproliferation and crypt fission^[80].

Under physiological conditions, the amount of stem cells within the niche remains constant, thus these processes need to be highly regulated, probably through negative feedback mechanisms^[40]. In fact, stem cells may divide: (1) asymmetrically, giving rise to another stem cell, which remains in the niche, and to a daughter cell which form a progenitor cell, that migrates upwards in the crypt and differentiate into a mature element; and (2) symmetrically, giving rise to two daughter stem cells, or two daughter non-stem progenitor cells, the latter phenomenon leading to stem cells exhaustion^[75].

Overall, the current scenario indicates a niche organized into a complex network of morphogenetic signals, each crucial for ISCs and crypt maintenance^[28].

now lends promise to the application of adult stem cell therapy in gastroenterology. The apparently unlimited scale at which these stem cells can be expanded *in vitro* offers particularly exciting therapeutic possibilities^[83-85].

In particular, organoids, derived from *in vitro* expansion of a single adult colonic stem cell, can be used to repair damaged colon tissue.

Indeed, as discussed above, intestinal organoid cultures, with a gut like structure, and containing all epithelial cell types, can be derived from single Lgr5+ sorted stem cells^[86]; the resulting organoids can be expanded efficiently and over long periods of time, without losing tissue identity. To date, protocols for the efficient generation of organoids from stomach, human small bowel and colon have been developed^[87-90].

Interestingly, the growth factors used to supplement the culture medium are the natural growth factors to which the stem cells are exposed *in vivo*, suggesting a high clinical-grade biocompatibility of this approach^[87]. Moreover, no genetic manipulation required, making the entire procedure extremely safe.

As a first step toward the development of stem cell transplantation, it has been shown that, using the colonic organoids culture system, significant amounts of tissue can be grown *in vitro* from a single adult colon stem cell^[91,92].

Colon organoids were reintroduced into superficially damaged recipient colons of immunocompromised (rag2^{-/-}) mice, pretreated with dextran sulphate sodium (DSS), which induces superficial mucosal lesions. The engrafted organoids RFP+, were able to readily integrate into the existing epithelium (RFP-), and generated histologically and functionally normal crypts containing all differentiated cell types, covering the area that lacked epithelium in recipient mice. At 4 wk after transplantation, the donor-derived cells constituted a single-layered epithelium, which formed self-renewing crypts that were functionally and histologically normal. In long term studies, carried out at 25 wk after transplantation, the grafts still contributed to the epithelium without any sign of adenomatous or dysplastic transformation^[91,93].

Moreover, transplanted mice displayed a significant lower weight loss than control mice^[91]. These data showed the feasibility of colon stem-cell therapy based on the *in vitro* expansion of a single adult colonic stem cell; graft rejection can be managed by standard approaches, *i.e.*, by leukocyte antigens matching of donor and acceptor and by immunosuppressive therapy, as currently used for organ transplantation.

Protocols have also been developed to expand human small intestine and colon organoids from small biopsies^[94]. As a first application, Cleavers and his collaborators have transplanted the organoid-derived small intestinal epithelium into the bowel of patients affected by the microvillus inclusion disease^[48]. This is a rare hereditary defect of the enterocyte brush border resulting in insufficient nutrients' assimilation^[95-97], requiring colon transplantation as the unique therapeutic strategy. Intes-

STEM CELLS DERIVED ORGANOID: THERAPEUTIC APPLICATIONS FOR GASTROINTESTINAL DISEASES

Rapid progress in the field of intestinal stem cell biology

tinal organoids technology may allow a novel venue into gene therapy approaches, that involves the introduction of DNA sequences into the genomes of cells of the pertinent patient. The achievement of a safe gene transfer represents the major hurdle, which has largely hampered the introduction of gene therapy into the clinic despite three decades of intensive efforts. On this regard, retrovirus- and lentivirus-mediated gene transfer has already been proven to be feasible in organoids systems^[98,99].

These viral vectors are though associated to documented risk for insertional mutagenesis. As organoids can be grown from single sorted stem cells, one could envisage an approach in which individual stem cells are analyzed after integration of the recombinant DNA sequences. Only stem cells with safe integrations could then be expanded clonally for subsequent transplantation.

Overall, adult stem-cell therapy holds promise for the treatment of gastrointestinal diseases, using tissues “harvested” from a single living donor, overcoming the difficulties of the organ transplantation, that is still limited by the availability of donor.

Clinical application of this protocol still waits the translation of the technical procedures to the good clinical practice standards, to generate the adequate amounts of tissue to treat human subjects, and the development of efficient transplantation approaches.

CONCLUSION

ISCs could be reasonably considered the key players that orchestrate the high-rate regenerative capacity of the intestinal epithelium. The understanding of the interplay between the ISCs and their niche, led by a complex molecular network, will pave the way for the future development ISCs based therapy especially to the application in gastroenterology.

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Hepatic arterial infusion chemotherapy in hepatocellular carcinoma with portal vein tumor thrombosis

Do Seon Song, Si Hyun Bae, Myeong Jun Song, Sung Won Lee, Hee Yeon Kim, Young Joon Lee, Jung Suk Oh, Ho Jong Chun, Hae Gyu Lee, Jong Young Choi, Seung Kew Yoon

Do Seon Song, Si Hyun Bae, Myeong Jun Song, Sung Won Lee, Hee Yeon Kim, Jong Young Choi, Seung Kew Yoon, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul 137-040, South Korea
 Young Joon Lee, Jung Suk Oh, Ho Jong Chun, Hae Gyu Lee, Department of Radiology, College of Medicine, The Catholic University of Korea, Seoul 137-040, South Korea

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Correspondence to: Si Hyun Bae, MD, PhD, Professor, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, #505 Banpo-dong, Seocho-gu, Seoul 137-040, South Korea. baesh@catholic.ac.kr

Telephone: +82-2-22582073 Fax: +82-2-34814025

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The median survival and time to progression were 7 and 2 mo, respectively. After 2 cycles of HAIC, CR was achieved in 1 patient (2%), PR in 10 patients (20%) and SD in 26 patients (52%). Significant pre-treatment prognostic factors were a tumor volume of $< 400 \text{ cm}^3$ ($P = 0.01$) and normal levels of protein induced by vitamin K absence or antagonist (PIVKA)-II ($P = 0.022$). After 2 cycles of treatment, disease control (CR + PR + SD) ($P = 0.001$), PVTT response ($P = 0.003$) and α -fetoprotein reduction of over 50% ($P = 0.02$) were independent factors for survival. Objective response (CR + PR), disease control, PVTT response, and combination therapy during the HAIC were also significant prognostic factors. Adverse events were tolerable and successfully managed.

CONCLUSION: HAIC may be an effective treatment modality for advanced HCC with PVTT in patients with tumors $< 400 \text{ cm}^3$ and good prognostic factors.

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Key words: Hepatocellular carcinoma; Hepatic arterial infusion chemotherapy; Portal vein tumor thrombosis

Abstract

AIM: To evaluate the prognostic factors and efficacy of hepatic arterial infusion chemotherapy in hepatocellular carcinoma with portal vein tumor thrombosis.

METHODS: Fifty hepatocellular carcinoma (HCC) patients with portal vein tumor thrombosis (PVTT) were treated using hepatic arterial infusion chemotherapy (HAIC) *via* a subcutaneously implanted port. The epirubicin-cisplatin-5-fluorouracil (ECF) chemotherapeutic regimen consisted of 35 mg/m^2 epirubicin on day 1, 60 mg/m^2 cisplatin for 2 h on day 2, and 500 mg/m^2 5-fluorouracil for 5 h on days 1-3. The treatments were repeated every 3 or 4 wk.

RESULTS: Three (6%) of the 50 patients achieved a complete response (CR), 13 (26%) showed partial responses (PR), and 22 (44%) had stable disease (SD).

Core tip: The aim of this study was to investigate the prognostic factors of hepatic arterial infusion chemotherapy in advanced hepatocellular carcinoma patients with portal vein tumor thrombosis. The primary findings of this study were as follows: (1) The median survival and time to progression were 7 and 2 mo, respectively; (2) A tumor volume of $< 400 \text{ cm}^3$ and protein induced by vitamin K absence or antagonist-II were independent pre-treatment prognostic factors; (3) Disease control and $\geq 50\%$ tumor marker reduction were significant prognostic factors after the second cycle of hepatic arterial infusion chemotherapy (HAIC); and (4) Objective tumor response, disease control and portal vein tumor thrombosis response were independent post-treatment prognostic factors at the end of the HAIC.

Song DS, Bae SH, Song MJ, Lee SW, Kim HY, Lee YJ, Oh JS, Chun HJ, Lee HG, Choi JY, Yoon SK. Hepatic arterial infusion chemotherapy in hepatocellular carcinoma with portal vein tumor thrombosis. *World J Gastroenterol* 2013; 19(29): 4679-4688 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4679.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4679>

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer globally and the third most common cause of cancer mortality^[1]. Surveillance of high-risk patients facilitates the early diagnosis of HCC^[2]. However, because many patients are diagnosed at intermediate or advanced stages, only 30% of patients benefit from curative therapies such as resection, transplantation, or percutaneous ablation^[3]. For patients with vascular invasion and/or extrahepatic spread, *i.e.*, whose tumors were classified as advanced stage according to the Barcelona Clinic Liver Cancer (BCLC) staging system, the multi-kinase inhibitor sorafenib is recommended^[4]. In recent randomized controlled trials, sorafenib significantly increased patient survival^[5,6]. However, contrary to our expectations, the survival and therapeutic advantages of sorafenib are modest, and the current cost of the drug precludes sorafenib from becoming a more generalized treatment tool for advanced HCC^[7]. Systemic chemotherapy also has limited utility in treating HCC due to frequent toxicity and is not associated with improved survival^[8,9]. Therefore, alternatives to sorafenib and systemic chemotherapy are often required for the treatment of advanced HCC, and hepatic arterial infusion chemotherapy (HAIC) could be an alternative modality.

HAIC using an implantable port system is theoretically more effective against HCC than systemic chemotherapy. HAIC enables anti-cancer agents to be delivered locally at high concentrations to hypervascular tumors, thereby keeping systemic concentrations of chemotherapeutic agents low due to the first-pass effect^[10]. Many studies using HAIC have reported that it is a useful modality for patients with advanced HCC^[10-14]. However, there are limited data defining the clinical factors predicting its efficacy. In this study, we investigated the efficacy and predictive factors of HAIC in patients with advanced HCC with portal vein tumor thrombus (PVTT) using the HAIC regimen, which was composed of epirubicin, cisplatin, and 5-fluorouracil.

MATERIALS AND METHODS

Patients

Between March 2009 and January 2012, 68 consecutive patients with advanced HCC underwent HAIC *via* an implantable port system with epirubicin, cisplatin and 5-fluorouracil (5-FU) in Seoul St. Mary Hospital, Seoul, South Korea. The patients were refractory to previous

treatments or not amenable to surgery or locoregional therapies such as ethanol injection, radiofrequency ablation, or transcatheter arterial chemoembolization due to metastasis or PVTT. HCC was diagnosed either histologically or using typical radiologic findings of HCC on two dynamic imaging examinations or one dynamic technique with an elevated serum α -fetoprotein (AFP) level ≥ 200 ng/mL^[15,16]. Among these 68 patients, 50 patients who had PVTT and received more than two cycles of HAIC were enrolled in this study. All tumor thromboses were radiologically confirmed in the main trunk or in the first or second branch of the portal vein. Additional inclusion criteria were a white blood cell count ≥ 3000 cells/mm³ or an absolute neutrophil count ≥ 1000 cells/mm³ and a platelet count ≥ 50000 cells/mm³. Other eligibility criteria included the following: ages 18-75 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and a Child-Pugh score ≤ 7 . Patients with extrahepatic metastasis were also included in this study because extrahepatic metastasis is common in HCC patients with a large tumor and PVTT due to high AFP levels and large tumor volumes. Exclusion criteria included another concurrent malignancy or other underlying serious medical condition such as renal or cardiopulmonary insufficiency. HCC was staged using the BCLC, modified Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) staging systems. Tumor volume was measured by a single radiologist using commercially available imaging software (Pinnacle³ with AcQSim³ v. 8.0, Philips, Fitchburg, WI, United States) and the summation-of-areas technique with a 5-mm reconstruction thickness.

Implantation of the arterial port system

After skin preparation and local anesthetic injection, the right common femoral artery was punctured using the Seldinger technique. The superior mesenteric and celiac arteries were selected under fluoroscopic guidance. After the selective angiographies were performed, the right gastric and gastroduodenal arteries were embolized with multiple microcoils to prevent reflux of the cytotoxic drug to the stomach and duodenum. After performing a follow-up celiac arteriography, a catheter was inserted and localized to the proper hepatic artery. The skin and right inguinal region was incised, and the subcutaneous pocket was prepared *via* dissection. The peripheral end of the catheter was connected to the infusion port, and the port device was implanted in a subcutaneous pocket in the right or left iliac fossa. To prevent the occlusion of the catheter, 10 mL of saline mixed with 10000 units of heparin were locked into the port after each cycle of chemotherapy. Hepatic angiography *via* the port system was performed every two cycles of treatment.

Chemotherapeutic regimen and additional therapy

The Epirubicin-Cisplatin-5-fluorouracil (ECF) chemotherapeutic regimen included 35 mg/m² epirubicin on day 1, 60 mg/m² cisplatin for 2 h on day 2, and 500 mg/m² 5-FU for 5 h on days 1-3. Intravenous hydration was

performed prior to cisplatin infusion to prevent nephrotoxicity, and all patients were given prophylactic anti-emetic treatment comprised of 5-hydroxytryptamine-3 antagonists. The treatment cycles were repeated every 3 or 4 wk until disease progression, unacceptable toxicity, or patient refusal to continue. The doses of chemotherapeutic agents or treatment intervals were adjusted at every treatment cycle depending on hepatic dysfunction or significant toxicity. The dose of subsequent treatment was reduced by 25% when repeated grade 2 or grade 3/4 toxicity occurred during the preceding cycle^[14].

During or after the HAIC treatment, additional therapies were performed as necessary, depending on the tumor responses to HAIC, performance status, and hepatic function. Additional treatment included targeted therapy with sorafenib, external radiation therapy, transarterial chemolipiodolization (TACL), systemic chemotherapy, local therapies such as radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI), or surgical treatment.

Study assessment

The primary endpoints were an objective response rate [complete response (CR) + partial response (PR)] and disease control rate [objective response rate + stable disease (SD)]. Response evaluations were performed after two cycles and at the end of the HAIC treatment. The overall survival (OS) and time to progression (TTP) were evaluated secondarily. The treatment response was classified according to the modified Response Evaluation Criteria In Solid Tumors (mRECIST). The pretreatment evaluation included medical history, physical examination, laboratory tests [complete blood count, blood chemistry, virologic marker, serum AFP and proteins induced by vitamin K absence or antagonist (PIVKA)-II], and imaging studies such as a computed tomography (CT) scan, magnetic resonance imaging (MRI) or positron emission tomography (PET) scan. During the treatment, toxicity assessment, laboratory tests, as well as chest and abdominal X-rays were repeated prior to each treatment cycle. CT scans were performed every two cycles or as needed to evaluate the tumor response or to confirm the disease progression. OS was defined as the time from the first treatment to death or the last follow-up visit, and TTP was the time from the first treatment to the radiologic progression. The patient's liver function was classified according to the scheme of Child-Pugh. AFP and PIVKA-II reduction were calculated according to the formula [(baseline level - level after the second cycle)/(baseline level) × 100] in patients whose AFP was elevated above 20 ng/mL^[17] and PIVKA-II was elevated above 40 mAU/mL^[18]. The treatment toxicity was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) v. 4.0^[19]. The PVTT response was evaluated using dynamic imaging. Response was defined as complete disappearance or at least a 30% decrease in the diameter of PVTT, and non-response was defined as any case that did not qualify for response.

Statistical analysis

The Kaplan-Meier methods and log-rank tests were used in the analysis of time-to-event variables, and a 95%CI for the median time to event were computed. Cox-proportional hazard regression models were used to determine the hazard ratios (HRs) of pre-treatment and post-treatment prognostic factors for survival. The variables with *P* values < 0.05 at univariate analysis were used as input variables in the multivariate model using the enter methods. In the multivariate analysis of post-treatment prognostic factors, hazard ratios were adjusted for the significant variables in the multivariate analysis of pre-treatment variables. For each covariate, the proportional hazard assumption was verified using a log minus log survival plot, and Cox-Snell residuals were used to evaluate the fit of the model. A plot of the estimated cumulative hazard rate versus Cox-Snell residuals followed a 45° line.

The χ^2 -test or Fisher's exact test were used for the analysis of clinical characteristics and prognostic factors between the disease control group (CR + PR + SD) and the disease progression group [progressive disease (PD)]. Statistical significance was defined as a *P* value < 0.05. All data were analyzed using the SPSS v. 14.0 software (SPSS, Chicago, IL, United States).

RESULTS

Patients characteristics

The characteristics of the 50 study patients are shown in Table 1. The median age was 54 years (range, 37-74), and 78% of the patients were male. The most common etiology of underlying liver disease was chronic hepatitis B (78%), and 84% had a Child-Pugh classification of A. All patients were BCLC stage C due to PVTT and extrahepatic metastasis, and 30 patients (60%) had main PVTT (Vp4). Sixteen patients (32%) had extrahepatic metastasis at the initiation of HAIC. Twenty-four patients (48%) received previous treatment, and the most common previous treatment was TACL.

Treatment efficacy

The patients received a total of 289 cycles of HAIC with a median of five cycles (range 2-25 cycles). The response rates are shown in Table 2. After two cycles of HAIC, 11 patients (22%) showed an objective response, and 37 patients (74%) achieved successful disease control. Based on the best response during HAIC, the objective response rate was 32% and disease control rate was 76%.

In total, the median OS was 7 mo (95%CI: 5.5-8.5) and TTP was 2 mo (95%CI: 1.3-2.7), as shown in Figure 1. We assessed the OS according to the presence of objective response and disease control (Figure 2). The treatment responses were evaluated after the second cycle of HAIC and at the end of the HAIC treatment. Based on the best response during HAIC, the median OS was 24 mo (95%CI: 12.9-35.1) in the objective responder group, 5 mo (95%CI: 3.6-6.4) in the non-responder group (*P* < 0.001), 8 mo (95%CI: 2.6-13.4) in the disease control

Table 1 Baseline patient characteristics

Patient characteristics	Statistic
Age (yr)	54 (37-74)
Gender (male/female)	39/11
Etiology	
HBV/HCV/non-viral	39/6/5
Child-Pugh classification	
A5/A6/B7	17/25/8
Staging	
BCLC staging C	50
Modified UICC (III/IVa/IVb)	9/31/10
Tumor type	
Nodular/massive/infiltrative	3/4/43
Portal vein thrombosis	
Vp2/Vp3/Vp4	7/13/30
Maximal tumor size (cm)	
< 10/≥ 10	26/24
Tumor volume (cm ³) ¹	492.7 (26.1-2746.6)
Extrahepatic metastases	16
Previous treatment	
TACL/TACL + RFA/TACL + ERT	19/3/2
Total bilirubin (mg/dL) ¹	0.87 (0.34-1.99)
PT (INR) ¹	1.14 (0.94-1.38)
ALT (IU/L) ¹	37 (15-345)
Platelet count (× 10 ³ /mL) ¹	133 (50-326)
AFP (ng/mL) ¹	3084.15 (7.94-426100)
PIVKA-II (mAU/mL) ¹	1190 (16-12000)

¹Expressed as the median (range). AFP: Alpha-fetoprotein; AJCC: American Joint Committee on Cancer; ALT: Alanine aminotransferase; BCLC: Barcelona Clinic Liver Cancer; ERT: External radiation therapy; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PIVKA: Protein induced by vitamin K absence or antagonist; PT: Prothrombin time; RFA: Radiofrequency ablation; TACL: Transarterial chemolipiodolization; UICC: Union for International Cancer Control.

Table 2 Tumor responses to hepatic arterial infusion chemotherapy treatment

	Response after two cycles	Best response during HAIC	Overall response after HAIC	Intra-hepatic tumor response
CR	2%	6%	6%	6%
PR	20%	26%	10%	26%
SD	52%	44%	12%	52%
PD	26%	24%	72%	16%
Objective response	22%	32%	16%	32%
Disease control rate	74%	76%	28%	84%

HAIC: Hepatic arterial infusion chemotherapy; CR: Complete response; PD: Progressive disease; PR: Partial response; SD: Stable disease.

group and 4 mo (95%CI: 2.9-5.1) in the progressive disease group ($P < 0.001$). Based on response after the second cycle of HAIC, the median OS was 17 mo (95%CI: 14.6-19.4) in the responder group and 7 mo (95%CI: 5.0-9.0) in the non-responder group ($P = 0.034$), 11 mo (95%CI: 6.7-15.3) in the disease control group and 5 mo (95%CI: 2.9-5.1) in the progressive disease group ($P < 0.001$).

Prognostic factors of survival

The prognostic factors affecting patient survival were analyzed by examining the pre-treatment and post-

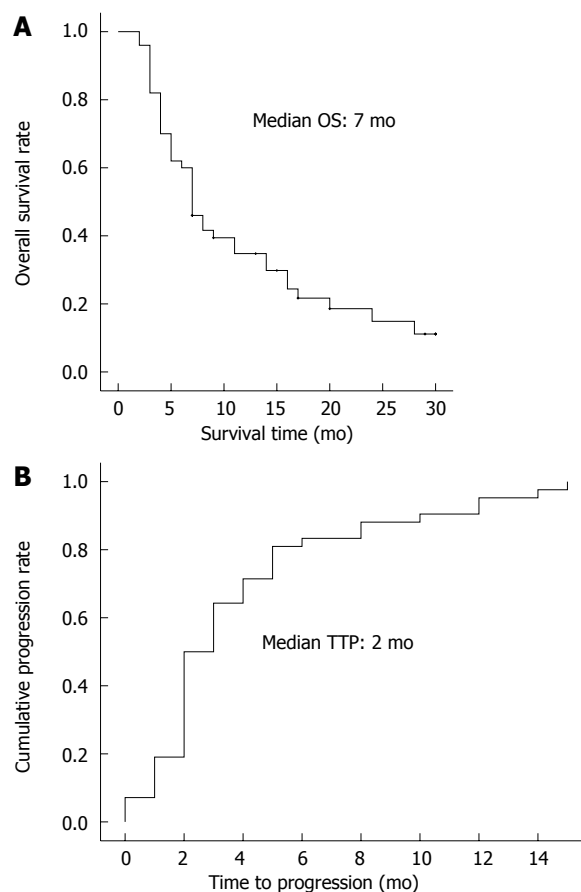


Figure 1 Overall survival rate (A) and time to disease progression (B) of the patients. OS: Overall survival; TTP: Time to disease progression.

treatment parameters shown in Tables 3 and 4. Univariate analysis revealed that four pre-treatment prognostic factors were significantly associated with survival: tumor volume ($< 400 \text{ cm}^3$), Child-Pugh score, pre-treatment PIVKA-II level, and AJCC stage. Based on multivariate analysis, a tumor volume of $< 400 \text{ cm}^3$ [$P = 0.01$, HR = 2.520 (95%CI: 1.252-5.072)] and PIVKA-II [$P = 0.022$, HR = 3.121 (95%CI: 1.177-8.274)] were independent prognostic factors among the pre-treatment parameters. There was no significant difference in overall survival according to the presence of extrahepatic metastasis; the median OS was 8 mo (95%CI: 5.192-10.808) in the patients without extrahepatic metastasis and 5 mo (95%CI: 2.387-7.613) in the patients with extrahepatic metastasis ($P = 0.201$) (Figure 3).

The post-treatment parameters were analyzed after the second cycle and at the end of HAIC. Univariate analysis after the second cycle of HAIC determined that objective response, disease control, portal vein tumor thrombosis response, and $\geq 50\%$ reduction of AFP and PIVKA-II level were significant post-treatment prognostic factors. Multivariate analysis after the second cycle of HAIC determined that disease control [$P = 0.001$, HR = 3.850 (95%CI: 1.768-8.381)], PVT response [$P = 0.003$, HR = 3.398 (95%CI: 1.529-7.552)] and $\geq 50\%$ AFP reduction [$P = 0.02$, HR = 3.031 (95%CI: 1.194-7.691)] were significant predictors for longer survival. At the

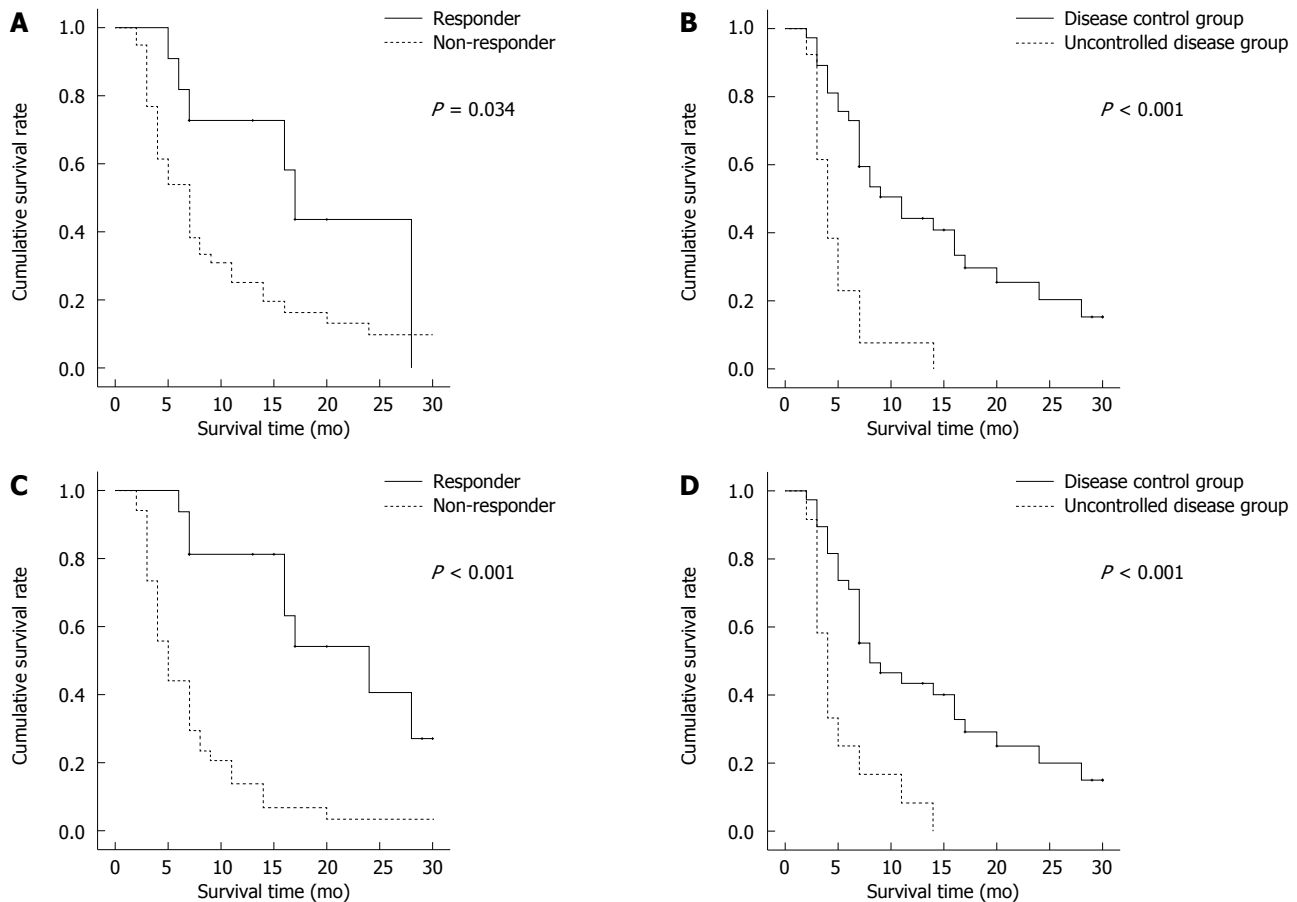


Figure 2 Overall survival of the objective response and disease control groups. A, B: After the second cycle of high-dose hepatic arterial infusion chemotherapy (HAIC); C, D: During HAIC.

Table 3 Pre-treatment prognostic factors for survival in hepatic arterial infusion chemotherapy treatment

Variables	Univariate		Multivariate ¹	
	HR (95%CI)		HR (95%CI)	P-value
Age (< 60/≥ 60 yr)	0.991 (0.514-1.912)			
Gender (male/female)	1.453 (0.687-3.076)			
Maximal tumor size (< 10/≥ 10 cm)	1.693 (0.894-3.204)			
Tumor volume (< 400/≥ 400 cm ³)	2.509 (1.289-4.885)	2.520 (1.252-5.072)	0.01	
Child-Pugh score (5/> 5)	2.099 (1.038-4.244)	1.812 (0.878-3.738)	0.108	
Stage				
mUICC stage (III/IV)	1.198 (0.502-2.863)			
AJCC (III/IV)	2.133 (1.085-4.193)	1.803 (0.895-3.634)	0.099	
Extrahepatic metastases	1.508 (0.772-2.948)			
Pre-HAIC treatment	0.939 (0.503-1.755)			
Portal vein thrombosis (Vp4 vs non-Vp4)	1.603 (0.829-3.100)			
AFP level (< 200/≥ 200 ng/mL)	1.707 (0.845-3.448)			
PIVKA-II (< 40/≥ 40 mAU/mL)	2.860 (1.110-7.368)	3.121 (1.177-8.274)	0.022	

¹Those variables with $P < 0.05$ in univariate analysis were included. AFP: Alpha-fetoprotein; AJCC: American Joint Committee on Cancer; HAIC: Hepatic arterial infusion chemotherapy; PIVKA: Protein induced by vitamin K absence or antagonist; mUICC: Modified Union for International Cancer Control.

end of the HAIC treatment, univariate analysis was performed with combination therapy and factors related to tumor response. Six factors were significant: the overall tumor response (including objective response and disease control), intrahepatic tumor response (including objective response and disease control), PVTT response, and combination therapy. A multivariate analysis at the end of HAIC treatment determined that objective tumor response [$P = 0.001$, HR = 4.445 (95%CI: 1.893-10.439)], disease control [$P = 0.003$, HR = 3.137 (95%CI: 1.494-6.591)], objective intrahepatic tumor response [$P = 0.001$, HR = 4.445 (95%CI: 1.893-10.439)], intrahepatic tumor control [$P = 0.01$, HR = 3.009 (95%CI: 1.302-6.958)], PVTT response [$P = 0.001$, HR = 8.188 (95%CI: 2.403-27.898)], and combination therapy [$P = 0.029$, HR = 2.164 (95%CI: 1.082-4.328)] were independent predictors for longer survival.

Toxicity

The toxicities observed in this study are summarized in Table 5. No treatment-related mortality was detected. The most common toxicities were anemia and aspartate aminotransferase (AST) elevation, and all patients showed a toxicity grade of at least 1. The most common grade 3/4 toxicities were thrombocytopenia and AST elevation (22 patients, 44%). The toxicities were transient, tolerable,

Table 4 Post-treatment prognostic factors for survival in hepatic arterial infusion chemotherapy treatment

Variables		Univariate HR (95%CI)	Multivariate ¹ Adjusted HR (95%CI)	P-value ²
After 2 nd HAIC cycle	Objective response			
	Responder	1		
	Non-responder	2.382 (0.995-5.704)		
	Disease control			
	Control group	1		
	Progressive group	3.708 (1.801-7.634)	3.850 (1.768-8.381)	0.001
	PVTT response			
	Response	1		
	Non-response	2.531 (1.164-5.505)	3.398 (1.529-7.552)	0.003
	AFP reduction			
	≥ 50%	1		
	< 50%	3.242 (1.297-8.102)	3.031 (1.194-7.691)	0.02
Response during HAIC	PIVKA-II reduction			
	≥ 50%	1		
	< 50%	3.164 (1.469-6.818)	2.254 (0.989-5.137)	0.053
	Best tumor response			
	Objective response			
	Responder	1		
	Non-responder	4.747 (2.111-10.672)	4.445 (1.893-10.439)	0.001
	Tumor control			
	Control group	1		
	Progressive group	3.274 (1.594-6.724)	3.137 (1.494-6.591)	0.003
	Intra-hepatic tumor response			
	Objective response			
	Responder	1		
	Non-responder	4.747 (2.111-10.672)	4.445 (1.893-10.439)	0.001
	Tumor control			
	Control group	1		
	Progressive group	3.032 (1.348-6.821)	3.009 (1.302-6.958)	0.01
	PVTT response			
	Response	1		
	Non-response	9.587 (2.879-31.927)	8.188 (2.403-27.898)	0.001
	Combination therapy			
	Yes	1		
	No	2.367 (1.218-4.601)	2.164 (1.082-4.328)	0.029

¹Adjusted for tumor volume and pre-treatment PIVKA-II level; ²P value for adjusted hazard ratio. AFP: Alpha-fetoprotein; HAIC: Hepatic arterial infusion chemotherapy; PIVKA: Protein induced by vitamin K absence or antagonist; PVTT: Portal vein tumor thrombosis.

and successfully managed using a conservative treatment; there were no discontinuation of the treatment due to toxicity. Hepatic arterial thrombosis developed in 4 of the 50 patients. However, thrombolysis by urokinase was effectively performed, and port removal was required in only 1 patient. Overall, 37 (74%) of the 50 patients in the treatment group died during the follow-up. The causes of death are listed in Table 6. The most common cause of death was tumor progression (57%), and six patients (16%) died from deteriorating hepatic function without any evidence of tumor progression, sepsis, or gastrointestinal bleeding. Five patients (14%) died from variceal bleeding, and three (8%) died from infection. Ten patients (20%) were still alive when the final analysis was performed, and three patients (6%) were lost to follow-up.

DISCUSSION

Portal vein tumor invasion is a common complication in HCC, reportedly observed in 64.7% of cases at au-

topsy^[20]. PVTT often leads to extensive spreading of the tumor and can increase portal venous blood pressure, resulting in the fatal rupture of esophageal varices. PVTT can also decrease portal flow that may lead to ascites, jaundice, hepatic encephalopathy, or liver failure. Therefore, the presence of PVTT is one of the most significant prognostic factors of poor prognosis^[21,22], and it has been reported that these patients survive only 2.7-4 mo if left untreated^[22,23]. In advanced HCC patients with PVTT, standard treatments have not been established, especially in the Asia-Pacific region. Though the BCLC staging system recommends sorafenib in these patients, its efficacy is limited. Thus, HAIC is considered an alternative treatment modality, especially in Japan and South Korea.

In this study, we analyzed the response rate and overall survival of HAIC using the ECF regimen. In previous reports, the response rate and disease control rate of HAIC in advanced HCC patients with PVTT^[11,24-26] were 33%-52% and 47%-77%, respectively. The median OS was 7-10 mo in those studies. The objective response

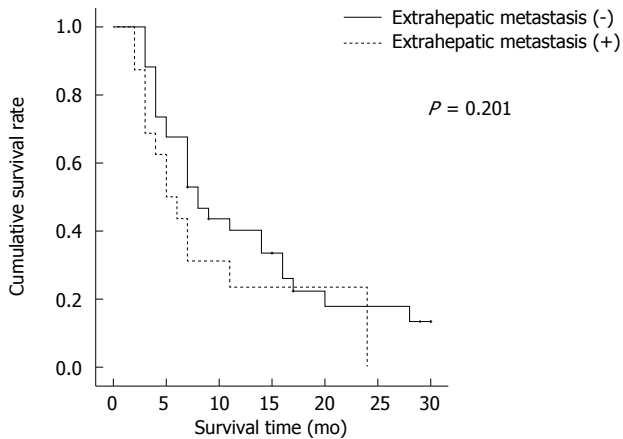


Figure 3 Cumulative survival rate according to the presence of extrahepatic metastasis.

rate and disease control rate of our study, which are 32% and 76%, respectively, are in accordance with the above results. In addition, the median OS of 7 mo was similar to the results of previous reports. However, our study included patients with extrahepatic metastasis at the beginning of HAIC, whereas the previous studies excluded those patients. HAIC treatment is primarily used for the local control of liver tumors in patients with minimal extrahepatic spread^[7]. Extrahepatic tumors would not respond well to HAIC. However, our data support the use of HAIC in HCC patients with extrahepatic metastasis because extrahepatic metastasis was not a significant factor in survival (Figure 3). The mortality in advanced HCC is related to intrahepatic tumors, and the leading cause of death in these patients is intrahepatic tumor progression^[27,28]. In this study, all patients had advanced intrahepatic HCC with vascular invasion. Because the survival of these patients was influenced by intrahepatic tumor progression, extrahepatic metastasis may not influence overall survival. If the treatment response evaluation is confined to intrahepatic tumor lesions, the disease control rate was as high as 84%. These response and disease control rates are significantly higher than those with sorafenib treatment^[5,29].

Several studies reported that the therapeutic effectiveness of HAIC was an important prognostic factor^[10,11,30], which is consistent with our results. In the present study, the median OS of the disease control group after two cycles of HAIC was significantly longer than patients showing PD. In addition, patients with an objective response to HAIC treatment also had significantly longer survival than non-responders. These results indicate that the responses to HAIC were independent prognostic factors. In addition, this study showed that the response after the second cycle of HAIC also significantly influences survival; thus, patients with CR, PR or SD after the second cycle of HAIC could continue HAIC treatment and expect favorable results.

PIVKA-II, also known as a des-gamma carboxy prothrombin (DCP), is an alternative tumor marker of AFP in diagnosing HCC. It is associated with aggressive features such as tumor size, vascular invasion, tumor stage

Table 5 Adverse events related to treatment

	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Leukopenia	26%	38%	8%	0%
Neutropenia	10%	26%	30%	12%
Anemia	26%	48%	26%	0%
Thrombocytopenia	30%	18%	44%	0%
Non-hematological				
Total bilirubin	30%	28%	10%	0%
AST	20%	36%	38%	6%
ALT	48%	12%	16%	2%

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 6 Causes of death

	Patient number (n = 37)
Disease progression	21
Hepatic dysfunction	6
Variceal bleeding	5
Infection	3
Unknown	2

and survival, and patients with high serum PIVKA-II levels have a poor prognosis^[31,32]. In the present study, the median OS in patients with a PIVKA-II level ≥ 40 and < 40 mAU/mL were 7 and 16 mo, respectively (Figure 4). Thus, patients with high serum PIVKA-II levels prior to HAIC treatment would be expected to have a poorer prognosis. Some reports state that, independent of pre-treatment level, tumor marker response to treatment was associated with survival. Park *et al.*^[18] reported that AFP and DCP response were independent factors associated with survival. Personeni *et al.*^[17] reported that AFP response is an independent surrogate end point for survival in patients treated with sorafenib. Similarly, a $\geq 50\%$ decline in AFP and PIVKA-II after the second cycle of HAIC treatment was associated with better outcomes among the patients with elevated AFP and PIVKA-II levels prior to the initiation of HAIC treatment (though PIVKA-II reduction is not statistically significant by multivariate analysis) (Figure 4). Therefore, tumor marker response (AFP and PIVKA-II) after the second cycle may be a useful surrogate endpoint for good outcomes in those receiving HAIC treatment for large HCC with PVTT.

Many studies have shown that tumor size is a major determinant of survival^[33,34]. However, few studies (with the exception of Hsu *et al.*) have analyzed survival according to tumor volume^[35]. In the current study, multivariate analysis of the pre-treatment parameters showed that a tumor volume < 400 cm³ was an independent pre-treatment prognostic factor, while the maximal tumor diameter was not. Along with tumor volume, we evaluated the Child-Pugh score as a predictive factor of survival. Though not a significant multivariate variable, a Child-Pugh score of 5 was an important factor predicting good outcome (median OS of Child class A5, A6 and B were 16, 7 and 3 mo, respectively). This result is consistent

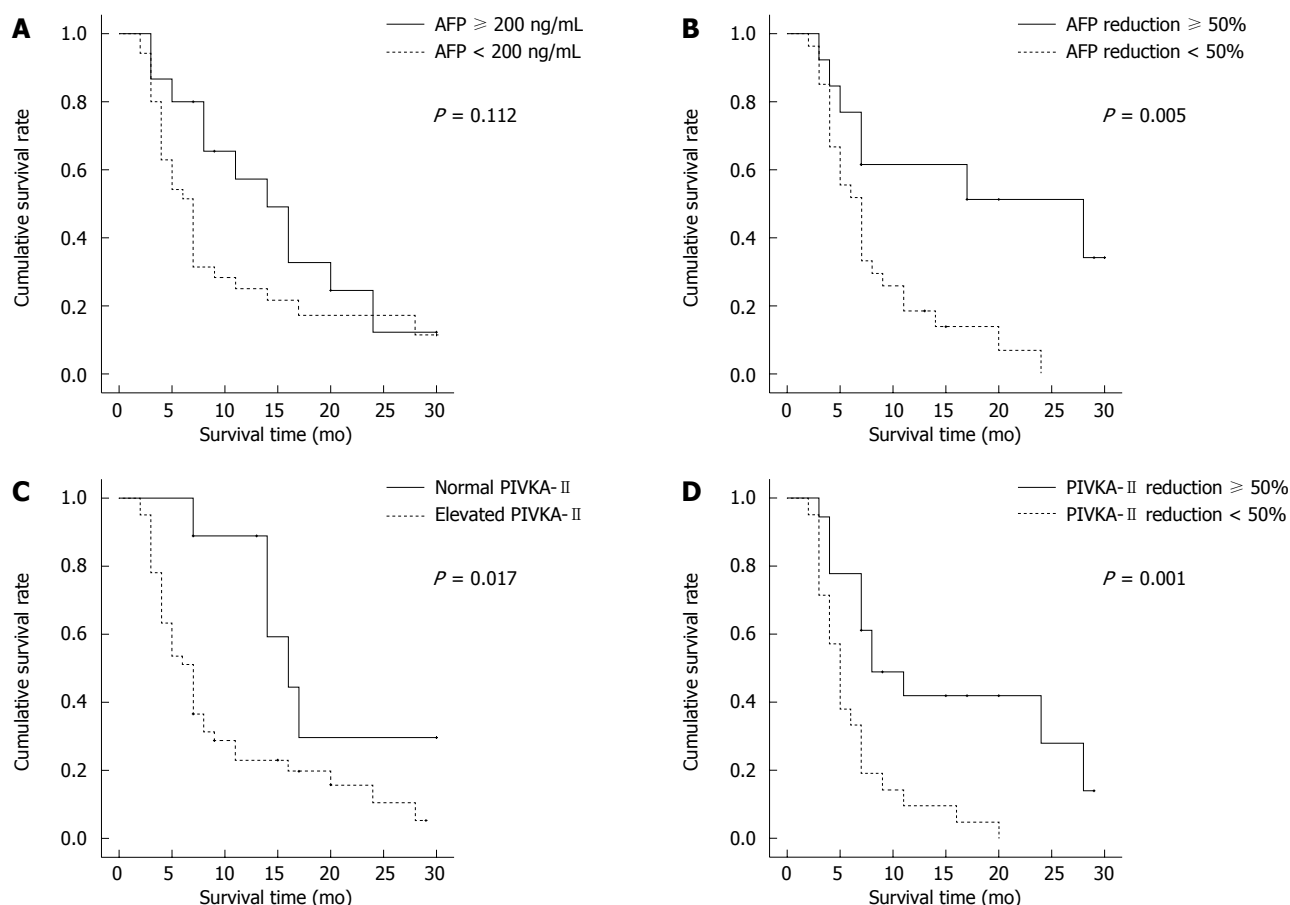


Figure 4 Cumulative survival rates. A: According to the pre-treatment α -fetoprotein (AFP) level; B: According to the AFP reduction after two cycles of hepatic arterial infusion chemotherapy (HAIC); C: According to the pre-treatment protein induced by vitamin K absence or antagonist (PIVKA)-II level; D: According to the PIVKA-II reduction after two cycles of HAIC.

with the results of HAIC studies showing that the Child-Pugh score was an independent survival factor^[30,36]. Thus, HAIC using ECF could be indicated in selected patients with tumor volumes $< 400 \text{ cm}^3$, good hepatic reserve function, and low PIVKA-II levels.

Although many studies using HAIC have been performed over the last decade, the therapeutic regimen of this treatment has not been standardized. Ando *et al.*^[11] reported that HAIC using low-dose cisplatin and 5-FU demonstrated a good response rate and survival time in 48 patients with PVTT. Park *et al.*^[10] showed that repetitive HAIC with high-dose 5-FU and cisplatin given for 3 d was effective and safe. In Japan, interferon-combined HAIC is also commonly used. The ECF chemotherapeutic regimen in this study consisted of high dose cisplatin and 5-FU in combination with epirubicin. Woo *et al.*^[14] compared high-dose HAIC with low-dose HAIC and reported that high-dose HAIC was safe and achieved better tumor response compared with that of low-dose HAIC. The addition of epirubicin to the high-dose HAIC regimen resulted in more effective control of the intrahepatic tumor in our study.

However, the ECF regimen appears to be more toxic than in previous reports of high-dose HAIC^[10,14]. While grade 3 hematologic or non-hematologic toxicities comprised less than 5% in previous studies, grade 3 leuko-

penia, anemia, and thrombocytopenia in this study comprised 8%, 26% and 44% of the toxicities, respectively, and hepatic toxicity was higher. In addition, hematologic toxicities tended to be more common in this study than in the trials using sorafenib such as the SHARP trial or an Asia-Pacific trial^[5,29]. The more frequent grade 3/4 toxicities in this study may be due to our use of different inclusion criteria. The hematologic inclusion criteria were lower than those used in the previous study, and there were no inclusion criteria related to hepatic function such as AST, ALT and bilirubin. As a result, our study included more patients with lower blood cell counts or higher liver enzyme levels, which could lead to more toxic adverse events. However, all hematologic and hepatic toxicity returned to baseline levels within several days.

This study has some limitations. First, we included patients with extrahepatic metastasis even though HAIC is only considered effective for the treatment of intrahepatic tumors^[7]. However, extrahepatic metastasis was not independently associated with survival, and the results were as good as those of previous studies despite the inclusion of patients with extrahepatic metastasis. Second, because most tumors were of the infiltrative type and margins were obscure, there was difficulty in accurately measuring tumor volume. Third, the retrospective nature of this study is underpowered due to a single arm regis-

try without a control group. In addition, this study may have inherent bias associated with a small sample size and heterogeneous treatments. Thus, large prospective studies are necessary to establish the efficacy of HAIC using the ECF regimen in patients with large tumors and PVTT.

In conclusion, HAIC with the ECF regimen may be a good option for advanced HCC with tumor volumes < 400 cm³ and a normal PIVKA-II level. In addition, this study suggests that response to chemotherapy after two cycles of HAIC, including radiologic tumor control and tumor marker reduction, is an independent predictor of longer survival. Therefore, HAIC treatment could be indicated in selected patients with favorable pre-treatment or post-treatment prognostic factors.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is a major health problem, accounting for more than 626000 new cases per year worldwide. Most HCC patients are diagnosed at an inoperable (intermediate or advanced) stage. The prognosis for advanced HCC is dismal. Sorafenib is the only approved drug for target therapy, and this drug is recommended for advanced HCC patients according to Barcelona Clinic Liver Cancer staging system. However, sorafenib shows only modest improvements in survival and tumor response.

Research frontiers

Hepatic arterial infusion chemotherapy (HAIC) has been used as a treatment modality for advanced HCC. Although sorafenib is regarded as the standard treatment of advanced HCC, HAIC can be an alternative treatment modality. The research hotspot in the area of HAIC treatment involves patients who will have good responses to HAIC.

Innovations and breakthroughs

Until now, the HAIC therapeutic regimen has not been standardized. This study is the first to use the epirubicin-cisplatin-5-fluorouracil (ECF) regimen, which consists of epirubicin, cisplatin, and 5-fluorouracil. HAIC treatment using the ECF regimen showed a good response, and its toxicity was tolerable. While previous studies on HAIC attempted to investigate the prognostic factors among the pre-treatment variables and the best response during treatment, this study revealed that the response after the second cycle of treatment, including radiologic response and tumor marker reduction, is also an important prognostic factor. The authors measured the actual tumor volume using imaging software and suggested a cut-off volume that could indicate a good response to HAIC.

Applications

Because the response after the second cycle is an important prognostic factor, those who showed a good response after the second cycle can continue the HAIC treatment and can expect favorable results.

Terminology

HAIC: HAIC is one type of arterial chemotherapy used in advanced hepatocellular carcinoma. HAIC requires an implantable port system for which a catheter is inserted and localized to the proper hepatic artery. HAIC enables the local delivery of high concentrations of anti-cancer agents to tumors, thereby maintaining a low systemic concentration of chemotherapeutic agent.

Peer review

This is a good article about the use of HAIC in the treatment of advanced HCC with portal vein tumor thrombosis. HAIC is shown to be an effective treatment modality.

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***Garcinia Cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation**

Young-Je Kim, Myung-Sook Choi, Yong Bok Park, Sang Ryong Kim, Mi-Kyung Lee, Un Ju Jung

Young-Je Kim, Myung-Sook Choi, Department of Food Science and Nutrition, Kyungpook National University, Daegu 702-701, South Korea

Myung-Sook Choi, Un Ju Jung, Center for Food and Nutritional Genomics Research, Kyungpook National University, Daegu 702-701, South Korea

Yong Bok Park, Sang Ryong Kim, School of Life Sciences and Biotechnology, Kyungpook National University, Daegu 702-701, South Korea

Sang Ryong Kim, Brain Science and Engineering Institute, Kyungpook National University, Daegu 702-701, South Korea

Mi-Kyung Lee, Department of Food and Nutrition, Sunchon National University, Jeonnam 540-742, South Korea

Author contributions: Kim YJ and Choi MS contributed equally to this work; Kim YJ performed experiments and analyzed the data; Choi MS designed the study and reviewed and revised the manuscript; Park YB, Kim SR and Lee MK contributed to the critical edition of the manuscript; Jung UJ designed the study, performed experiments, analyzed the data and wrote the manuscript. **Supported by** The Basic Science Research Program, No. 2011-0022387; and the SRC program, Center for Food and Nutritional Genomics: No. 2012-0000644 through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology

Correspondence to: Un Ju Jung, PhD, Center for Food and Nutritional Genomics Research, Kyungpook National University, 1370 San-Kyuk Dong, Puk-Ku, Daegu 702-701, South Korea. jungunju@naver.com

Telephone: +82-53-9507937 Fax: +82-53-9581230

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Abstract

AIM: To investigate long-term effects of *Garcinia Cambogia* (GC), weight-loss supplement, on adiposity and non-alcoholic fatty liver disease in obese mice.

METHODS: Obesity-prone C57BL/6J mice were fed a high-fat diet (HFD, 45 kcal% fat) with or without GC

(1%, w/w) for 16 wk. The HFD contained 45 kcal% fat, 20 kcal% protein and 35 kcal% carbohydrate. They were given free access to food and distilled water, and food consumption and body weight were measured daily and weekly, respectively. Data were expressed as the mean $\pm$ SE. Statistical analyses were performed using the statistical package for the social science software program. Student's *t* test was used to assess the differences between the groups. Statistical significance was considered at $P < 0.05$.

RESULTS: There were no significant changes in body weight and food intake between the groups. However, the supplementation of GC significantly lowered visceral fat accumulation and adipocyte size *via* inhibition of fatty acid synthase activity and its mRNA expression in visceral adipose tissue, along with enhanced enzymatic activity and gene expression involved in adipose fatty acid β -oxidation. Moreover, GC supplementation resulted in significant reductions in glucose intolerance and the plasma resistin level in the HFD-fed mice. However, we first demonstrated that it increased hepatic collagen accumulation, lipid peroxidation and mRNA levels of genes related to oxidative stress (superoxide dismutase and glutathione peroxidase) and inflammatory responses (tumor necrosis factor- α and monocyte chemoattractant protein-1) as well as plasma alanine transaminase and aspartate transaminase levels, although HFD-induced hepatic steatosis was not altered.

CONCLUSION: GC protects against HFD-induced obesity by modulating adipose fatty acid synthesis and β -oxidation but induces hepatic fibrosis, inflammation and oxidative stress.

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Key words: *Garcinia Cambogia*; Anti-adiposity; Metabolic changes; Hepatic collagen accumulation; Hepatic inflammation; Hepatic oxidative stress

Core tip: *Garcinia Cambogia* (GC) is a popular dietary supplement for weight loss. However, little is known about the efficacy and hepatotoxicity of long-term GC supplementation in mice fed a high-fat diet (HFD). We observed that GC ameliorated HFD-induced adiposity by modulating enzymatic activity and gene expression involved in fatty acid metabolism. GC also reduced the plasma resistin level and glucose intolerance. However, GC caused hepatic collagen accumulation, inflammation and oxidative stress without affecting hepatic steatosis.

Kim YJ, Choi MS, Park YB, Kim SR, Lee MK, Jung UJ. *Garcinia Cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation. *World J Gastroenterol* 2013; 19(29): 4689-4701 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4689.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4689>

INTRODUCTION

Obesity is one of the global public health problems commonly associated with metabolic diseases including insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and dyslipidemia^[1]. According to the World Health Organization global estimates from 2008, more than 1.4 billion adults are overweight and at least 500 million adults are obese^[2]. Although the use of dietary supplements for weight loss becomes common^[3], the optimal dose and safety profiles of many dietary supplements are poorly studied. The United States Food and Drug Administration (FDA) do not regulate dietary supplements in the same manner as pharmacological agents^[4,5]. While pharmaceutical companies are required to obtain FDA approval, which involves assessing the risks and benefits prior to their entry into the market, dietary supplements are not subject to the same scientific scrutiny and are not required to demonstrate any scientific safety and efficacy pertaining to the claims made by manufacturers.

Several studies have shown that *Garcinia Cambogia* (GC), a fruit native to southeastern Asia and Western Africa, has beneficial effects on body weight and fat loss in both experimental animals and human^[6-10]. Its main component hydroxycitric acid (HCA) not only inhibits ATP-citrate lyase, the enzyme response for *de novo* fatty acid synthesis, but also increases hepatic glycogen synthesis, reduces food intake by suppressing appetite and decreases body weight gain^[6-9]. Although extensive experiments reported the weight loss and body fat-lowering effects of GC, some animal and clinical studies have shown controversial findings^[6,10-13] and no studies have shown whether these effects persist beyond 13 wk of GC treatment. Furthermore, some studies have reported the potential for hepatotoxicity of hydroxycut, a formulation that contains GC among other ingredients^[14,15].

The present study was therefore done to investigate

the effect of long-term GC supplementation on adipogenesis and obesity-related metabolic changes, such as glucose intolerance and hepatic steatosis, in mice fed a high fat diet (HFD). We also examined the effect of GC on liver dysfunction, collagen accumulation, inflammation and oxidative stress.

MATERIALS AND METHODS

Animals and diets

Male C57BL/6J mice (4-wk-old) were purchased from Jackson Laboratories (Bar Harbor, ME, United States). The mice were individually housed in polycarbonate cages, which were kept in a room maintained at a constant temperature (24 °C) with a 12-h light/dark cycle. The mice were fed a normal chow diet for acclimation for 1 wk after delivery. At 5 wk of age, they were randomly divided into two groups of 10 mice each and fed a HFD (D12451, Research Diets, New Brunswick, NJ, United States) with or without GC (1%, w/w, 60% hydroxyl citric acid; Newtree Inc., United States) for 16 wk. The HFD contained 45 kcal% fat, 20 kcal% protein and 35 kcal% carbohydrate. They were given free access to food and distilled water, and food consumption and body weight were measured daily and weekly, respectively. At the end of the experimental period, all the mice were anesthetized with isoflurane (5 mg/kg body weight, Baxter, United States) after a 12-h fast, and blood samples were collected from the inferior vena cava into heparin-coated tube for the measurement of plasma parameters. The blood was centrifuged at 1000 *g* for 15 min at 4 °C, and the plasma was separated.

Fasting blood glucose, intraperitoneal glucose tolerance test and homeostatic index of insulin resistance

The blood glucose concentration was measured with whole blood obtained from the tail veins after withholding food for 12 h using a glucose analyzer (OneTouch Ultra, Lifescan Inc., United States) based on the glucose oxidase method. The intraperitoneal glucose tolerance test was performed on the 15th week. After a 12-h fast, the mice were injected intraperitoneally with glucose (0.5 g/kg body weight). The blood glucose level was measured from the tail vein at 0, 30, 60 and 120 min after glucose injection. Area under the curve (AUC) was calculated for all glucose levels as an index of glucose tolerance. Homeostatic index of insulin resistance (HOMA-IR) was calculated according to the homeostasis of the assessment as follows: $HOMA-IR = [\text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{L U/mL)}] / 22.51$.

Plasma biomarkers

Plasma adipokines were measured with a multiplex detection kit (171-F7001M, Bio-Rad, Hercules, CA, United States). Capture antibodies directed against the adipokines (resistin, leptin) were covalently coupled to the beads, and the coupled beads reacted with plasma. After a series of washes to remove unbound protein,

a biotinylated detection antibody was added to create a sandwich complex. The final detection complex was formed with the addition of streptavidin-phycoerythrin conjugate. Phycoerythrin served as a fluorescent indicator, or reporter. All samples were assayed in duplicate and analyzed with a Luminex 200 Labmap system (Luminex, Austin, TX, United States). Data analyses were done with Bio-Plex Manager software version 4.1.1 (Bio-Rad, Hercules, CA, United States).

Plasma cytokines were measured with a multiplex detection kit (M60-009RDPD, Bio-Rad, Hercules, CA, United States). Capture antibodies directed against the cytokines [insulin, tumor necrosis factor- α (TNF- α) and monocyte chemoattractant protein-1 (MCP-1)] were covalently coupled to the beads, and the same procedure for plasma adipokine analysis as described above was used to determine the plasma cytokines levels.

Plasma lipid and apolipoprotein concentrations were determined with commercially available kits: Plasma free fatty acid (01120301.HE98), phospholipid (01120251), apolipoprotein A (14535014) and apolipoprotein B (14537014) levels were measured using the Nittobo enzymatic kit (Nittobo medical Co., Tokyo, Japan), and triglyceride (AM157S-K), total cholesterol (AM202-K) and high-density lipoprotein (HDL)-cholesterol (AM203-K) levels were determined using Asan enzymatic kits (Asan, Seoul, South Korea).

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using enzymatic kits (AM101-K, Asan, Seoul, South Korea).

Hepatic lipids contents

Hepatic lipids were extracted^[16], and then the dried lipid residues were dissolved in 1 mL of ethanol for triglyceride and cholesterol assays. Triton X-100 and a sodium cholate solution in distilled water were added to 200 μ L of the dissolved lipid solution for emulsification. The hepatic triglyceride and cholesterol contents were analyzed with the same enzymatic kit used for the plasma analysis.

Lipid-regulating enzyme activity

To measure the lipid-regulating enzymes activities in the epididymal white adipose tissue (WAT) and liver, samples were prepared and analyzed as previously described^[17]. Briefly, fatty acid synthase (FAS) activity was determined with a spectrophotometric assay according to the method by Carl *et al.*^[18]; one unit of FAS activity represented the oxidation of 1 nmol of NADPH per minute at 30 °C. Carnitine palmitoyltransferase (CPT) activity was determined according to the method by Markwell *et al.*^[19] and the results were expressed as nmol/min per milligram protein. Fatty acid β -oxidation was measured spectrophotometrically by monitoring the reduction of NAD to NADH in the presence of palmitoyl-CoA as described by Lazarow^[20], with slight modification. Protein concentration was measured by the Bradford method using BSA as the standard^[21].

Lipid peroxidation assay

The hepatic thiobarbituric acid-reactive substances (TBARS) concentration, as a marker of lipid peroxide production, was measured spectrophotometrically by the method of Ohkawa *et al.*^[22]. Hepatic homogenates containing 8.1% sodium dodecyl sulfate were mixed with 20% (w/v) acetic acid (pH 3.5), distilled water and 0.8% (w/v) TBA. The reaction mixture was heated at 95 °C for 60 min. After cooling the mixture, n-butanol: pyridine (15:1, v/v) was added and centrifuged at 3000 rpm for 15 min. The resulting colored layer was measured at 535 nm.

Analysis of gene expression

Epididymal WAT and liver were homogenized in TRIzol reagent (15596-026, Invitrogen Life Technologies, Grand Island, NY, United States) and total RNA was isolated according to the manufacturer's instructions. The total RNA was converted to cDNA using the QuantiTect Reverse Transcription Kit (205313, QIAGEN GmbH, Hilden, Germany). The RNA expression was quantified by quantitative real-time PCR using the QuantiTect SYBR green PCR kit (204143, QIAGEN GmbH, Hilden, Germany) and the SDS7000 sequence-detection system (Applied Biosystems, CA, United States). Each cDNA sample was amplified using primers labeled with SYBR Green dye for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The amplification was performed as follows: 10 min at 90 °C, 15 s at 95 °C and 60 s at 60 °C for a total of 40 cycles. The cycle threshold values obtained were those cycles at which a statistically significant increase in the SYBR green emission intensity occurred. Ct data were normalized using GAPDH, which was stably expressed in mice. Relative gene expression was calculated with the $2^{-\Delta\Delta C_t}$ method^[23]. The following gene-specific primers were used: for catalase (CAT), 5'-GCGTCCGTCCT-GCTGTC-3' (forward), 5'-TGCTCCTTCCACT-GCTTCATCTG-3' (reverse); for cell death-inducing DNA fragmentation factor- α -like effector A (CIDEA), 5'-TTTCAAACCATGACCGAAGTAGCC-3' (forward), 5'-CCTCCAGCACCAGCGTAACC-3' (reverse); for CPT, 5'-ATCTGGATGGCTATGGTCAAGGTC-3' (forward), 5'-GTGCTGTCATGCGTTGGAAGTC-3' (reverse); for FAS, 5'-CGCTCCTCGCTTGTCTGTCGTCG-3' (forward), 5'-AGCCTTCCATCTCCTGTTCATCATC-3' (reverse); for fatty acid translocase/cluster of differentiation 36 (FAT/CD36), 5'-ATTGGTCAAGC-CAGCT-3' (forward), 5'-TGTAGGCTCATCCACTAC-3' (reverse); for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-ACAATGAATACGGCTACAGCAA-CAG-3' (forward), 5'-GGTGGTCCAGGGTTTCT-TACTCC-3' (reverse); for glutathione peroxidase (GHS-Px), 5'-TCGCAATGAGCCAAAAGTACG-3' (forward), 5'-GCCAGGATTCGTAAACCACACTC-3' (reverse); for MCP-1, 5'-TTCTCTCCACCACCATG-CAG-3' (forward), 5'-CCAGCCGGCAACTGTGA-3' (reverse); for peroxisome proliferator-activated receptors (PPAR) α , 5'-CCTGAACATCGAGTGTCTGAATAT (forward), 5'-GGTCTTCTTCTGAATCTTGCAGCT-3'

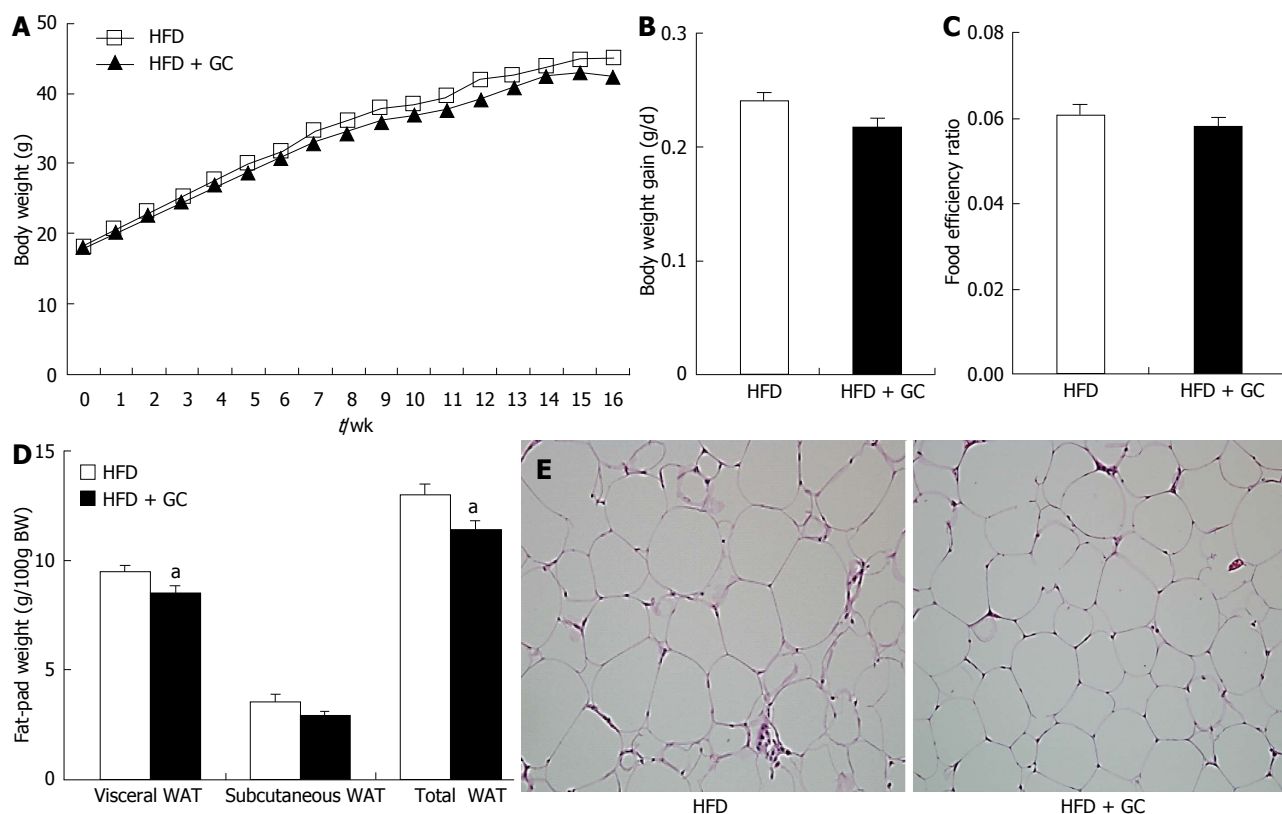


Figure 1 Effects of *Garcinia Cambogia* supplementation on body weight gain, food intake, fat-pad weight and adipocyte size in mice fed a high-fat diet for 16 wk. A-D: Data are expressed as the mean $\pm$ SE ($n = 10$); E: HE staining is shown. Representative photographs of epididymal white adipose tissue (WAT) (original magnification $\times 200$). High-fat diet (HFD), mice fed a high-fat diet alone; HFD + *Garcinia Cambogia* (GC), mice fed a high-fat diet containing GC (1%, w/w). ^a $P < 0.05$ vs control group.

(reverse); for TNF- α , 5'-GCAGGTCTACTTTAGAGT-CATTGC-3' (forward), 5'-TCCCTTTGCAGAACTCAGGAATGG-3' (reverse); for stearoyl-CoA desaturase (SCD1), 5'-CCCCTGCGGATCTTCCTTAT-3' (forward), 5'-AGGGTCGGCGTGTGTTTCT-3' (reverse); for superoxide dismutase (SOD), 5'-TG GTTGAGAA-GATAGGCGACA-3' (forward), 5'-CATCTCG-GCAGCATCCACCTC-3' (reverse); and for sterol-regulatory-element-binding protein 1c (SREBP1c), 5'-GGAGCCATGGATTCACAT-3' (forward), 5'-CCTGTCTCACCCCCAGCATA-3' (reverse).

Histological analysis

Epididymal WAT and liver were fixed in a buffer solution of 10% formalin and embedded in paraffin for staining with hematoxylin and eosin (HE) and Masson's trichrome. Stained areas were viewed using an optical microscope (Nikon, Tokyo, Japan) with a magnifying power of $\times 200$.

Ethics

After blood collection, epididymal WAT, perirenal WAT, retroperitoneal WAT, mesentery WAT, subcutaneous WAT and liver were promptly removed, rinsed with physiological saline and weighed. Among them, epididymal WAT and liver were snap-frozen in liquid nitrogen and stored at -70°C until enzyme activity and RNA analyses. All experimental procedures were performed in accor-

dance with the protocols for animal studies approved by the Kyungpook National University Ethics Committee (Approval No. KNU-2011-49).

Statistical analysis

Data were expressed as the mean $\pm$ SE. Statistical analyses were performed using the statistical package for the social science software (SPSS) program. Student's *t* test was used to assess the differences between the groups. Statistical significance was considered at $P < 0.05$.

RESULTS

Long-term GC supplementation did not alter body weight but significantly lowered body fat weight in HFD-induced obese mice

To investigate the effects of long-term GC supplementation in diet-induced obese mice, we provided 5-wk-old male C57BL/6J mice with HFD or 1% (w/w) GC supplemented HFD for 16 wk. During the experimental period, there was no significant difference in daily food intake between the groups (HFD, 3.96 ± 0.14 g; GC, 3.87 ± 0.07 g). The body weight gain was slightly lower in the GC-supplemented mice compared to the HFD control mice but the effects of GC were not significant (Figure 1A and B). Thus, the food efficiency ratio was not significantly different between the groups (Figure 1C). However, the weight of the visceral WAT including

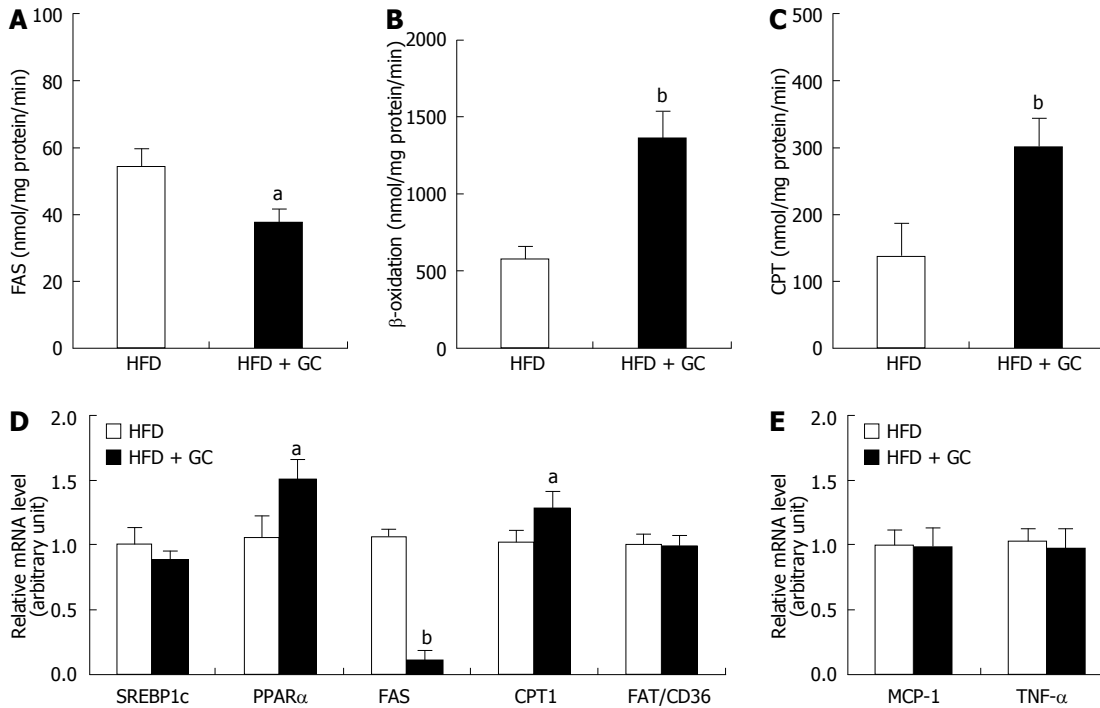


Figure 2 Effects of *Garcinia Cambogia* supplementation on fatty acid-regulating enzyme activity and gene expression in epididymal white adipose tissue of mice fed a high-fat diet for 16 wk. Data are expressed as the mean $\pm$ SE ($n = 10$). A: Fatty acid synthase (FAS); B: β -oxidation; C: Carnitine palmitoyltransferase (CPT); D, E: Relative mRNA level. High-fat diet (HFD), mice fed a high-fat diet alone; HFD + *Garcinia Cambogia* (GC), mice fed a high-fat diet containing GC (1%, w/w). ^a $P < 0.05$, ^b $P < 0.01$ vs control group. WAT: White adipose tissue; PPAR α : Peroxisome proliferator-activated receptor α .

the epididymal, perirenal, retroperitoneal and mesentery WAT was significantly lower in the GC-supplemented mice than in the HFD control mice (Figure 1D). The GC supplementation also tended to lower the subcutaneous WAT weight compared to the HFD control group by 17% although it was not significantly different. Hence, the weight of the total WAT (visceral and subcutaneous WAT) was significantly lower in mice fed a GC supplemented HFD. Morphological observations also indicated the epididymal adipocyte size was smaller in the GC-supplemented mice than in the HFD control mice (Figure 1E). However, GC supplementation did not alter the extent and degree of fibrosis in the epididymal WAT of HFD-fed mice (data not shown).

Long-term GC supplementation alters the activity of enzymes and expression of genes related to fatty acid synthesis and fatty acid oxidation in visceral WAT

To examine the mechanism through which GC supplementation reduces the visceral WAT weight, we measured the activity of enzymes that regulate lipid accumulation in visceral WAT. The GC supplementation resulted in a significant decrease in the activity of FAS in the epididymal WAT of mice fed a HFD (Figure 2A). Furthermore, GC-supplemented mice showed a significant increase in the activity of CPT and β -oxidation in the epididymal WAT (Figure 2B and C).

We also examined the expression of genes that regulate adipogenesis and inflammation. Consistent with the activity of adipose enzymes, GC supplementation

significantly down-regulated FAS mRNA expression, whereas it markedly up-regulated CPT mRNA expression in the epididymal WAT of HFD-fed mice (Figure 2D). Moreover, GC-supplemented mice showed a significant increase in the mRNA expression of transcription factor PPAR α in the epididymal WAT compared to the control mice. However, there were no significant differences in the mRNA expression of SREBP1c, FAT/CD36, MCP-1 and TNF- α between the two groups (Figure 2D and E).

Long-term GC supplementation improved HFD-induced glucose intolerance but did not alter plasma lipid, apolipoprotein and pro-inflammatory cytokine levels

We next determined whether GC influenced HFD-induced glucose intolerance. The fasting blood glucose, plasma insulin and HOMA-IR levels were not significantly altered by GC supplementation (data not shown). However, GC supplementation significantly lowered the blood glucose level compared to the control group at 120 min after glucose loading (Figure 3A). The level of AUC was also markedly decreased in the GC-supplemented mice compared to the control obese mice.

No significant differences were observed in the levels of plasma lipids (triglycerides, total cholesterol, HDL-cholesterol, phospholipids and free fatty acids) and apolipoproteins (apolipoproteins A and B) between the two groups (Table 1). GC supplementation also did not affect the plasma leptin, TNF- α and MCP-1 levels in the HFD-fed mice; however, it significantly lowered the plasma resistin level (Figure 3B and C).

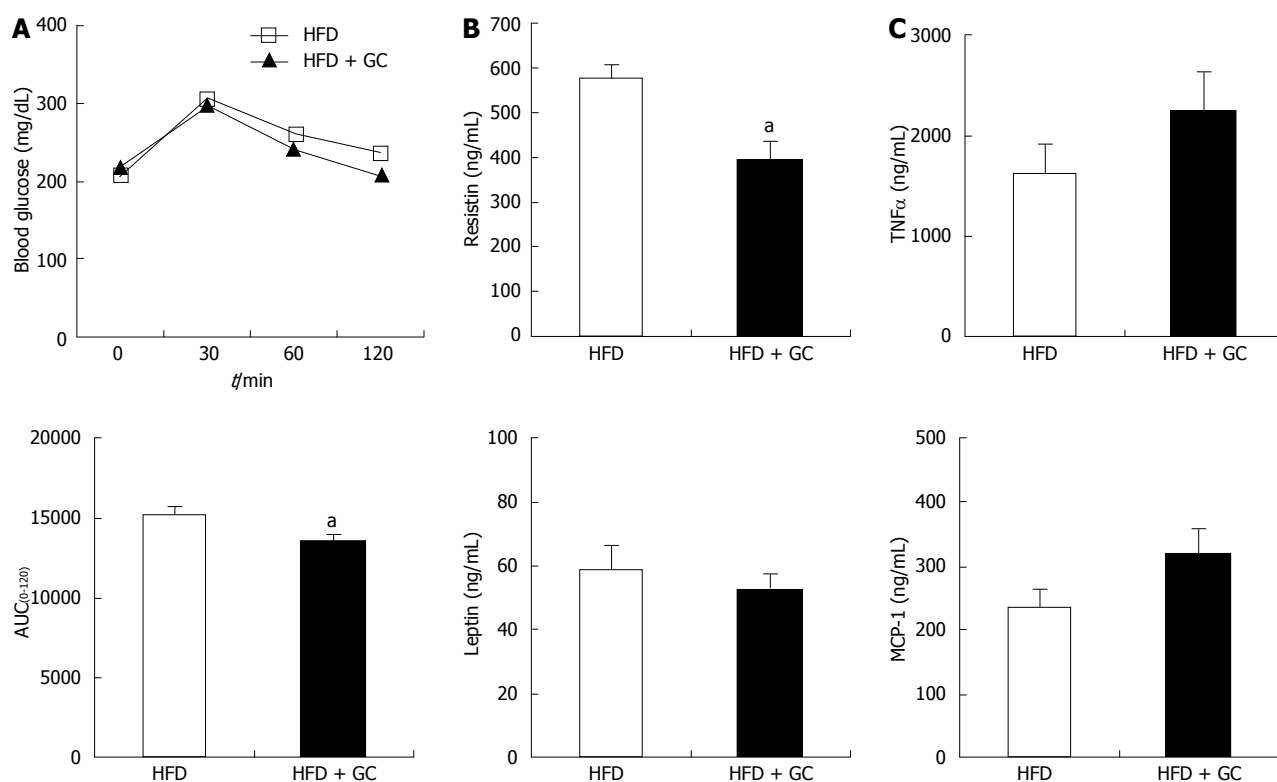


Figure 3 Effects of *Garcinia Cambogia* supplementation on glucose tolerance and plasma adipocytokine levels in mice fed a high-fat diet for 16 wk. Data are expressed as the mean $\pm$ SE ($n = 10$). A: The intraperitoneal glucose tolerance test was performed on the 15th week of *Garcinia Cambogia* (GC) supplementation in high-fat diet (HFD)-fed mice. Following a 12-h fast, the mice were injected intraperitoneally with glucose (0.5 g/kg body weight). Blood glucose was then measured via the tail vein at the indicated time [above: Blood glucose values; below: Areas under the curves (AUC)]; B, C: Plasma levels of leptin, resistin, monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor- α (TNF- α) were assayed after 16 wk of GC supplementation in HFD-fed mice. HFD, mice fed a high-fat diet alone; HFD + GC, mice fed a high-fat diet containing GC (1%, w/w). ^a $P < 0.05$ vs control group.

Table 1 Effects of *Garcinia Cambogia* supplementation on plasma lipids and apolipoproteins levels in mice fed a high-fat diet for 16 wk

	HFD	HFD + GC
Triglyceride (mg/dL)	97.47 $\pm$ 5.62	83.56 $\pm$ 4.51
Total cholesterol (mg/dL)	162.65 $\pm$ 10.16	167.73 $\pm$ 14.40
HDL-cholesterol (mg/dL)	76.27 $\pm$ 6.05	76.06 $\pm$ 6.37
Phospholipid (mmol/L)	2.35 $\pm$ 0.15	2.05 $\pm$ 0.15
Free fatty acid (mmol/L)	0.95 $\pm$ 0.08	1.02 $\pm$ 0.17
Apolipoprotein B (mmol/L)	5.32 $\pm$ 0.59	5.97 $\pm$ 0.39
Apolipoprotein A (mmol/L)	50.87 $\pm$ 1.24	45.22 $\pm$ 1.35

Data are expressed as the mean $\pm$ SE ($n = 10$). HFD: High-fat diet; GC: *Garcinia Cambogia*; HDL: High-density lipoprotein.

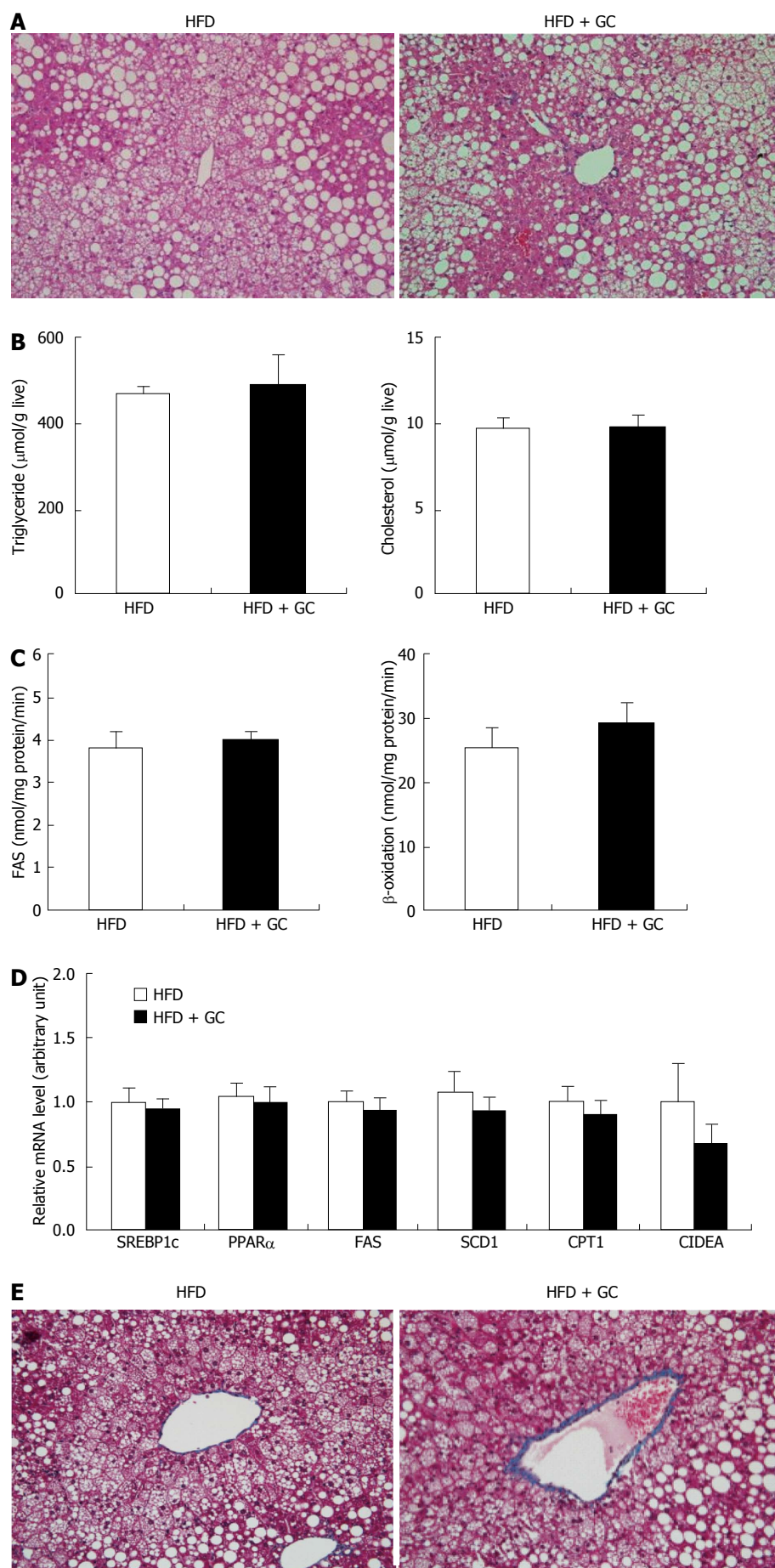
Long-term GC supplementation did not affect HFD-induced hepatic steatosis but increased hepatic collagen accumulation, inflammation and oxidative stress

Next, we examined the effect of GC supplementation on NAFLD induced by HFD. GC supplementation did not alter the hepatic triglyceride and cholesterol contents as well as the accumulation of hepatic lipid droplets in HFD-fed mice (Figure 4A and B). There were also no significant changes in the activities of hepatic FAS and β -oxidation and in the mRNA levels of the genes involved in lipogenesis and fatty acid oxidation, includ-

ing FAS, SCD1, CPT, CIDEA, SREBP1c and PPAR α , between the two groups (Figure 4C and D). However, trichrome staining of the liver revealed GC supplementation increased collagen deposition (blue staining) compared to the control mice (Figure 4E). Furthermore, Plasma ALT and AST levels were significantly increased in the GC group compared to the control mice (Figure 4F). The mRNA levels of TNF- α and MCP-1, pro-inflammatory markers, were significantly increased in the liver of GC-supplemented mice compared to the control mice (Figure 4G). GC supplementation also caused significant increases of hepatic SOD and glutathione peroxidase (GSH-Px) mRNA levels as well as TBARS level compared to the control mice, although there was no significant difference in hepatic CAT mRNA level between the two groups (Figure 4H and I).

DISCUSSION

Since unhealthy eating habits combined with limited activity are a major contributor to obesity and its related metabolic disease, lifestyle changes may present a cost-effective first-line of intervention for obesity^[24-26]. Dietary supplements seemed to be an inefficient agent for dietary intervention in obese subjects^[26]. Such supplements are not recommended by the position papers/guidelines for management of NAFLD^[27-32], and have been associated



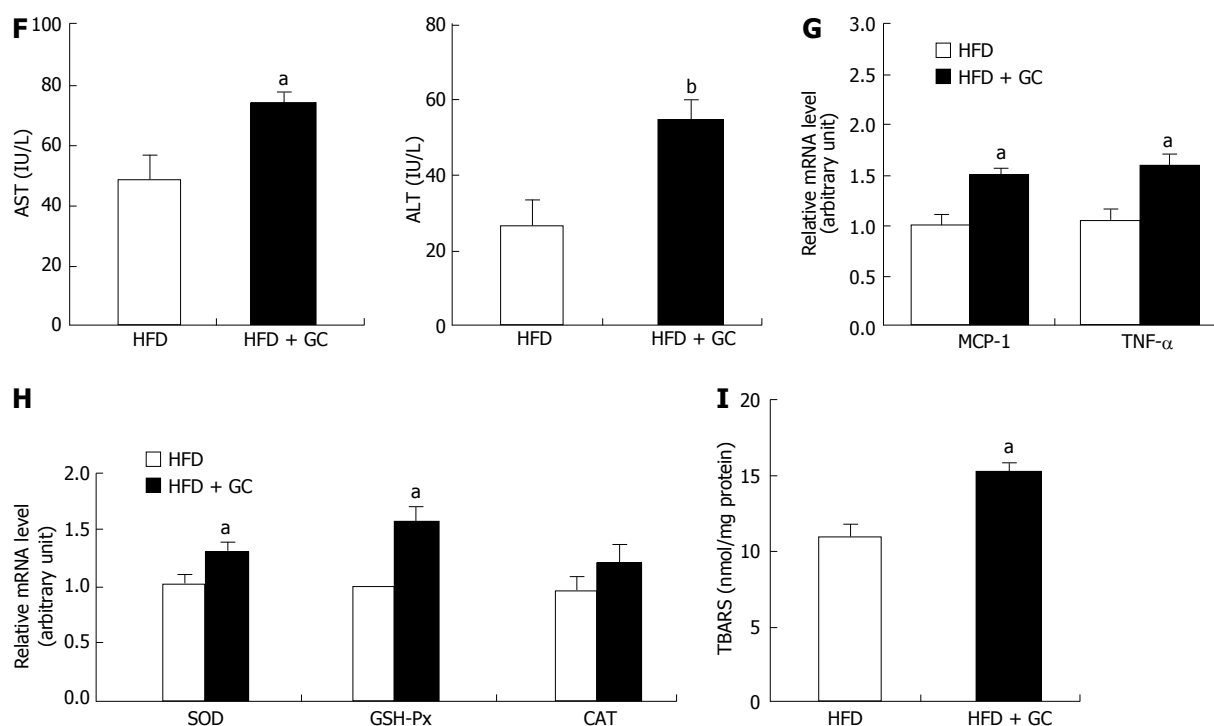


Figure 4 Long-term *Garcinia Cambogia* supplementation did not affect high-fat diet-induced hepatic steatosis but increased hepatic collagen accumulation, inflammation and oxidative stress. A: The liver tissue sections were stained with hematoxylin and eosin; B: Triglyceride and cholesterol level; C: Fatty acid synthase (FAS) and β -oxidation level; D: Relative mRNA level; E: The liver tissue sections were stained with Masson's trichrome; F: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level; G, H: Relative mRNA level; I: thiobarbituric acid-reactive substances (TBARS) concentration. Representative images are shown (original magnification $\times 200$). Data are expressed as the mean $\pm$ SE ($n = 10$). High-fat diet (HFD), mice fed a high-fat diet alone; HFD + *Garcinia Cambogia* (GC), mice fed a high-fat diet containing GC (1%, w/w). ^a $P < 0.05$, ^b $P < 0.01$ vs control group. CAT: Catalase; CIDEA: Cell death-inducing DNA fragmentation factor- α -like effector A; CPT: Carnitine palmitoyltransferase; FAS: Fatty acid synthase; GSH-Px: Glutathione peroxidase; MCP-1: Monocyte chemoattractant protein-1; PPAR α : Peroxisome proliferator-activated receptor α ; SCD1: Stearoyl-CoA desaturase; SOD: Superoxide dismutase; SREBP1c: Sterol-regulatory-element-binding protein 1c; TNF- α : Tumor necrosis factor- α .

with increased mortality in some studies^[33]. However, there are a number of natural dietary supplements for weight management, including GC, guar gum and chitosan^[34]. Among them, HCA-containing GC has been shown to be efficacious in lowering body weight and body fat^[6,10-13]. But, some clinical trials and animal studies have shown conflicting results^[6,12], and a case series on hepatotoxicity has been reported in patients taking GC-containing hydroxycut, although the individual chemical component underlying liver injury remains poorly understood^[14,15]. Therefore, the aim of this study was first to determine the effects from long-term GC supplementation on obesity and related metabolic diseases as well as the hepatotoxicity in mice fed a HFD.

In the present study, GC supplementation (1%, w/w) in a HFD for 16 wk did not lead to significant changes in body weight and food intake in mice. However, it resulted in significant decreases in visceral WAT weight and adipocyte size in HFD-induced obese mice. The anti-adiposity effect of GC was partly associated with marked decreases in FAS activity and its gene expression in the epididymal WAT. FAS is a key enzyme involved in *de novo* fatty acid synthesis and WAT is a major site of fatty acid synthesis and storage. We also found that the activity of CPT as well as fatty acid oxidation in epididymal WAT was elevated by GC supplementation. Furthermore, the

enhanced adipose fatty acid oxidation in GC-supplemented mice was accompanied by the up-regulated mRNA expression of genes involved in fatty acid oxidation such as CPT and PPAR α in the epididymal WAT. The CPT is a major rate-limiting enzyme for fatty acid oxidation, and its gene expression is regulated by PPAR α in adipocytes^[35]. The PPAR α mRNA expression was decreased in the WAT of both genetic and HFD-induced obese mice, and the down-regulation of PPAR α in obese WAT was involved in obesity-induced mitochondrial dysfunction and metabolic disorders^[36]. Taken together, our findings suggest that in HFD-fed mice, a significant reduction in visceral fat accumulation by GC supplementation could be partly due to decreased fatty acid synthesis as well as increased fatty acid oxidation in adipose tissue. The results of this study are supported by the findings of a previous study which suggested GC supplementation significantly lowered body fat mass, but not body weight and food intake, by inhibiting adipose ATP-citrate lyase (ACL) activity in Zucker obese rats^[12]. The ACL is another lipogenic enzyme catalyzing the cleavage of citrate to oxaloacetate and acetyl-CoA for *de novo* fatty acid synthesis^[37]. The inhibitory action of HCA on ACL reduces the acetyl-CoA pool, which can lead to a decreased concentration of malonyl-CoA, a physiological inhibitor of CPT^[38], and thus results in the suppression of body

fat accumulation through stimulation of fatty acid oxidation^[39]. Kim *et al.*^[11] also reported that HCA-containing GC (1%, w/w) supplementation in HFD-fed mice for 12 wk significantly lowered body fat accumulation by modulating multiple genes associated with adipogenesis in mice fed a HFD.

Along with the anti-obesity effects of HCA, previous studies have reported on the beneficial effects of HCA on insulin resistance^[40,41]. HCA increases the cellular pool of citrate by inhibiting ACL, which in turn can increase glycogen production^[42]. Recently, HCA supplementation enhanced the glycogen synthesis rate in skeletal muscles and improved post-meal insulin sensitivity^[41]. Although we did not measure the level of glycogen in the liver, HCA-containing GC supplementation improved glucose tolerance in HFD-induced obese mice. Furthermore, the plasma resistin level was significantly lowered by GC supplementation in the current study. Resistin is one of the adipokines proposed to link obesity with insulin resistance. Circulating resistin level was elevated in obesity and insulin resistance^[43], and HFD significantly increased plasma resistin levels in mice compared to a standard low-fat/high-carbohydrate diet^[44]. Resistin deficiency ameliorated glucose homeostasis in mice^[45], whereas administration of resistin impaired glucose tolerance and insulin action^[43,46]. Thus, the beneficial effect of GC on glucose intolerance might be associated with the decreased resistin level in plasma. Another mechanism in which GC could contribute to improve glucose tolerance is the lowered body fat mass because excess adiposity, especially in visceral WAT, is considered to promote insulin resistance^[47]. However, there was no significant difference in HOMA-IR which estimates insulin sensitivity from fasting glucose and insulin concentrations. Tripathy *et al.*^[48] reported a significant relationship between hepatic insulin sensitivity and HOMA-IR regardless of the stage of glucose tolerance, suggesting that the HOMA-IR is dependent upon hepatic insulin sensitivity rather than peripheral insulin sensitivity. Another clinical study also demonstrated that HOMA-IR did not accurately predict insulin sensitivity^[49].

Increased adiposity with the consequences of inflammation and insulin resistance has been linked to the development of NAFLD, which refers to a wide spectrum of liver damage, ranging from simple steatosis to steatohepatitis and cirrhosis. Steatosis represents the accumulation of fat within the liver through multiple mechanisms including an altered balance in fatty acid uptake and triglycerides secretion, increased *de novo* lipogenesis, and decreased fatty acid oxidation^[50,51]. Steatohepatitis is the combination of steatosis with hepatic inflammation and fibrosis. Liver fibrosis is excess synthesis and deposition of extracellular matrix proteins including collagen^[52], and pro-inflammatory factors including MCP-1 and TNF- α that contribute to the second hit in the pathogenesis of steatohepatitis. Among the inflammatory mediators, MCP-1 is a pro-inflammatory chemokine which coordinates leukocyte recruitment to the liver by activation of

the CC chemokine receptor 2 (CCR2) on inflammatory cells including monocytes and macrophages promoting the inflammatory response^[53]. Several studies indicate that MCP-1 is an important mediator of liver fibrosis^[54-56]. MCP-1 mRNA expression was markedly increased in the livers of patients with steatohepatitis and in murine models of steatohepatitis such as mice fed a HFD or methionine-choline deficient diet^[57-63]. CCR2 inhibitor suppressed the early and late features of steatohepatitis including fibrosis^[64], and chronic exposure of HFD induced hepatic MCP-1 mRNA expression in mice before induction of other pro-inflammatory cytokine mRNAs including TNF- α and prior to the onset of steatohepatitis, suggesting that MCP-1 plays a major role in initiating the inflammatory process in steatohepatitis^[65]. Moreover, MCP-1 deficiency reduced liver fibrosis (collagen deposition) and pro-fibrogenic gene expression in mice fed a methionine-choline deficient diet although it did not affect liver steatosis in this model^[66].

Similarly, we found that GC supplementation did not affect hepatic lipogenesis and lipid droplet formation, but it markedly increased collagen deposition as well as pro-inflammatory MCP-1 and TNF- α mRNA expression in the liver of HFD-fed mice. Furthermore, GC-supplemented mice exhibited impaired liver function indicated by the elevations of plasma ALT and AST, suggesting that GC possibly promotes liver injury in HFD-fed mice. Several case reports have suggested HCA-containing hydroxycut has potential hepatotoxicity but the underlying mechanism remains unknown^[14,15,67,68]. Our experiments provide new information regarding hepatotoxicity after long-term GC supplementation in HFD-induced obese mice. Accordingly, GC supplementation contributes to steatohepatitis by increasing hepatic collagen accumulation and hepatic MCP-1 and TNF- α expression in mice fed a HFD, which are independent of its effects on HFD-induced hepatic steatosis.

On the other hand, oxidative stress is considered to play an important role in progression of nonalcoholic steatohepatitis and hepatocellular injury^[69]. Reactive oxygen species (ROS) can damage DNA, lipids and proteins, induce necrosis and apoptosis of hepatocytes and amplify the inflammatory response. The ROS also stimulate the production of pro-fibrogenic mediators from Kupffer cells and inflammatory cells and directly induce hepatic stellate cells proliferation, resulting in the initiation of fibrosis^[70]. Antioxidant enzymes such as SOD and GSH-Px ameliorate the damaging effects of ROS. SOD converts superoxide radicals into hydrogen peroxide, which is then further metabolized by GSH-Px, where it catalyzes the destruction of hydrogen peroxide and lipid hydroperoxide. We observed that the supplementation of GC significantly up-regulated hepatic SOD and GSH-Px mRNA expression with concomitant increase in lipid peroxidation in the liver, suggesting that the increases in antioxidant gene expression by GC seems to be a compensatory response of the liver to cope with oxidative stress.

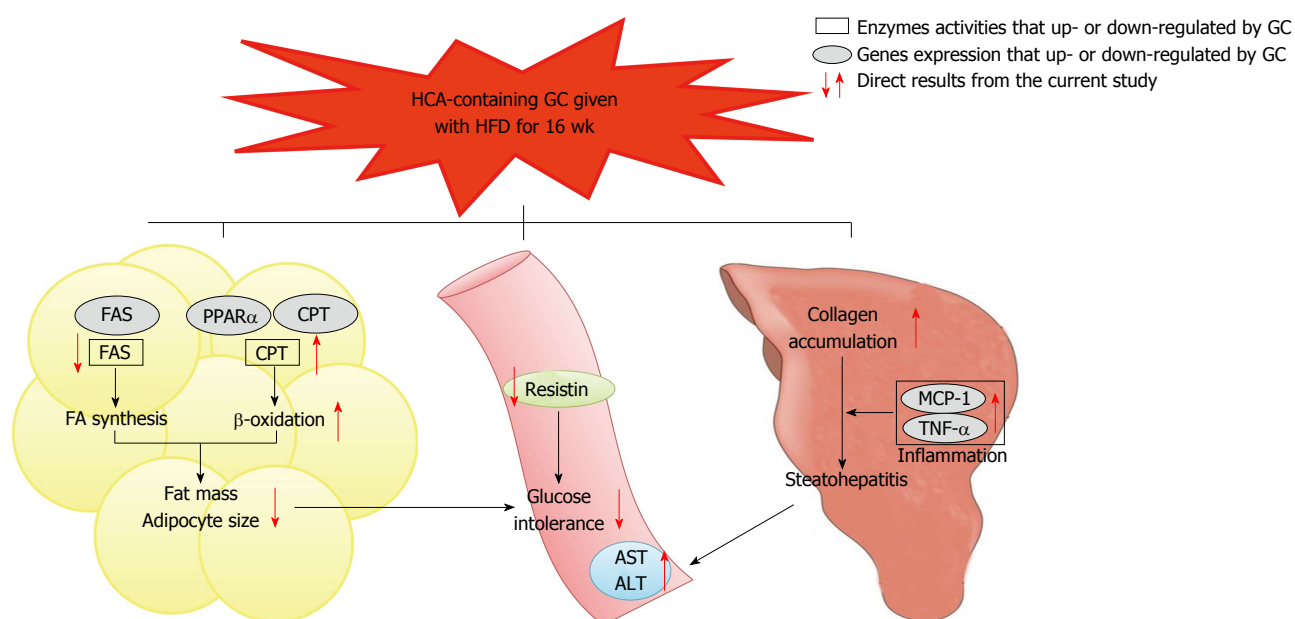


Figure 5 Summary of the long-term *Garcinia Cambogia* supplementation effects on adiposity, glucose tolerance and steatohepatitis in high-fat diet-induced obese mice. The *Garcinia Cambogia* (GC) supplementation decreased fatty acid synthase (FAS) mRNA expression and its activity, while peroxisome proliferator-activated receptor α (PPAR α) and carnitine palmitoyltransferase (CPT) mRNA expression along with the activities of CPT and β -oxidation were increased in the visceral adipose tissue, indicating that these changes may be potential mechanisms for reducing body fat accumulation and glucose intolerance induced by high-fat diet (HFD). Furthermore, GC supplementation decreased the plasma resistin level, which may be also related to improved glucose tolerance. There were no significant changes in hepatic lipid accumulation as well as in hepatic gene expression and enzymatic activity involved in fatty acid synthesis, oxidation and storage. However, GC increased pro-inflammatory monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor- α (TNF- α) mRNA expression, lipid peroxidation and collagen accumulation in the liver. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were also increased by GC supplementation in HFD-induced obese mice, thus suggesting that GC may negatively affect liver function by increasing hepatic fibrosis, inflammation and oxidative stress without affecting hepatic fat accumulation. FA: Fatty acid; HCA: Hydroxycitric acid. Open square means enzymes activities that up- or down-regulated by GC. Solid circle means genes expression that up- or down-regulated by GC.

In conclusion, this study demonstrated that long-term GC supplementation ameliorated adipogenesis in mice fed a HFD by promoting fatty acid oxidation with a simultaneous decrease in fatty acid synthesis in visceral WAT. Furthermore, GC exhibited a protective role against glucose intolerance induced by HFD. Moreover, this study provides the first evidence that long-term GC supplementation significantly increased hepatic collagen accumulation, lipid peroxidation and MCP-1 and TNF- α mRNA expression as well as plasma AST and ALT levels, thereby contributing partly to the exacerbation of steatohepatitis in HFD-induced obese mice at the doses given. The observations described above are summarized in Figure 5. Although further research is required to elucidate the efficacy and safety of long-term use of GC in humans, caution is needed when using GC supplements for weight management.

COMMENTS

Background

Garcinia Cambogia (GC) is a popular dietary supplement for weight loss, but the efficacy and hepatotoxicity of long-term GC supplementation remain poorly understood. Thus, authors investigated the long-term supplementation effects of GC on adiposity and non-alcoholic fatty liver disease (NAFLD) in mice fed a high-fat diet (HFD).

Research frontiers

A number of experiments reported GC has beneficial effects on weight loss and body fat in some animals and human. However, there are controversial findings

and little studies have reported whether these effects persist beyond 13 wk of GC supplementation.

Innovations and breakthroughs

This is first report which shows the supplementation of GC increased hepatic collagen accumulation, inflammatory genes expression and lipid peroxidation as well as plasma alanine aminotransferase and aspartate aminotransferase levels, although HFD-induced hepatic steatosis did not change. Thus their findings suggest that long-term supplementation of GC induces hepatic fibrosis and inflammation, although it protects against HFD-induced adiposity and glucose intolerance in mice fed a high fat diet.

Applications

Extensive dietary supplements are popular and widely used for weight management. However, the optimal dose and safety profiles of many dietary supplements are poorly studied and they are not regulated by the Food and Drug Administration in a manner observed for pharmacological agents. The authors suggest that caution is needed when using GC supplements for weight management, although further research is required to elucidate the efficacy and safety of long-term use of GC in humans.

Peer review

The authors investigated long-term supplementation effects of GC on adiposity and NAFLD in diet-induced obese mice. They reported that long-term GC supplementation improved adipogenesis by promoting fatty acid oxidation along with a decrease in fatty acid synthesis in visceral white adipose tissue. They also reported a protective role of GC against glucose intolerance induced by HFD. However, they also showed that long-term GC supplementation increased hepatic collagen accumulation and cytokine expression, thereby exacerbating steatohepatitis. The manuscript has no structural flaws. The hypothesis is relevant and methods to test the hypothesis were up-to-date.

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Mesenchymal stem cells alleviate TNBS-induced colitis by modulating inflammatory and autoimmune responses

Qian-Qian Chen, Li Yan, Chang-Zheng Wang, Wei-Hua Wang, Hui Shi, Bin-Bin Su, Qing-Huan Zeng, Hai-Tao Du, Jun Wan

Qian-Qian Chen, Li Yan, Chang-Zheng Wang, Wei-Hua Wang, Hui Shi, Bin-Bin Su, Qing-Huan Zeng, Hai-Tao Du, Department of Gastroenterology, Chinese PLA General Hospital, Medical College of PLA, Beijing 100853, China
Jun Wan, Department of Gastroenterology, Chinese PLA General Hospital, Beijing 100853, China

Author contributions: Chen QQ and Yan L done this study and wrote the paper; Wang CZ and Wang WH gave experimental instructions; Shi H and Su BB collected data; Zeng QH and Du HT analyzed and interpreted data; Wan J designed this study.

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Correspondence to: Jun Wan, MD, Professor, Department of Gastroenterology, Chinese PLA General Hospital, No. 28, Fu Xing Road, Haidian District, Beijing 100853, China. wanjun301@126.com

Telephone: +86-10-66876266 Fax: +86-404-68295664

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Abstract

AIM: To investigate the potential therapeutic effects of mesenchymal stem cells (MSCs) in inflammatory bowel disease (IBD), we transplanted MSCs into an experimental model of IBD.

METHODS: A rectal enema of trinitrobenzene sulfonic acid (TNBS) (100 mg/kg body weight) was administered to female BALB/c mice. Bone marrow mesenchymal stem cells (BMSCs) were derived from male green fluorescent protein (GFP) transgenic mice and were transplanted intravenously into the experimental animals after disease onset. Clinical activity scores and histological changes were evaluated. GFP and Sex determining region Y gene (*SRY*) expression were used for cell tracking. Ki67 positive cells and Lgr5-expressing cells were determined to measure proliferative activity. Inflammatory response was determined by mea-

suring the levels of different inflammatory mediators in the colon and serum. The inflammatory cytokines included tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin-2 (IL-2), IL-6, IL-17, IL-4, IL-10, and transforming growth factor (TGF- β). Master regulators of Th1 cells (T-box expressed in T cells, T-bet), Th17 cells (retinoid related orphan receptor gamma(t), ROR γ t), Th2 cells (GATA family of transcription factors 3, GATA3) and regulatory T cells (forkhead box P3, Foxp3) were also determined.

RESULTS: Systemic infusion of GFP-BMSCs ameliorated the clinical and histopathologic severity of colitis, including body weight loss, diarrhea and inflammation, and increased survival ($P < 0.05$). The cell tracking study showed that MSCs homed to the injured colon. MSCs promoted proliferation of intestinal epithelial cells and differentiation of intestinal stem cells ($P < 0.01$). This therapeutic effect was mainly mediated by down-regulation of both Th1-Th17-driven autoimmune and inflammatory responses (IL-2, TNF- α , IFN- γ , T-bet; IL-6, IL-17, ROR γ t), and by up-regulation of Th2 activities (IL-4, IL-10, GATA-3) ($P < 0.05$). MSCs also induced activated CD4⁺CD25⁺Foxp3⁺ regulatory T cells (TGF- β , IL-10, Foxp3) with a suppressive capacity on Th1-Th17 effector responses and promoted Th2 differentiation *in vivo* ($P < 0.05$).

CONCLUSION: MSCs are key regulators of immune and inflammatory responses and may be an attractive candidate for cell-based therapy of IBD.

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Key words: Mesenchymal stem cells; Transplantation; Inflammatory bowel disease; Inflammatory response; Immunomodulation; Trinitrobenzene sulfonic acid colitis; Therapy

Core tip: In this study, the following factors were identi-

fied: (1) The differentiation of intestinal stem cells in injured gut was determined by detecting Lgr5+ cells; (2) Th1-Th2-Th17-Tregs-related inflammatory and immune cytokine expressions in serum and local intestinal tissues were determined; (3) A Th2 shift and correction of the imbalanced Th17/Tregs were found; (4) Master regulators of Th1, Th2, Th17 and Tregs were detected in bone marrow mesenchymal stem cells -treated trinitrobenzene sulfonic acid-induced colitis; and (5) The pathways of Th1-T-bet, Th2-GATA family of transcription factors 3, Th17-retinoid related orphan receptor gamma(t) and Tregs-Foxp3 which serve as important immunoregulators in the correction of immune disorders and enhance the healing of injured intestinal mucosa were identified.

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INTRODUCTION

Inflammatory bowel diseases (IBD) are comprised of Crohn's disease (CD), ulcerative colitis (UC) and indeterminate colitis, and are found mainly in Western countries^[1]. Recent studies indicated that enhanced abnormalities of the immune system, normal gut flora and environmental influences may play central roles in these diseases^[2,3]. Conventional therapy for IBD includes corticosteroids, 5-aminosalicylates, 6-mercaptopurine, sulfasalazine, antimicrobial therapy, immunosuppressive agents and monoclonal antibodies (mAbs)^[4]. Surgery is indicated for complications and failure of medical treatment^[5]. Although the above therapies are effective for maintaining remission, they also have many side effects^[6,7]. Several studies have reported that the transplantation of mesenchymal stem cells (MSCs) could temper IBD both in clinical trials and in animal models, which indicated that MSCs may be a promising therapeutic option for IBD^[8-12]. However, the molecular mechanisms of this effect are still unclear.

Trinitrobenzene sulfonic acid (TNBS)-induced colitis (CD-like) is a well-established animal model for studying IBD. The model is well characterized by inflammatory cell infiltration accompanied by heightened Th1-Th17 response both systematically and locally in the gut mucosa^[13]. T-lymphocytes secrete cytokines (cytokines can be both regulators and effectors) which can be divided into Th1 (characterized by secretion of [tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin-2 (IL-2)], Th17 (characterized by secretion of IL-17), Th2 (characterized by secretion of IL-4 and IL-10), and regulatory T cells [Tregs, characterized by expression of forkhead box P3 (Foxp3) and CD25]^[14]. These T cells can be identified by the expression of specific transcription fac-

tors, T-bet for Th1, GATA family of transcription factors 3 (GATA3) for Th2, FoxP3 for Tregs, and retinoid related orphan receptor gamma(t) (ROR γ t) for Th17 cells, respectively^[15]. In this study, we used the TNBS-induced colitis animal model to investigate the therapeutic role of MSCs in CD-like diseases.

Many recent studies have demonstrated that bone marrow mesenchymal stem cells (BMSCs) were seen in injured intestinal mucosa. These cells may repopulate and differentiate into intestinal stem cells (ISCs)^[16]. ISCs are the progenitors of various functional intestinal cell types, including intestinal epithelial cells (IECs), interstitial cells, endothelial cells, vascular smooth muscle cells, and ill-defined inflammatory cells^[17,18]. *Lgr5* gene product can be used as a specific marker for identifying these ISCs^[19,20]. However, it is not clear which factors from the transplanted BMSCs promote the repopulation of cells in recipient intestinal tissues. Several experiments have shown that MSCs can release soluble factors (cytokines, chemokines, and growth factors) that result in cell cycle arrest in pro-inflammatory lymphocytes and induce T cell apoptosis^[21]. Reports also indicated that the regenerative, immunomodulatory, and anti-inflammatory effects of MSCs may provide a potential remedy for autoimmune diseases, such as focal cerebral ischemia, multiple sclerosis (MS), systemic sclerosis (SSc), type I diabetes and juvenile idiopathic arthritis^[22-24]. However, there have been few reports regarding the roles of MSCs in IBD as well as the molecular mechanisms of MSCs in alleviating IBD.

In this study, we focused on the trafficking of transplanted GFP-BMSCs into diseased colon tissues and on their immune modulating effects in TNBS-induced colitis. We investigated the inhibitory effect of BMSCs on Th1 and Th17 mediated inflammatory response. We also examined the enhancement of anti-inflammatory immune activities of Th2 and Tregs by BMSCs in an animal model of TNBS-induced colitis.

MATERIALS AND METHODS

Animals

Male Green fluorescent protein (GFP) transgenic mice [TgN (β -act-EGFP) Osb; 2-3 wk] on a C57BL/6 background were kindly provided by the Molecular Biology Laboratory of Chinese PLA General Hospital. Female BABL/c mice (6-8 wk) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences of China (Beijing). All studies were performed under approval of the Ethics Committee of the Animal Facility of Chinese PLA General Hospital and were in agreement with the Guidance suggestion of caring for laboratory animals^[25]. Mice were group-housed under controlled temperature (25 °C) and a 12-h light/dark cycle, fed standard mouse chow and tap water and maintained for 2 wk in our animal facilities before the start of the experiments.

Establishment of TNBS model

Colitis was induced in female BABL/c mice (6-8 wk,

18–22 g) using 2,4,6-trinitrobenzene sulfonic acid (2,4,6-TNBS; Sigma-Aldrich, Deisenhofen, Germany) in 50% ethanol^[26]. Then 0.1 mL of TNBS (100 mg/kg body weight) was administered *via* a vinyl catheter positioned 2.5 cm from the anus. To ensure distribution of TNBS within the entire colon and rectum, mice were held in a vertical position for 2 min after instillation of the TNBS enema. The control group was administered 0.1 mL of 50% ethanol.

Culture and identification of putative BMSCs

Isolation, culture, and expansion of BMSCs: Male GFP-mice (2–3 wk) were sacrificed by cervical dislocation and their femurs and tibia were carefully flushed with Dulbecco's modified Eagle's medium-low glucose (DMEM-LG; HyClone Lab, Inc. Logan, UT) using a 0.45-mm syringe needle until the bones become pale. The released cells were discarded and the bones were dissected into fragments of 1–3 mm³ and digested with collagenase II (Sigma) for 1–2 h in a shaking incubator at 37 °C and a shaking speed of 200 rpm. The collagenase was removed by dilution with DMEM-LG containing 10% heat inactivated fetal bovine serum (FBS; Gibco-Invitrogen, Carlsbad, CA). In addition, the digested bone fragments were washed by centrifuging twice for 5 min at 1000 rpm and then cultivated in a 60 mm dish (Corning International, Tokyo, Japan) with DMEM-LG containing 10% FBS and penicillin/streptomycin (100 U/mL and 100 g/mL; Sigma, St. Louis, MO) at 37 °C in a 5% CO₂ humidified incubator. To isolate putative MSCs, after 72 h of culture, nonadherent cells and tissue debris were removed with phosphate-buffered saline (PBS, HyClone), and adherent cells were maintained. On reaching 70%–80% confluence, these adherent cells were replated using 0.25% (wt/v) trypsin/0.02% (wt/v) EDTA (Gibco) for 2–3 min. The medium was changed every 2–3 d^[27].

Flow cytometric analysis: The cultured MSCs were retrieved by trypsin-EDTA digestion. Cell aliquots (1 × 10⁶) were washed with cold PBS and resuspended in 100 µL of PBS per EP tube and stained with FITC-conjugated anti-mouse CD45 (Becton Dickinson), or PE-conjugated anti-mouse CD11b (eBioScience, San Diego, CA) and CD90.2 (eBioScience) at a concentration of 2 µg/mL at 4 °C for 30 min. One tube of unstained cells was prepared as a control for the antibodies. Cells were examined using a Becton Dickinson Fluorescence Activated Cell Sorting (BD FACScalibur) cytometer (Becton Dickinson, San Jose, CA, USA) and analyzed using a FACS Vantage cytometer and CellQuest software (BD).

Differentiation of BMSCs: The potential of BMSCs to differentiate into osteogenic and adipogenic lineages has been examined^[27,28]. For osteogenesis, the isolated cells (passage 3) were plated in osteogenic conditioned medium supplemented with 10 mmol/L β-glycerol phosphate (Sigma), 50 µg/mL ascorbate-2-phosphate (Sigma) and 10 nmol/L dexamethasone (Sigma). The culture medium was changed every 3 d for 3 wk. Alizarin red staining was

used to examine the culture mineralization. For staining, the cultures were first fixed using 4% paraformaldehyde for 20 min and then subjected to alizarin red solution at 37 °C for 30 min.

For adipogenesis, the cultured cells (passage 3) were incubated in adipogenic medium supplemented with 100 µmol/L indomethacin (Sigma), 1 µmol/L dexamethasone (Sigma), and 0.5 mmol/L 1-methyl-3-isobutylxanthine (Sigma). The culture medium was changed every 3 d for 8 d. At the end of this period, the cells were then fixed in 4% paraformaldehyde for 1 h and stained with Oil Red (Sigma) for 1–2 h at room temperature.

Identification of fluorescence distribution in intestinal mucosa

GFP-BMSCs were harvested from male GFP-mice as described above. Then 1 × 10⁶ cells in 100 µL PBS were transplanted on the indicated day *via* the tail vein on the second day after TNBS administration, while control mice received 100 µL PBS without BMSCs. In addition, the mice were killed on day 1, 2, 3, 5, 7 and 9 and their colons were washed with PBS. The colonic tissues were embedded in Tissue-Tek OCT compound (Sakura Finechemical Co., Tokyo, Japan) and circumferential sections 3 µm thick were prepared for fluorescent microscopy examination (Olympus, Tokyo, Japan). Paraffin-embedded tissue slides were stained with hematoxylin and eosin (HE) to assess the intensity of colitis. Additional specimens were frozen in liquid nitrogen for further use.

Isolation of DNA and detection of Y chromosomal DNA by polymerase chain reaction

DNA was isolated from snap-frozen colon specimens using the QIAamp DNA mini kit (Qiagen, CA) and Wizard DNA purification resin (Promega, Madison, WI). Polymerase chain reaction (PCR) was used to investigate the transplanted male donor cells using primers specific for the sex-determining region of the mouse Y-chromosome (Sry). The male group acts as a positive control, while the female group acted as a negative control. PCR amplification was performed as described below. The reaction mixture included 2 µL of genomic DNA, 2 µL Taq DNA polymerase (Takara, Japan), 5 µL of each oligonucleotide primer, 1 µL of a 20 mmol/L solution of each dNTP, 5 µL of 10 × PCR buffer, and 5 µL of 25 mmol/L MgCl₂ in a final volume of 25 µL. The primer sequences used were as follows: mouse *Sry* gene, 5'-GGTGTG-GTCCCGTGGTGAGAG-3' and 5'-ATGGCATGTGGGTTCCTGTCC-3'. PCR was performed in a thermal cycler (PerkinElmer Cetus) for 35 cycles of denaturation (9 °C, 20 s), annealing (64 °C, 20 s), and extension (72 °C, 20 s). The product was analyzed by electrophoresis on a 2% agarose gel followed by ethidium bromide staining.

Assessment of inflammation and colitis severity

Clinical activity score of colitis: Mice were observed daily for weight, water/food consumption, morbidity, stool consistency, piloerection, and the presence of rectal bleeding. The clinical activity score of colitis was calculated

Table 1 Sequence of gene-specific primers used for real-time polymerase chain reaction analysis

mRNA target	Forward primer sequences 5'→3'	Reverse primer sequences 5'→3'	bp
Actin	CGTTGACATCCGTAAGAGACC	CTAGGAGCCAGAGCAGTAATC	111
IL-2	TGAGTGCCAATTCGATGATGAG	TTATTGAGGGCTTGTGAGATGAT	91
IL-4	CAGCAACGAAGAACACCACAG	CGAAAAGCCCGAAAAGAGTC	148
IL-6	AATTTCCTCTGGTCTTCTGG	ACTCTGGCTTTGTCTTCTTGTT	93
IL-10	AGTGGAGCAGGTGAAGAGTGATT	CTATGCAGTTGATGAAGATGTC	92
IL-17A	GAGAGCTGCCCTTCACTTTCA	GGCTGCTGGCGGACAAT	87
IFN- γ	TGGTGACATGAAAATCCTG	TTGCTGTTGCTGAAGAAG	141
TNF- α	AGTTCCTCCAAATGGCCTCCCT	ACTTGGTGGTTTGCTACGAC	115
GATA3	AGGTGCATGACGCGCTGGAG	GGAGTGGCTGAAGGGAGAG	107
Foxp3	GAAGAATGCCATCCGCCACAA	TGCTCCCTTCTCGCTCTCCA	70
TGF- β	TGCCCTCTACAACCAACACAAC	GCAGGAGCGCACAATCAT	142
T-bet	ACCTCTTCTATCCAACCAAGTATCC	GAGGTGTCCCCAGCCAGTA	104
ROR γ t	GAAGGCAAATACGGTGGTGTGG	GCTGAGGAAGTGGGAAAAGTC	90

IL: Interleukin; IFN: Interferon; TNF: Tumor necrosis factor; GATA3: GATA family of transcription factors 3; Foxp3: Forkhead box P3; TGF: Transforming growth factor; T-bet: T-box expressed in T cells; ROR γ t: Retinoid related orphan receptor gamma(t).

ed independently by two blinded investigators using the disease activity index (DAI)^[13]. The length of the colon, which was measured from the cecum to the anus, served as an indirect marker of the intensity of inflammation.

Histological scoring of the colon: The sections were HE stained and were used to assess colonic damage microscopically. The criteria were graded as follows: 0 point = no ulcer, no inflammation; 1 point = no ulcer, local hyperemia; 2 points = ulceration without hyperemia; 3 points = ulceration and inflammation at one site only; 4 points = two or more sites of ulceration and inflammation; and 5 points = ulceration extending more than 2 cm.

Immunohistochemistry for Ki67 and Lgr5: The slides of 4 μ m colon sections were incubated with a rabbit anti-mouse primary antibody to Ki67 (1:500, ab66155, Abcam,) or a rabbit anti-human primary antibody to Lgr5 (1:400, LS-A1236, MBL International Co., Woburn, MA) in PBS at 4 °C overnight. After 3 washes in PBS, the sections were incubated with a goat anti-rabbit secondary antibody (1:1000, Beijing Zhongshan Biotechnology, Beijing, China) at 37 °C for 30 min. Finally, the sections were visualized by incubating in 3,3'-diaminobenzidine tetrahydrochloride with 0.05% H₂O₂ (Liquid DAB + Substrate Chromogen System; Dako) for 3 min to induce a color reaction. The expression and localization of Ki67 and Lgr5 were examined under a light microscope (Olympus, Japan), and a brown color indicated a positive result.

Images were acquired using a digital camera connected to a light microscope (both from Olympus, Tokyo, Japan). Five immunostained sections (1280 × 960 pixels/per section) were selected from each image (× 200, 3 mice per group) for analysis. Ki-67 positivity and mean optical density of Lgr5 (mean optical density = total optical density/area) that best discriminated staining from the background was obtained using the NIH Image J 1.36b imaging software (NIH, Bethesda, MD). For immunohistochemistry analysis, immunostained sections were evaluated by two investigators following the principle of “blinded”.

Quantification of Th1/Th2/Th17 cytokines by FACS:

The BD Cytometric Bead Array Mouse Th1/Th2/Th17 Cytokine Kit (Becton Dickinson, San Jose, CA) was used to quantify TNF- α , IFN- γ , IL-2, IL-4, IL-6, IL-10 and IL-17A secretions in murine peripheral blood and colon proteins according to the manufacturer's instructions using the BD FACScalibur cytometer (Becton Dickinson). Fifty microliters of supernatant were collected at each of the time points indicated above. Data were acquired on a FACS ARIA and samples were analyzed using FCAP Array Software (BD Biosciences).

RNA extraction and real-time PCR: RNA was extracted from colon (100 mg) using the Total RNA Kit II (Qiagen) following the manufacturer's instructions. The quantity of extracted RNA was evaluated using the Nanodrop ND1000 (ThermoFisher Scientific). Complementary DNA (cDNA) was created using DNase I /RNase-free (EN0521, Fermentas). Single intra-exon gene-specific primers listed in Table 1 were generated using Primer Express Software (Perkin Elmer Applied Biosystems). Applied Biosystems PRISM[®] 7300 (Applied Biosystems) and SYBR green fluorescent dye were used to detect amplification under the following amplification conditions: (1) 1 warm-up cycle for 2 min at 50 °C; (2) 1 pre-amplification cycle for 10 min at 95 °C, 40 amplification cycles for 30 s at 95 °C and for 31 s at 60 °C; and (3) end-amplification cycle for 15 s at 95 °C, 30 s at 60 °C and 15 s at 95 °C. Quantitative PCR crossing threshold (Ct) values were obtained during the exponential amplification phase using SDS 2.3 Software (Applied Biosystems).

Western blotting analysis for Foxp3, T-bet, GATA-3, TGF- β 3 (III) and ROR gamma (t)

Total proteins (100 μ g) were added to lysis buffer containing protease inhibitors (Sigma; St. Louis, MO) and boiled at 95 °C for 5 min. The proteins were then routinely processed for Western blotting. Briefly, the proteins were respectively separated on 10% SDS polyacrylamide gels and blotted onto nitrocellulose membranes which

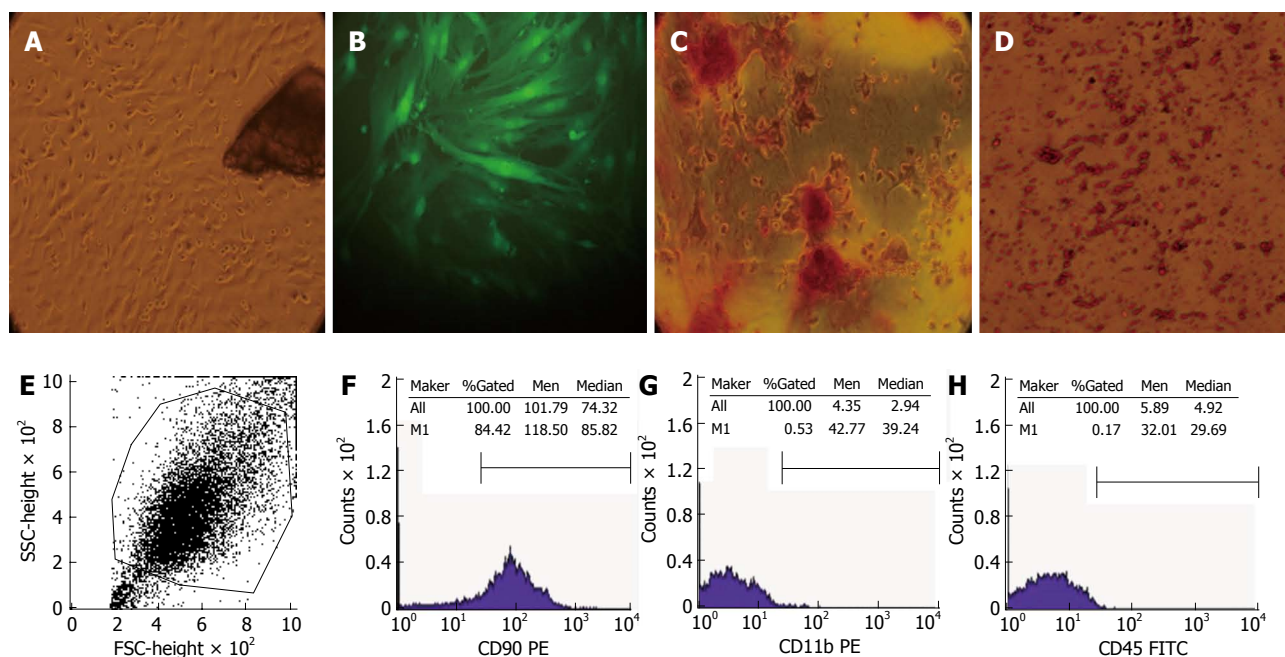


Figure 1 Morphological features, immunophenotypic characterization and differentiation assays of compact bone-derived mouse mesenchymal stem cells. A: At 72 h after initial culture, the cells migrate out from the enzyme-digested bone chips ($\times 100$); B: Green fluorescent protein - bone marrow mesenchymal stem cells (BMSCs) arranged in a bunched or radiated shape without weakening of the green fluorescence after passages ($\times 400$); C: Osteoplastic differentiation of BMSCs ($\times 400$); D: Apoplastic differentiation of BMSCs ($\times 400$); E-H: BMSCs' phenotypes detected by flow cytometry.

were incubated in PBST-milk (PBS buffer containing 0.1% Tween-20 and 5% milk), followed by primary antibodies (1 h) for mouse FoxP3 (1:400 dilution, PM024S, MBL International Co., Woburn, MA); T-bet (1:250 dilution, 14-5825, eBioscience, San Diego, CA); GATA-3 (1:250 dilution, 14-9966, eBioscience); transforming growth factor (TGF- β 3) (III) (1:1000 dilution, sc-83, Biotechnology, Santa Cruz, CA); ROR gamma(t) (1:250 dilution, 14-6981, eBioscience). Loading controls were obtained using a goat anti-rabbit antibody (1:3000, CoWin Biotech Co., Beijing, China). Blots were then washed with PBST 3 times (10 min each) and subsequently incubated (1 h) with actin antibody (1:3000, Biotechnology, Santa Cruz, CA) diluted in PBST milk. Each Western blot was repeated at least twice. Bands were detected after exposure to Hyperfilm-MP (1:10000, Amersham International PLC, Buckinghamshire, United Kingdom). The bands were then detected with scanning densitometry using a Desaga Cab UVIS scanner and Desaga ProViDoc software (Desaga, Wiesloch, Germany).

Statistical analysis

Data are represented as mean $\pm$ SD. Statistical comparisons between experimental groups were performed with one-way ANOVA using SPSS 17.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Characterization of the putative GFP-BMSCs

The bone marrow-derived GFP-BMSCs were obtained by cultivation of collagenase II digested bone fragments. At 72 h after initial culture, fibroblast-like cells were

observed to migrate out from the bone fragments and adhere to the dish (Figure 1A). Some fibroblast colonies were observed after removing nonadherent cells and tissue debris when changing the culture medium. After 5 consecutive passages, the cell population started to demonstrate clustering and a radial pattern maintaining strong GFP expression (Figure 1B). The differentiation assays showed that these cells could differentiate into osteoblasts and adipocytes. For osteogenic differentiation, alizarin red staining was used after 21 d of culture and mineralized nodules were formed after induction (Figure 1C). For adipogenic differentiation, fat red particles were seen following Oil-red-O-staining after 8 d of culture (Figure 1D). Flow cytometry showed that these cells were homogeneously positive for the mesenchymal marker CD90, but negative for hematopoietic markers CD11b and CD45 (Figure 1E-H).

Transplanted GFP-BMSCs migrated into the inflamed colon in TNBS-induced colitis

The transplanted GFP-BMSCs were found to be localized in the inflamed colon. Green fluorescence was mainly observed in the lamina propria, near the bottom of crypts 48 h after GFP-BMSCs transplantation (Figure 2A). No GFP-labeled MSCs were observed in non-inflamed tissues. *Sry* gene was detected in the TNBS-MSC-female group and the male-control group, but not in the TNBS-PBS-female group on day 9 (Figure 2B).

Clinical and mucosa healing of TNBS-induced colitis

TNBS instillation in 50% ethanol led to a substantial wasting disease caused by severe diarrhea, weight loss, decreased water/food consumption, piloerection and the

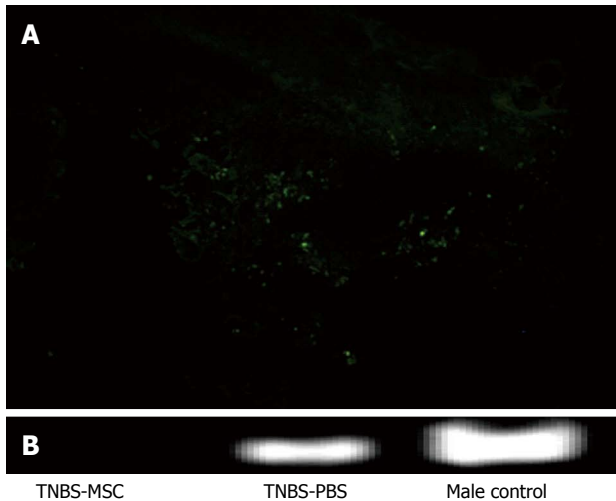


Figure 2 Localization of transplanted green fluorescent protein-bone marrow mesenchymal stem cells. A: Fluorescence in the lamina propria of colon using fluorescence microscopy ($\times 100$); B: Sry gene expression (1188 bp) in the colons of 3 groups by polymerase chain reaction. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered saline.

presence of rectal bleeding with about 55% mortality (Figure 3E). BMSCs significantly reduced colitis intensity in acute TNBS-induced colitis compared with the TNBS-PBS group assessed by DAI scores (Figure 3F). In the TNBS-MSC group, weight loss was improved from day 3 (-1.60 ± 0.45 g *vs* day 2 -4.71 ± 1.42 g, $P < 0.05$), but not in the TNBS-PBS group from day 5 (-4.40 ± 0.76 g *vs* day 3 -8.78 ± 1.08 g, $P < 0.01$) (Figure 3D). In the TNBS-MSC group, bloody stools decreased and became solid, while food and water intake also improved. We assessed the colon length in these 3 groups on day 0, 1, 2, 3, 5, 7 and 9. On days 3-7, macroscopic findings in the colon in the TNBS-PBS group included severe shortening, and wine-colored tissue with bloody stools compared to the TNBS-MSC group (Figure 3B). On day 3, a significant difference in colon length was observed between the TNBS-MSC group and TNBS-PBS group (11.05 ± 0.31 cm *vs* 7.43 ± 0.33 cm, $P < 0.01$) (Figure 3A and G). This significant difference was also seen in the TNBS-PBS group compared with the control group (7.43 ± 0.33 cm *vs* 11.68 ± 1.01 cm, $P < 0.01$) (Figure 3A and G).

In addition, histological changes in TNBS-induced colitis were mainly observed in the colon-rectum with severity progressively less towards the proximal site. In the TNBS-MSC group, the histological colitis score significantly decreased compared with the TNBS-PBS group on day 3 (3.00 ± 0.82 *vs* 8.75 ± 0.96 , $P < 0.01$) (Figure 3C and H). BMSCs treatment reduced the extent of the inflamed area in the intestine. Compared with the TNBS-PBS group, TNBS-MSC reduced TNBS-induced crypt damage and infiltration of inflammatory cells composed mainly of neutrophils and macrophages (Figure 3H).

Expression of Ki67 and Lgr5 in colon tissues

To determine whether administration of BMSCs could promote proliferation and differentiation of intestinal

epithelial cells into ISCs, the expression of Ki67 (one of the markers of cell proliferation) and Lgr5 (one of the markers of ISCs), were examined by immunohistochemistry. Four days after transplantation of BMSCs, the expression of Ki67 and Lgr5 in damaged colonic tissues increased significantly compared with TNBS-PBS (Ki67: $12.09\% \pm 3.95\%$ *vs* $8.33\% \pm 3.1\%$, $P < 0.01$; Lgr5: $52.54 \pm 14.77 \times 10^3$ *vs* $30.00 \pm 8.08 \times 10^3$) (Figure 4).

BMSCs corrected the imbalance in T cell disorders in mice with TNBS-induced colitis. Since BMSCs are known to have *in vitro* immunosuppressive and anti-inflammatory properties^[29], we investigated the potential therapeutic effects of BMSCs in an *in vivo* experimental model of IBD induced by TNBS. In TNBS-induced colitis, Th1-Th17 cells were activated to promote an exaggerated macrophage and neutrophil infiltration, giving rise to a prolonged severe transmural inflamed intestinal mucosa and immune response, characterized by the production of inflammatory cytokines and their transcription factors^[30]. BMSCs can rapidly reduce inflammation by affecting the differentiation of T cells, such as promoting Th2 cells and enhancing regulatory T cell functions.

Transplanted MSCs affect immune responses systemically in IBD models

Cytokines are principal mediators of the systemic and local immune responses in immunological or inflammatory diseases. We examined the expression levels of 7 cytokines: IL-2, TNF- α , IFN- γ , IL-4, IL-10, IL-6, and IL-17. In acute TNBS-induced colitis, the level of proinflammatory cytokines (including IL-2, TNF- α , IFN- γ , IL-6, and IL-17) increased significantly ($P < 0.01$) when compared to the control group (Figure 5A-C, E and F). Following the transplantation of MSCs, the level of proinflammatory cytokines decreased significantly ($P < 0.01$), while the level of IL-4 and IL-10 increased ($P < 0.05$) when compared to the TNBS-PBS group (Figure 5A-G).

BMSCs inhibit the differentiation of Th1 cells in the gut in TNBS-induced colitis

The differentiation of Th1 lymphocytes is known to be associated with a specific transcription factor, T-bet, which is a key component regulating the expression of Th1 cytokines^[31]. The up-regulation of Th1 activities in TNBS-induced colitis and the reduction of Th1 activities after BMSCs transplantation were further confirmed by the analysis of T-bet expression using both real-time PCR and Western blotting (Figure 6D and H). In addition, we analyzed the expression of IL-2, IFN- γ , and TNF- α by real-time PCR. There was an increase in Th1 activities in acute TNBS-induced colitis (*vs* the control group) and a decrease in Th1 activities after treatment with BMSCs (*vs* the TNBS-PBS group) (Figure 6A-C). FACS analysis of IL-2, IFN- γ and TNF- α proteins (days 1-9) also showed the same trend. In the TNBS-PBS group, IL-2, IFN- γ and TNF- α expressions were significantly up-regulated (*vs* the control group, IL-2: 4.37 ± 0.27 pg/mL *vs* 2.30 ± 0.03 pg/mL, $P < 0.01$; IFN- γ : 4.82 ± 0.11 pg/mL *vs* 3.64 ± 0.39 pg/mL, $P < 0.01$; TNF- α : 32.45 ± 3.52 pg/mL *vs*

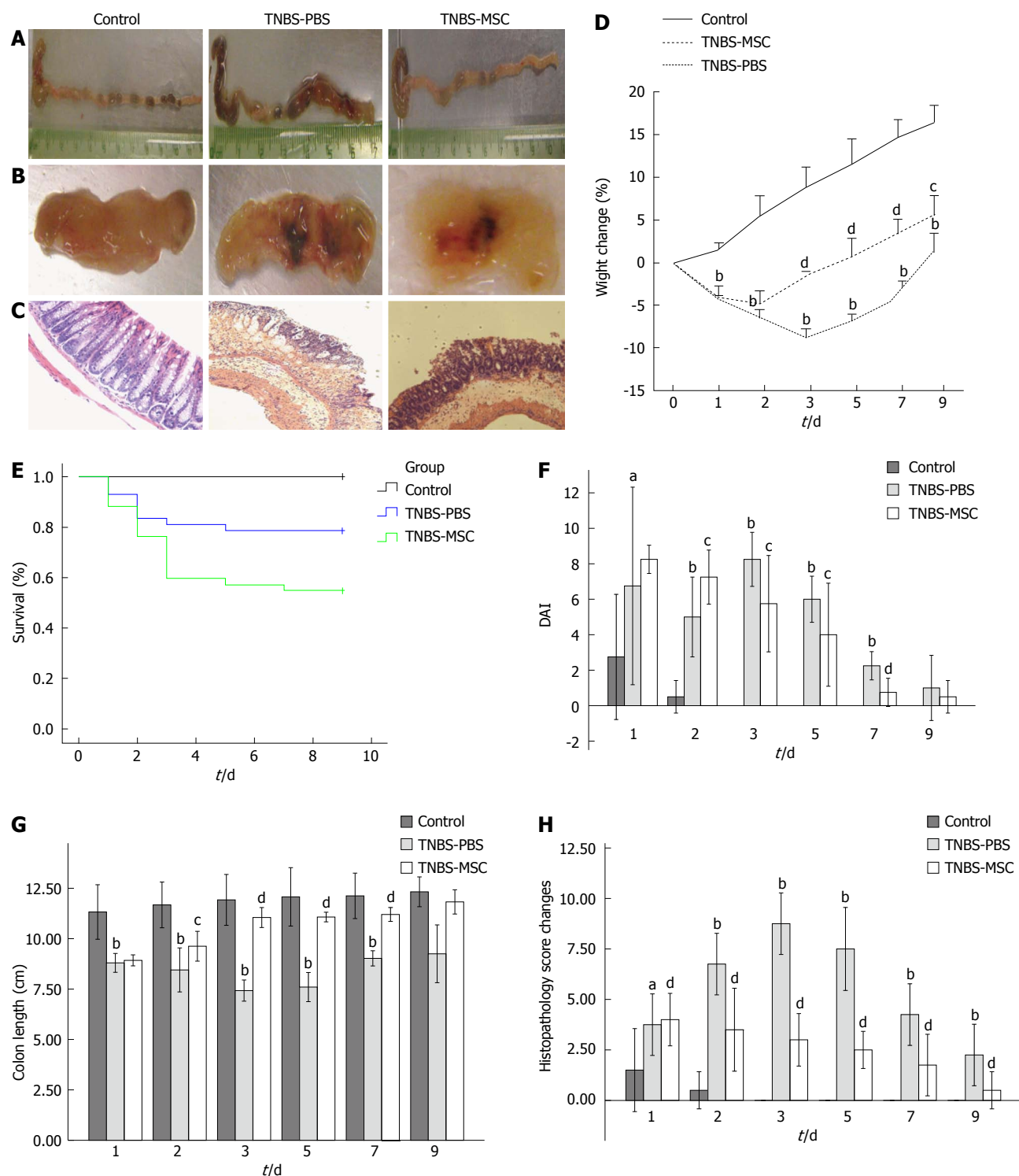


Figure 3 Therapeutic efficacy of bone marrow mesenchymal stem cells treatment. A: Colon length at day 3; B: Macroscopic colonic damage at day 3; C: Histopathologic colonic damage by hematoxylin and eosin staining at day 3 ($\times 100$); D-F: Clinical evolutions were monitored by body weight changes, colitis score and survival; G) Colon length changes; H: Histopathology score changes. ^a $P < 0.05$, ^b $P < 0.01$ vs control; ^c $P < 0.05$, ^d $P < 0.01$ vs TNBS-PBS. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered saline.

13.91 ± 0.94 pg/mL, $P < 0.01$). However, BMSCs treatment led to a distinct reduction in the above factors (*vs* the TNBS-PBS group, IL-2: 2.37 ± 0.20 pg/mL *vs* 2.87 ± 0.25 pg/mL, $P < 0.05$; IFN- γ : 3.71 ± 0.17 pg/mL *vs* 4.44 ± 0.07 pg/mL, $P < 0.01$; TNF- α : 13.12 ± 1.76 pg/mL *vs* 19.45 ± 0.82 pg/mL, $P < 0.01$) (Figure 6E-G).

BMSCs promote Th2 differentiation in the gut in TNBS-induced colitis

A possible role for BMSCs in promoting the Th2 subset was investigated by analyzing IL-4 and IL-10 production as well as the Th2 lineage transcription factor GATA3. There was no significant change in GATA3 (day 3) ex-

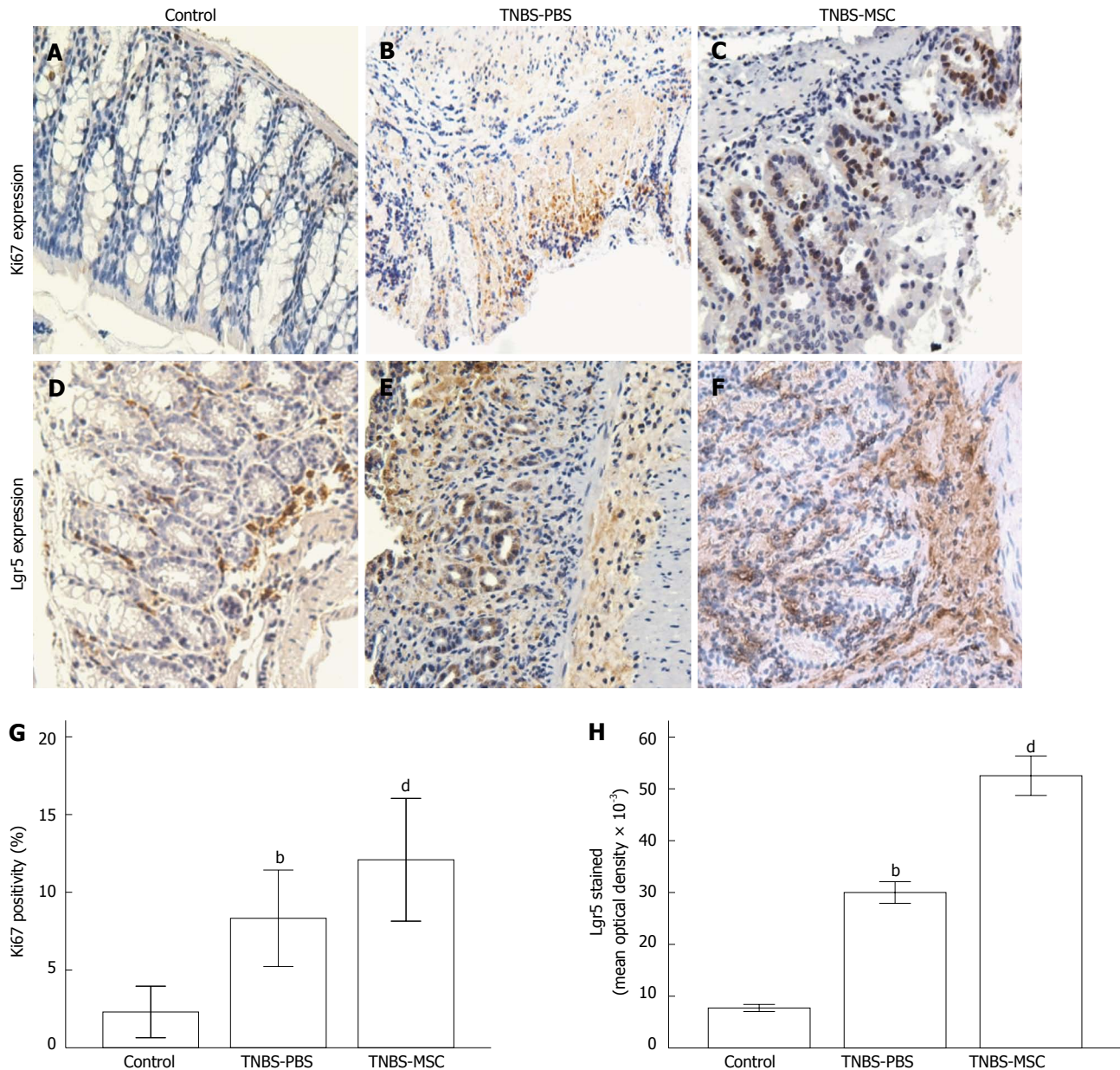


Figure 4 Immunohistochemistry of Ki67 and Lgr5 in colon tissues ($\times 200$). A-C: Ki67; D-F: Lgr5; G, H: Five days after MSCs transplantation, Ki67 and Lgr5 expressions were significantly increased (vs control, ^b $P < 0.01$; vs TNBS-PBS, ^d $P < 0.01$). Ki67 staining was evaluated by positivity, and Lgr5 expression was represented by mean optical density. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered

pression in the TNBS-PBS group, however, GATA3 expression was increased in the TNBS-MSC group confirmed by either real-time PCR or Western blotting (Figure 7C and F). IL-4 and IL-10 were also up-regulated at both the mRNA and protein levels in the TNBS-MSC group, while no significant changes were observed in TNBS-induced colitis (Figure 7A and B). The expression of IL-4 and IL-10 at the protein level (days 1-9) was further determined by FACS analysis and showed a similar pattern to that of the mRNAs. In the TNBS-MSC group, IL-4 and IL-10 were up-regulated (vs the TNBS-PBS group, IL-4: 2.15 ± 0.16 pg/mL vs 1.90 ± 0.15 pg/mL, $P < 0.05$; IL-10: 25.93 ± 0.63 pg/mL vs 19.09 ± 2.85 pg/mL $P < 0.01$). There were no significant changes in IL-4 and IL-10 in the TNBS-PBS group (vs the control group,

$P > 0.05$) (Figure 7D and E).

BMSCs inhibit Th17 cell differentiation in the gut of mice with TNBS-induced colitis

Th17 cell differentiation requires at least two cytokine signals that transmit through the ROR γ t and Smad-dependent signaling pathways^[32]. Real-time PCR and Western blot results of ROR γ t expression at day 7 revealed that BMSCs clearly inhibited the differentiation of pathogenic Th17 effector cells (Figure 8C and F). In this study, the expressions of IL-6 and IL-17 were significantly up-regulated in the TNBS-PBS group, but were down-regulated in the TNBS-MSC group shown by real-time PCR (Figure 8A and B). The protein expressions of IL-6 and IL-17 (days 1-9) were further determined by FACS and the re-

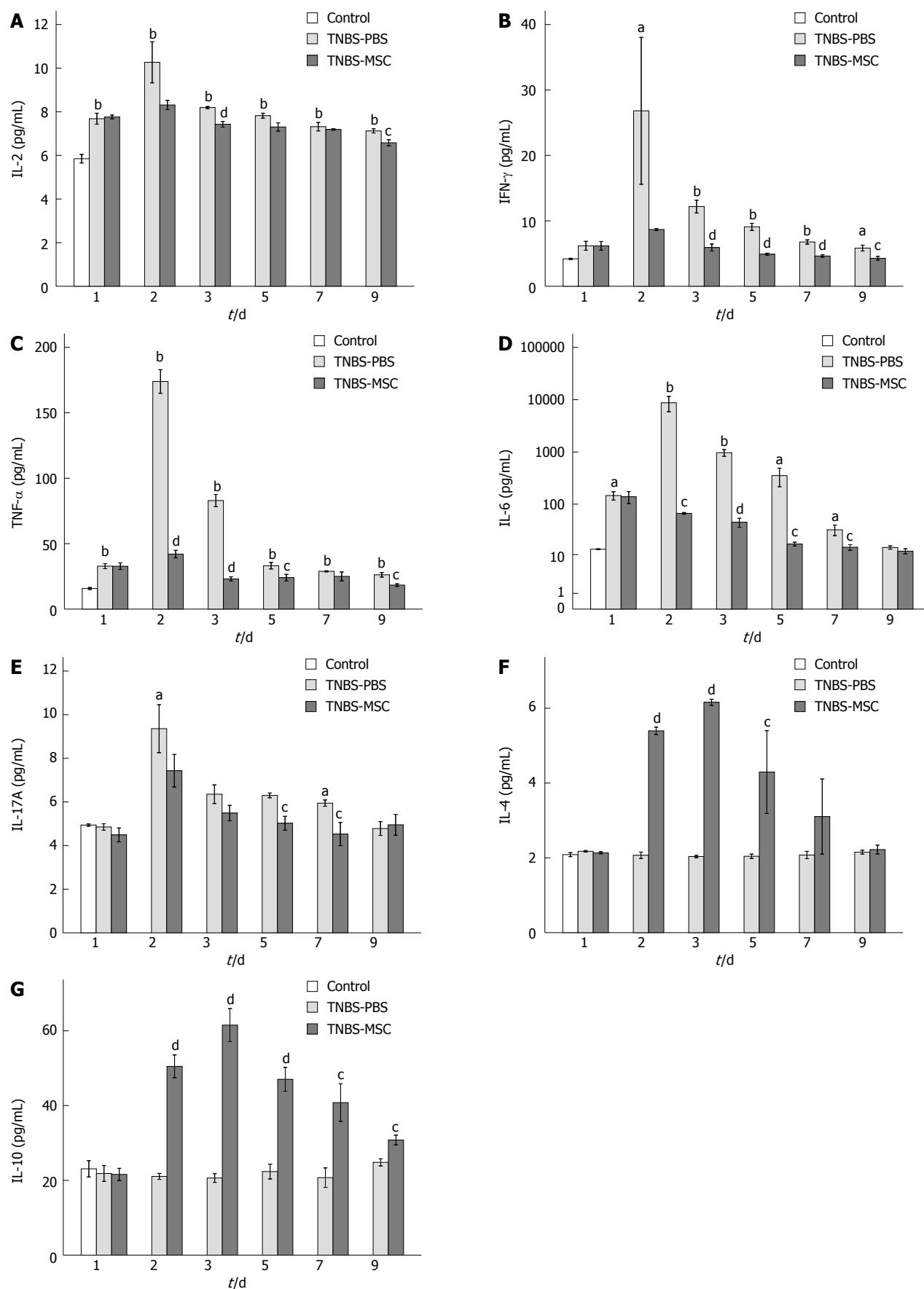


Figure 5 Levels of 7 cytokines were measured simultaneously from mouse serum using Fluorescence Activated Cell Sorting. A-E: TNBS-induced colitis exhibits heightened Th1-Th17 response of a complex, dynamic cytokine network [interleukin (IL)-2, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IL-6, IL-17], and MSCs maintain the balance of T-lymphocytes. F, G: In the TNBS-MSC group, transplanted MSCs promote T-lymphocytes to secrete cytokines (IL-4, IL-10) which regulate their functions. ^a $P < 0.05$, ^b $P < 0.01$ vs control; ^c $P < 0.05$, ^d $P < 0.01$ vs TNBS-PBS. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered saline.

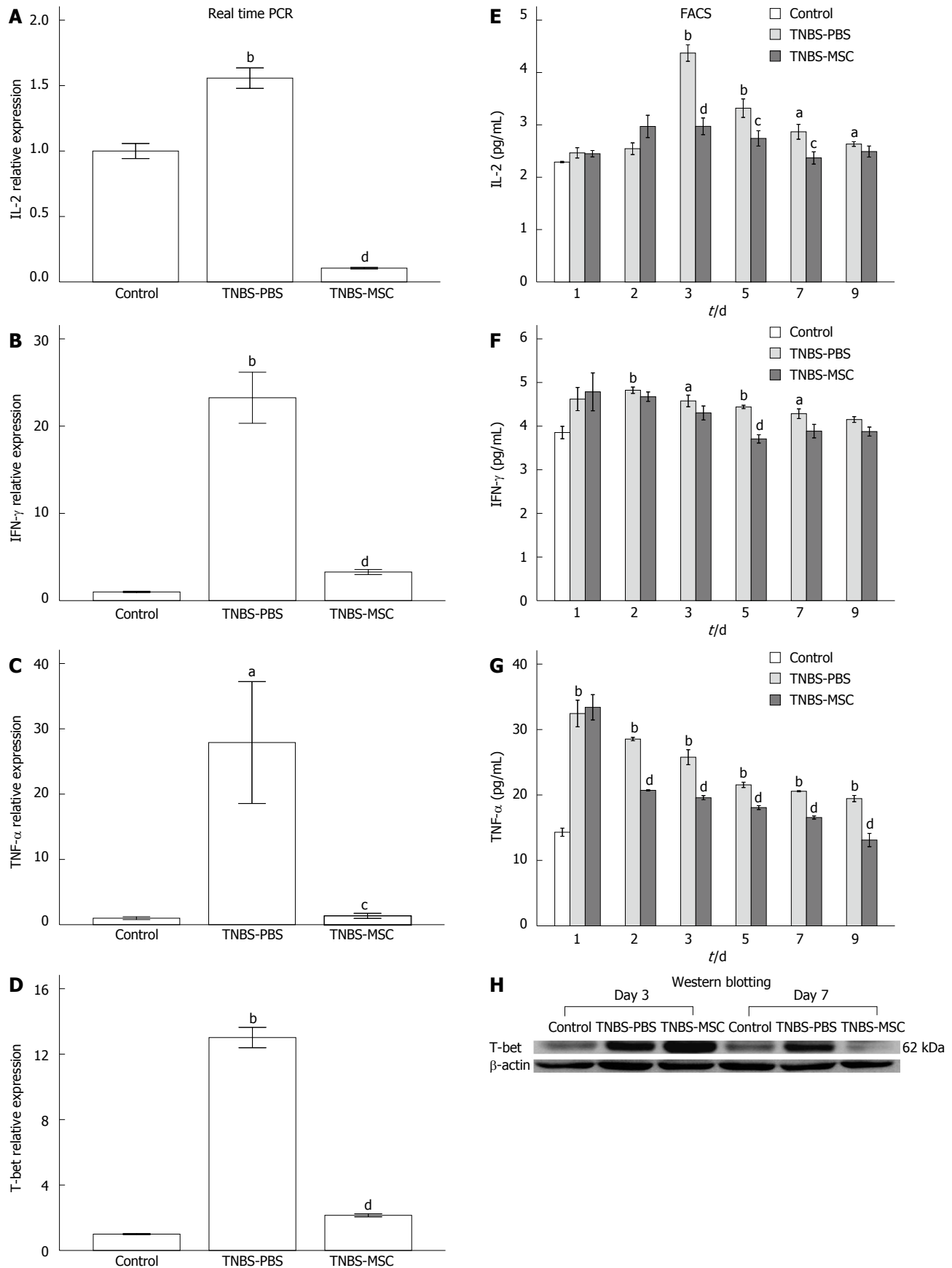


Figure 6 Changes in mRNA expression and colonic protein levels of Th1-related inflammatory mediators following treatment with bone marrow mesenchymal stem cells. A-D: Real-time polymerase chain reaction. Acute TNBS colitis demonstrated a cytotoxic and chemotactic profile with significantly elevated mRNA expressions of interleukin (IL)-2, interferon (IFN)- γ , tumor necrosis factor (TNF)- α and T-bet when compared to controls. Preventive treatment with BMSCs reduced the levels of the above cytokines; E-G: Flow cytometry. The changes in colonic protein levels of IL-2, IFN- γ and TNF- α followed the same trend; H: Western blotting. The changes in colonic protein levels of T-bet followed the same trend. ^a $P < 0.05$, ^b $P < 0.01$ vs control; ^c $P < 0.05$, ^d $P < 0.01$ vs TNBS-PBS. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered saline.

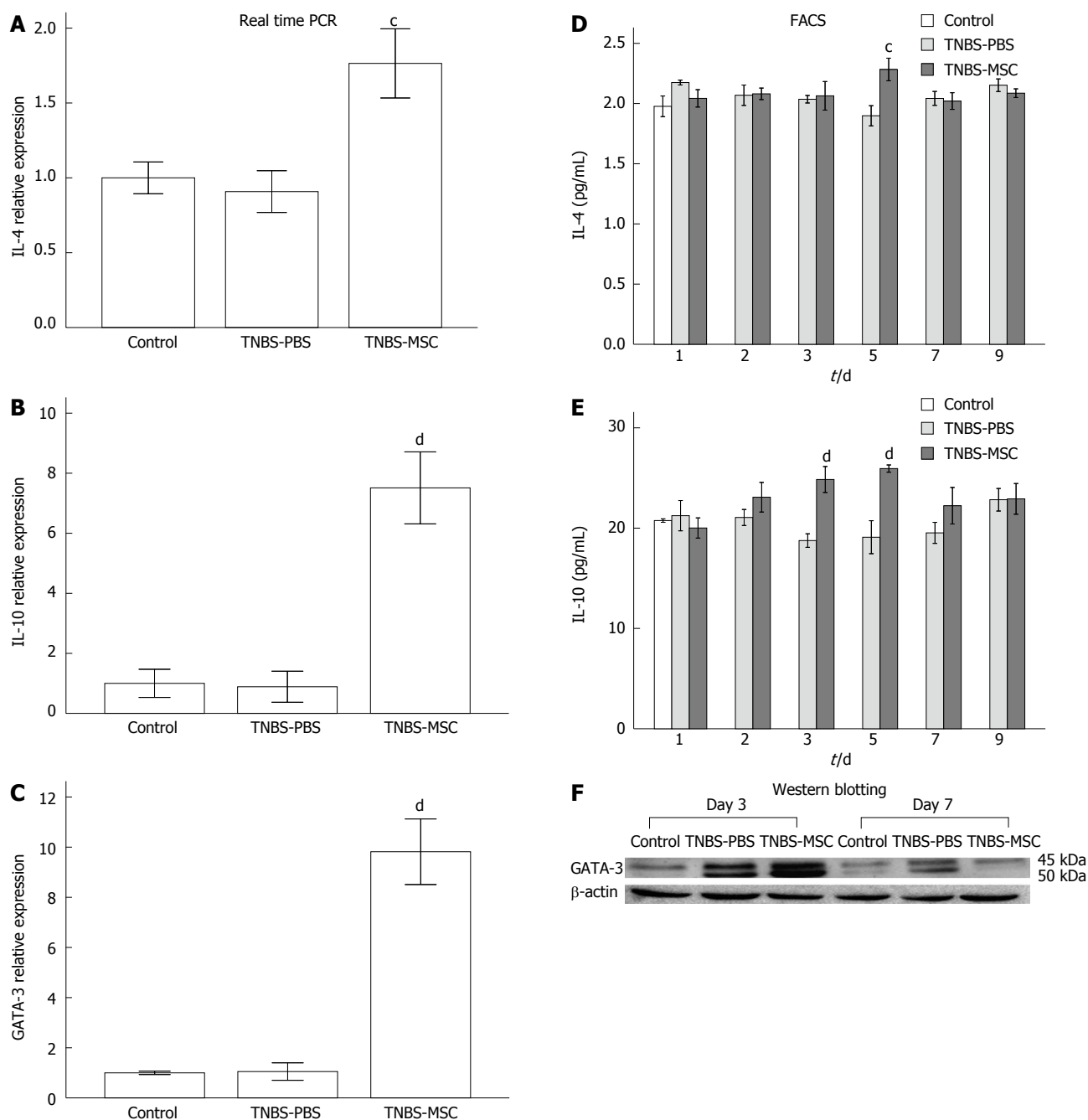


Figure 7 Changes in mRNA expression and colonic protein levels of Th2-related inflammatory mediators following treatment with bone marrow mesenchymal stem cells. A-C: Real-time polymerase chain reaction. No significant differences in mRNA expressions of interleukin (IL)-4, IL-10 and GATA family of transcription factors 3 (GATA-3) were observed when compared to controls ($P > 0.05$). After treatment with bone marrow mesenchymal stem cells, the mRNA expressions of IL-4, IL-10 and GATA-3 were elevated. D, E: Flow cytometry. Colonic protein levels of IL-4 and IL-10 followed the same trend; F: Western blotting. Colonic protein level of GATA-3 followed the same trend. ^a $P < 0.05$, ^b $P < 0.01$ vs control; ^c $P < 0.05$, ^d $P < 0.01$ vs TNBS-PBS. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered saline.

sults showed the same trend as their mRNA expressions. In the TNBS-PBS group, IL-6 and IL-17A expressions were significantly up-regulated (*vs* the control group, IL-6: 14.52 ± 0.96 pg/mL *vs* 3.80 ± 0.52 pg/mL, $P < 0.01$; IL-17A: 4.97 ± 0.19 pg/mL *vs* 3.61 ± 0.29 pg/mL, $P < 0.01$). However, BMSCs treatment led to a distinct reduction in the above factors (*vs* the TNBS-PBS group, IL-6: 4.22 ± 0.40 pg/mL *vs* 7.50 ± 0.51 pg/mL, $P < 0.05$; IL-17A: 2.87 ± 0.21 pg/mL *vs* 4.54 ± 0.14 pg/mL, $P < 0.01$) (Figure 8D and E).

BMSCs enhance regulatory T cell functions

The suppressor cytokines IL-10 and TGF- β are also produced by CD4⁺Foxp3⁺ Tregs which are involved in the control of colitis as indicated in recent investigations^[10,33]. BMSCs significantly up-regulated IL-10 as well as TGF- β levels ($P < 0.01$, Figure 7B, E, Figure 9A and C). The expression of Foxp3 also increased in the TNBS-MSC group when compared to the TNBS-PBS group ($P < 0.01$, Figure 9B and D). However, the production of IL-10, TGF- β and Foxp3 using real-time PCR and Western

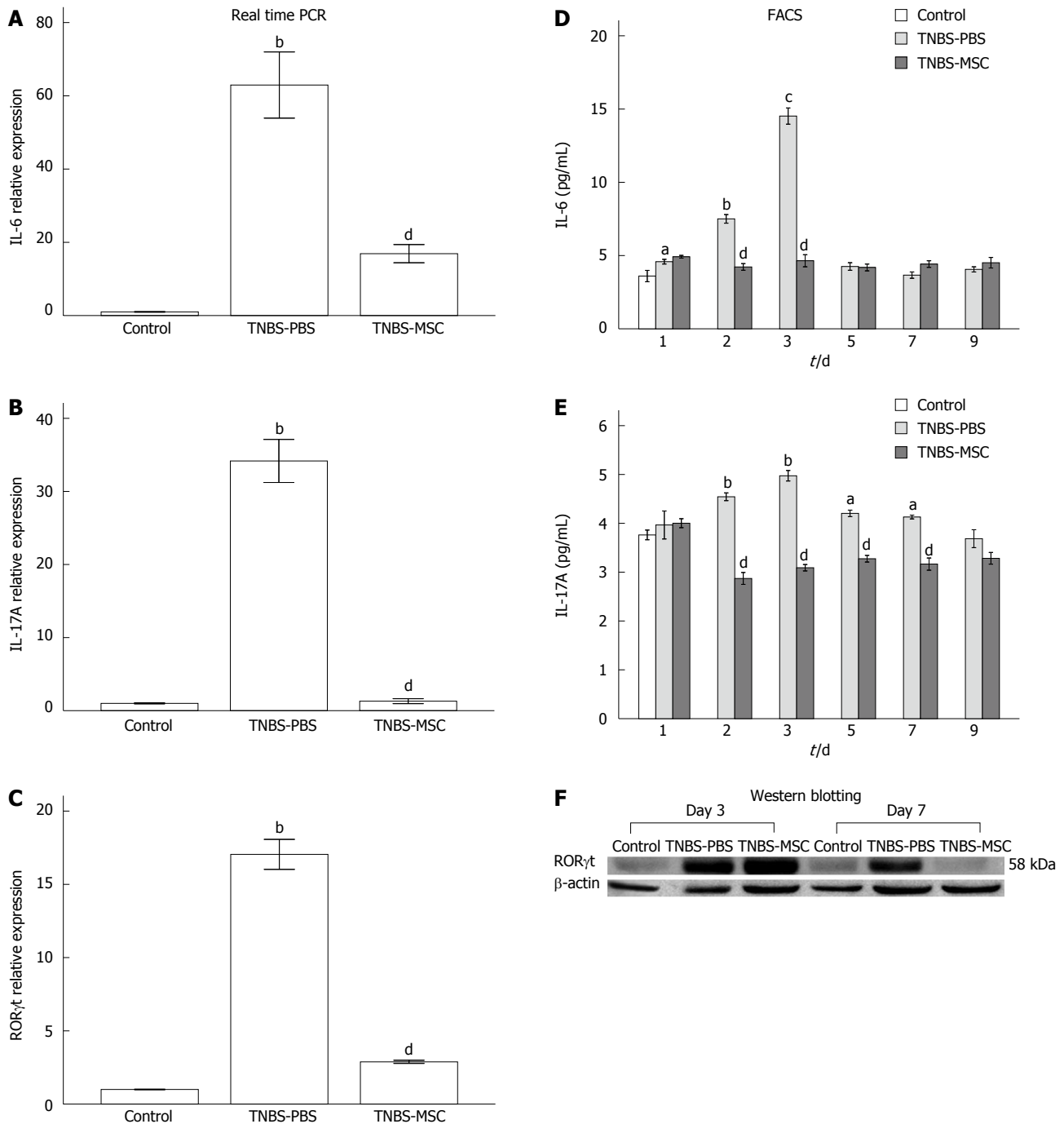


Figure 8 Changes in mRNA expression and colonic protein levels of Th17-related inflammatory mediators following treatment with bone marrow mesenchymal stem cells. A-C: Real-time polymerase chain reaction. The results showed a significant increase in mRNA expressions of interleukin (IL)-6, IL-17A and retinoid related orphan receptor gamma(t) (RORγt) when compared to controls ($P < 0.01$). After treatment with bone marrow mesenchymal stem cells, the levels of the above cytokines were reduced ($P < 0.01$); D, E: Flow cytometry. The same changes in colonic protein levels of IL-6 and IL-17A were further confirmed; F: Western blotting. The same changes in colonic protein levels of RORγt were further confirmed. ^a $P < 0.05$, ^b $P < 0.01$ vs control; ^c $P < 0.05$, ^d $P < 0.01$ vs TNBS-PBS. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered saline.

blotting showed no changes in the TNBS-MSC group compared with the control group ($P > 0.05$, Figure 7B and E and Figure 9A-D).

DISCUSSION

Kashyap *et al.*^[34] reported a patient with non-Hodgkin lymphoma and CD who was maintained for more than 7 years in clinical remission after autologous hematopoietic stem cell transplantation.

Since then, more and more studies have focused on the use of stem cell therapy in IBD. The results from our study were consistent with previous reports, which demonstrated that intravenously transplanted BMSCs may home to injured intestinal tissues, promote intestinal cell regeneration, ameliorate the inflammatory symptoms in an experimental IBD mouse model and increase survival^[35,36]. However, our study also identified the following: (1) We showed the differentia-

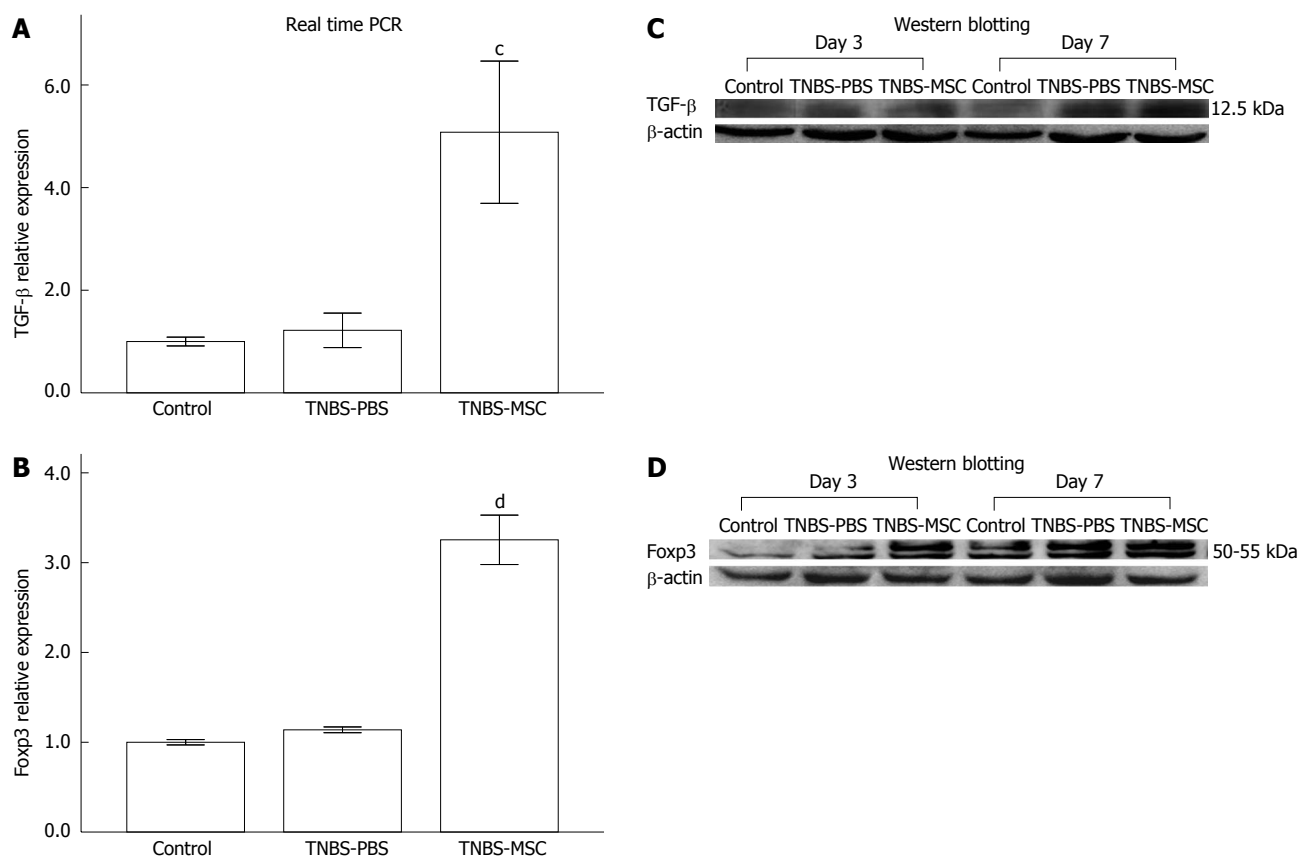


Figure 9 Changes in mRNA expression and colonic protein levels of Tregs-related inflammatory mediators following treatment with bone marrow mesenchymal stem cells. A, B: Real-time polymerase chain reaction. No significant changes in mRNA expressions of transforming growth factor (TGF)- β and Foxp3 were observed when compared to controls ($P > 0.05$). While the mRNA expressions of TGF- β and Foxp3 increased after BMSCs transplantation ($P < 0.01$); C, D: Western blotting. The colonic protein level of TGF- β and Foxp3 were confirmed to show the same results as real-time polymerase chain reaction. ^c $P < 0.05$, ^d $P < 0.01$ vs TNBS-PBS. TNBS-MSC: Trinitrobenzene sulfonic acid-mesenchymal stem cells; TNBS-PBS: Trinitrobenzene sulfonic acid-phosphate-buffered saline.

tion of ISCs in injured gut by detecting Lgr5 positive cells; (2) We examined the expression levels of Th1-Th2-Th17-Tregs-related inflammatory and regulatory cytokines in peripheral blood and in local intestinal tissues; (3) We found a Th2 shift and subsequent correction of imbalanced Th17/Tregs; (4) Master regulators of Th1, Th2, Th17 and Tregs were detected in BMSCs-treated TNBS-induced colitis; and (5) We showed that the signaling pathways of Th1/T-bet, Th2/GATA-3, Th17/ROR γ t and Tregs/Foxp3 may serve as important immunoregulators in the correction of immune disorders and can enhance the healing of injured intestinal mucosa.

In our study, we established a mouse model of TNBS-induced colitis and transplanted BMSCs into the mice. On day 1, 2, 3, 5, 7 and 9 after transplantation, the mice were sacrificed. Moreover, 48 h after BMSCs transplantation, we found that a small proportion of the infused BMSCs had homed to the inflammatory or injured tissues by observing GFP and detecting *Sry* gene (only located on the Y chromosome). In addition, we investigated the role BMSCs played in TNBS-induced colitis at different time points by assessing the expression of factors, pathological changes, and clinical symptoms. We found that BMSCs had a strong therapeutic effect on TNBS-induced colitis. We also detected the expression of Ki67 (one of the markers of cell proliferation) and Lgr5 (an

intestinal stem cell marker) using immunohistochemistry. The significantly increased expression of Ki67 and Lgr5 after BMSCs transplantation suggests that these BMSCs in recipient colonic tissues may have trans-differentiated into ISCs. Taken together, we showed that there are two possible therapeutic mechanisms of BMSCs treatment. First, some of these BMSCs could directly differentiate into pluripotent stem cells, such as ISCs. Second, MSCs could secrete cytokines or chemokines to influence the imbalanced intestinal micro-environment and stimulate the tissue-specific ISCs to proliferate. However, these experimental findings might be an indirect consequence of the general improvement in tissue regeneration of acute colitis mediated by BMSCs and the detailed mechanism involved requires further clarification.

The mechanism could be as follows: First, when BMSCs were intravenously transplanted into the experimental animal model, they first circulated around the whole body through blood circulation. During this process, BMSCs secrete related cytokines to activate the systemic immune system. Second, these BMSCs migrated, scatter implanted and survived within the injured gut mucosa and subsequently reversed the imbalance in Th1/Th2/Th17/Tregs, which is important in maintaining the intestinal mucosa microenvironment.

In IBD, the ratio of pro-inflammatory (IL-2, TNF- α ,

IFN- γ , IL-6 and IL-17) and anti-inflammatory cytokines (IL-4 and IL-10) are imbalanced systematically. In our study, we found that intravenously transplanted MSCs were able to modulate the release and/or expression of pro-inflammatory cytokines in the serum by effectively inducing remission and promoting the release and/or expression of anti-inflammatory cytokines in the serum to balance inappropriate immune system disorders systematically. These appropriate responses would finally slow down and/or reverse the natural course of the disease and even prevent complications such as fistulae and colorectal cancer.

Little work has been carried out on how BMSCs display their immune regulatory and anti-inflammatory properties in damaged colonic tissues. According to previous investigations, BMSCs can efficiently suppress the proliferative response of T cells *in vitro*^[37,38]. Our study showed that the complex immune-modulating effects of GFP-BMSCs may result from the differential down-regulation of proinflammatory signaling of Th1 and Th17 lymphocytes as assessed by analysis of IL-2, TNF- α , IFN- γ (Th1-related) and IL-6, IL-17 (Th17-related), and the up-regulation of anti-inflammatory signaling of Th2 activities with IL-4 and IL-10. This also led to a moderate induction of induced/activated regulatory T cells (IL-10, TGF- β), which may influence Th1 and Th17 cell function. Naive T helper cells (Th0) can be induced to differentiate into Th1, Th2, Th17 and regulatory (Treg) phenotypes based on the mode of stimulation, antigen concentration, co-stimulation and cytokine milieu^[39]. These factors exert their functions by cross-regulating one another and are selectively expressed in the corresponding cell populations^[31,40]. Our experiments showed that when MSCs migrated into the inflamed colon, they began to produce cytokines, chemokines, growth factors and adhesion molecules by themselves or promoted intestinal lymphocytes to regulate the inappropriate inflammatory responses.

In this study, we found significant inhibition of the production of Th1-cytokines such as IL-2, TNF- α , IFN- γ and the Th1-specific transcription factor, T-bet, using real-time PCR, FACs and/or Western blotting, suggesting that MSCs can alter the inflammatory process by down-regulating Th1-driven autoimmune and inflammatory responses. We also found increased expression of IL-4 and GATA-3 using the same methods. In conjunction with the changes in Th1-cytokines, this showed that MSCs may shift the pathways of differentiation towards Th1 and Th2 cells with IL-4 signaling. This is possibly due to the fact that T-bet expression inhibits GATA-3 activity and Th2 cytokines block the differentiation of Th1 cells^[41,42]. In this study, the scenarios are very complex. (1) The transplanted MSCs had their own effect in secreting cytokines; (2) Endogenous MSCs can have similar or different effects; (3) The transplanted or endogenous MSCs may migrate to inflamed tissues and recruit Th1/Th2/Th17/Tregs; (4) The transplanted or endogenous MSCs may secrete cytokines/chemokines/growth factors and exert remote effects; (5) Transplanted or endog-

enous MSCs may affect local ISCs and change the local environment; and (6) Transplanted MSCs could recruit endogenous MSCs or ISCs to the local colon; and even more complex, the combination of all of them or some of them.

In addition to suppressive effects on Th1-activated immune and inflammatory responses, there is ample evidence from our experiments to suggest that MSCs also mediate the modulation of Th17-cytokines such as IL-17 and IL-6 to ameliorate TNBS-induced colitis. Our data showed high mRNA and protein levels of IL-17, ROR γ t and IL-6 in colonic tissue, but decreased levels in the MSCs-treated group. The reason for this may be that Th17 cells, characterized by expression of IL-17 (also known as IL-17A), differentiate from naive T helper cells in the presence of IL-6 and TGF- β ^[43]. ROR γ t is an essential factor for Th17 differentiation^[43]. Previous experiments showed that TGF- β and IL-6 enhanced ROR γ t mRNA expression in naive T cells, which in turn induced IL-17A expression. We also demonstrated that the mRNA and protein levels of IL-10, TGF- β and Foxp3 were increased after administration of MSCs. This indicated that MSCs could restore the balance between Th17 cells and CD4⁺CD25⁺Foxp3⁺ Treg in the intestinal tissues. Foxp3 directly interacts with ROR γ t to inhibit its function, resulting in decreased IL-17 expression^[44,45]. IL-10 produced by regulatory subsets of T cells exerts a variety of anti-inflammatory and immunoregulatory functions linked with Foxp3 *in vivo*^[46].

In conclusion, we have shown that BMSCs have a possible therapeutic effect in Th1- or Th17-driven IBD, including (1) homing to and surviving in the injured location; (2) exerting an immunoregulatory effect and controlling inflammation systematically; (3) accelerating colon mucosa regeneration; and (4) orchestrating a shift from Th1 and Th17 toward Th2 and the enhanced activities of Tregs to suppress local inflammation in colon tissues. Thus, exogenous MSCs transplantation is a novel therapeutic strategy for human IBD. However, for further clinical application, there are some unresolved questions owing to the complexity of human IBD. These include: (1) When is the best time for BMSCs treatment? (2) What dosage of BMSCs should be used to achieve optimal therapeutic results with least side effects? and (3) Should a combination of other drugs be used?

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COMMENTS

Background

Inflammatory bowel disease (IBD) is a chronic disease characterized by severe

T-helper cell-driven inflammation and immune disorder. Mounting evidence suggests that mesenchymal stem cells (MSCs) have properties including low immunogenicity, immunomodulation and anti-inflammatory activity both *in vitro* and *in vivo*, and especially regulate T-cell responses. However, the molecular mechanisms involved in these effects are still unclear. Therefore, we transplanted MSCs into an experimental model of IBD to investigate their potential therapeutic effects *in vivo*.

Research frontiers

MSCs can release soluble factors (cytokines, chemokines, and growth factors) which result in cell cycle arrest in pro-inflammatory lymphocytes and induce T cell apoptosis. Many studies have shown that these cells are a potential treatment for autoimmune diseases. However, there have been few reports on the roles of MSCs in IBD and the molecular mechanisms of MSCs in alleviating IBD.

Innovations and breakthroughs

The results demonstrated changes in Th1-Th2-Th17-Tregs-related inflammatory and immune cytokine expressions and master regulators of these immune cells in serum and local intestinal tissues after MSCs transplantation. The authors found that MSCs resulted in a Th2 shift and correction of the imbalanced Th17/Tregs to enhance the healing of injured intestinal mucosa.

Applications

This data will contribute to future research on the immunomodulatory properties of MSCs and support a rationale for the clinical application of stem cell therapy in IBD.

Terminology

T-bet is a T-isolated box gene family of transcription factors, which is selectively expressed on Th1 cells. Retinoic acid-related orphan receptor γ t (ROR γ t) is the orphan nuclear receptor that regulates the development of Th17 cells. GATA family of transcription factors 3 (GATA3) plays a central role in Th2 differentiation. Forkhead box P3 (Foxp3) acts as a master switch governing the development and function of CD4⁺ regulatory T cells.

Peer review

In this manuscript, the authors studied the influence of bone marrow stem cells on colitis. This is an interesting study showing the mechanisms by which MSCs attenuate colitis. However, the direct contribution of transplanted MSCs in Ki67⁺ proliferation assay and the data showing an increase in interleukin (IL)-4, IL-10, tumor growth factor- β and Foxp3 in inflamed tissue is not expressed in transplanted MSCs, but in infiltrating immune cells.

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Probiotic supplementation decreases intestinal transit time: Meta-analysis of randomized controlled trials

Larry E Miller, Arthur C Ouwehand

Larry E Miller, Miller Scientific Consulting, Inc., Arden, NC 28704, United States

Arthur C Ouwehand, DuPont Nutrition and Health, FIN-02460 Kantvik, Finland

Author contributions: Miller LE and Ouwehand AC contributed equally to this work; Miller LE designed the research; Miller LE analyzed the data; Miller LE and Ouwehand AC performed the research, wrote the paper, and approved the final draft of the paper.

Correspondence to: Larry E Miller, PhD, Miller Scientific Consulting, Inc., 26 Portobello Road, Arden, NC 28704, United States. larry@millerscientific.com

Telephone: +1-928-6079657 Fax: +1-928-2683563

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Abstract

AIM: To determine the efficacy of probiotic supplementation on intestinal transit time (ITT) and to identify factors that influence these outcomes.

METHODS: A systematic review of randomized controlled trials (RCTs) of probiotic supplementation that measured ITT in adults was conducted by searching MEDLINE and EMBASE using relevant key word combinations. Main search limits included RCTs of probiotic supplementation in healthy or constipated adults that measured ITT. Study quality was assessed using the Jadad scale. A random effects meta-analysis was performed with standardized mean difference (SMD) of ITT between probiotic and control groups as the primary outcome. Meta-regression and subgroup analyses were conducted to examine the impact of moderator variables on ITT SMD.

RESULTS: A total of 11 clinical trials with 13 treatment effects representing 464 subjects were included in this analysis. Probiotic supplementation was associated with decreased ITT in relation to controls, with an SMD

of 0.40 (95%CI: 0.20-0.59, $P < 0.001$). Constipation ($r^2 = 39\%$, $P = 0.01$), higher mean age ($r^2 = 27\%$, $P = 0.03$), and higher percentage of female subjects ($r^2 = 23\%$, $P < 0.05$) were predictive of decreased ITT with probiotics in meta-regression. Subgroup analyses demonstrated statistically greater reductions in ITT with probiotics in subjects with *vs* without constipation and in older *vs* younger subjects [both SMD: 0.59 (95%CI: 0.39-0.79) *vs* 0.17 (95%CI: -0.08-0.42), $P = 0.01$]. Medium to large treatment effects were identified with *Bifidobacterium Lactis* (*B. lactis*) HN019 (SMD: 0.72, 95%CI: 0.27-1.18, $P < 0.01$) and *B. lactis* DN-173 010 (SMD: 0.54, 95%CI: 0.15-0.94, $P < 0.01$) while other single strains and combination products yielded small treatment effects.

CONCLUSION: Overall, short-term probiotic supplementation decreases ITT with consistently greater treatment effects identified in constipated or older adults and with certain probiotic strains.

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Key words: Constipation; Gastrointestinal; Intestinal transit time; Meta-analysis; Probiotics

Core tip: Clinical trials of probiotics for gut health often utilize intestinal transit time (ITT) as a measure of clinical success although treatment effects are not consistent across studies. We performed the first systematic review and meta-analysis of randomized controlled trials to investigate the efficacy of probiotic supplementation on ITT in adults and to identify factors that influence these outcomes. Overall, short-term probiotic supplementation decreases ITT with consistently greater treatment effects identified in constipated or older adults and with certain probiotic strains.

Miller LE, Ouwehand AC. Probiotic supplementation decreases intestinal transit time: Meta-analysis of randomized controlled

trials. *World J Gastroenterol* 2013; 19(29): 4718-4725 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4718.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4718>

INTRODUCTION

Functional gastrointestinal (GI) disorders are symptom-based conditions that are not explained by definable structural or biochemical causes^[1]. The prevalence of at least one functional GI disorder in the last 3 mo has been reported to be as high as 69% in the general population^[2]. Slow intestinal transit is a common symptom of functional GI disorders, particularly those involving the bowel^[3]. Therapies intended to ameliorate GI-related symptoms by decreasing intestinal transit time (ITT), such as laxatives, are a mainstay treatment of slow-transit bowel disorders although no known therapy is highly efficacious, safe, and cost effective^[4].

Probiotics are live micro-organisms that confer a health benefit on the host when administered in adequate dosages^[5], which have been extensively studied for treatment of functional GI disorders^[6,7]. Additionally, there is speculation that probiotics may even improve gut health in healthy adults. For example, the European Food Safety Authority (EFSA) guidance on health claims related to gut function states that reduced ITT may be considered a beneficial physiological effect in the non-diseased general population, provided that diarrhea does not develop^[8]. Consequently, ITT often serves as a primary study endpoint in probiotic clinical trials of gut health.

Based on the recent emphasis in this study endpoint in clinical trials and because accurate estimates of ITT effect size are mandatory for performing power calculations and estimating sample size in clinical trials, we performed the first systematic review and meta-analysis on the efficacy of probiotic supplementation on ITT in adults.

MATERIALS AND METHODS

The main objective of this systematic review and meta-analysis of RCTs was to assess the efficacy of probiotic supplementation on ITT in adults. The PRISMA Statement for reporting systematic reviews and meta-analyses served as a template for this report^[9].

Eligibility criteria and information sources

Studies that were eligible for consideration in this systematic review were RCTs published in English-language journals and indexed in MEDLINE or EMBASE with no date restrictions on the effects of probiotic supplementation on ITT in adults. The following search terms were used for probiotic supplementation (with “*” characterizing a wildcard and “OR” being used as a Boolean function): probiotic*; lactobacill*; bifidobacteri*; yogurt; yoghurt; fermented milk. The following search terms were used for ITT: gastrointestinal; transit; gut; motility;

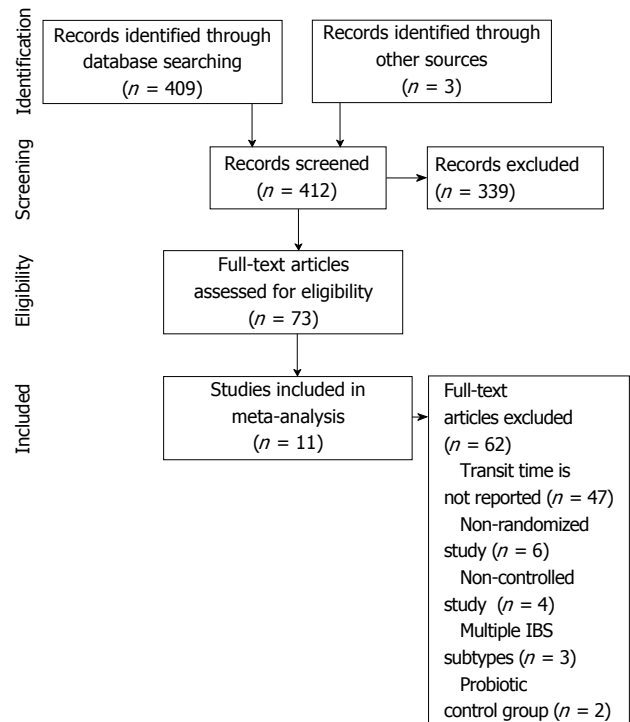


Figure 1 PRISMA flow diagram. IBS: Irritable bowel syndrome.

colonic; constipation; irritable bowel. To identify clinical trials, we applied the filters Clinical Trial or Randomized Controlled Trial. The results of each of the three sections were combined by utilizing the “AND” Boolean. In addition, we attempted to identify additional studies by hand-searching references of included studies and relevant review articles.

Study selection

One reviewer (Miller LE) initially assessed study eligibility. Titles and abstracts were screened to exclude all manuscripts published in non-English journals. Next, review articles, commentaries, letters, and case reports were excluded. We also excluded obviously irrelevant articles. Lastly, we excluded studies of subjects where ITT reduction was undesirable or uninterpretable (*i.e.*, subjects with diarrhea or cohorts with multiple IBS subtypes). Full-text of the remaining manuscripts was retrieved and reviewed. Publications that failed to report ITT or that described non-randomized, non-controlled, or otherwise irrelevant studies were excluded. The last search was performed in December 2012.

Data collection process

Data were extracted and entered into a pre-designed database by one reviewer (Miller LE) and the entries were checked by the other reviewer (Ouweland AC). Disagreements were settled by consensus.

Data items

The following variables were recorded in a pre-designed database: general manuscript information (author, in-

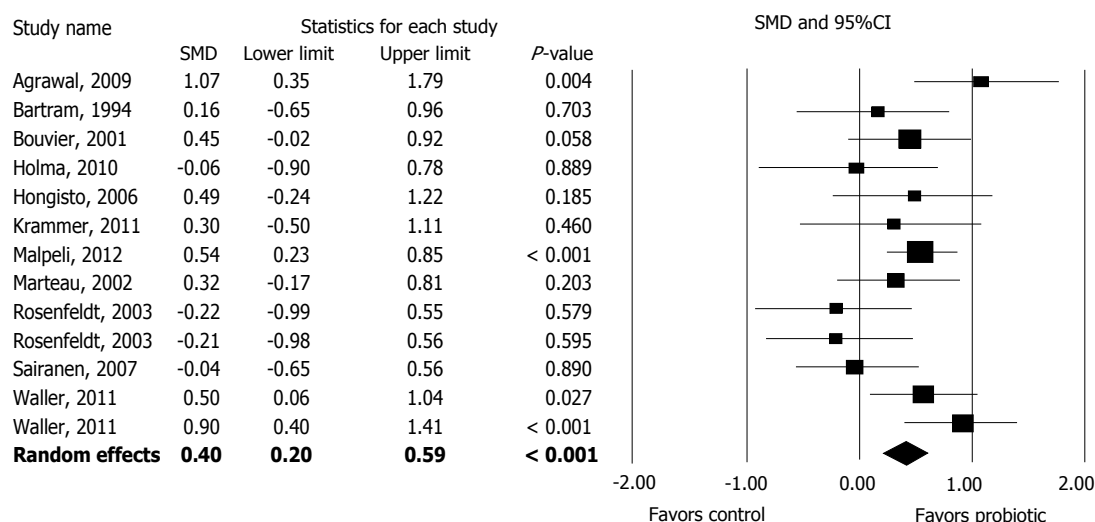


Figure 2 Forest plot of standardized mean difference in intestinal transit time across studies. Random effects model. $I^2 = 29\%$, $P = 0.15$. SMD: Standardized mean difference.

stitution name and location, journal, year, volume, page numbers), study design characteristics (study quality, study design, sample size, method of IIT assessment, probiotic strain, daily dosage, product delivery method, and treatment duration), subject characteristics (age, gender, body mass index, and condition), and IIT before and after probiotic supplementation.

Quality assessment

We used the Jadad scale to assess study quality of RCTs^[10]. Studies were scored according to the presence of three key methodological features: randomization, blinding and subject accountability. Randomization was scored from 0 to 2 with 2 implying appropriate methods of randomization were described, 1 if the study was merely described as “randomized”, and 0 when no details were provided to evaluate randomization. A score of 0 was given if the study was described as randomized, but the method of randomization was clearly inappropriate. Similarly, blinding was scored from 0 to 2 with 2 points awarded if subjects and investigators were blinded using appropriate methods, 1 point if the study was described merely as blinded, and 0 points if the study was described as blinded, but the method of blinding was clearly inappropriate. Subject accountability was scored 0 or 1 with 1 point awarded if all subjects were accounted for in the analysis and reasons for withdrawals were provided. A score of 0 was given when information regarding withdrawals was incomplete. *A priori*, studies with a Jadad score of 3 to 5 were deemed higher quality and those with a score of 0 to 2 were classified as lower quality.

Statistical analysis

A random effects meta-analysis model was selected *a priori* based on the assumption that the true effect may vary among studies based on known differences in probiotic strain, study design characteristics, and subject characteristics. The standardized mean difference (SMD)

and 95%CI was selected to report treatment effects because different measures of IIT (*e.g.*, whole gut, colonic, oro-cecal, *etc.*) were utilized in the included studies. The SMD is a measure of effect size for continuous outcomes defined as the mean difference between groups divided by the pooled standard deviation. SMD values of 0.2, 0.5 and 0.8 are defined as small, medium, and large, respectively^[11]. A forest plot was used to illustrate the individual study findings and the random effects meta-analysis results. We used the I^2 statistic to estimate heterogeneity of effects across studies with values of $\leq 25\%$, 50% , and $\geq 75\%$ representing low, moderate, and high inconsistency, respectively^[12]. An alpha error $P < 0.05$ and/or $I^2 \geq 50\%$ were taken as indicators of substantial heterogeneity of treatment effects. Publication bias was visually assessed with a funnel plot (not shown) and quantitatively assessed using Egger's test^[13]. Meta-regressions and pre-defined subgroup analyses were undertaken to quantify the relationship of individual moderators on IIT SMD. All analyses were performed using Comprehensive Meta-analysis (version 2.2, Biostat, Englewood, NJ, United States).

RESULTS

Study selection

Our initial database search retrieved 409 titles and abstracts and hand searching relevant bibliographies identified 3 additional records. After screening records for inclusion criteria, 73 full text articles were reviewed for eligibility. Ultimately, 11 RCTs with 13 treatment effects representing 464 distinct subjects were included in the final analysis^[14-24]. A flow chart of study identification and selection is shown in Figure 1.

Study characteristics

Sample sizes were generally small, ranging from 10 to 36 per treatment group for parallel groups designs and

Table 1 Study characteristics

Ref.	Study design	Active: control (n:n)	Transit time outcome, method	Probiotic strain	Daily dosage (10 ⁹ cfu)	Delivery method	Treatment duration (d)
Agrawal <i>et al</i> ^[14]	Parallel groups	17:17	CTT, radiopaque markers	<i>B. lactis</i> DN-173 010	25.0	Active: Yogurt + probiotic Control: Nonfermented milk-based product	28
Bartram <i>et al</i> ^[15]	Cross-over	12	OATT, radiopaque markers	<i>B. longum</i>	> 0.5	Active: Yogurt with 2.5 g lactulose + probiotic Control: Yogurt	21
Bouvier <i>et al</i> ^[16]	Parallel groups	36:36	CTT, radiopaque markers	<i>B. lactis</i> DN-173 010	97.5	Active: Probiotic fermented milk Control: Heat-treated probiotic fermented milk	11
Holma <i>et al</i> ^[17]	Parallel groups	12:10	TITT, radiopaque markers	<i>L. rhamnosus</i> GG	20	Active: Buttermilk + probiotic and white wheat bread Control: White wheat bread	21
Hongisto <i>et al</i> ^[18]	Parallel groups	16:14	TITT, radiopaque markers	<i>L. rhamnosus</i> GG	15	Active: Yogurt + probiotic and low fiber toast Control: Low fiber toast	21
Malpeli <i>et al</i> ^[19]	Cross-over	83	OCTT, carmine red dye	<i>B. lactis</i> BB12 <i>L. casei</i> CRL 431	2-20 2-12	Active: Yogurt with 0.625 g inulin and oligofructose + probiotic Control: Yogurt	15
Marteau <i>et al</i> ^[20]	Cross-over	32	CTT, radiopaque markers	<i>B. lactis</i> DN-173 010	18.75	Active: Yogurt + probiotic Control: Yogurt	10
Rosenfeldt <i>et al</i> ^[21]	Cross-over	13	GTT, radiopaque markers	<i>L. rhamnosus</i> 19070-2, <i>L. reuteri</i> DSM 12246	20 20	Active: Freeze-dried powder + probiotic Control: Skimmed milk powder w/dextrose	18
Rosenfeldt <i>et al</i> ^[21]	Cross-over	13	GTT, radiopaque markers	<i>L. casei subsp. alactus</i> CHCC 3137, <i>L. delbrueckii subsp. lactis</i> CHCC 2329, <i>L. rhamnosus</i> GG	20 20 20	Active: Freeze-dried powder + probiotic Control: Skimmed milk powder w/dextrose	18
Sairanen <i>et al</i> ^[22]	Parallel groups	22:20	CTT, radiopaque markers	<i>B. longum</i> BB536, <i>B. lactis</i> 420, <i>L. acidophilus</i> 145	2.4-18 ¹ 0.48	Active: Probiotic fermented milk Control: Fermented milk	21
Waller <i>et al</i> ^[23]	Parallel groups	33:34	WGTT, radiopaque markers	<i>B. lactis</i> HN019	1.8	Active: Capsule, maltodextrin, probiotic Control: Capsule, maltodextrin	14
Waller <i>et al</i> ^[23]	Parallel groups	33:34	WGTT, radiopaque markers	<i>B. lactis</i> HN019	17.2	Active: Capsule, maltodextrin, probiotic Control: Capsule, maltodextrin	14
Krammer <i>et al</i> ^[24]	Parallel groups	12:12	CTT, radiopaque markers	<i>L. casei</i> Shirota	6.5	Active: Probiotic fermented milk drink Control: Nonfermented milk drink	28

¹Represents the reported range of total *Bifidobacterium* spp. cfu: Colony-forming units; CTT: Colonic transit time; GTT: Gastrointestinal transit time; OATT: Oro-anal transit time; OCTT: Oro-cecal transit time; TITT: Total intestinal transit time; WGTT: Whole gut transit time. *L. rhamnosus*: *Lactobacillus rhamnosus*; *B. lactis*: *Bifidobacterium lactis*; *L. casei*: *Lactobacillus casei*.

from 12 to 83 for cross-over designs. The average detectable effect size, based on sample size and study design by assuming $P = 0.05$ and statistical power = 80%, was 0.8 (range: 0.3 to 1.3). Eleven RCTs contributed one treatment effect each. The study of Rosenfeldt *et al*^[21] contributed two treatment effects (two different probiotic formulations) and the study of Waller *et al*^[23] contributed two treatment effects (same probiotic strain, two different dosages). Eight of the 11 studies were parallel groups designs while 3 were cross-over studies. The

most commonly studied probiotic strains were *Bifidobacterium lactis* (*B. lactis*) DN-173 010 (3 treatment effects), *B. lactis* HN019 (2 treatment effects), and *Lactobacillus rhamnosus* (*L. rhamnosus*) GG (2 treatment effects). Daily probiotic dosages varied substantially across studies, ranging from 5×10^8 to 9.75×10^{10} cfu per day (median 1.72×10^{10} cfu per day). Supplementation periods ranged from 10 to 28 d (median 18 d). Intestinal transit time was quantified using radiopaque markers in 10 studies and with carmine red dye in 1 study^[19]. The most commonly

Table 2 Subject characteristics

Ref.	Age (yr)	Female gender	BMI (kg/m ²)	Condition
Agrawal <i>et al</i> ^[14]	40	100%	25	IBS-C
Bartram <i>et al</i> ^[15]	23	58%	-	None
Bouvier <i>et al</i> ^[16]	33	50%	22	None
Holma <i>et al</i> ^[17]	44	92% ¹	24	Constipation
Hongisto <i>et al</i> ^[18]	43	100%	24	Constipation
Malpeli <i>et al</i> ^[19]	41	100%	-	Constipation
Marteau <i>et al</i> ^[20]	27	100%	21	None
Rosenfeldt <i>et al</i> ^[21]	25	0%	-	None
Rosenfeldt <i>et al</i> ^[21]	25	0%	-	None
Sairanen <i>et al</i> ^[22]	39	64%	25	None
Waller <i>et al</i> ^[23]	44	65%	31	Constipation
Waller <i>et al</i> ^[23]	44	65%	32	Constipation
Krammer <i>et al</i> ^[24]	50	100%	-	Constipation

¹Percentage estimated from larger study cohort. BMI: Body mass index; IBS-C: Irritable bowel syndrome, constipation predominant; “-”: Represents missing data.

tested product format was yogurt or other forms of fermented milk. Two studies were confounded by inclusion of other components in the active product that may influence ITT such as lactulose^[15] and the combination of inulin and oligofructose^[19] (Table 1). Seven treatment effects were calculated based on subjects with constipation or irritable bowel syndrome-C while 6 were based on healthy subjects. Subjects were predominantly female with a mean age ranging from 23 to 50 years and mean body mass index ranging from 21 to 32 kg/m² (Table 2).

Study quality assessment

Overall, the quality of RCT reporting was medium with a median Jadad score of 3 (range: 1-5). Eight of 13 treatment effects were based on higher quality (Jadad score 3-5) trials. The method of randomization was unclear in most studies. Descriptions of blinding were adequate overall. Subject accountability in RCTs was mentioned in only 7 of 13 cases (Table 3).

Synthesis of results

Overall, probiotic supplementation was associated with reduced ITT, with an SMD of 0.40 (95%CI: 0.20-0.59, $P < 0.001$) (Figure 2). There was low heterogeneity among studies ($I^2 = 29\%$, $P = 0.15$) with no evidence of publication bias (Egger's regression test: $P = 0.13$). Only 4 of 13 individual treatment effects statistically favored probiotic supplementation.

Additional analyses

We performed meta-regression analysis including pre-defined covariates to explore the potential predictors of SMD. Constipation ($r^2 = 39\%$, $P = 0.01$), higher mean age ($r^2 = 27\%$, $P = 0.03$), and higher percentage of female subjects ($r^2 = 23\%$, $P < 0.05$) were predictive of decreased ITT with probiotics in meta-regression (Table 4). Additionally, we performed a pre-defined subgroup analysis to observe the influence of study- and subject-related characteristics on SMD (Table 5). Subgroup

Table 3 Assessment of study quality

Ref.	Jadad scale			
	Randomization (range: 0-2)	Double blinding (range: 0-2)	Subject account (range: 0-1)	Total score ¹ (range: 0-5)
Agrawal <i>et al</i> ^[14]	1	2	1	4
Bartram <i>et al</i> ^[15]	1	2	0	3
Bouvier <i>et al</i> ^[16]	1	2	0	3
Holma <i>et al</i> ^[17]	1	0	1	2
Hongisto <i>et al</i> ^[18]	1	0	0	1
Malpeli <i>et al</i> ^[19]	0	2	1	3
Marteau <i>et al</i> ^[20]	1	2	1	4
Rosenfeldt <i>et al</i> ^[21]	1	1	0	2
Rosenfeldt <i>et al</i> ^[21]	1	1	0	2
Sairanen <i>et al</i> ^[22]	1	1	0	2
Waller <i>et al</i> ^[23]	2	2	1	5
Waller <i>et al</i> ^[23]	2	2	1	5
Krammer <i>et al</i> ^[24]	1	1	1	3

¹Higher scores represent better study quality.

Table 4 Meta-regression of study- and subject-related factors on intestinal transit time

Variable	Unit of measure	Intercept	Point estimate	Explained variance	P-value
Constipation	0 = no, 1 = yes	0.171	0.415	39%	0.01
Age	Per 10 years	-0.445	0.230	27%	0.03
Female gender proportion	Per 10%	0.024	0.053	23%	< 0.05
Body mass index ¹	Per 5 kg/m ²	-0.544	0.200	25%	0.11
Daily probiotic dosage	Per 10 × 10 ⁹ cfu	0.454	-0.013	1%	0.62
Treatment duration	Per 1 wk	0.535	-0.048	1%	0.67

¹Body mass index not reported for 5 treatment effects.

analyses demonstrated statistically greater reductions in ITT with probiotics in subjects with *vs* without constipation and in older *vs* younger subjects (both SMD: 0.59 *vs* 0.17, $P = 0.01$). Study design, body mass index, treatment duration, and daily probiotic dosage had no influence on probiotic treatment effects in any analysis. Analysis of outcomes by probiotic strain identified medium to large treatment effects with *B. lactis* HN019 (SMD: 0.72, $P < 0.01$) and *B. lactis* DN-173 010 (SMD: 0.54, $P < 0.01$) while treatment effects with other single strains and combination products were small (SMD: 0.17-0.25) and not statistically significant (Table 6).

DISCUSSION

Summary of evidence

Clinical trials of probiotic supplementation often utilize ITT as a primary efficacy outcome. However, inconsistent treatment effects among trials have been observed, likely due to differences among study designs, probiotic strains, dosing regimens, and subject characteristics. We performed the first systematic review and meta-analysis on this

Table 5 Subgroup analysis of study- and subject-related factors on intestinal transit time

Study	SMD	95%CI	P-value (within groups)	P-value (between groups)
Subject condition				
Constipation/IBS-C (n = 7)	0.59	0.39-0.79	< 0.001	0.01
Healthy (n = 6)	0.17	-0.08-0.42	0.18	
Age				
≥ 40 years (n = 7)	0.59	0.39-0.79	< 0.001	0.01
< 40 years (n = 6)	0.17	-0.08-0.42	0.18	
Study design				
Parallel groups (n = 8)	0.49	0.24-0.75	< 0.001	0.23
Cross-over (n = 5)	0.25	-0.06-0.56	0.11	
Body mass index ¹				
≥ 25 kg/m ² (n = 4)	0.61	0.27-0.95	< 0.001	0.29
< 25 kg/m ² (n = 4)	0.34	-0.02-0.70	0.06	
Female gender proportion				
≥ 75% (n = 6)	0.44	0.17-0.76	< 0.01	0.47
< 75% (n = 7)	0.32	0.05-0.60	0.02	
Treatment duration				
< 20 d (n = 7)	0.43	0.18-0.67	< 0.001	0.62
≥ 20 d (n = 6)	0.32	-0.02-0.66	0.07	
Daily probiotic dosage				
≥ 10 ¹⁰ cfu (n = 8)	0.41	0.14-0.68	< 0.01	0.84
< 10 ¹⁰ cfu (n = 5)	0.36	0.05-0.68	0.02	

¹Body mass index not reported for 5 treatment effects. IBS-C: Irritable bowel syndrome, constipation predominant; SMD: Standardized mean difference.

Table 6 Subgroup analysis of probiotic strains on intestinal transit time

Probiotic strain	Treatment effects (n)	SMD	95%CI	P-value
<i>B. lactis</i> HN019	2	0.72	0.27-1.18	< 0.01
<i>B. lactis</i> DN-173 010	3	0.54	0.15-0.94	< 0.01
<i>L. rhamnosus</i> GG	2	0.25	-0.38-0.87	0.44
Other single strains	2	0.23	-0.41-0.87	0.48
Strain combinations	4	0.17	-0.18-0.52	0.34

SMD: Standardized mean difference. *B. lactis*: *Bifidobacterium lactis*; *L. rhamnosus*: *Lactobacillus rhamnosus*.

Table 7 Sample size requirements for randomized controlled trials based on standardized mean difference

SMD	Study design	
	Parallel groups ¹	Cross-over
0.2	786	156
0.3	350	71
0.4	198	41
0.5	128	27
0.6	90	19
0.7	66	15
0.8	52	12

¹Total sample size, assuming 1:1 active-to-control group ratio. Assumes two-sided alpha of 0.05 and statistical power of 80%. Attrition estimate not included. SMD: Standardized mean difference.

topic and demonstrated that, overall, short-term (10-28 d) probiotic supplementation is able to reduce ITT in adults.

We also demonstrated that the treatment effect of probiotics is strongly dependent on: (1) the presence or absence of constipation; (2) subject age; and (3) probiotic strain.

Clinical relevance of findings

Presence of constipation and older age were predictive of greater ITT treatment effects with probiotic supplementation. Constipation was the primary influencer of probiotic treatment effects on ITT, explaining 39% of the variance in SMD. The independent influence of subject age, after accounting for constipation, is unknown and may be confounded since the seven studies that enrolled the oldest subjects were the same studies that enrolled constipated subjects. The number of treatment effects per strain is limited; *B. lactis* DN-173 010 (3), *B. lactis* HN019 (2) and *L. rhamnosus* GG (2). Drawing definite conclusions is therefore perilous, but the finding that the former two strains have notably greater treatment effects on ITT suggests that these strains could be considered when aiming to relieve slow intestinal transit.

The clinical importance of ITT is highly dependent on the underlying pathology. In healthy adults with no evidence of GI disturbances or delayed transit, there is arguably little benefit in lowering ITT^[25]. In contrast to this position, EFSA considers that a reduction in ITT within the normal range to be a possibly beneficial physiological effect in healthy adults^[8]. Overall, probiotic supplementation for this sole purpose cannot be strongly recommended given the questionable clinical benefit and the small effect size (SMD: 0.17) identified in this meta-analysis. In adults with constipation or IBS, a reduction in ITT is moderately associated with improvements in stool form and frequency^[25,26]. Therefore, probiotic supplementation appears to be a reasonably effective option to achieve this therapeutic goal provided diarrhea does not develop. Current evidence suggests that probiotics contribute to lowering intestinal pH, decreasing colonization and invasion by pathogenic organisms, and modifying the host immune response with few known side effects^[27]. However, there is no strong evidence from RCTs that probiotics improve symptoms such as abdominal pain or bloating in these patients^[28]. The clinical importance of decreased ITT in the absence of symptom amelioration is controversial and requires further exploration.

Relevance of findings to clinical trial designs

Interestingly, this meta-analysis identified a positive benefit of probiotic supplementation on ITT although only 4 of 13 treatment effects demonstrated such a benefit. This is likely because the majority of clinical trials were underpowered due to small sample size. In fact, only 1 treatment effect was identified from a study with a minimum detectable effect size ≤ 0.5 (moderate effect) and only 5 had a minimum detectable effect size ≤ 0.8 (large effect). Considering the overall ITT SMD with probiotic supplementation is only 0.4, it is clear that small sample size and, consequently, inadequate statistical power was

the main driver of the high failure rate of individual studies.

The use of estimated SMD is an integral component of study design development and sample size estimation for RCTs. Sample sizes for RCTs based on estimated SMD are shown in Table 7. Based on the SMDs calculated in this meta-analysis, enrollment of approximately 90 subjects would be required in a study of probiotics for constipation or irritable bowel syndrome-C with a parallel groups design or 19 subjects if utilizing a cross-over design. In comparison, for a trial of healthy volunteers, required sample sizes would be 786 and 156 for parallel groups and cross-over designs, respectively, in order to achieve adequate statistical power. Although cross-over trials always require a smaller sample size for a given SMD since subjects serve as their own controls, the main disadvantages of this design include a longer time on study, higher attrition rates due to the extended trial duration, and difficulties in estimating an appropriate washout duration. As such, cross-over designs are inappropriate for clinical trials with extended treatment durations or long or unknown washout periods.

Strengths and limitations

The strengths of this systematic review and meta-analysis include selection of only RCTs to minimize bias and the comprehensive assessment of the impact of moderator variables on the primary outcome. Nevertheless, our analysis was associated with several limitations. First, treatment duration in the reviewed studies ranged from 10 to 28 d and, therefore, the treatment effect of longer term probiotic supplementation on ITT is unknown. Second, the therapeutic benefit of probiotics is considered to be strain-specific; however, the small number of studies performed with each strain prevented robust strain-specific comparisons. Third, there was a significant over-representation of subjects who were young to middle-aged, female, and with a normal body mass index. Abundant caution must be exercised when extrapolating the treatment effects observed in this review to a broader population. Finally, we noted significant heterogeneity among ITT measurement methods as well as product delivery methods and additional included ingredients (*e.g.*, prebiotics) among studies. There is potential for these differences to confound the results of our analysis.

In conclusion, short-term probiotic supplementation decreases ITT with consistently greater treatment effects identified in constipated or older adults and with certain probiotic strains.

COMMENTS

Background

Functional gastrointestinal (GI) disorders are common in the general population, with slow intestinal transit a common symptom. No known therapy is highly efficacious, safe, and cost effective for treatment of slow-transit bowel disorders. Probiotics are live micro-organisms that confer a health benefit on the host when administered in adequate dosages and have been extensively studied for treatment of functional GI disorders.

Research frontiers

Clinical trials of probiotic supplementation on intestinal transit time (ITT) yield widely

variable outcomes. The reasons for these discrepant outcomes have not been explored to date. Authors performed the first systematic review and meta-analysis on the efficacy of probiotic supplementation on ITT in adults with a secondary focus on identifying the factors that influence these outcomes.

Innovations and breakthroughs

Authors demonstrated that, overall, short-term (10-28 d) probiotic supplementation reduces ITT in adults. However, the treatment effect of probiotics is strongly dependent on: (1) the presence or absence of constipation; (2) subject age; and (3) probiotic strain.

Applications

The effect of probiotics on ITT is highly dependent on probiotic strain and patient characteristics. The reason for these differences requires exploration in future clinical trials. Thus far, no evidence supports the use of probiotics to decrease ITT in younger subjects or in those without constipation.

Terminology

Probiotics are live micro-organisms that confer a health benefit on the host when administered in adequate dosages. Intestinal transit time is a general term that refers to the time taken for a food bolus to travel through the gastrointestinal system. The standardized mean difference is a statistical measure of effect size for continuous outcomes, defined as the mean difference between groups divided by the pooled standard deviation.

Peer review

Considering the high prevalence of functional GI disorders nowadays and the numerous studies on the role of probiotics in treating such conditions, it is important to know where we stand. This meta-analysis demonstrates the efficacy of probiotic supplementation in improving intestinal transit time. It is very well written, with methods clearly presented. Conclusions are drawn regarding the clinical importance of these findings and their relevance to clinical trials design, representing valuable information.

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Comparison of double balloon enteroscopy in adults and children

Odul Egritas Gurkan, Tarkan Karakan, Ibrahim Dogan, Buket Dalgic, Selahattin Unal

Odul Egritas Gurkan, Buket Dalgic, Department of Pediatric Gastroenterology, School of Medicine, Gazi University, 06500 Ankara, Turkey

Tarkan Karakan, Ibrahim Dogan, Selahattin Unal, Department of Gastroenterology, School of Medicine, Gazi University, 06500 Ankara, Turkey

Author contributions: Gurkan OE designed the study; Gurkan OE, Karakan T and Dogan I acquired, collected and elaborated the data; Gurkan OE and Karakan T wrote the article; Gurkan OE, Karakan T and Dogan I performed enteroscopies; Dalgic B and Unal S drafted the article and revised it critically for important intellectual content; Karakan T revised and approved the final version.

Correspondence to: Tarkan Karakan, MD, Associate Professor, Department of Gastroenterology, School of Medicine, Gazi University, TR-06510 Besevler, 06500 Ankara, Turkey. tkarakan@gmail.com

Telephone: +90-312-2025819 Fax: +90-312-2236365

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Abstract

AIM: To compare results of double balloon enteroscopy (DBE) procedures in pediatric and adult patients.

METHODS: The medical files of patients who underwent DBE at Gazi University School of Medicine, Ankara, Turkey between 2009 and 2011 were examined retrospectively. Adult and pediatric patients were compared according to DBE indications, procedure duration, final diagnosis, and complications. DBE procedures were performed in an operating room under general anesthesia by two endoscopists. An oral or anal approach was preferred according to estimated lesion sites. Overnight fasting of at least 6 h prior to the start of the procedure was adequate for preprocedural preparation of oral DBE procedures. Bowel cleansing was performed by oral administration of sennosides A and B solution, 2 mL/kg, and anal saline laxative en-

ema. The patients were followed up for 2 h after the procedure in terms of possible complications.

RESULTS: DBE was performed in 35 patients (5 pediatric and 30 adult). DBE procedures were performed for abdominal pain, chronic diarrhea, bleeding, chronic vomiting, anemia, and postoperative evaluation of anastomosis. Final diagnosis was diffuse gastric angiodysplasia ($n = 1$); diffuse jejunal angiodysplasia ($n = 1$); ulceration in the bulbous ($n = 1$); celiac disease ($n = 1$); low differentiated metastatic carcinoma ($n = 1$); Peutz-Jeghers syndrome ($n = 1$); adenomatous polyp ($n = 1$) and stricture formation in anastomosis line ($n = 1$). During postprocedural follow-up, abdominal pain and elevated amylase levels were noted in three patients and one patient developed abdominal perforation.

CONCLUSION: With the help of technological improvements, we may use enteroscopy as a safe modality more frequently in younger and smaller children.

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Key words: Double-balloon enteroscopy; Small bowel disease; Polyp; Angiodysplasia; Peutz-Jeghers syndrome

Core tip: Small bowel diseases are encountered frequently in adults and children. For diagnosing small bowel disease, endoscopy, barium series, ultrasound, and computed tomography methods were used before advanced techniques such as capsule endoscopy and double balloon enteroscopy (DBE). Many centers began to use DBE widely in adults and children. Interventional procedures of DBE in children are also as safe as in adult patients. Although a small number of children were included in the present study, this is believed to be the first comparison of DBE in adult and pediatric patients.

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of double balloon enteroscopy in adults and children. *World J Gastroenterol* 2013; 19(29): 4726-4731 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4726.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4726>

INTRODUCTION

Considerable progress has been made in the area of diagnostic or therapeutic endoscopic interventions since Hirschowitz *et al* introduced the fiberoptic endoscope in 1950^[1]. Endoscopic imaging of the whole small intestine is more difficult than the colon due to its length, free mobility and tortuosity within the abdominal cavity. Capsule endoscopy (CE) is one of the latest techniques, and provides single pass visualization of the intestines but without biopsy or therapeutic capabilities. Small bowel diseases are common in children and adults. However, surgical approaches were the first choice for diagnostic and therapeutic applications before using techniques like CE and double balloon enteroscopy (DBE). DBE provides significant progress in diagnosis and management of small intestinal diseases. The diagnostic and therapeutic benefits of DBE in adults are well documented and DBE is widely used in many centers. Although DBE is a safe method in adults, its use in children was limited to case reports, which were followed by center reports about the DBE experience^[2-6].

In the present study, we report our preliminary comparison of DBE in pediatric and adult patients, focusing on the evolving indications, limitations, associated risks, and complications of this endoscopic therapeutic technique, in the setting of a tertiary care referral center.

MATERIALS AND METHODS

Patients

The medical files of the patients who underwent DBE at Gazi University School of Medicine, Ankara, Turkey between 2009 and 2011 were examined retrospectively. A total of 36 oral or anal procedures were performed in 35 patients. Endoscopy and colonoscopy were performed in all patients prior to DBE. The indications for DBE in each patient were based on clinical, laboratory, radiology, endoscopy and colonoscopy findings, and biopsy results. Unexplained chronic abdominal pain, chronic diarrhea, gastrointestinal bleeding, anemia, and postoperative evaluation of anastomosis line were the indications for DBE.

DBE procedure

DBE included a complete small bowel enteroscope, an overtube, balloons and a special air pump. The Fujinon EN-450T5 DBE system (Tokyo, Japan) used a 200-cm working length enteroscope with an outer diameter of 8.5 and 2.8-mm working channel. The flexible overtube had a length of 140 cm and outer diameter of 12 mm. The enteroscope and overtube had balloons fitted at the distal tip. An air pressure controlled pump system had

a maximum inflatable pressure of 45 mmHg. For oral DBE procedures, there was no difference in preprocedural preparation of adult and pediatric patients and overnight fasting of at least 6 h prior to the start of the procedure was adequate. Bowel cleansing was performed by oral administration of sennosides A and B solution, 2 mL/kg, and anal saline laxative enema. DBE procedures were performed in an operating room under general anesthesia by two endoscopists. The patients were followed up for 2 h after the procedure to assess possible complications.

Ethics

Information about the DBE procedure, including its requirements and potential complications, were explained to all adult patients and parents of the children before the procedure. Informed consent was obtained from all the participants.

Statistical analysis

The SPSS 16.0 software package was used for statistical analysis. Numerical data were expressed as a percentage, and measurement data were expressed as the mean $\pm$ SD. Differences were evaluated with the χ^2 test.

RESULTS

DBE procedures were performed in 35 patients (5 children and 30 adults). Fourteen of the adult patients were female (46.7%), and 16 were male (53.3%). Among pediatric patients, four were male (80%) and one was female (20%). The mean ages in the adult and pediatric patients were 48.63 ± 12.70 and 12 ± 3 years, respectively. Demographic data of the patients are listed in Table 1.

Indications for DBE procedures were abdominal pain ($n = 11$), chronic diarrhea ($n = 8$), anemia ($n = 4$), bleeding ($n = 9$), postoperative evaluation of anastomosis line ($n = 2$), and chronic vomiting in a patient with percutaneous endoscopic jejunostomy tube ($n = 1$). During postprocedural follow-up, three patients developed abdominal pain and elevated amylase levels, thus follow-up periods were extended. One patient developed intestinal perforation.

Diagnosis was made by enteroscopy, evaluation of biopsy or surgical material, or clinical and laboratory data. Results were as follows: diffuse gastric angiodysplasia ($n = 1$); diffuse jejunal angiodysplasia ($n = 1$); ulceration in the bulbous ($n = 1$); scalloping of the folds in the bulbous and bowel mucosa compatible with celiac disease ($n = 3$); ulceration crater in the jejunum with 8-9 mm diameter covered with white exudate (low differentiated metastatic carcinoma, $n = 1$); polyps (3 patients with Peutz-Jeghers syndrome and 1 with adenomatous polyp); and stricture formation in anastomosis line ($n = 1$) (Table 2). In 23 patients, monitoring of the small bowel mucosa throughout the process was normal. Demographic characteristics of pediatric patients are given in Table 3.

Table 1 Demographic features of double balloon enteroscopy patients

Indications for DBE	Procedure time (mean, min)	n	Sex	Oral/anal
Chronic abdominal pain	64.8	11	3 F/8 M	Oral approach DBE ¹ Patient oral + anal approach DBE
Anemia	62.5	4	1 F/3 M	Oral approach DBE
Diarrhea	73.5	8	4 F/4 M	Oral approach DBE
Bleeding	66	9	6 F/3 M	Oral approach DBE
Evaluation of anastomosis line	38	2	1 F/1 M	Oral approach DBE
Chronic vomiting	64	1	1 M	Oral approach DBE

¹Diffuse jejunal angiodysplasia was noted in a female patient being investigated for anemia etiology. F: Female; M: Male; DBE: Double balloon enteroscopy.

Table 2 Comparison of double balloon enteroscopy findings and final diagnosis of study population

	Pediatric (n = 5)	Adult (n = 30)	P
Male/female	4/1	16/14	< 0.05
Procedure time (min, 95%CI)	74 (65-89)	114 (76-125)	< 0.05
Route	5 oral	29 oral, 1 anal + oral	
Findings/intervention (n)	Multiple polyps (2) / polypectomy	Diffuse gastric angiodysplasia (1)/APC ¹ Diffuse jejunal angiodysplasia in (1) / APC Ulceration in bulbus (1) Scalloping of the folds in bulbus and bowel mucosa (3) Ulceration crater in jejunum with 8-9 mm diameter covered with white exudate (1) Multiple polyps (3)/polypectomy Polyp (1)/polypectomy anastomotic stricture (1)/balloon dilation Angiodysplasia (2)	
Final diagnosis (n)	Irritable bowel syndrome (3) PJS (2)	Peptic ulcer (1) Celiac disease (3) Metastatic carcinoma (1) Anastomotic stricture (1) PJS (3) Adenomatous polyp (1) No definitive diagnosis (18) Abdominal discomfort (21) Hyperamylasemia (5)	
Minor complications (n)	Sore throat (4) Abdominal discomfort (3)	Sore throat (4) Perforation (1)	
Major complications (n)	None		

Argon plasma coagulation (APC) was performed, unfortunately intestinal perforation identified after the procedure. PJS: Peutz-Jeghers syndrome.

Table 3 Demographic features of pediatric patients

Patient	Complaint	Age (yr) /gender	Enteroscopy findings	Intervention	Final diagnosis
1	Abdominal pain / Diarrhea	12/male	Normal mucosa	-	Irritable bowel syndrome
2	Abdominal pain	11.6/male	Multiple polyps	Polypectomy	Peutz-Jeghers syndrome
3	Anemia	12/female	Multiple polyps	Polypectomy	Peutz-Jeghers syndrome
4	Abdominal pain	10/male	Normal mucosa	-	Irritable bowel syndrome
5	Abdominal pain	10 yr and 5 mo/male	Normal mucosa	-	Irritable bowel syndrome

and children. For diagnosing small bowel disease, endoscopy, barium series, ultrasound, computed tomography (CT) methods were used before advanced techniques such as CE and DBE. Segments of the small intestine that cannot be reached are now viewed *via* DBE and progress has been made in diagnosis and treatment of lesions in this region. Although a small number of children were included in the present study, this is believed to be the first comparison of DBE in pediatric and adult patients. Demographic characteristics of pediatric patients.

Analyzing pediatric patients, patient 1 had abdominal pain and diarrhea whereas patients 4 and 5 had abdominal pain only. In all three patients acute phase reactants and leukocyte count were normal and stools were negative for blood. There were no pathological imaging signs and endoscopic and colonoscopic examinations were considered as normal. Abdominal pain was the most common indication for enteroscopy in our limited number of pediatric patients. Evaluating the adult group of patients, abdominal pain constituted the most common indication for enteroscopy, with 11 patients (33%). Reviewing the literature for adult enteroscopy, Pata *et al*^[7] reported that obscure bleeding constituted the major indication, with 42.1%, whereas abdominal pain ranked last with 9.47%. He *et al*^[8] reported that abdominal pain with 15 patients (25.4%) ranked second below melena with 36 patients (61.01%) as the major indications. In our adult series, three of the 11 patients with abdominal pain had special features. Stool examinations of all three patients were normal. Endoscopic and colonoscopic examinations did not reveal any clear pathological sign. However, due to visualization of local irregularities and scalloping in jejunal bowel mucosa in some areas, celiac serology was performed and biopsy results were compatible with celiac disease.

According to the classical knowledge on celiac disease, it is known that four or more biopsy specimens from the bulbus and duodenum are sufficient for diagnosis. Enteroscopy was not needed for diagnosis of celiac disease. Today, we are aware of using enteroscopy

DISCUSSION

Small bowel diseases are encountered frequently in adults

for viewing the whole small bowel in refractory or complicated celiac disease. Höroldt *et al*^[9] reported that endoscopic duodenal biopsies of 31 patients with suspected celiac disease were evaluated as normal. However, 60% of these patients were diagnosed with celiac disease, with the biopsies taken from the jejunum by enteroscopy. Similarly, Rondonotti *et al*^[10] reported that among 23 patients with suspected celiac disease, four were diagnosed with celiac disease with the help of enteroscopic jejunal biopsies. Reviewing the literature, and as shown in our study group, duodenal biopsies may not be sufficient in 10%-17% of patients with suspected celiac disease, and biopsies have to be taken from distal parts of the small intestine with the help of an enteroscope^[11-13].

In our study, four patients had polyps and resections were done. Patient 2 was being followed for Peutz-Jeghers syndrome in a different center and was referred to our department due to development of ileus symptoms. In abdominal CT scans, a mass of 3 cm × 2.5 cm was noted. Enteroscopy was performed and a giant three-leaf-clover-shaped polyp completely filling the intestinal lumen was seen (Figure 1). Complete resection was done without postprocedural complications. Patient 3 was also being followed for Peutz-Jeghers syndrome and prominent anemia. The patient was positive for occult blood in stool examination. During endoscopy, a jejunal polyp was seen in an area that was out of reach. Polypectomy was performed with the help of an enteroscope without any complications.

Enteroscopy was performed in one adult patient who had Peutz-Jeghers syndrome in childhood due to anemia and recurrent abdominal pain. Polypectomy was performed for multiple polyps with the help of an enteroscope, without any complications. One adult patient being investigated for anemia with normal endoscopy and colonoscopy was referred to our center for enteroscopy. Jejunal polyps were seen and complete resection was performed without any complications. Biopsies were found to be compatible with adenomatous polyps.

Yoon *et al*^[14] reported the resection of a giant ileal polyp of 2.5 cm × 2.5 cm *via* enteroscopy. Miyata *et al*^[15] reported a case of small bowel intussusception due to inflammatory polyps that was preoperatively diagnosed using DBE. Thus, minimal laparoscopic resection could be performed by shortening intussusception. Until recently, primary surgical resection and intraoperative endoscopy were the only available possibilities to treat polyps in the mid-small bowel in patients with polyps. DBE has changed this approach and now it is possible not only perform endoscopic surveillance and diagnose these lesions, but also resect them.

One of our adult patients investigated for abdominal pain and anemia presented with occult blood. Endoscopy and colonoscopy did not reveal any pathological findings. During enteroscopy, an ulceration crater in the jejunum of 8-9 mm diameter covered with white exudate was noted. Biopsy evaluation was reported as low differentiated metastatic carcinoma.

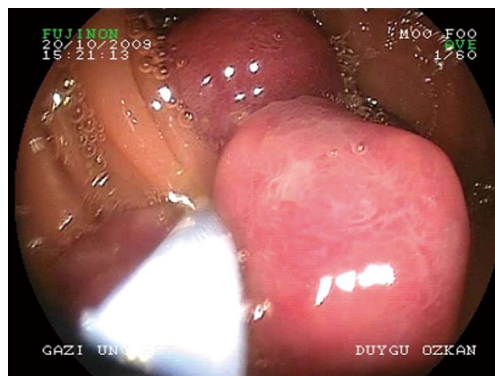


Figure 1 A giant three-leaf cloverleaf shaped polyp completely filling the intestinal lumen.

Fry *et al*^[16] reported a relative incidence of 9.6% for small bowel tumors encountered during DBE in a series of 40 patients. Nakatani *et al*^[17] reported 10 years DBE experience with 705 patients. All patients were investigated for obscure gastrointestinal bleeding and 12 intestinal tumors were identified. According to the above mentioned study, detection rates for intestinal tumors with DBE, CE and CT were 92%, 60% and 67%, respectively. Considering adult small bowel tumors, probably one of the major benefits of DBE may be defined as diagnostic and/or therapeutic capability without the need for surgery.

Although not met in our pediatric patients, diffuse gastric and jejunal angiodysplasia was noted in two adult patients, and argon plasma coagulation (APC) therapy was performed. May *et al*^[18] reported 44 cases of angiodysplasia in a series of 66 patients and APC was the treatment option. As in patients with small bowel tumors, DBE may be compared better alternative to surgery in patients with angiodysplasia^[19,20].

Initially, studies about DBE in childhood were limited to case reports, which were followed by center reports. To date, there have been seven studies about DBE procedures in childhood. In 2007, Leung^[21] performed DBE in 15 pediatric patients; five of whom were < 10 years old. Liu *et al*^[22] performed DBE with a diagnostic rate of 77.8% in 31 pediatric patients aged 3-14 years. In 2010, Lin *et al*^[23] reported 13 successful DBE procedures in 11 pediatric patients ranging from 8 to 20 years. Thomson *et al*^[24] and Nishimura *et al*^[25] reported DBE in 14 and 48 pediatric patients, respectively. In 2012, Shen *et al*^[26] reported 35 DBE procedures performed in 30 pediatric patients, whereas Uchida *et al*^[27] performed 17 DBE procedures to 12 children. Evaluation of obscure gastrointestinal bleeding, surveillance and treatment of polyposis syndromes, investigation of abdominal pain, chronic diarrhea, and suspected mucosal inflammation comprised the indications in all the above-mentioned pediatric DBE patients. Reported complications encountered in these patients were post-polypectomy bleeding, sore throat, abdominal discomfort, and oral secretion aspiration. One intestinal perforation was reported af-

ter resection of a Peutz-Jeghers polyp in a 3-year-old boy during enteroscopy. Reviewing the studies and case reports, it seems that the younger children with lower weight have a higher possibility of complications such as perforation. In our study, perforation occurred in an adult patient with diffuse angiodysplasia after APC. Post-procedural sore throat and abdominal discomfort were noted in all our pediatric patients, whereas abdominal discomfort was the major complaint in adult patients.

The mean procedure time was significantly shorter for pediatric patients (74 min *vs* 114 min). The possible explanation for this is the relatively shorter small bowel length of pediatric patients. Although the majority of the pediatric patients had polypectomy, none of them had major complications. Although the lumen of the small bowel is narrower in children, interventional endoscopic procedures are relatively safe. Only one patient in the adult group had perforation (possibly due to APC) and the clinical course was uneventful.

As a result, many centers have begun to use DBE widely in adults and children. DBE is a safe method for small bowel lesions in children, as in adults. Interventional procedures are also as safe in pediatric as adult patients; however, larger trials are needed to reach a firm conclusion.

COMMENTS

Background

Small bowel diseases are encountered frequently in adults and children. For diagnosing small bowel disease, endoscopy, barium series, ultrasound, and computed tomography were used before advanced techniques such as capsule endoscopy and double balloon enteroscopy (DBE). Segments of the small intestine that cannot be reached are now viewed via DBE and progress has been made in diagnosis and treatment of lesions in this region.

Research frontiers

Studies about DBE in childhood were limited to case reports, which were followed by center reports. To date, there have been seven studies about DBE procedures in childhood. This is believed to be the first comparison of DBE in pediatric and adult patients.

Innovations and breakthroughs

Postprocedural sore throat and abdominal discomfort were noted in all our pediatric patients, whereas abdominal discomfort was the main complaint in the adult patients. The mean procedure time was significantly shorter for pediatric patients. The possible explanation for this is the relatively shorter small bowel length in pediatric patients. Although the lumen of the small bowel is narrower in pediatric patients, interventional endoscopic procedures are relatively safe. DBE is a safe method for small bowel lesions, as in adults.

Applications

DBE is a reliable method in children as well as adults. Diagnostic and therapeutic use of DBE will gradually increase in the future.

Peer review

The study is interesting in that it evaluated the use of DBE in children, which has not yet been universally adopted. It appears to be safe and clinically effective.

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Utility of single and double balloon endoscopy in patients with difficult colonoscopy: A randomized controlled trial

Atsuo Yamada, Hirotsugu Watabe, Noriyuki Takano, Goichi Togo, Yutaka Yamaji, Haruhiko Yoshida, Takao Kawabe, Masao Omata, Kazuhiko Koike

Atsuo Yamada, Hirotsugu Watabe, Noriyuki Takano, Goichi Togo, Yutaka Yamaji, Haruhiko Yoshida, Takao Kawabe, Masao Omata, Kazuhiko Koike, Department of Gastroenterology, University of Tokyo, Tokyo 113-8655, Japan

Author contributions: Togo G, Yamaji Y, Yoshida H, Kawabe T and Omata M designed the research; Yamada A, Watabe H and Takano N performed the research and analyzed the data and wrote the paper; Yoshida H and Koike K had final approval of the version to be published.

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Correspondence to: Dr. Atsuo Yamada, MD, Department of Gastroenterology, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. yamada-a@umin.ac.jp

Telephone: +81-3-38155411 Fax: +81-3-38140021

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Abstract

AIM: To compare the utility of single-balloon colonoscopy (SBC) or double-balloon colonoscopy (DBC) for difficult colonoscopies.

METHODS: Between August 2008 and June 2010, patients in whom total colonoscopy failed within 30 min of insertion were assigned randomly to undergo either SBC or DBC. No sedatives were used. After the endoscopy, all patients were asked to evaluate pain during the procedure on a 10-point analog scale (1 = no pain; 10 = worst imaginable pain) with a questionnaire. The study outcomes were the cecal intubation rate and time, endoscopic findings, complications, and pain score.

RESULTS: The SBC and DBC groups included 11 and 10 patients, respectively. All but one SBC patient achieved total colonoscopy successfully. The cecal intubation times were 18 min (range: 10-85 min) and 12.8 min (range: 9.5-42 min) in the SBC and DBC groups, respectively (P

= 0.17). No difference was observed in the prevalence of colon polyps between the SBC and DBC groups (45% vs 30%, P = 0.66). SBC showed advanced colon cancer in the ascending colon, which was inaccessible using conventional colonoscopy. The respective pain scores were 5 (1-10) [median (range)] and 5 (1-6) in the SBC and DBC groups (P = 0.64). No complications were noted in any patient.

CONCLUSION: The utility of single- and double-balloon endoscopy for colonoscopy seems comparable in patients with incomplete colonoscopy using a conventional colonoscope.

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Key words: Difficult colonoscopy; Double-balloon endoscopy; Single-balloon endoscopy; Double-balloon colonoscopy; Single-balloon colonoscopy

Core tip: We compared the utility of single-balloon colonoscopy and double-balloon colonoscopy for difficult colonoscopy. Both single-balloon endoscopy (SBE) and double-balloon endoscopy (DBE) make possible performance of total colonoscopy in patients with incomplete colonoscopy using a conventional colonoscope. The utility of SBE and DBE for colonoscopy seems to be comparable. We recommend that patients with incomplete total colonoscopy undergo SBE or DBE.

Yamada A, Watabe H, Takano N, Togo G, Yamaji Y, Yoshida H, Kawabe T, Omata M, Koike K. Utility of single and double balloon endoscopy in patients with difficult colonoscopy: A randomized controlled trial. *World J Gastroenterol* 2013; 19(29): 4732-4736 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4732.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4732>

INTRODUCTION

Optical colonoscopy is the gold standard for colorectal examination. Despite advances in colonoscopes and endoscopy techniques, total colonoscopy is still demanding technically. Total colonoscopy is unsuccessful in 5%-10% of patients for a number of reasons^[1]. Difficult cecal intubation is associated with female gender, old age, a low body mass index, diverticular disease, and previous abdominal surgery^[2-5]. Solutions to this problem are the use of pediatric colonoscopes^[6] or a transparent hood^[7].

Balloon endoscopy is an effective method for investigating the small intestine^[8,9]. Two different types of balloon endoscopy are available: single-balloon endoscopy (SBE) and double-balloon endoscopy (DBE). Both can be performed using either the antegrade or retrograde approach. A retrograde approach might facilitate successful total colonoscopy and allow endoscopic therapy in patients who had incomplete colonoscopy with a conventional colonoscope. Although several studies have assessed the utility of single-balloon colonoscopy (SBC)^[10-12] or double-balloon colonoscopy (DBC)^[13-15] for colorectal examination, a difference between SBC and DBC has not yet been identified.

Therefore, we compared the utility of SBC and DBC for difficult colonoscopy in an exploratory randomized controlled trial.

MATERIALS AND METHODS

Study protocol

Consecutive patients after a prior incomplete colonoscopy with a conventional colonoscope were candidates for this study. Incomplete colonoscopy was defined as failure to identify two landmarks, the ileocecal valve and appendiceal orifice, within 30 min or cancellation of colonoscopy due to intolerable pain during the procedure. The exclusion criteria were the following: incomplete colonoscopy due to poor bowel preparation or colonic stenosis, prior colectomy, inflammatory bowel disease, malignant tumor, poor general condition, pregnancy, having undergone successful total colonoscopy within 1 year, age younger than 20 years, and refusal to provide written informed consent.

To eliminate patient selection bias, the enrolled patients were assigned randomly to either the SBC or DBC group in a 1:1 ratio. Randomization was performed using a computer-generated list of random numbers. The endoscopists and patients were not blinded to the group assignment.

The study was conducted according to the Declaration of Helsinki and approved by the ethics committee of our institution. The Japanese clinical trial registration scheme (UMIN-CTR) registration number for the study was UMIN000001684. Written informed consent was obtained from each study participant.

Endoscopic procedure

Each balloon endoscopic examination was conducted by

Table 1 Baseline of characteristics of the study patients *n* (%)

	SBC group (<i>n</i> = 11)	DBC group (<i>n</i> = 10)	<i>P</i> value
Male/female	7/4	6/4	0.99 ¹
Age (yr)	71.7 ± 8.0	71.5 ± 7.8	0.94 ²
BMI (kg/m ²)	22.3 ± 4.4	22.6 ± 3.3	0.87 ²
Past history of abdominal surgery	4 (36)	2 (20)	0.64 ¹

¹Fisher's exact test; ²Student's *t*-test. All variables are means ± SD. SBC: Single-balloon colonoscopy; DBC: Double-balloon colonoscopy; BMI: Body mass index.

an endoscopist who had performed at least 30 balloon endoscopies. SBC was performed using an SIF-Q260 (Olympus Medical Systems, Tokyo, Japan), and DBC was performed using an EN-450T5 (FUJIFILM Medical, Tokyo, Japan). The study patients were administered 2 L of polyethylene glycol (PEG) solution before the procedure. Scopolamine butylbromide (20 mg) or glucagon (1 IU) was administered. No sedatives were used. Air insufflation was used during the both procedures. Fluoroscopy was used when stretching the scope or when the scope was stacked. We withdrew the endoscope when either the insertion time exceeded 90 min or the patient requested that the procedure be stopped. Cecal intubation was defined as successful when the ileocecal valve and appendiceal orifice were identified. Ancillary procedures such as polypectomy and biopsy were performed while withdrawing the scope after cecal intubation. While the patients were in the recovery room after the examination, they were asked to evaluate the pain during the examination on a 10-point analog scale (1 = no pain, 10 = worst imaginable pain) in a questionnaire.

Study outcomes

The primary outcome was the successful cecal intubation rate. Secondary outcome measures were the cecal intubation time, endoscopic findings, complications, and pain score during the examination.

Statistical analysis

Categorical data including the total enteroscopy rate and diagnosis rate were compared using Fisher's exact test. Continuous variables were compared using Student's *t*-test. The cecal insertion time and X-ray fluoroscopy time were compared with the Mann-Whitney *U*-test. Differences with *P* < 0.05 were considered to indicate statistical significance. All statistical analyses were performed using JMP, ver. 9.0 (SAS Institute, Cary, NC, United States).

RESULTS

Baseline characteristics

During the study period from August 2008 to June 2010, 21 patients were enrolled and assigned randomly to undergo either SBC (*n* = 11) or DBC (*n* = 10). Table 1 shows the baseline characteristics of the study patients. There was no significant difference between the SBC and

Table 2 Cecal intubation rate and endoscopy-related outcomes of balloon colonoscopy

	SBC group (<i>n</i> = 11)	DBC group (<i>n</i> = 10)	<i>P</i> value
Successful cecal intubation, <i>n</i> (%)	10 (91)	10 (100)	0.99 ¹
Cecal intubation time ³ (min) [median (range)]	18.0 (10-85)	12.8 (9.5-42)	0.17 ²
Total X-ray fluoroscopic time (min) [median (range)]	3 (1-7)	1 (1-5)	0.12 ²
Pain score ⁴ [median (range)]	5 (1-10)	5 (1-6)	0.64 ²

¹Fisher's exact test; ²Mann-Whitney *U*-test; ³The cecal intubation time was compared for cases with successful cecal intubation; ⁴After balloon endoscopy, the patients were asked to evaluate the pain during the procedure on a 10-point analog scale (1 = no pain, 10 = worst imaginable pain). SBC: Single-balloon colonoscopy; DBC: Double-balloon colonoscopy.



Figure 1 Failed cecal intubation with single-balloon colonoscopy. The patient had previously undergone abdominal surgery for cholecystitis. Fluoroscopy shows adhesion between the sigmoid and transverse colons (arrow).

DBC groups.

Cecal intubation rate and endoscopic procedural results

Table 2 shows the cecal intubation rate and endoscopy results. Using balloon endoscopy, cecal intubation was achieved in all cases but one patient who previously had abdominal surgery for cholecystitis and fluoroscopy showed adhesion between the sigmoid and transverse colons (Figure 1). The cecal intubation rate was 91% in the SBC group and 100% in the DBC group ($P = 0.99$). The median cecal intubation time of the successful cases did not differ between the SBC and DBC groups [18.0 min (range: 10-85 min) *vs* 12.8 min (range: 9.5-42 min), respectively, $P = 0.17$], neither did the X-ray fluoroscopy time [3 min (range: 1-7 min) *vs* 1 min (range: 1-5 min), $P = 0.12$]. There was no difference in the pain score during the endoscopic procedure between the SBC and DBC groups [median (range), 5 (range: 1-10) *vs* 5 (range: 1-6), $P = 0.64$].

Diagnostic yield

The diagnostic yield in each group is shown in Table 3. Colorectal polyps were detected in 8 of the 21 (38%) pa-

Table 3 Comparison of the diagnostic yields of single-balloon colonoscopy and double-balloon colonoscopy *n* (%)

	SBC group (<i>n</i> = 11)	DBC group (<i>n</i> = 10)	<i>P</i> value ¹
Advanced cancer	1 (9)	0 (0)	0.99
Colon polyp	5 (45)	3 (30)	0.66
Diverticulosis	3 (27)	4 (40)	0.66

¹Fisher's exact test. SBC: Single-balloon colonoscopy; DBC: Double-balloon colonoscopy.

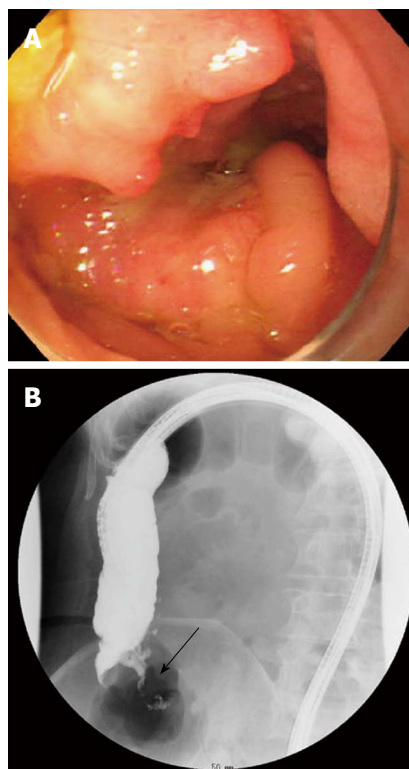


Figure 2 Advanced colon cancer detected at single-balloon colonoscopy. A: Endoscopic image; B: Selective contrast image. The lesion was located in the ascending colon, which was not accessible by conventional colonoscopy. With single-balloon colonoscopy, the lesion was detected in 11 min.

tients. All polyps were histologically confirmed as adenoma. The detection rate was 45% and 30% with SBC and DBC, respectively; the difference was not significant ($P = 0.66$). Moreover, SBC showed advanced colon cancer in the ascending colon, which was inaccessible by conventional colonoscopy (Figure 2).

Complications

No complications were noted in this study.

DISCUSSION

In our series, both SBE and DBE had high total colonoscopy rates in patients with incomplete colonoscopy using a conventional colonoscope. The utility of SBE and DBE for colonoscopy seems comparable.

Both SBE and DBE were initially designed for small

bowel endoscopy and have proved their value for small bowel examination^[9]. The endoscope and overtube are advanced sequentially with serial fixing and shortening of the small intestine using the balloons, in order to minimize looping and advance the scope. This balloon technique has already been used successfully for patients in whom colonoscopy is difficult. The reported total colonoscopy rate is 95%-100% for SBE^[10-12], and 88%-100% for DBE^[13-16]. Our total colonoscopy rates were comparable for SBE and DBE (91% *vs* 100%, *P* = 0.99). Previously, we reported that the total enteroscopy rate for the small intestine was higher with DBE than with SBE (57% *vs* 0%, *P* = 0.002)^[17]. DBE grips the small intestine at the tip of the endoscope more easily than SBE, which makes possible deep insertion of the endoscope without redundant loops. When used for colonoscopy, the potential disadvantage of SBE seen in small intestinal endoscopy is negligible.

In our series, the cecal intubation time was comparable between SBC and DBC. Teshima *et al.*^[12] reported that SBE was faster than DBE because only one balloon cycle is used, as opposed to two. Compared with small intestinal endoscopy, colonoscopy is faster and requires fewer balloon cycles. Consequently, the simpler manipulation with SBE does not shorten the cecal intubation time.

The importance of total colonoscopy is well recognized, especially for older patients because of the increase in right-sided colon cancer with age^[18,19]. Indeed, SBE detected advanced colon cancer in the ascending colon, which was inaccessible to conventional colonoscopy. In addition, we performed all procedures without any sedatives or complications. The study indicated that SBE and DBE can be performed safely in patients with incomplete total colonoscopy using a conventional colonoscope. Therefore, we recommend that patients with incomplete total colonoscopy undergo SBE or DBE.

The present study showed that SBC and DBC can be performed safely without sedation even in patients with incomplete total colonoscopy using a conventional colonoscope. In terms of colonoscopy, several papers reported that conventional colonoscopy without sedation is feasible, effective and well tolerated^[20,21]. On the other side, most of previous papers regarding SBC and DBC used sedative drugs^[11,12,14-16]. Although the pain score during for procedure was slightly high, all of the present study patients did not request to stop the procedure.

There were some limitations to this study. The number of participants was relatively small and a larger prospective non-inferiority trial is needed to elucidate any difference in the utility of SBE and DBE. However, the procedures had very high diagnostic yields in both groups, suggesting that both SBC and DBC are effective modalities for colonic examination.

In conclusion, both single- and double-balloon endoscopy make possible performance of total colonoscopy in patients with incomplete colonoscopy using a conventional colonoscope. The utility of SBE and DBE for colonoscopy seems to be comparable.

COMMENTS

Background

Despite advances in colonoscopes and endoscopy techniques, total colonoscopy still fails in some patients. Balloon endoscopy is an effective tool for investigating the small intestine. Two different types of balloon endoscopy are available commercially: single-balloon endoscopy (SBE) and double-balloon endoscopy (DBE). A retrograde approach might facilitate successful total colonoscopy and allow endoscopic therapy in patients who had incomplete colonoscopy with a conventional colonoscope. A difference between single-balloon colonoscopy (SBC) and double-balloon colonoscopy (DBC) has not yet been identified.

Research frontiers

SBE and DBE can be used to complete examination of the colon in patients with incomplete colonoscopy using a conventional colonoscope. It also allows therapeutic interventions.

Innovations and breakthroughs

SBE and DBE have been an important endoscopic breakthrough for successful total colonoscopy and endoscopic therapy in patients who had incomplete colonoscopy with a conventional colonoscope. In this study, the authors compared the utility of SBC and DBC for difficult colonoscopy in an exploratory randomized controlled trial. The study indicated that both SBE and DBE make possible performance of total colonoscopy in patients with incomplete colonoscopy using a conventional colonoscope without any sedation. The utility of SBE and DBE for colonoscopy seems to be comparable.

Applications

This study suggests that patients with incomplete total colonoscopy undergo SBE or DBE.

Terminology

DBE consists of an endoscope and a soft overtube. A latex balloon is attached to the tip of the endoscope and another to the tip of the overtube. Each balloon can be inflated and deflated by a pressure controlled air pump system. SBE is simpler to perform than DBE because it has only 1 balloon at the tip of the overtube. The equipment and techniques are different between DBE and SBE. However, the principle of insertion is the same; gripping the intestine by using balloon inflation prevents redundant loop formation and thus facilitates deep insertion of the endoscope.

Peer review

The authors compared SBE with DBE in patients with previous incomplete colonoscopy because of several reasons. They achieved excellent total colonoscopy rates (91% *vs* 100%) even in these difficult cases. These results indicate the utility of SBE and DBE in patients with incomplete conventional colonoscopy.

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L- Editor A **E- Editor** Zhang DN



Clinical outcome in patients with hepatocellular carcinoma after living-donor liver transplantation

Ho Joong Choi, Dong Goo Kim, Gun Hyung Na, Jae Hyun Han, Tae Ho Hong, Young Kyoung You

Ho Joong Choi, Dong Goo Kim, Gun Hyung Na, Jae Hyun Han, Tae Ho Hong, Young Kyoung You, Hepatobiliary-Pancreas Surgery and Liver Transplantation Division, Department of Surgery, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul 137-701, South Korea

Author contributions: Choi HJ designed the study, analyzed the data, performed the study, and wrote the paper; Na GH, Han JH, Hong TH and You YK collected the data; Kim DG designed the study, collected the data, and wrote the paper.

Correspondence to: Dong Goo Kim, MD, PhD, Hepatobiliary-Pancreas Surgery and Liver Transplantation Division, Department of Surgery, Seoul St. Mary's Hospital, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, South Korea. kimgd@catholic.ac.kr

Telephone: +82-2-22586102 Fax: +82-2-5952992

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Abstract

AIM: To investigate risk factors for hepatocellular carcinoma (HCC) recurrence after living donor liver transplantation (LDLT) and efficacy of various criteria.

METHODS: From October 2000 to November 2011, 233 adult patients underwent LDLT for HCC at our institution. After excluding nine postoperative mortality cases, we analyzed retrospectively 224 patients. To identify risk factors for recurrence, we evaluated recurrence, disease-free survival (DFS) rate, survival rate, and various other factors which are based on the characteristics of both the patient and tumor. Additionally, we developed our own criteria based on our data. Next, we compared our selection criteria with various tumor-grading scales, such as the Milan criteria, University of California, San Francisco (UCSF) criteria, TNM stage, Barcelona Clinic Liver Cancer (BCLC) stage and Cancer of the Liver Italian Program (CLIP) scoring system. The median follow up was 68 (6-139) mo.

RESULTS: In 224 patients who received LDLT for HCC, 37 (16.5%) experienced tumor recurrence during the follow-up period. The 5-year DFS and overall survival rates after LDLT in all patients with HCC were 80.9% and 76.4%, respectively. On multivariate analysis, the tumor diameter {5 cm; $P < 0.001$; exponentiation of the B coefficient [Exp(B)], 11.89; 95%CI: 3.784-37.368} and alpha fetoprotein level [AFP, 100 ng/mL; $P = 0.021$; Exp(B), 2.892; 95%CI: 1.172-7.132] had significant influences on HCC recurrence after LDLT. Therefore, these two factors were included in our criteria. Based on these data, we set our selection criteria as a tumor diameter ≤ 5 cm and AFP ≤ 100 ng/mL. Within our new criteria (140/214, 65.4%), the 5-year DFS and overall survival rates were 88.6% and 81.8%, respectively. Our criteria ($P = 0.001$), Milan criteria ($P = 0.009$), and UCSF criteria ($P = 0.001$) showed a significant difference in DFS rate. And our criteria ($P = 0.006$) and UCSF criteria ($P = 0.009$) showed a significant difference in overall survival rate. But Milan criteria did not show significant difference in overall survival rate ($P = 0.137$). Among stages 0, A, B and C of BCLC, stage C had a significantly higher recurrence rate ($P = 0.001$), lower DFS ($P = 0.001$), and overall survival rate ($P = 0.005$) compared with the other stages. Using the CLIP scoring system, the group with a score of 4 to 5 showed a high recurrence rate ($P = 0.023$) and lower DFS ($P = 0.011$); however, the overall survival rate did not differ from that of the lower scoring group. The TNM system showed a trend of increased recurrence rate, decreased DFS, or survival rate according to T stage, albeit without statistical significance.

CONCLUSION: LDLT is considered the preferred therapeutic option in patients with an AFP level less than 100 ng/mL and a tumor diameter of less than 5 cm.

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Key words: Hepatocellular carcinoma; Living donor

liver transplantation; Selection criteria; Milan criteria; University of California, San Francisco criteria; Barcelona Clinic Liver Cancer; Cancer of the Liver Italian Program

Core tip: Liver transplantation (LT) for hepatocellular carcinoma (HCC) is known to be the best therapeutic option. To obtain a good result, it is important to select appropriate LT candidates from among HCC patients. For living-donor liver transplantation (LDLT), investigation of the efficacy of such criteria will facilitate adoption of extended criteria in LDLT. We defined the selection criteria according to risk factors for recurrence based on our results and compared them with other criteria or scoring systems, such as the Milan and University of California, San Francisco criteria, tumor node metastasis and Barcelona Clinic Liver Cancer staging, and the Cancer of the Liver Italian Program scoring system.

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INTRODUCTION

On the hepatocellular carcinoma (HCC), two alternative treatment options exist with curative intention, such as liver resection and liver transplantation (LT). LT, unlike hepatic resection, has the advantage that it can treat not only the tumor but also the underlying liver cirrhosis. Most LT candidates in the past have been patients with advanced HCC. The high tumor recurrence rates and low survival rates of these patients were discouraging^[1,2]. However, LT was established as a suitable treatment for HCC since Mazzaferro *et al.*^[3] reported the Milan criteria in 1996. The Milan criteria improved the overall survival and disease-free survival (DFS) rates. Thereafter, LT has achieved the best results in well-selected candidates, and most international transplantation communities have adopted the Milan criteria for the treatment of HCC.

However, unfortunately, about 70% of HCC patients are diagnosed with advanced stage disease^[4]. Even among patients who meet the Milan criteria, 20% or more will be removed from the waiting list because progression of HCC excludes them from the Milan criteria^[5-7]. Additionally, the treatment outcome of a patient excluded from the waiting list because of HCC progression who selects other treatments is known to be very poor. Furthermore, many centers have reported good results despite expansion of the selection criteria. Therefore, the current Milan criteria are too strict, and most centers agree on the need for their expansion. Additionally, in Asian countries, potential donors for de-

ceased donor liver transplantation (DDLT) are lacking. Therefore, living donor liver transplantation (LDLT) is emerging as an additional therapeutic option. In LDLT, it is possible to match the donor and recipient, so the decision to operate is based on the risk to the donor and the expected benefit to the recipient. Patient selection in LDLT is generally wider compared with that in DDLT. However, ethical issues exist concerning LDLT, such as the risk of the donor and the high recurrence rate of the recipients due to advanced-stage disease. Appropriate criteria are necessary for these issues. Therefore, criteria for LDLT and DDLT that are suitable for each center are necessary.

To date, various groups have attempted to expand the Milan criteria while maintaining long-term survival rates^[8-12]. One was the proposal of the University of California, San Francisco (UCSF) criteria by Yao *et al.*^[8] in 2001. Most centers agree on expanding the criteria because more patients can benefit from transplantation, and the DFS and overall survival are comparable to the Milan criteria. Other systems include the TNM system that reflects cancer stage, and the Barcelona clinic liver cancer (BCLC) staging and the cancer of the liver Italian program (CLIP) staging systems that are well-known guidelines for therapy and prognosis prediction that are used in the United States and Europe. Regarding LT, investigation of the efficacy of these criteria for therapy of HCC is important.

The purpose of the present study was to evaluate the characteristics of patients and tumors according to recurrence. Subsequently, we defined the selection criteria according to our results and compared our criteria with other criteria or scoring systems, such as the Milan and UCSF criteria, TNM and BCLC staging, and the CLIP scoring system.

MATERIALS AND METHODS

From October 2000 to November 2011, 233 patients underwent LDLT for HCC at our center. After excluding nine postoperative mortality cases, 224 patients were evaluated. We defined the postoperative mortality as expire of patients within a month after transplantation. All LDLT patients were adults, and the right lobe was used for transplantation. The Institutional Review Board of our center approved the study design.

All patients with HCC planned for transplantation were evaluated preoperatively by computed tomography (CT) of the abdomen and chest, enhanced magnetic resonance image (MRI), positron emission tomography-CT (PET-CT), bone scintigraphy, gastrofiberscopy, and colonoscopy. Alpha fetoprotein (AFP) and protein induced by vitamin K absence or angiotensin-II (PIVKA-II) were also evaluated as tumor markers. Contraindications of LT in patients with HCC included tumor thrombus in the main portal vein, regional lymph node metastasis, and distant metastasis. We reviewed the tumor diameter and number of tumors based on the pathologic report.

LDLT was performed according to a standard technique using a modified right lobe with middle hepatic vein reconstruction. For ascites, aspiration and cytology were performed before beginning the operation. When lymph node enlargement was present, or in cases with suspicious metastatic disease, an intraoperative biopsy was performed. The operation was performed only in cases with negative biopsy results. Immunosuppression treatment included a regimen of a calcineurin inhibitor (Cyclosporine or Tacrolimus) as part of a dual- or triple-drug regimen with prednisone and mycophenolate mofetil (MMF). An interleukin-2 receptor blocker was administered on both the day of the operation and the fourth postoperative day. Steroids were withdrawn 1 mo after surgery, and MMF was withdrawn 6 mo after surgery. Only a low dose of a single calcineurin inhibitor was administered after this period. The immunosuppression protocol did not differ from other benign diseases. When recurrence was detected during the follow-up period, the immunosuppressive agent was changed or decreased.

For early detection of cancer recurrence, AFP and PIVKA-II were checked monthly during the first year, and then bimonthly thereafter. Abdomen CT, chest CT, and bone scintigraphy were routinely performed every 6 mo during the first 2 years, and then were performed annually. When tumor recurrence was suspected, MRI and/or PET-CT were performed.

We evaluated recurrence, the DFS rate, the survival rate, and various other factors to identify the risk of recurrence. Additionally, based on our data, we developed patient selection criteria suitable for our center. Furthermore, using our LDLT data, we evaluated and assessed criteria, such as the most frequently used Milan criteria, the UCSF criteria, and important therapeutic guidelines such as TNM staging, BCLC staging, and the CLIP scoring system.

Statistical analysis

Numeric data were presented as means and standard deviations or as medians and ranges. Continuous variables (means, standard deviations, medians, and ranges) were analyzed using an independent *t*-test or χ^2 test. Multiple regression analyses were performed using Cox proportional hazards models for identification of factors independently associated with recurrence in 95%CI. DFS and 5-year survival rates were estimated using the Kaplan-Meier method, and survival curves were compared using the log-rank test. Statistical analysis was performed using SPSS version 19.0 (IBM SPSS statistics 19). Statistical significance was accepted for *P* values less than 0.05.

RESULTS

Patient characteristics

The mean age of all patients was 51.9 ± 6.92 years (range, 34-66 years), and 184 patients (82.1%) were male. Underlying liver disease was caused by HBV infection (87.9%) most commonly, followed by HCV infection (5.8%) and

other causes (6.3%). The average Child-Pugh score was 8.15 ± 2.40 (range, 5-15), and the average Model for end-stage liver disease (MELD) score was 12.8 ± 7.63 (range, 1-37). The graft vs recipient weight ratio (GRWR) was $1.21\% \pm 0.27\%$ (range, 0.6%-2.3%). Preoperative treatments were performed in 167 patients (74.6%). Of the 224 patients, 133 (59.4%) met Milan criteria, and 154 patients (68.8%) met UCSF criteria. During the follow-up period, 50 patients expired. The cause of death was HCC recurrence in 31 patients (62%), technical complications in nine patients (18%), sepsis in five patients (10%), graft failure in three patients (6%), and other causes in two patients (4%). The median follow-up duration was 68 (range, 6-139) mo.

Recurrence and related factors

Of the 224 patients, recurrence occurred in 37 (16.5%) during the follow-up period. The number of patients who met the Milan criteria was 16 of 133 (12.0%), and of those who did not meet the Milan criteria 20 of 83 (24.1%) (*P* = 0.021). Most recurrences occurred within 2 years, with 26 patients (76%) experiencing recurrence within 1 year, and 30 (81%) within 2 years. Two patients experienced recurrence 5 years after transplantation. The primary recurrence site was intrahepatic in 10 patients (27%) and extrahepatic in 27 (73%). Of the extrahepatic metastasis sites, the lung was the most common primary recurrence site [10 patients (27%)]. Other recurrence sites were the brain, bone, adrenal gland, diaphragm, omentum, para-aortic lymph nodes, and neck lymph nodes.

Patient demographics and tumor characteristics were compared between the group without recurrence and the group with recurrence. The levels of AFP and PIVKA-II were compared to the averages of 50, 100 and 200 ng/mL. The AFP level showed a significant difference when compared to that of 100 ng/mL (*P* = 0.025). Additionally, although the compared number of PIVKA-II cases was small, the comparison itself was significant at 50, 100 and 200 ng/mL. Furthermore, the mean AFP and PIVKA-II levels seemed higher in the recurrent patient group; however, no significant differences were noted (AFP, *P* = 0.568; PIVKA-II, *P* = 0.576). The group that had received preoperative treatment showed a higher recurrence rate (*P* = 0.025). However, age, gender, cause of disease, Child-Pugh scores, MELD scores, and GRWRs showed no statistically significant difference. The mean maximal and total tumor diameters were higher in the recurrent group, being 5.54 ± 5.65 and 7.82 ± 6.56 cm, respectively, in the recurrent group and 2.76 ± 1.98 and 4.21 ± 2.88 cm, respectively, in the non-recurrent group (*P* = 0.006 and 0.005, respectively). Additionally, when the recurrent and non-recurrent groups were divided according to maximal diameter and total diameter over 5 cm, both groups had significantly higher recurrence rates (*P* < 0.001 and *P* = 0.011, respectively). The tumor number was significantly different between the non-recurrent and recurrent groups when the cutoff numbers were 5 and 7 (*P* = 0.046 and *P* = 0.049, respectively). Microvascular invasion was

Table 1 Patient and tumor characteristics according to recurrence rate, univariate analysis

Variables	Non-recurrent (<i>n</i> = 187)	Recurrent HCC (<i>n</i> = 37)	<i>P</i> -value
Patient characteristics			
Age (yr)	52.05 ± 6.93	50.89 ± 6.88	0.354
Gender (male: female)	151 (80.7):36 (19.3)	33 (89.2):4 (10.8)	0.221
HBV: HCV: Other cause	164:11:12	33:2:2	0.965
Child-Pugh score	8.15 ± 2.41	8.14 ± 2.37	0.972
MELD score	12.76 ± 7.25	13.05 ± 9.45	0.858
GRWR (%)	1.20 ± 0.28	1.24 ± 0.24	0.454
≤ 1 (<i>n</i> = 29)	26 (89.7)	3 (10.3)	0.327
> 1 (<i>n</i> = 193)	159 (82.4)	34 (17.6)	
AFP (ng/mL)	170.5 ± 806.2	249.94 ± 481.37	0.568
≤ 100 (<i>n</i> = 163)	142 (87.1)	21 (12.9)	0.025
> 100 (<i>n</i> = 59)	44 (74.6)	15 (25.4)	
PIVKA-II (mAU/mL)	189.6 ± 1150.3	397.2 ± 674.5	0.576
≤ 100	99 (94.3)	6 (5.7)	0.018
> 100	14 (77.8)	4 (22.2)	
Pre-transplant treatment			0.025
No (<i>n</i> = 57)	53 (93.0)	4 (7.0)	
Yes (<i>n</i> = 167)	134 (80.2)	33 (19.8)	
Pathologic characteristics			
Maximal diameter (cm)	2.76 ± 1.98	5.54 ± 5.65	0.006
≤ 5 (<i>n</i> = 196)	170 (86.7)	26 (13.3)	< 0.001
> 5 (<i>n</i> = 20)	10 (50.0)	10 (50.0)	
Total diameter (cm)	4.21 ± 2.88	7.82 ± 6.56	0.005
≤ 9 (<i>n</i> = 185)	162 (87.6)	23 (12.4)	0.001
> 9 (<i>n</i> = 19)	11 (57.9)	8 (42.1)	
Tumor number	2.46 ± 2.18	3.06 ± 3.11	0.282
≤ 5 (<i>n</i> = 193)	164 (85.0)	29 (15.0)	0.046
> 5 (<i>n</i> = 22)	15 (68.2)	7 (31.8)	
Microvascular invasion			0.039
No (<i>n</i> = 163)	140 (85.9)	23 (14.1)	
Yes (<i>n</i> = 44)	32 (72.7)	12 (27.3)	
E-S grade			0.300
Low (I, II) (<i>n</i> = 108)	93 (86.1)	15 (13.9)	
High (III, IV) (<i>n</i> = 81)	65 (80.2)	16 (19.8)	

Data are expressed as absolute *n* (%) or mean ± SD. HBV: Hepatitis B virus; HCV: Hepatitis C virus; MELD: Model for End-Stage Liver Disease; GRWR: Graft vs recipient weight ratio; AFP: Alpha fetoprotein; PIVKA-II: Protein induced by vitamin K absence or angiotensin-II; E-S grade: Edmondson-Steiner grade.

significant different between the non-recurrent and recurrent groups (*P* = 0.039). There was no difference in the Edmondson-Steiner (E-S) grade (*P* = 0.3; Table 1).

To identify factors related to recurrence, a multivariate analysis of factors that had shown statistical significance in univariate analysis was performed. The prognostic factors affecting recurrence included a serum AFP level > 100 ng/mL, a PIVKA-II level > 100 mAU/mL, a tumor diameter > 5 cm, a total tumor diameter > 9 cm, a tumor number > 5, microvascular invasion, and pretransplantation treatment in univariate analyses. In multivariate analysis, a maximal tumor diameter > 5 cm {exponentiation of the B coefficient [Exp(B)], 11.89; 95%CI: 3.784-37.368; *P* < 0.001} and an AFP level > 100 ng/mL [Exp(B), 2.892; 95%CI: 1.172-7.132; *P* = 0.021] had a significant influence on recurrence (Table 2). Based on these data, we set our selection criteria [Catholic Medical Center (CMC) criteria] as a tumor diameter ≤ 5 cm and AFP ≤ 100 ng/mL. When both

Table 2 Risk factors for recurrence; multivariate analysis

Variables	<i>P</i> -value	Exp(B)	95%CI
AFP (100 ng/mL)	0.021	2.892	1.172-7.132
Pre-transplant treatment	0.114	2.421	0.742-7.897
Maximal diameter (5 cm)	< 0.001	11.891	3.784-37.368
Total diameter (9 cm)	0.142	2.633	0.754-9.194
Tumor number (5)	0.712	1.373	0.262-7.203
Microvascular invasion	0.27	1.768	0.653-4.784

AFP: Alpha fetoprotein; Exp(B): Exponentiation of the B coefficient.

criteria were met, the case was classified as within criteria (*n* = 138, 66.0%), and when at least one criterion was not met, the case was classified as beyond criteria (*n* = 71, 34.0%).

Impacts of the various criteria

We applied our data to various conventional criteria and therapeutic guidelines and compared the results with those of our CMC criteria. The data were applied to the Milan and UCSF criteria, TNM and BCLC stagings, the CLIP scoring system, and our CMC criteria were evaluated in terms of recurrence. In the Milan criteria and UCSF criteria, the patient group not meeting the criteria demonstrated a significantly higher recurrence rate than the group meeting the criteria (Milan, *P* = 0.021; UCSF, *P* = 0.002). In the CMC criteria specified above, the within-criteria group showed a recurrence rate of 10.0%, and the beyond-criteria group showed a recurrence rate of 28.4%. The difference was statistically significant (*P* = 0.001). In BCLC staging, the C stage showed a significantly higher recurrence rate compared with the 0, A, and B stages (*P* < 0.001); however, the differences among the 0, A, and B stages were not significant. In evaluating the CLIP scoring system, scores of 0 and 1, 2 and 3, and 4 and 5 were classified as three groups. The group with scores of 4 and 5 had a significantly higher recurrence rate than the other two groups (*P* = 0.023). The group with scores of 0 and 1, as well as that with scores of 2 and 3, showed no difference in recurrence. However, in the TNM staging, although recurrence showed an increasing trend with higher T status, there were no significant differences in recurrence (*P* = 0.325; Table 3).

The DFS and overall survival rates according to various criteria were compared. The 5-year DFS and survival rates of total patients were 80.9% and 76.4%, respectively. The Milan criteria showed a statistically significant difference in only the DFS rate (*P* = 0.009). However, the UCSF criteria showed a statistically significant difference in the DFS rate (*P* = 0.001) and overall survival rate (*P* = 0.009). Regarding CMC criteria, the 5-year DFS rate (*P* < 0.0001) and survival rate (*P* = 0.0006) showed statistically significant differences. Classification according to T status showed a decreasing trend in the 5-year DFS rate (*P* = 0.190) and survival rate (*P* = 0.394) with increasing T stage, albeit without statistical significance (*P* = 0.190). In the BCLC staging, the 5-year DFS and survival rates among stages 0, A, and B showed no statistically sig-

Table 3 Recurrence rates according to various criteria *n* (%)

Variables	Non-recurrent	Recurrent	P-value
Milan criteria			0.021
Within (<i>n</i> = 133)	117 (88.0)	16 (12.0)	
Beyond (<i>n</i> = 83)	63 (75.9)	20 (24.1)	
UCSF criteria			0.002
Within (<i>n</i> = 154)	136 (88.3)	18 (11.7)	
Beyond (<i>n</i> = 62)	44 (71.0)	18 (29.0)	
CMC criteria			0.001
Within (<i>n</i> = 140)	126 (90.0)	14 (10.0)	
Beyond (<i>n</i> = 74)	53 (71.6)	21 (28.4)	
TNM			0.325
T1 (<i>n</i> = 81)	71 (87.7)	10 (12.3)	
T2 (<i>n</i> = 127)	103 (81.1)	24 (18.9)	
T3 (<i>n</i> = 7)	5 (71.4)	2 (28.6)	
BCLC			< 0.001
0 (<i>n</i> = 36)	33 (91.7)	3 (8.3)	
A (<i>n</i> = 82)	73 (89.0)	9 (11.0)	
B (<i>n</i> = 53)	47 (88.7)	6 (11.3)	
C (<i>n</i> = 45)	27 (60.0)	18 (40.0)	
CLIP			0.023
0, 1 (<i>n</i> = 89)	73 (82.0)	16 (18.0)	
2, 3 (<i>n</i> = 110)	97 (88.2)	13 (11.8)	
4, 5 (<i>n</i> = 12)	7 (58.3)	5 (41.7)	

UCSF: University of California, San Francisco; CMC: Catholic Medical Center; BCLC: Barcelona Clinic Liver Cancer; CLIP: Cancer of the Liver Italian Program; HCC: Hepatocellular carcinoma.

Table 4 Disease-free survival and overall survival rates according to various criteria

Criteria		Disease-free survival			Overall survival		
		3 yr	5 yr	P-value	3 yr	5 yr	P-value
Milan	Within (<i>n</i> = 130)	0.880	0.867	0.009	0.808	0.798	0.137
	Beyond (<i>n</i> = 81)	0.730	0.704		0.733	0.706	
UCSF	Within (<i>n</i> = 150)	0.881	0.869	0.001	0.822	0.813	0.009
	Beyond (<i>n</i> = 61)	0.681	0.645		0.677	0.643	
CMC	Within (<i>n</i> = 138)	0.899	0.886	< 0.001	0.840	0.818	0.006
	Beyond (<i>n</i> = 71)	0.682	0.656		0.663	0.663	
TNM	T1 (<i>n</i> = 80)	0.890	0.870	0.190	0.830	0.830	0.394
	T2 (<i>n</i> = 123)	0.788	0.773		0.753	0.725	
	T3 (<i>n</i> = 7)	0.571	0.571		0.556	0.556	
BCLC	0 (<i>n</i> = 35)	0.943	0.902	< 0.001	0.882	0.882	0.005
	A (<i>n</i> = 80)	0.878	0.878		0.805	0.787	
	B (<i>n</i> = 52)	0.854	0.854		0.878	0.826	
	C (<i>n</i> = 44)	0.578	0.544		0.549	0.549	
CLIP	0,1 (<i>n</i> = 88)	0.795	0.771	0.011	0.776	0.776	0.272
	2,3 (<i>n</i> = 106)	0.885	0.870		0.819	0.792	
	4,5 (<i>n</i> = 12)	0.556	0.556		0.509	0.509	

UCSF: University of California, San Francisco; CMC: Catholic Medical Center; BCLC: Barcelona Clinic Liver Cancer; CLIP: Cancer of the Liver Italian Program.

nificant differences. However, the 5-year DFS rate ($P < 0.001$) and survival rate ($P = 0.005$) of stage C were significantly lower than those of the other stages. The CLIP scoring system showed a statistically significant difference in only DFS ($P = 0.011$; Table 4).

The Milan criteria showed significant differences regarding DFS, but not survival rate. We subdivided each factor of the Milan criteria and compared them with re-

spect to the recurrence rate, and DFS and survival rates. Additionally, we subdivided factors of the CMC criteria and compared them with those of the Milan criteria (Table 5). Of the total, 133 (59.4%) patients were within the Milan criteria, and 140 (62.5%) were within the CMC criteria. When applying Milan criteria, the difference was significant when the cutoff value was a single tumor under 5 cm but was not significant when the cutoff value was less than three tumors, or two or three tumors less than 3 cm. When factors of the CMC criteria were subdivided, the recurrence rate, DFS, and overall survival rates were all significantly different. Although the number was small, most cases with a tumor size over 5 cm and an AFP level over 100 ng/mL recurred with a very poor prognosis; no patients survived beyond 3 years (Figure 1).

DISCUSSION

LT is a curative treatment modality for HCC. LT is particularly important in cases where resection is impossible due to factors such as liver cirrhosis. In countries with a shortage of deceased donor organs, LDLT can be the mainstay of therapy rather than DDLT. However, there is concern that LDLT has disadvantages regarding HCC recurrence compared with DDLT. The LDLT selection criteria are likely applied more widely than DDLT. Due to the short waiting time, LDLT candidates have no opportunity to be screened for an aggressive tumor biology. Because of their relatively small size LDLT grafts are subject to additional mechanical injury at the start of reperfusion, and angiogenesis and cell division signaling pathways may be initiated more frequently. The rapid graft regeneration in LDLT may also be associated with acceleration of tumor growth^[13]. In fact, some studies have reported higher recurrence rates of LDLT compared with DDLT^[14,15]. However, a recent meta-analysis concluded that the DFS rates of LDLT and DDLT do not differ significantly^[16]. In our study, the recurrence rate was 16.5%, and for DDLT performed on patients within the Milan criteria, the recurrence rate was 12.0%. The total 5-year DFS rate and overall survival rate were 80.9% and 76.4%, respectively, and these results were generally acceptable.

The Milan criteria (MC) are often used to determine which patients will benefit from LT. However, when LT is strictly confined to those within MC, many patients who may benefit from LT will be lost. In advanced-stage III HCC patients, survival rates are about 59% after LT, which is comparable to patients with benign disease (65%)^[17]. LT is undoubtedly superior to transarterial chemoembolization or chemotherapy in patients beyond MC, who may still benefit from transplantation. Unlike DDLT, LDLT is private, not public, and is performed in beyond-MC patients more easily. As reported by four major LT centers, 29.6% of LDLT procedures were performed in beyond MC patients^[18]. At our institution, 37.5% of LDLT patients exceeded MC. Many centers have center-based criteria for selection of patient

Table 5 Comparison of the Milan and Catholic Medical Center criteria

Criteria		Recurrence rate	P-value	5-yr DFS	P-value	5-yr survival	P-value
Milan criteria							
Single	≤ 5 cm (n = 91)	13%	< 0.001	0.862	< 0.001	0.829	< 0.001
	> 5 cm (n = 12)	61.5%		0.333		0.333	
No. 2 or 3	≤ 3 cm (n = 39)	9.8%	0.889	0.878	0.891	0.711	0.251
	> 3 cm (n = 22)	8.7%		0.848		0.747	
Number	≤ 3 (n = 164)	15.4%	0.209	0.824	0.228	0.781	0.316
	> 3 (n = 47)	21.7%		0.737		0.698	
CMC criteria							
Size ≤ 5 cm	AFP ≤ 100 (n = 138)	9.6%	0.001	0.886	< 0.001	0.818	< 0.001
Size ≤ 5 cm	AFP > 100 (n = 52)	20.0%		0.765		0.749	
Size > 5 cm	AFP ≤ 100 (n = 15)	38.9%		0.514		0.508	
Size > 5 cm	AFP > 100 (n = 4)	75.0%		0		0	

CMC: Catholic Medical Center; DFS: Disease free survival; AFP: Alpha fetoprotein (mg/dL).

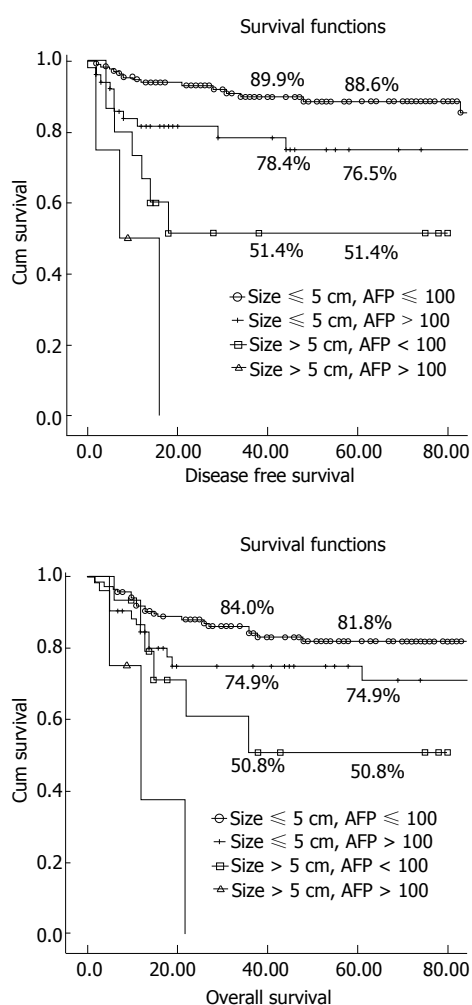


Figure 1 Comparison of the disease free survival and overall survival rates in patients with hepatocellular carcinoma according to Catholic Medical Center criteria. AFP: Alpha fetoprotein (mg/dL).

who exceed MC. The UCSF, Tokyo, and up-to-seven criteria are based on HCC size and number, which are surrogate markers of tumor volume^[8-10]. Recently, the expression level of pre-operative serum tumor markers that affect HCC recurrence, such as pre-operative AFP and PIVKA-II levels, have become a hot topic of

research. Some authors have reported that an AFP level prior to LT of > 200 or > 1000 ng/mL affected HCC recurrence^[8,19,20]. Conversely, the Kyoto and Kyushu University criteria included the PIVKA-II level as a factor affecting tumor recurrence^[11,12].

According to our results, a largest tumor diameter > 5 cm and AFP level > 100 ng/mL significantly influenced recurrence. Tumor number, which is important in the Milan criteria, did not show statistical significance. Indeed, many tumors discovered postoperatively are around 1 cm in size and are difficult to identify preoperatively. A discrepancy between the preoperatively and postoperatively discovered tumor numbers exists. Therefore, using tumor number as an important criterion may be problematic. Our results showed significant differences with the Milan criteria regarding recurrence and DFS; however, there was no significant difference regarding the survival rate, and so we subdivided each factor. There was a significant difference with a “single tumor smaller than 5 cm”, a finding that ascribes importance to size. However, no significant difference was found regarding the two other factors, a finding that confers importance on the tumor number. These findings correlate with the finding that tumor number was not a significant factor in multivariate analysis. Additionally, biologic factors are more important than morphologic factors in terms of predicting tumor behavior. Thus, inclusion of AFP, which is a biologic factor rather than representative of the tumor number, seems to be more appropriate. Therefore, we decided that, for our criteria (CMC criteria), the largest diameter of the tumor should be less than 5 cm, and the AFP level should be less than 100 ng/mL.

Next, we applied our data to current HCC guidelines, staging systems, and the Milan and UCSF criteria, which are relatively widely used LT criteria, and compared the recurrence, DFS, and overall survival rates with those of our criteria. The Milan and UCSF criteria both adequately reflected recurrence, DFS, and survival. However, when compared with only our data, the UCSF criteria included more patients and showed a better correlation between criteria and prognosis. CMC criteria showed statistically significant differences in recurrence, DFS, and overall

survival rates. Notably, although the case number was small, in cases beyond both criteria, the recurrence rate was as high as 75%, and the 5-year DFS and overall survival rates were 0%, suggesting a contraindication to LT.

Many HCC staging systems are extant; however, the most commonly used is TNM staging, the 7th edition of which was recently published by the American Joint of Committee on Cancer (AJCC). The TNM system effectively stratifies post-hepatectomy HCC patients into stages I, II, and III^[21]. According to this system, we analyzed our results to evaluate the efficacy of the TNM system for LDLT. The recurrence, DFS, and overall survival rates worsened with increasing T stage; however, no statistically significant difference was found. T3 cases seemed to show a stronger trend, although the number was small. A statistically significant difference may be found if additional cases are examined.

Because HCC patients also have liver cirrhosis, not only the stage of HCC but also liver function and the general condition of the patient must be considered when deciding the treatment modality. Many HCC guidelines exist, but the most commonly used are the BCLC and CLIP scoring systems. The BCLC scoring system is used frequently in the United States and Europe for HCC therapy and considers factors such as the patient's general condition, Child-Pugh score, portal pressure, and tumor size and number. LT is recommended only for early stage A (single, 3 nodules, < 3 cm). Because LT can be performed regardless of Child-Pugh score or portal pressure, only the tumor factor is considered. Regarding transplantation, it is important to investigate the results according to BCLC stage. Vitale *et al.*^[22] reported that LT showed a survival benefit for patients with HCC and advanced liver cirrhosis (BCLC stage D) and in those with intermediate tumors (BCLC stage B-C). In our study, the recurrence, DFS, and overall survival rates showed no significant differences among BCLC stages 0, A, and B. However, these rates showed significant differences between stage C, which includes gross portal vein thrombosis, and the other stages. Therefore, although LT is recommended only for stage A according to the BCLC guidelines, LT may be performed on stage 0, A, and B patients with comparable results. Additionally, our study showed a stage C recurrence rate of 40.0% and 5-year DFS and survival rates of 54.4% and 54.9%, respectively. Although those data indicate a poor prognosis, as the recommended minimum prerequisite 5-year survival is 50%, LDLT may be performed in select stage C patients when the patient and family agree.

The CLIP scoring system was established in 1993. It considers Child-Pugh stage, tumor morphology, AFP level, and degree of portal vein thrombosis, and is considered an important index of prognosis. The CLIP scoring system for HCC is accurate and easy to implement^[23]. However, to our knowledge, few studies have investigated the association between the CLIP scoring system and LDLT. In our study, we divided patients into three groups according to CLIP scores. In the lower-scoring groups (scores 0-3), recurrence rates, DFS rates, and overall survival rates showed

no statistically significant differences. However, the group with scores of 4 and 5 showed a significant difference compared with the lower-scoring groups. Although it is difficult to reach a definite conclusion due to the small number of cases, patients with a CLIP score of 3 or lower may be suitable for LT, whereas those with a score of 4 or 5 may demonstrate worse results.

In conclusion, the recurrence rate was 16.5%, and the 5-year DFS and overall survival rates were 80.9% and 76.4%, respectively, after performance of LDLT in HCC patients. Factors influencing recurrence were a maximal tumor diameter greater than 5 cm and an AFP level greater than 100 ng/mL. When both criteria are not met, LT is contraindicated. Milan criteria, UCSF criteria, TNM staging, BCLC staging, and the CLIP scoring system showed different outcomes depending on the degree of criteria. Therefore, all of these staging and scoring systems are useful for determining LDLT for HCC patients. These findings should be confirmed by future prospective studies that include larger numbers of cases.

COMMENTS

Background

Liver transplantation (LT) for hepatocellular carcinoma (HCC) is known to be the best therapeutic option. To obtain a good result, it is important to select appropriate LT candidates from among HCC patients. Currently, many centers have their own criteria or treatment guidelines, which generally consist of factors that influence survival.

Research frontiers

The Milan criteria is a well-established tool for assessing the prognosis of HCC. We expanded the criteria while maintaining long-term survival rates. Authors decided that, for our criteria (CMC criteria), the largest diameter of the tumor should be less than 5 cm, and the alpha fetoprotein (AFP) level should be less than 100 ng/mL. When both criteria are not met, authors think LT is contraindicated.

Innovations and breakthroughs

Most centers agree on expanding the criteria because more patients can benefit from transplantation. Other systems include the TNM system that reflects cancer stage, and the Barcelona Clinic Liver Cancer (BCLC) and the Cancer of the Liver Italian Program (CLIP) staging systems that are well-known guidelines for therapy and prognosis prediction that are used in the United States and Europe. Regarding LT, investigation of the efficacy of these criteria for therapy of HCC is important. Authors defined the selection criteria according to risk factors for recurrence based on authors' results and compared them with other criteria or scoring systems, such as the Milan and UCSF criteria, TNM and BCLC staging, and the CLIP scoring system.

Applications

For living donor liver transplantation (LDLT), investigation of the efficacy of such criteria will facilitate adoption of extended criteria in LDLT.

Peer review

It is an important study which establishes a new and useful scoring system for HCC patients undergoing living donor liver transplantation. The paper is interesting, as it introduces a new (biochemical) parameter for the evaluation of HCC candidates to LDLT.

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Risk of sedation for diagnostic esophagogastroduodenoscopy in obstructive sleep apnea patients

Jae Myung Cha, Jung Won Jeun, Kwan Mi Pack, Joung Il Lee, Kwang Ro Joo, Hyun Phil Shin, Won-Chul Shin

Jae Myung Cha, Jung Won Jeun, Kwan Mi Pack, Joung Il Lee, Kwang Ro Joo, Hyun Phil Shin, Department of Internal Medicine, Kyung Hee University Hospital at Gang Dong, Seoul 134-727, South Korea

Won-Chul Shin, Department of Neurology, Kyung Hee University Hospital at Gang Dong, Seoul 134-727, South Korea

Author contributions: Cha JM contributed to conception and design, analysis and interpretation of data; Jeun JW, Pack KM, Lee JI, Joo KR, Shin HP and Shin WC contributed to acquisition of data; all authors drafted the article or revised it critically for important intellectual content and made the final approval of the version to be published.

Correspondence to: Jae Myung Cha, MD, PhD, Department of Internal Medicine, Kyung Hee University Hospital at Gang Dong, 149 Sangil-dong, Gangdong-gu, Seoul 134-727, South Korea. drcha@khu.ac.kr

Telephone: +82-2-4406110 Fax: +82-2-4406295
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Abstract

AIM: To investigate whether patients with obstructive sleep apnea (OSA) are at risk of sedation-related complications during diagnostic esophagogastroduodenoscopy (EGD).

METHODS: A prospective study was performed in consecutive patients with OSA, who were confirmed with full-night polysomnography between July 2010 and April 2011. The occurrence of cardiopulmonary complications related to sedation during diagnostic EGD was compared between OSA and control groups.

RESULTS: During the study period, 31 patients with OSA and 65 controls were enrolled. Compared with the control group, a higher dosage of midazolam was administered ($P = 0.000$) and a higher proportion of deep sedation was performed ($P = 0.024$) in the OSA group. However, all adverse events, including sedation fail-

ure, paradoxical responses, snoring or apnea, hypoxia, hypotension, oxygen or flumazenil administration, and other adverse events were not different between the two groups (all $P > 0.1$). Patients with OSA were not predisposed to hypoxia with multivariate logistic regression analysis ($P = 0.068$).

CONCLUSION: In patients with OSA, this limited sized study did not disclose an increased risk of cardiopulmonary complications during diagnostic EGD under sedation.

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Key words: Conscious sedation; Obstructive sleep apnea; Endoscopy; Complications; Safety

Core tip: Patients with obstructive sleep apnea (OSA) are known to be vulnerable to cardiopulmonary complications during deep sedation and anesthesia; however, little is known about the risk of conscious sedation during esophagogastroduodenoscopy (EGD). This prospective study evaluated the cardiopulmonary complications related to conscious sedation during diagnostic EGD between OSA group ($n = 31$) and control group ($n = 65$). All adverse events, including sedation failure, paradoxical responses, snoring or apnea, hypoxia, hypotension, oxygen or flumazenil administration, and other adverse events were not different between groups. Therefore, the risk of cardiopulmonary complications during diagnostic EGD under sedation may not be increased in patients with OSA.

Cha JM, Jeun JW, Pack KM, Lee JI, Joo KR, Shin HP, Shin WC. Risk of sedation for diagnostic esophagogastroduodenoscopy in obstructive sleep apnea patients. *World J Gastroenterol* 2013; 19(29): 4745-4751 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4745.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4745>

INTRODUCTION

Esophagogastroduodenoscopy (EGD) is generally a safe procedure, and has been routinely performed with sedation in the general population. Sedation reduces patient discomfort by inducing analgesia and amnesia and improves patient tolerance during EGD^[1-3]. However, sedation itself is also the cause of many potentially serious adverse events during EGD. The frequency of serious adverse events associated with EGD is about 0.5%^[4,5], and over 50% of these adverse events are cardiopulmonary complications related to the sedation^[4-6]. The most common cardiopulmonary adverse event is hypoxia, although its incidence is variable depending on the definition of hypoxia, the patient population, and the level of sedation^[7-9].

Obstructive sleep apnea (OSA) is characterized by intermittent and recurrent episodes of partial or complete obstruction of the upper airway during sleep. The administration of sedatives in patients with OSA may worsen obstruction of the pharynx and depression of the upper airway muscles^[10]. Therefore, patients with OSA are known to be vulnerable to cardiopulmonary complications during deep sedation and anesthesia^[10-13]. However, little is known about the adverse events of moderate sedation for diagnostic EGD in patients with OSA. Khiani *et al.*^[14] reported that there was no significant difference in the rates of hypoxia during EGD with sedation between high- and low-risk OSA cases, but patients with confirmed OSA were not included in this study.

The purpose of this study was to determine whether patients with confirmed OSA undergoing diagnostic EGD under sedation are more likely to become hypoxic. A prospective, case-control study was performed to compare the rate of hypoxia between an OSA group and a control group during diagnostic EGD under sedation.

MATERIALS AND METHODS

Patient population

A prospective study was performed in consecutive patients with OSA, who were recruited from a sleep center laboratory at the Kyung Hee University Hospital in Gang Dong, Seoul, Republic of Korea between July 2010 and April 2011. Patients with confirmed OSA who underwent EGD under moderate sedation for their routine health checkup at initial diagnosis or at a follow-up visit in an outpatient clinic were considered eligible for this study. Consecutive healthy subjects, who underwent EGD under moderate sedation for a routine health checkup between February 2011 and April 2011, were also enrolled as a control group. As the prevalence of gastric cancer is high in South Korea, healthy subjects often routinely undergo screening EGD for a regular medical checkup without any gastrointestinal symptoms in Korea. The occurrence of hypoxia and other cardiopulmonary complications related to sedation for diagnostic EGD were compared between an OSA and a control groups.

Patients with confirmed OSA between 18 and 70 years old who provided informed consent were eligible for this study. The diagnosis of OSA was confirmed based on the results of full-night, in-laboratory polysomnography. Asymptomatic patients who scored higher than 15 on the apnea hypopnea index or respiratory disturbance index were diagnosed with OSA, as were patients with symptoms or signs of disturbed sleep who scored higher than 5 on the hypopnea index or respiratory disturbance index^[11]. Patients who declined to participate in the study; were younger than 18 years or older than 70 years; had an EGD examination within the last 12 wk; were pregnant; had a history of substance abuse; were in poor health as determined by a score greater than grade III in the American Society of Anesthesiologists classification; had lung disease requiring home oxygen; had a baseline oxygen saturation less than 90% as measured by pulse oximetry without sedation; or had previous gastric surgery were excluded from the study.

A study coordinator administered a questionnaire to patients, which included questions about alcohol consumption, cigarette smoking, or co-morbidities such as hypertension or diabetes mellitus (DM). Current smokers were defined as those who smoked at least one cigarette per day for the previous 12 mo and alcohol consumption was defined as drinking over 40 g of alcohol per day. Hypertension was defined as a blood pressure of ≥ 140 mmHg or taking anti-hypertensive medication. DM was defined as fasting glucose of ≥ 126 mg/dL or previously diagnosed DM. All data were collected and stored securely.

EGD and sedation

EGDs were performed by an expert staff endoscopist (Cha JM) in a standard manner using a standard video scope (EG-590WR; Fujinon Inc., Saitama, Japan). Sedation was performed in accordance with guidelines published by the American Society of Gastroenterological Endoscopy^[2]. An individualized dose of midazolam was administered by registered nurses according to study protocol based on patient age and weight. In this study, sedation was initiated with a standard dose of midazolam at 0.07 mg/kg^[15,16]. Those not adequately sedated 180 s later were provided an additional 1-2 mg of midazolam until the patient reached a state of moderate sedation or until the maximum dose of 0.1 mg/kg had been administered. The goal was to achieve moderate sedation (*i.e.*, conscious sedation), which was defined as depression of consciousness during which the patient responds purposefully to verbal commands, either alone or accompanied by light tactile stimulation^[3]. The level of sedation was evaluated by an independent nurse using the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale^[17], 2 min after initiation of sedation and when the EGD was inserted. MOAA/S scores range from 0 to 6, and moderate sedation was defined as a MOAA/S score from 3 to 4. The total duration of the EGD was recorded with a stopwatch, from the beginning of sedation until the end of the procedure. During EGD, the following data

were collected: midazolam dose, MOAA/S score, total duration of EGD, oxygen administration, patient physiologic parameters (*e.g.*, SaO₂ and systolic blood pressure), whether the patient snored during the procedure, and episodes of hypoxia or other cardiopulmonary complications. Potential complications were described to patients before the procedure, and all patients provided verbal and written consent prior to the EGD under sedation. After EGD, patients were observed for at least 30 min in a recovery room for possible complications.

Monitoring and data collection

All outcome parameters during the procedure were assessed by the endoscopist and registered nurses. Baseline blood pressure and SaO₂ were recorded before administration of midazolam. All patients were continuously monitored for blood pressure, SaO₂, respiratory activity, and electrocardiography during the procedure. The assistant nurse's responsibilities were limited to sedating and monitoring ventilatory effort, which was visually monitored by chest excursions, respiratory effort, and respiratory rate at regular intervals. A change in tone denoted a rise or fall in saturation, and alarms were set to sound if the value fell below 90%. Oxygen was supplemented when the oxygen saturation level dropped to between 81%–89% for more than 15 s, or below 80% more than 5 s.

Sedation failure was defined as any procedure that required the use of flumazenil for successful completion of the procedure, or that was terminated by the endoscopist due to patient agitation. Hypoxia was defined as a pulse oximetry measurement of SaO₂ less than 90% for at least 5 s. Paradoxical response was defined as hostility, rage and even physical violence, necessitating the restraint of such patients after the administration of midazolam. Hypotension was defined as systolic blood pressure less than 90 mmHg or a drop in systolic blood pressure of more than 20 mmHg from baseline systolic blood pressure.

Ethics

This work was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved ethically by the Institutional Review Board of the Kyung Hee University Hospital at Gangdong (KHNMC IRB 2010-013). All patients provided informed written consent.

Statistical analysis

The prevalence of hypoxia related to moderate sedation for diagnostic EGD was assumed to be approximately 0.5% in control group, as the risk of hypoxia in diagnostic EGD with moderate sedation was approximately 0.5% in previous studies^[4,5,8,18]. We assumed that a difference in hypoxia rate of 20% between the OSA and control groups was clinically significant and would be sufficient for clinicians to avoid sedation in patients with OSA. It was calculated that 56 participants (28 in each group) would be required to have an 80% chance of ruling out a 20% difference with 95% confidence (one-sided analysis).

Continuous data are described by mean and SD. Categorical data are presented as numbers and percentages. Continuous variables were compared using the *t* test. Categorical variables were compared using the χ^2 test or Fisher's exact test, when appropriate. We computed OR and 95%CI using logistic regression analysis. A *P* value < 0.05 was considered significant. All statistical analyses were performed using SPSS statistical software version 13.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

During the study period, 61 patients with OSA were eligible. Data of 31 patients with OSA were analyzed after exclusion of 30 patients: unwillingness to participate in the study (*n* = 22), EGD examination within the last 12 wk (*n* = 6) and previous gastric surgery (*n* = 2). For the control group, 69 subjects were eligible and data of 65 subjects were analyzed after exclusion of 4 subjects due to unwillingness to participate (*n* = 2) and an EGD examination within the last 12 wk (*n* = 2). In total, 31 patients with confirmed OSA and 65 control subjects were enrolled in this study. The study group of 96 subjects included 50 men (52.1%) and 46 women (47.9%), with a mean age of 48.3 years. Mean body mass index (BMI) was 24.2 kg/m²; 24.0% (*n* = 23) of subjects were classified as overweight and 37.5% (*n* = 36) of subjects were classified as obese according to Asia-Pacific guidelines. In total, a mean dose of 5.0 mg midazolam was used for sedation of diagnostic EGD.

Table 1 shows the patient characteristics and baseline clinical data of the two study groups, including age, sex, height, weight, BMI, smoking and drinking status, Charlson's comorbidity score, history of hypertension or DM, baseline blood pressure and SaO₂. As expected, a higher proportion of males, subjects with a higher BMI and subjects with hypertension were more frequently included in the OSA group than in the control group. However, other clinical variables and baseline SaO₂ were not different between the two groups.

Table 2 shows the procedural characteristics and adverse events in the two study groups. A higher dosage of midazolam was administered in the OSA group than in the control group (*P* < 0.001), which makes sense considering midazolam doses were based on weight, and patients with OSA weighed more on average. MOAA/S scores were not significantly different between two groups. The target level of sedation for this study was moderate sedation, and 58.3% of subjects reached moderate sedation while 41.7% reached deep sedation. For the level of sedation, the proportion of deep sedation was significantly higher in the OSA group than in the control group (*P* = 0.024). Most patients (99.0%) reached the target level of sedation within 2 min, and only one in the OSA group needed an additional dose of sedatives. The frequency of all adverse events, including sedation failure, paradoxical responses, snoring or apnea, hypoxia, hypotension, oxygen or flumazenil administration, and other

Table 1 Characteristics and baseline clinical data of subjects in the obstructive sleep apnea and control groups

	OSA group (<i>n</i> = 31)	Control group (<i>n</i> = 65)	<i>P</i> value
Patient characteristics			
Age (yr)	51.3 ± 9.6	47.1 ± 11.8	0.082
Sex (male)	23 (74.2)	27 (41.5)	0.003
Height (meters)	167.1 ± 6.8	164.4 ± 8.0	0.104
Weight (kg)	74.3 ± 11.2	62.6 ± 12.0	0.000
BMI (kg/m ²)	26.5 ± 3.0	23.1 ± 3.3	0.000
Smoking	9 (29.0)	9 (13.8)	0.075
Drinking	11 (35.5)	24 (36.9)	0.891
Charlson score (points)	1.0 ± 0.2	1.0 ± 0.0	0.325
Hypertension	10 (32.3)	9 (13.8)	0.034
Diabetes mellitus	4 (12.9)	7 (10.8)	0.759
Baseline clinical data			
Systolic BP (mmHg)	127.6 ± 16.9	114.3 ± 22.2	0.004
Diastolic BP (mmHg)	79.3 ± 10.6	66.1 ± 14.4	0.000
SaO ₂ (%)	97.6 ± 1.7	98.0 ± 2.2	0.418

Data are expressed as absolute *n* (%) or mean ± SD. OSA: Obstructive sleep apnea; BMI: Body mass index; BP: Blood pressure; SaO₂: Arterial oxygen saturation.

Table 3 Multivariate logistic regression analysis of possible risk factors for a hypoxia

Parameter	OR (95%CI)	<i>P</i> value
Age (continuous)	1.056 (0.958-1.164)	0.276
Sex (female <i>vs</i> male)	1.003 (0.153-6.563)	0.997
Smoking (no <i>vs</i> yes)	0.753 (0.056-10.127)	0.830
Alcohol (no <i>vs</i> yes)	0.592 (0.095-3.706)	0.576
Diabetes mellitus (no <i>vs</i> yes)	1.842 (0.187-18.128)	0.601
Hypertension (no <i>vs</i> yes)	1.102 (0.120-10.117)	0.932
Body mass index (< 25 kg/m ² <i>vs</i> ≥ 25 kg/m ²)	1.304 (0.192-8.845)	0.786
Midazolam dose (< 5 mg <i>vs</i> ≥ 5 mg)	10.726 (0.815-141.100)	0.071
OSA (no <i>vs</i> yes)	0.117 (0.012-1.168)	0.068

OSA: Obstructive sleep apnea.

adverse events was not different between two groups (all *P* > 0.1). In cases of hypoxia, only one (3.2%) patient became transiently hypoxic in the OSA group, and seven cases (10.8%) became transiently hypoxic in the control group (*P* = 0.211). In total, 96.8% of patients remained stable without oxygenation supplementation in the OSA group, and 99.8% remained stable without oxygenation supplementation in the control group (*P* = 0.588).

To determine independent predictors of hypoxia, we performed logistic regression analysis adjusted for age, sex, smoking, alcohol, DM, hypertension, BMI (< 25 kg/m² *vs* ≥ 25 kg/m²), midazolam dose (< 5 mg *vs* ≥ 5 mg), and OSA (Table 3). In this analysis, all variables including the confirmed diagnosis of OSA were not predisposed to risk of hypoxia (OR = 0.117, 95%CI: 0.012-1.168, *P* = 0.068).

DISCUSSION

This is the first prospective study to evaluate the risk of sedation for diagnostic EGD in patients with OSA.

Table 2 Procedural characteristics and adverse events in the obstructive sleep apnea and control groups

	OSA group (<i>n</i> = 31)	Control group (<i>n</i> = 65)	<i>P</i> value
EGD data			
Duration of EGD (s)	261.4 ± 79.6	304.4 ± 154.2	0.074
Midazolam dosage (mg)	5.3 ± 0.9	4.5 ± 0.8	0.000
MOAA/S score (points, at 2 min)	2.5 ± 1.2	2.9 ± 1.0	0.146
Level of sedation for EGD			0.024
Moderate sedation	13 (41.9)	43 (66.2)	
Deep sedation	18 (58.1)	22 (33.8)	
Adverse events			
Sedation failure	0 (0.0)	0 (0.0)	-
Paradoxical responses	4 (12.9)	4 (6.2)	0.268
Snoring or apnea	5 (16.1)	7 (10.8)	0.458
Hypoxia (SaO ₂ < 90%)	1 (3.2)	7 (10.8)	0.211
Hypotension	0 (0.0)	0 (0.0)	-
Other adverse events	0 (0.0)	0 (0.0)	-
Oxygen administration	1 (3.2)	1 (0.2)	0.588
Flumazenil administration	0 (0.0)	0 (0.0)	-

Data are expressed as absolute *n* (%) or mean ± SD. OSA: Obstructive sleep apnea; EGD: Esophagogastroduodenoscopy; MOAA/S: Modified Observer's Assessment of Alertness/Sedation; SaO₂: Arterial oxygen saturation.

Although higher doses of midazolam were administered and a higher proportion of deep sedations were performed in the OSA group than in the control group, all adverse events associated with sedation for diagnostic EGD were not significantly different between the two study groups. In addition, the confirmed diagnosis of OSA was not predisposed to hypoxia with multivariate logistic regression analysis.

Anesthesia and deep sedation have been shown to increase pharyngeal collapse, decrease ventilator response, and impair the arousal response, leading to cardiopulmonary complications in patients with OSA^[10-13,19-22]. However, little is known about the risk of sedation for diagnostic EGD, which is usually targeted for moderate sedation, in patients with OSA. The risk of hypoxia associated with sedation for diagnostic EGD in patients with OSA might be lower than expected due to the following three reasons. First, the sedation level for diagnostic EGD is generally targeted for moderate sedation. The risk of hypoxia in patients with OSA might be lower in cases of moderate sedation than deep sedation or anesthesia because increasing depth of sedation is associated with a progressive increase in upper airway collapsibility^[23,24]. Second, diagnostic EGD is a relatively short procedure, which may be associated with a lower risk of hypoxia, as longer endoscopic procedures were associated with increased risk of hypoxia compared to shorter endoscopic procedures^[25]. Finally, the position of the patient during diagnostic EGD is in the left lateral decubitus position, which may be associated with a lower risk of hypoxia. Patients who undergo anesthesia or surgery in the supine position may be at greater risk of upper airway collapsibility than those who are in the left lateral decubitus position. Therefore, the risk of hypoxia associated with sedation for diagnostic EGD in patients with OSA might

not be high.

As patients with OSA are classified at increased risk for sedation-related complications^[26], physicians are often reluctant to recommend sedation for diagnostic EGD. However, the risk of sedation for diagnostic EGD in patients with OSA is not supported by the direct evidence. Our findings suggest that the risk of sedation for diagnostic EGD in patients with OSA might be rare. In the literature, hypoxia has been used as a surrogate for cardiopulmonary outcomes, and its reported incidence during gastrointestinal endoscopy under sedation ranges widely from 10% to 70%^[9,18,27-38]. Although the study methodology, definition of hypoxia, patient populations, type of endoscopy, use of supplemental oxygen, and type of sedatives should be considered for the interpretation of risk factors of hypoxia during endoscopy under sedation, a confirmed diagnosis of OSA is rarely pointed out as a risk factor of hypoxia during endoscopy under moderate sedation.

This study is unique as this is the first prospective study that evaluated the risk of sedation for diagnostic EGD in patients with OSA. Khiani *et al*^[14] suggested that patients at risk for OSA can safely undergo sedation for routine endoscopic procedures; however, only the Berlin questionnaire was used for OSA risk stratification and patients with OSA were not included in this study. Sharara *et al*^[39] suggested that snoring during colonoscopy is a strong predictor of OSA, however, only a sleep questionnaire was also used in this study. Mador *et al*^[40] showed that presence of OSA does not clearly increase the risk of cardiopulmonary complications in endoscopy procedures under moderate sedation, which is a consistent finding with ours. Although all patients with OSA were confirmed with overnight polysomnography in this study, it was also limited due to its retrospective nature and nonstandard definition of hypopnea. Furthermore, the method of sedation is not controlled, and EGD and colonoscopy were analyzed together in this retrospective analysis. In contrast, our prospective study included all patients with confirmed OSA with a polysomnography and evaluated adverse effects with pre-defined criteria. As an OSA diagnosis is confirmed in only 50%-60% of subjects suspected of having OSA with questionnaire^[41,42], subjects without OSA might be included in previous questionnaire based studies^[14,39].

The present study has several limitations. First, 30 OSA cases were excluded from 61 eligible patients with confirmed diagnosis of OSA. However, there was minimal selection bias, if any, as data was prospectively collected and patients were excluded based on criteria determined before starting enrollment. One reason for the high rate of exclusions in our study is that most patients with OSA were concerned about sedation risks. They have been recommended for a diagnostic EGD without sedation due to hypoxia risk for a long time. Therefore, our study may have a helpful clinical implication for them. Second, the sample size was small because we only included confirmed OSA cases. At the inception of this

study, we estimated that the OSA group would comprise a much larger number of cases and improve the power of this study. The sample size of our study was small ($n = 96$), however; the statistical significance may be sufficiently verified with our study design as only 56 participants were required in our sample size calculation. Third, there is a limitation for the generalizability of our results to all patients with OSA because our patients were recruited from a single university outpatient clinic, and procedures were purely diagnostic EGD with midazolam alone. Our results cannot be generalized to EGDs that include therapy or more extensive diagnosis such as Barrett's surveillance or sedation with propofol or opioids.

In conclusion, this limited sized study did not disclose an increased risk of cardiopulmonary complications during diagnostic EGD with moderate sedation in patient with OSA. This suggests that the majority of patients with OSA would safely undergo diagnostic EGD with sedation. However, our findings should be more clearly established in the future in large studies.

COMMENTS

Background

Obstructive sleep apnea (OSA) is characterized by intermittent and recurrent episodes of partial or complete obstruction of the upper airway during sleep. The administration of sedatives in patients with OSA may worsen obstruction of the pharynx and cause depression of the upper airway muscles. Therefore, patients with OSA are known to be vulnerable to cardiopulmonary complications during deep sedation and anesthesia. However, little is known about the adverse events of moderate sedation for diagnostic esophagogastroduodenoscopy (EGD) in patients with OSA.

Research frontiers

The research hotspot is to investigate whether patients with OSA are at risk of sedation-related complications during diagnostic EGD.

Innovations and breakthroughs

This is the first prospective study to evaluate the risk of sedation for diagnostic EGD in patients with OSA. Although higher doses of midazolam were administered and a higher proportion of deep sedation was performed in the OSA group than in the control group, all adverse events associated with sedation for diagnostic EGD were not significantly different between the two study groups. In addition, the confirmed diagnosis of OSA was not predisposed to hypoxia with multivariate logistic regression analysis. This study is outstanding in that the risk of sedation for diagnostic EGD in patients with OSA was investigated in a prospective design with pre-defined criteria and the diagnosis of all OSA patients was confirmed with full-night, in-laboratory polysomnography. Although the diagnosis of OSA was made with a questionnaire in previous studies, subjects without OSA might be included as an OSA diagnosis is confirmed in only 50%-60% of subjects suspected of having OSA with a questionnaire.

Applications

As patients with OSA are classified at increased risk for sedation-related complications, physicians are often reluctant to recommend sedation for diagnostic EGD. In fact, most patients with OSA were concerned about sedation risks in our study, as they have been recommended for a diagnostic EGD without sedation for a long time. However, the risk of sedation for diagnostic EGD in patients with OSA is not supported by the direct evidence. Therefore, this study may have a helpful clinical implication for patients with OSA when they perform diagnostic EGD under sedation.

Terminology

OSA: The diagnosis of OSA was confirmed based on the results of full-night, in-laboratory polysomnography. Asymptomatic patients who scored higher than 15 on the apnea hypopnea index or respiratory disturbance index were diagnosed with OSA, as were patients with symptoms or signs of disturbed sleep who scored higher than 5 on the hypopnea index or respiratory disturbance index.

Moderate sedation: The level of sedation was evaluated by an independent nurse using the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale, and moderate sedation was defined as a MOAA/S score from 3 to 4. Hypoxia: Hypoxia was defined as a pulse oximeter measurement of SaO₂ less than 90% for at least 5 s.

Peer review

It is generally a well-written paper: it has an excellent core tip section to "advertise" the paper, well-summarized results section adding meaningfully to the existing literature on an important subject and the rest of the sections are also well-written. The bibliography is focused and well-researched. The results are interesting and suggest that the majority of patients with OSA would safely undergo diagnostic EGD with sedation.

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A feasible modified biopsy method for tissue diagnosis of gastric subepithelial tumors

Jung Ho Kim, Jun-Won Chung, Minsu Ha, Min Young Rim, Jong Joon Lee, Jungsuk An, Yoon Jae Kim, Kyoung Oh Kim, Kwang An Kwon, Dong Kyun Park, Yeon Suk Kim, Duck Joo Choi

Jung Ho Kim, Jun-Won Chung, Minsu Ha, Min Young Rim, Jong Joon Lee, Yoon Jae Kim, Kyoung Oh Kim, Kwang An Kwon, Dong Kyun Park, Yeon Suk Kim, Duck Joo Choi, Department of Internal Medicine, Gachon University Gil Medical Center, Incheon 405-760, South Korea

Jungsuk An, Department of Pathology, Gachon University Gil Medical Center, Incheon 405-760, South Korea

Author contributions: Chung JW designed the research and performed procedure of modified biopsy; Kim JH and Ha M performed research; Rim MY, Lee JJ and Kim KO collected data; An J and Kim YJ analysed data; Kwon KA, Park DK and Kim YS coordinated and supported the statistical analysis; Kim JH wrote the paper; Choi DJ prepared the figures.

Correspondence to: Jun-Won Chung, MD, PhD, Department of Internal Medicine, Gachon University Gil Medical Center, 1198 Guwol-dong, Namdong-Gu, Incheon 405-760, South Korea. drgreen@gilhospital.com

Telephone: +82-32-4603778 Fax: +82-32-4603408

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the SETs was 21.8 (range, 11-30) mm. The number of biopsy specimens was 6.3 (range 5-8). The mean procedure time was 9.0 min (range, 4-17 min). The diagnostic yield of MIF biopsies was 90.9% (10/11). The histological diagnoses were leiomyoma (4/11, 36.4%), aberrant pancreas (3/11, 27.3%), gastrointestinal stromal tumors (2/11, 18.2%), an inflammatory fibrinoid tumor (1/11, 9.1%); one result was non-diagnostic (1/11, 9.1%). There were six mesenchymal tumors; the specimens obtained in each case were sufficient for an immunohistochemical diagnosis. There was no major bleeding, but one perforation occurred that was successfully controlled by endoscopic clipping.

CONCLUSION: The MIF biopsy was simple to perform, safe, and required a shorter procedure time, with a high diagnostic yield for small SETs.

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Key words: Subepithelial tumors; Stomach; Biopsy; Endoscopy; Diagnostic techniques

Abstract

AIM: To evaluate the diagnostic yield and safety of a modified technique for the histological diagnosis of subepithelial tumors (SETs).

METHODS: A retrospective review of patients who underwent a modified technique for the histological diagnosis of gastric SETs, consisting of a mucosal incision with a fixed flexible snare (MIF) and deep-tissue biopsy under conventional endoscopic view, from January 2012 to January 2013 was performed. Eleven patients with gastric SETs 10-30 mm in diameter and originating from the third or fourth layer on endoscopic ultrasonography were included.

RESULTS: The mean age was 59.8 (range, 45-76) years, and 5 patients were male. The mean size of

Core tip: Tissue acquisition from subepithelial tumors (SETs) is essential for a differential diagnosis. Several techniques have been introduced to obtain SET tissue samples. However, the diagnostic efficacy was limited or the procedure was complex and difficult. We investigated a modified technique for the histological diagnosis of SETs, consisting of a mucosal incision with a fixed flexible snare (MIF) and deep-tissue biopsy at the incision site under a conventional endoscopic view. The results of this study suggest that the MIF biopsy is simple to perform, safe, fast, and provides a high diagnostic yield for small SETs.

Kim JH, Chung JW, Ha M, Rim MY, Lee JJ, An J, Kim YJ, Kim KO, Kwon KA, Park DK, Kim YS, Choi DJ. A feasible modified biopsy method for tissue diagnosis of gastric subepithelial tumors.

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INTRODUCTION

Gastric subepithelial tumors (SETs) are typically found incidentally during screening endoscopies. The exact incidence of SETs on routine endoscopy is unknown, although one retrospective study reported a prevalence of 0.36%^[1]. A wide range of diseases may present as SETs in the upper gastrointestinal tract, including lipoma, leiomyoma, aberrant pancreas, varices, carcinoid, gastrointestinal stromal tumors (GISTs), and lymphomas. Thus, tissue diagnosis for SET differentiation is particularly important because these lesions may have different prognoses and have different therapeutic protocols, such as resection or observation.

Gastric SETs are difficult to definitively diagnose by conventional imaging studies, such as ultrasonography, computed tomography, and magnetic resonance imaging. Endoscopic ultrasonography (EUS) is currently the most effective diagnostic tool for the differential diagnosis of SETs because it can help determine the depth and originating layer of the gastrointestinal wall of the lesion^[2]. However, EUS morphological characteristics alone do not provide an accurate diagnosis. EUS has limited utility in distinguishing between benign and malignant lesions^[3]. In particular, if the SET is found to be a hypoechoic lesion located in the third or fourth layer on EUS findings, tissue acquisition should be strongly considered for a histological diagnosis^[3].

Generally, histological diagnosis may not be necessary in large SETs (more than 3 cm in diameter) or symptomatic lesions because such SETs require resection regardless of the pathological confirmation^[4,5]. In contrast, small SETs, such as GISTs less than 3 cm in diameter, do not usually require resection because most are benign. However, the current concept is that every GIST has at least malignant potential, even small GISTs of 1 cm in diameter^[6,7].

Presently, there is no consensus regarding the management strategy and surveillance of asymptomatic and small SETs^[5,8]. For a definitive diagnosis of SETs, tissue acquisition from a subepithelial lesion is essential for a differential diagnosis and an assessment of the malignant potential.

However, conventional endoscopic biopsies do not typically provide sufficient submucosal tissue specimens for diagnosis because SETs are located deep and are covered with normal mucosa. Thus, several techniques have been introduced to obtain SET tissue samples. However, the diagnostic efficacy seems to be limited for immunohistological diagnosis with these methods, such as EUS-guided fine-needle aspiration (EUS-FNA), EUS-guided trucut biopsy (EUS-TCB), and stacked biopsy^[9-15].

We thus investigated a modified technique for the his-

tological diagnosis of SETs, consisting of mucosal incision with a fixed flexible snare (MIF) and deep-tissue biopsy at the incision site under a conventional endoscopic view.

MATERIALS AND METHODS

Patients

A retrospective review of patients who underwent MIF biopsies from January 2012 to January 2013 was conducted. Among the patients with incidental SETs 10-30 mm in diameter, the inclusion criteria were SETs found in the third and fourth layers, with hypoechoic or mixed echogenic patterns on EUS. We excluded patients with typical findings of a vessel, cyst, or lipoma on EUS. We also excluded patients with EUS characteristics suggestive of malignancy, including those with hyperechogenic foci, anechoic necrotic zones, irregular extraluminal borders, or adjacent malignant-appearing lymphadenopathy^[16].

Informed consent, with adequate explanation of the biopsy and possible complications, was obtained from each patient. This study was approved by the Institutional Review Board of Gachon University Gil Medical Center (IRB No. GDIRB2013-05).

Procedure details

All procedures were performed by one endoscopist (Chung JW) using a conventional single-channel endoscope (GIF Q260 or H260; Olympus Optical Co., Ltd., Tokyo, Japan) with patients under conscious sedation without a transparent hood. An endoscopic-knife (fixed flexible snare; Kachu Technology, Seoul, Korea) connected to an electrosurgical unit (VIO 300D; ERBE, Tübingen, Germany) in "ENDO CUT 1" mode was used for the incision of the mucosa covering the SETs (Figure 1). The length of the tip in this endoscopic knife was 1.5 mm. Under a direct conventional endoscopic view, a mucosal incision was made over the convex zone of the lesion (Figure 2). After the mucosal incision using the fixed flexible snare, we performed a conventional forceps (FB-25K-1; Olympus) biopsy, deep into the incision site of the covering mucosa. Finally, we obtained 5-8 biopsy samples. According to the judgment of the endoscopist, incision site bleeding was controlled using argon plasma coagulation (APC 2; ERBE); the site was closed prophylactically with 2-4 endoclips (HX-610-90L or HX 610-135L; Olympus) in some patients.

Before the MIF biopsy, EUS was performed to characterize the SETs using conventional radial EUS (UM2000; Olympus). All patients were closely monitored for any procedure-related complication in the recovery room and were discharged 2-3 h after the procedure was finished. Oral intake was started 8 h after the procedure. Patients who underwent MIF biopsy empirically received proton pump inhibitors for 1 wk after the procedure. If there was no symptom and/or sign associated with complications, routine follow-up endoscopy was not performed. All patients were instructed to visit our hos-

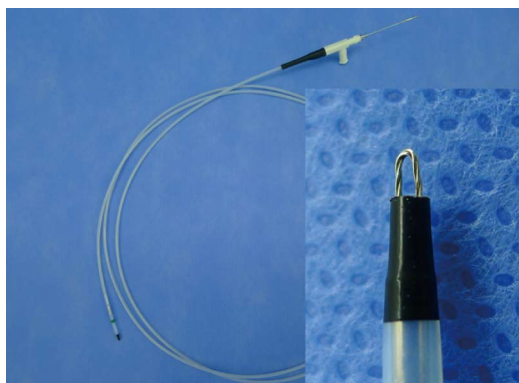


Figure 1 The fixed flexible snare is an endoscopic instrument for mucosal incision and dissection.

pital immediately if they had symptoms and/or signs of complications (abdominal pain, hematemesis, melena, dizziness). Patients without symptoms and/or signs of complications visited the outpatient clinic 1-2 wk after the procedure.

Perforation was defined as a split in the muscle layer that occurred during the procedure or the presence of free air detected in post-procedure imaging studies. Major bleeding was defined as bleeding that resulted in a drop in hemoglobin of 2 g/dL or more, that required blood transfusion and/or endoscopic re-intervention, or if surgical intervention caused the hemorrhage. Minor bleeding was defined as bleeding that was controlled by endoscopic hemostasis (argon plasma coagulation or clip) during the procedure.

Pathologic examination

The forceps biopsy specimens were fixed in a 10% formalin solution and embedded in paraffin wax. The pathologic examinations included identification of cell type, overall cellularity, cytoplasmic features, nuclear atypia, mitotic index, and immunohistochemical findings. The mitotic index was determined on 50 consecutive high-power fields (HPFs). Immunohistochemical analyses of CD117 (c-kit), CD34, desmin, smooth muscle actin, S-100, and Ki-67 markers were performed with commercially available antibodies to classify the tumor subtype. Positive reactions for CD117 and CD34 were considered diagnostic of a GIST. Mesenchymal lesions that were positive for desmin and smooth muscle actin and negative for CD117 and CD34 were diagnosed as smooth muscle tumors such as leiomyoma. Positivity for S-100 protein and negativity for desmin, smooth muscle actin, and CD117 were diagnostic of neural tumors.

Statistical analysis

Statistical analyses were performed using SPSS software (Ver. 12.0 for Windows; SPSS, Chicago, IL, United States). Continuous data are presented as the means (range), and categorical data are presented as absolute numbers and percentages.

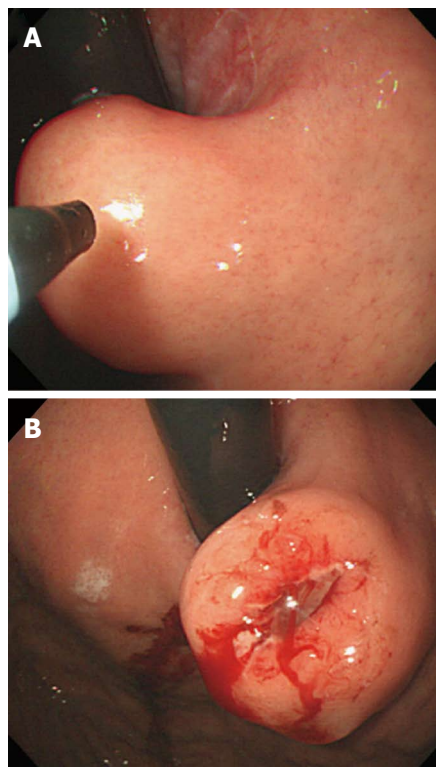


Figure 2 Mucosal incision with a fixed flexible snare and conventional forceps biopsy. A: A fixed flexible snare performing a mucosal incision over the center of a subepithelial lesion in the cardia of the stomach; B: The lesion after the forceps biopsy following the mucosal incision.

RESULTS

The patient characteristics, location and size of the SETs, histological results, and procedure details are summarized in Table 1. In total, 11 patients were enrolled during the study period. The mean age was 59.8 years (range, 45-76 years); there were five males and six females. The mean size (longest diameter) of the tumors was 21.8 mm (range, 11-30 mm). The number of biopsy specimens was 6.3 (range, 5-8). The mean procedure time was 9.0 min (range 4-17 min).

The MIF biopsy provided specimens that were sufficient for a definitive histological diagnosis in 90.9% (10/11) of cases. The histological diagnoses were leiomyoma (36.4%, 4/11), aberrant pancreas (27.3%, 3/11), GIST (18.2%, 2/11), and inflammatory fibrinoid tumor (9.1%, 1/11), and one result was non-diagnostic (9.1%, 1/11; Table 1). There were six mesenchymal tumors (4 leiomyomas, 2 GISTs), and the specimens obtained were large enough for immunohistochemical diagnoses. Both cases (case No. 3, 5) with GISTs had a spindle cell-type tumor with intermediate mitotic activity (mitotic index 5-10/50 HPFs). These patients had undergone surgical resection (wedge resection), and the results of the biopsy and the surgical resection were consistent.

The patient with a non-diagnostic result (case No. 6) refused a re-biopsy and did not want further evaluation or surgical resection. Thus, this patient was followed an-

Table 1 Endoscopic and clinicopathological characteristics of the patients and subepithelial tumors lesions

Case	Gender	Age (yr)	Location	EUS			MIF biopsy ¹			
				Layer	Echogenicity	Size (mm)	Procedure time (min)	Biopsy number (pieces)	Additional procedure	Pathology
1	Male	71	Angle	Fourth	Hypoechoic	21	12	7	Clip	IFT
2	Female	46	LB	Third	Mixed	15	10	6	APC	Aberrant pancreas
3	Female	69	Fundus	Fourth	Hypoechoic	20	5	5	Clip	GISTs
4	Male	76	Cardia	Fourth	Hypoechoic	21	10	6	Clip	Leiomyoma
5	Female	65	HB	Fourth	Mixed	27	7	6	No	GISTs
6	Female	47	LB	Third	Hypoechoic	30	11	8	No	CAG
7	Female	45	Angle	Third	Mixed	28	4	7	No	Aberrant pancreas
8	Male	71	Angle	Fourth	Mixed	26	9	6	Clip	Aberrant pancreas
9	Female	46	Cardia	Fourth	Hypoechoic	11	7	7	No	Leiomyoma
10	Male	62	HB	Fourth	Mixed	22	17	6	Clip	Leiomyoma
11	Male	60	Cardia	Fourth	Hypoechoic	19	7	5	Clip	Leiomyoma

¹MIF biopsy is defined as a modified technique for the histological diagnosis of SETs: consisting of a mucosal incision with a fixed flexible snare (MIF) and deep-tissue biopsy at the incision site under a conventional endoscopic view. EUS: Endoscopic ultrasonography; SETs: Subepithelial tumors; IFT: Inflammatory fibrinoid tumor; LB: Low body; APC: Argon plasma coagulation; GISTs: Gastrointestinal stromal tumors; HB: High body; CAG: Chronic atrophic gastritis.

nually, and a final histological diagnosis was not reached. One perforation (case No. 10) was observed, and it was successfully controlled by endoscopic clipping. No major bleeding was recorded, but 63.6% (7/11) of patients showed minor bleeding.

DISCUSSION

We present a modified biopsy technique for the histological diagnosis of SETs. The diagnostic accuracy of MIF biopsies was 90.9% in our study. Adequate samples for diagnosis were obtained from 10 of 11 patients. The success rate was higher than other previously reported conventional methods.

Despite an endoscopist's intention to obtain tissue from submucosal lesions, conventional methods such as large-capacity "jumbo" forceps biopsies acquire submucosa for diagnosis with an approximately 17% yield^[17]. Recent studies have investigated EUS-based methods, which have several limitations, despite a higher success rate than previously reported methods. EUS-FNA can obtain only a limited number of cells and cannot determine the structure of the organization, although the method typically has a 60%-80% success rate^[10,14,18,19]. EUS-TCB generally has a similar yield to EUS-FNA, with no additional benefit^[10]. Additionally, with EUS-TCB, it is not easy to obtain sufficient tissue with intact tissue architectural details for determining the mitotic index. However, it can provide a higher success rate than EUS-FNA in some patients requiring immunostaining. Combined EUS-FNA and EUS-TCB has been reported to have a diagnostic yield as high as 77%, although with a longer procedure time and higher cost^[14].

The MIF biopsy is a simple technique, first making an incision in the mucosa covering the SETs, followed by acquiring SET tissues at the incision site with conventional biopsy forceps. Another advantage of this method

is that it is not difficult regardless of the location of the lesion. In contrast, EUS-FNA and EUS-TCB are limited by technical problems in approaching the antrum and at angles because of the stiffness of the device and the rubbery consistency of the subepithelial mass^[9,20].

Recently, there have been efforts to resect gastric SETs using ESD techniques, which provided successful resection of SETs in 74.3%-81.1% of cases, with a mean procedure time of 60.9 (range, 20-170) min^[21-23]. There has been no report of life-threatening complications, although the incidence of complications was relatively high, at 12%-17%. In our study, the mean procedure time of the MIF biopsy was short (9 min), and the success rate was high (90.9%). Large GISTs with high potential for malignancy should be removed using surgical or endoscopic approaches. However, resection of all small SETs may be an unnecessarily invasive and money-wasting treatment, considering the risk of complications and cost effectiveness. Thus, a pre-resection histological evaluation is essential for SETs, and the MIF biopsy may provide a useful alternative technique in this regard.

One reported method for the adequate tissue acquisition of SETs is to remove the mucosa covering the SETs using an endoscopic knife for an endoscopic submucosal dissection (ESD) and to then to perform a partial resection of the SET^[24]. This method provides a 93.7% diagnostic yield, but the procedure is more complex and difficult than the MIF biopsy we describe. Another ESD technique is mucosal incision-assisted biopsy (MIAB), which allows a mucosal incision at the circumferential margin of the lesion using an ESD-associated technique, followed by submucosal dissection to expose the SETs and then biopsy. This method differs from MIF biopsy, which involves an incision in the mucosa covering the top of the convex zone. MIAB appears to be much more complex and difficult than the MIF biopsy^[25].

Another method, similar to the MIF biopsy, was re-

ported recently and involves performing a mucosal incision using a needle-knife sphincterotome (Microknife XL; Boston Scientific Inc., Natick, MA, United States), followed by sampling of the tissues inside and then prophylactic clipping (a SINK biopsy)^[26]. The difference between the two methods is that we used a fixed flexible snare and did not routinely perform prophylactic endoscopic clipping. We performed APC or clipping for minor bleeding in 63.6% (6/11) of cases, which did not require additional endoscopy or re-admission for post-procedural bleeding. The mucosal incision with multiple deep biopsies appeared to be relatively safe in terms of bleeding, even without prophylactic APC or clipping.

The MIF biopsy needs to be performed carefully, depending on the shape of the SETs. Mucosal incision with deep biopsies should not be technically difficult if the SET is an exophytic “ball shape” growing toward the gastric lumen. However, a slightly elevated lesion, not a ball-shaped protruding lesion, necessitates a careful procedure. One (case No. 6) of our patients could not be diagnosed after the MIF biopsy, and another patient (case No. 10) experienced perforation; the SETs were slightly elevated, *i.e.*, mounded, in both cases. We suggest that these lesions were difficult to target because they were movable, and it was therefore difficult to identify the correct location when making the mucosal incision. Thus, SETs with such shapes require special attention.

Our data suggest that the MIF biopsy is a safe and effective method for the tissue diagnosis of small SETs. However, we recognize the limitations of this study. This was a retrospective study at a single tertiary academic center, and the sample size was small.

In conclusion, MIF biopsy was simple to perform, safe, required a shorter procedure time, and provided a high diagnostic yield for small SETs. Further comparative, prospective studies with larger sample sizes are required.

COMMENTS

Background

Gastric subepithelial tumors (SETs) are difficult to definitively diagnose by conventional imaging studies. Therefore, tissue acquisition from SETs is essential for a differential diagnosis. Conventional endoscopic biopsies do not typically provide sufficient submucosal tissue specimens for diagnosis. Several techniques have been introduced to obtain SET tissue samples. However, their diagnostic efficacies were limited, or the procedures were complex and difficult. Authors investigated a modified technique for the histological diagnosis of SETs, consisting of a mucosal incision with a fixed flexible snare (MIF) and deep-tissue biopsy at the incision site under a conventional endoscopic view.

Research frontiers

Presently, there is no consensus regarding the management strategy and surveillance of asymptomatic and small SETs. For a definitive diagnosis of SETs, tissue acquisition from a subepithelial lesion is essential for a differential diagnosis and an assessment of the malignant potential. Authors present a modified biopsy technique for the histological diagnosis of small SETs. The diagnostic accuracy of MIF biopsies was 90.9% in our study. The success rate was higher than other previously reported conventional methods.

Innovations and breakthroughs

The results of this study suggest that the MIF biopsy is simple to perform, safe, requires a shorter procedure time, and provides a high diagnostic yield for small SETs. Another advantage of this method is that it is not difficult regardless of

the location of the lesion.

Applications

Their data suggest that the MIF biopsy is a safe and effective method for the tissue diagnosis of small SETs. A pre-resection histological evaluation is essential for SETs, and the MIF biopsy may provide a useful alternative technique.

Terminology

The modified technique that uses the MIF biopsy consists of two steps. Under direct conventional endoscopic view, the incision of the mucosa covering the SETs is made over the convex zone of the lesion using an endoscopic knife. After the mucosal incision is created using the fixed flexible snare, a conventional forceps biopsy is performed, deep in the incision site of the covering mucosa.

Peer review

This paper reports the usefulness of a mucosal incision with a fixed flexible snare and a deep-tissue biopsy for the histological diagnosis of gastric subepithelial tumors. A modified biopsy method is feasible for the tissue diagnosis of gastric subepithelial tumors. The article is well written, and the findings are of practical importance.

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A novel technique for endoscopic ultrasound-guided biliary drainage

Varayu Prachayakul, Pitulak Aswakul

Varayu Prachayakul, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
 Pitulak Aswakul, Liver and Digestive Institute, Samitivej Sukhumvit Hospital, Bangkok 10110, Thailand

Author contributions: Prachayakul V developed the concept; Prachayakul V and Aswakul P performed the data acquisition and wrote and revised the paper.

Correspondence to: Dr. Varayu Prachayakul, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkok 10700, Thailand. kaiyjr@gmail.com

Telephone: +66-2-4121088 Fax: +66-2-4196101

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Abstract

AIM: To describe a successful endoscopic ultrasound (EUS)-guided biliary drainage technique with high success and low complication rates.

METHODS: The recorded data of consecutive patients who presented to Siriraj Gastrointestinal Endoscopy Center, Siriraj Hospital in Bangkok, Thailand for treatment of malignant obstructive jaundice but failed endoscopic retrograde cholangiopancreatography and underwent subsequent EUS-guided biliary drainage were retrospectively reviewed. The patients' baseline characteristics, clinical manifestations, procedure details, and post-procedure follow-up data were recorded and analyzed. Clinical outcomes were assessed by physical exam and standard laboratory tests. Technical success of the procedure was defined as completion of the stent insertion. Clinical success was defined as improvement of the patient's overall clinical manifestations, in terms of general well-being evidenced by physical examination, restoration of normal appetite, and adequate biliary drainage. Overall median survival time was calculated as the time from the procedure

until the time of death, and survival analysis was performed by the Kaplan-Meier method. The Student's *t*-test and the χ^2 test were used to assess the significance of inter-group differences.

RESULTS: A total of 21 cases were enrolled, a single endoscopist performed all the procedures. The mean age was 62.8 years (range: 46-84 years). The sex distribution was almost equal, including 11 women and 10 men. Patients with failed papillary cannulation (33.3%), duodenal obstruction (42.9%), failed selective cannulation (19.0%), and surgical altered anatomy (4.8%) were considered candidates for EUS-guided biliary drainage. Six patients underwent EUS-guided choledochoduodenostomy and 15 underwent EUS-guided hepaticogastrostomy. The technique using non-cauterization and no balloon dilation was performed for all cases, employing the in-house manufactured tapered tip Teflon catheter to achieve the dilation. The technical success and clinical success rates of this technique were 95.2% and 90.5%, respectively. Complications included bile leakage and pneumoperitoneum, occurred at a rate of 9.5%. None of the patients died from the procedure. One patient presented with a biloma, a major complication that was successfully treated by another endoscopic procedure.

CONCLUSION: We present a highly effective EUS-guided biliary drainage technique that does not require cauterization or balloon dilation.

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Key words: Endoscopic ultrasound; Biliary drainage; Hepaticogastrostomy; Choledochoduodenostomy; Endoscopic ultrasound-guided

Core tip: A total of 21 patients who underwent endoscopic ultrasound (EUS)-guided biliary drainage following failure of endoscopic retrograde cholangiopancreatography were analyzed. The EUS-guided biliary drain-

age technique, which does not require cauterization or balloon dilation, was found to be effective and safe. The rates of technical and clinical success were 95.2% and 90.5%, respectively. Complications occurred at a relatively low rate (9.5%) and included bile leakage and pneumoperitoneum. No procedure related deaths occurred during the procedure, hospital recovery, or follow-up period. However, one patient developed the major complication of iatrogenic biloma due to stent mal-position, which was successfully resolved by another endoscopic procedure.

Prachayakul V, Aswakul P. A novel technique for endoscopic ultrasound-guided biliary drainage. *World J Gastroenterol* 2013; 19(29): 4758-4763 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4758.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4758>

INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) is a standard treatment for benign and malignant biliary obstruction. ERCP has a failure rate of about 3%-10% due to a combination of the following factors: improper cannulation due to periampullary diverticulum or anatomical variation, biliary obstruction from impacted large common bile duct stones, tight malignant obstruction, gastrointestinal anatomical changes, surgically altered anatomy or gastroduodenal obstruction^[1]. Common management techniques following a failed ERCP include an additional ERCP performed by a more experienced endoscopist or the implementation of alternative methods for biliary decompression such as surgical bypass or percutaneous transhepatic biliary drainage (PTBD). PTBD is superior to surgical bypass techniques in terms of morbidity and complications^[1-5]. However, PTBD still resulted in complications (9%-30%) and a 2%-15% mortality rate was reported. Furthermore, patients reported symptoms including discomfort, unusual physiology and some limitations of the procedure, especially in patients with large amount of ascites^[2,3].

Endoscopic ultrasound (EUS), a medical procedure in which endoscopy is combined with ultrasound to provide imaging of internal organs, has expanded its therapeutic potential since the invention of the linear-array echoendoscope. EUS guided biliary access (EUS guided cholangiography) was first reported by Wiersema *et al*^[6], followed by EUS-guided choledochoduodenostomy (EUS-CD) by Giovannini *et al*^[7], and lastly EUS guided hepatocystogastrostomy (EUS-HG) was introduced by Burmester *et al*^[8]. There have been many reports regarding the efficacy, safety and feasibility of EUS guided biliary drainage (EUS-BD)^[9-24]. The two most common EUS-BD procedures are EUS-CD and EUS-HG^[2-10]. Both procedures are initiated by identifying and targeting the biliary tract, followed by puncturing the bile duct depended on the location of obstruction and the reasons for performing drainage, manipulating the guidewire, dilating the created

tract, and placing the stent. The success rate of EUS-BD was 72%-98% while the complication rate is 15%-35%, with complications such as peritonitis and bile leakage being fatal^[9-16]. The details of different endoscopic techniques, instruments and accessories used in the procedures are described elsewhere^[17-19]. Reasonably, the most important issue for high success and low complication rate of EUS-BD is an appropriate technique for biliary access. The techniques for biliary access were classified into two groups, cauterized or non-cauterized, while the dilation maneuver was also classified as graded dilation or balloon dilation. At present, there are no data directly comparing these two methods. Therefore, the aim of this study was to describe the techniques for EUS-BD used in our institute, which provided good clinical outcomes.

MATERIALS AND METHODS

We retrospectively reviewed data from patients with advanced malignant bile duct obstruction who failed ERCP and underwent EUS-guided biliary drainage in our institute from October 2010 to July 2012. The medical records and electronic based medical records were reviewed. Prachayakul V was the endoscopist who performed all the procedures. We analyzed the following clinical outcomes during the follow-up period: patient demographic data, baseline clinical characteristics, indications for EUS-BD, procedure details, and clinical and laboratory results. We defined clinical outcomes using several different criteria. Technical success was defined as the ability to complete the procedure until stent insertion. Clinical success was defined as the improvement of the overall clinical manifestations of the patients in terms of clinical well-being, loss of appetite, and adequate biliary drainage. Adequate biliary drainage was defined as the reduction of total bilirubin more than 50% of pre-treatment bilirubin. Median survival time was defined as the time from the procedure until the time of death. While most patients experiencing complications were followed-up until they passed away, some were contacted telephonically in order to obtain any missing or additional information related to specific complications.

All participating patients gave written informed consent. The procedures were performed in the endoscopic suite at the Siriraj Hospital's Endoscopy Center with propofol administered total intravenous anesthesia. Patients were positioned in left lateral decubitus, with the punctured site chosen depending on the site of biliary obstruction. The site was chosen after EUS survey, EUS-CD for distal biliary obstruction and EUS-HG for hilar obstruction. Curvilinear echoscope (GF UC-140P, Olympus, Tokyo, Japan) were used in all cases. Either the common bile duct or the intrahepatic bile duct was punctured by using 19-gauge needle (EchoTip® Ultra, Cook Ireland, Limerick, Ireland) and bile was aspirated to confirm the correct position. Contrast medium was injected to get more cholangiography details. The 0.035 jag wire was inserted through a channel in the intrahepatic bile duct and then the tapered tip Teflon catheters which were made by

the author (Aswakul P) (Figure 1) were used for graded dilatation starting from 7 or 8.5 up to 10 Fr in diameter. Next, the fully covered self-expandable metal stent (FCSEMS) with varying sizes (60, 80 or 100 mm) were inserted depending on the location of EUS-BD. We generally used FCSEMS size 60 mm in diameter for EUS-CD and 80-100 mm for EUS-HG. All the procedures were done under fluoroscopic monitoring. The positions of the stents were checked and the bleeding was secured. Patients were observed in the recovery room for 1-2 h and transferred to the regular ward care. Most achieved uneventful discharge within 3-5 d after the procedure. Due to awareness of the positions of the stents and stent shortening, abdominal X-rays were done in all patients within 48-72 h. The patients' physical examination, laboratory tests, and clinical status were recorded. The procedure sequence is shown in Figure 2.

Statistical analysis

Data were analyzed using SPSS version 13.0 software (SPSS, Inc., Chicago, IL, United States). Overall median survival time was evaluated from the time of procedure until the time of death. The data was analyzed using survival analysis with the Kaplan-Meier method. The descriptive data were reported as mean $\pm$ SD and percentage. The Student's *t*-test and the χ^2 test were used to assess the inter-group differences according to the clinical data. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

A total of 21 patients were enrolled in the present study. The mean age was 62.8 years (range 46-84 years), and ten were men (47.6%). The most common diagnoses were advanced pancreatic cancer (45.5%), cholangiocarcinoma (18.2%), gallbladder cancer (18.2%) and others (18.1%). The most common clinical manifestation was obstructive jaundice, which occurred in 86.4% of patients. The mean pre-treatment total bilirubin, alkaline phosphatase and carbohydrate antigen 19-9 level were 18.1 ± 7.9 mg/dL, 716.5 ± 395.6 IU/mL and 242.0 ± 325.6 IU/mL respectively. The indications for EUS-BD were failed papillary cannulation (33.3%), duodenal obstruction (42.9%), failed selective cannulation (19.0%) and surgical altered anatomy (4.8%). Six patients underwent EUS-CD while another 15 patients underwent EUS-HG. One patient underwent the procedure twice due to a first time technical failure where during guide wire manipulation the wire slipped out and bile leakage occurred. This event led to reduction of intrahepatic duct size and it was too small for puncturing. The patient underwent another procedure two weeks later and achieved successful biliary drainage. Therefore, only 21 patients would be analyzed for clinical outcomes following treatment.

There were immediate complications reported in two cases (9.5%); a case of pneumoperitoneum related with neotract creation and dilation technique that recovered after conservative treatment and another case of bile leakage. Here, a 53-year-old female who had a known case of

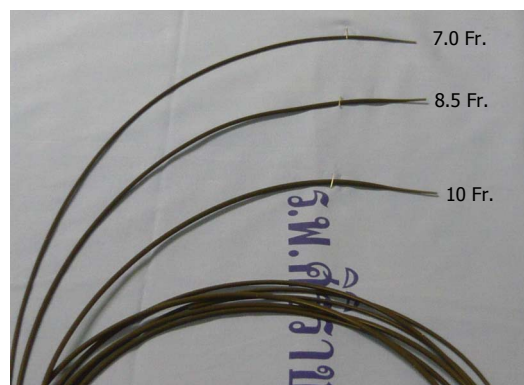


Figure 1 Self-made tapered tip Teflon catheters.

advanced cholangiocarcinoma and underwent EUS-guided hepaticogastrostomy, showed higher level of bilirubin within 2 wk after stent insertion due to malposition of the stent. The stent was slipped out of the gastric wall causing acute angulation at hepatogastric site that occurred during stent deployment and led to biloma formation. The patient was managed conservatively without surgery because there was no evidence of clinical peritonitis. A computed tomography scan was done 10 d later and revealed biloma at the intra-peritoneum site of the stent. The patient underwent EUS-guided biloma drainage and passed away one month later due to disease progression.

Technical success occurred in 95.5% of patients while clinical success was achieved in 90.5%. Adequate biliary drainage was achieved in 85.7% of the patients within two weeks except only one case where drainage did not occur until the fourth week after the procedure. There were two patients who did not benefit from the drainage procedures; the first case showed no improvement of bilirubin level after the stent insertion due to liver failure from massive liver metastasis and a case of bile leakage where the patient eventually died due to disease progression. The median survival of the patients in this series was 93 d. There were three patients still alive at the time that we finished the study.

DISCUSSION

The result of EUS-BD in the present study was similar to previous case reports in terms of technical and clinical success, at about 75.0%-96.5%^[1-13]. However the complications found in this series were lower than those reported in other studies^[16-29]. More than 90% of the patients were clinically improved after the procedures even though the median survival time was only 93 d. Most patients passed away because of advancement of the primary malignancy. Regarding of the complications occurring with EUS-BD technique that we used in this study, only one case (4.8%) related with neotract creation and dilation technique. We did not use cauterization because we hypothesized that it might cause more tissue injury and is technically dangerous, especially when the tip of the knife was not in the appropriate position. Difficulty

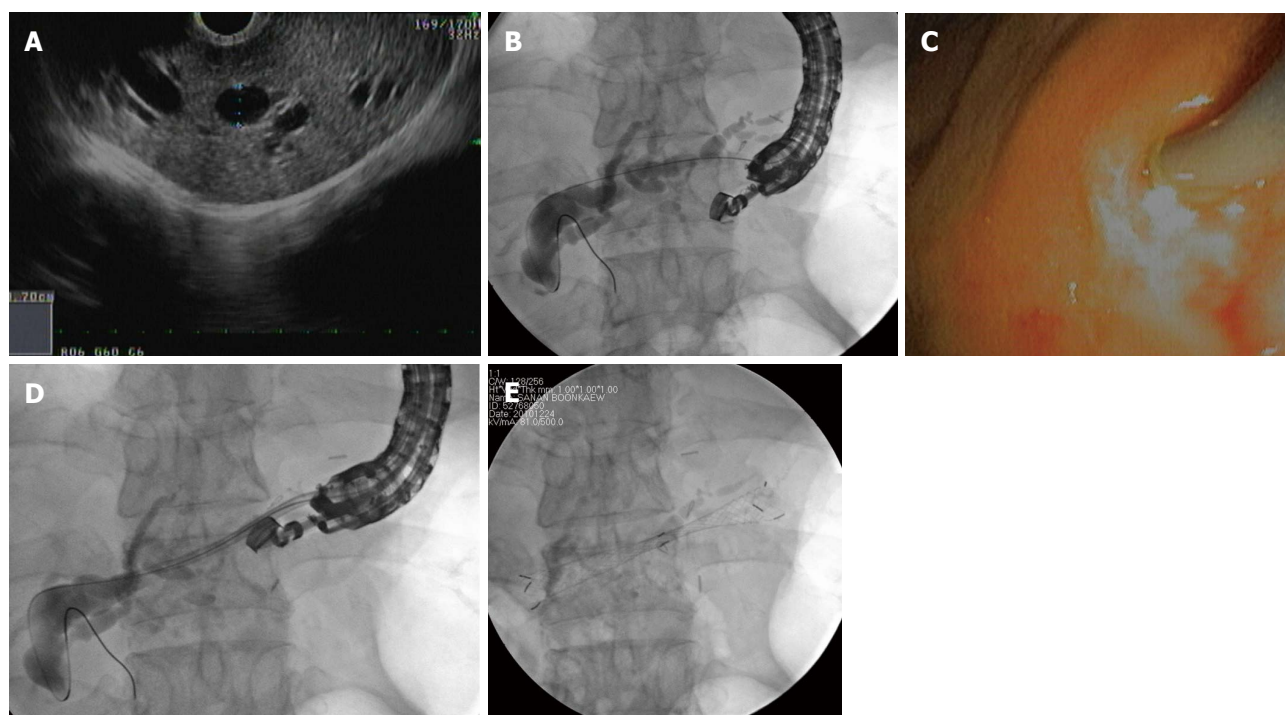


Figure 2 The procedure sequence. A: Endosonographic view of dilated intra hepatic duct; B: Endoscopic ultrasound guided cholangiography; C: Endoscopic view showing dilatation using tapered tip Teflon catheter; D: Fluoroscopic view showing dilatation tapered tip Teflon catheter; E: Post self-expandable metal stent insertion.

inserting the catheter was overcome by using a self-made Teflon catheter with a small the tapered tip diameter (as small as the 0.035 guidewire). We also chose to not use the balloon dilatation technique to create the neo-tract that was slightly larger than the size of the introducer of the SEMS. This worked to minimize the risk of bile leakage and symptomatic gastric perforation. Further, we found this technique was feasible and easy to perform because of the specific characteristic of dilator tip. While a potential challenge to this technique is the need for more frequent guidewire exchange, the presence of a well-trained assistant allowed us to complete the procedure without encountering any problems. While we did encounter some bile leakage, it was due to a mistake during SEMS deployment, not during the neotract creation and dilation. Fortunately, because of smaller dilated tract, the SEMS did not immediately expand, so the bile leakage in this particular case was minimal and gradually occurred leading only to a localized formation of biloma without peritonitis. Another complication of EUS-BD was early stent migration. This led to peritonitis, but was corrected by surgery^[30]. We did not experience this complication because the SEMS did not immediately over-expanded due to the smaller diameter of the neo-tract. This made the SEMS fit the tract appropriately under good radial SEMS force, and the tract was slowly formed and fully expanded by 72 h^[31]. This technique also minimized the risk of early stent migration. We had four cases of late stent migration (partial migration) when the tract was already well formed four to eight weeks after the procedure and thus no morbidity was found. Consistent with findings of Park *et al.*^[11], the only one risk factor associ-

ated with postprocedure adverse events in performing EUS-BD was the use of a needle knife for fistula tract creation. This study did have some limitations. First, although the tapered tip Teflon catheter used in this case series were very unique, the small sample size may not be reproducible. Second, this was the retrospective review of the data. Therefore, multi-center, larger population, prospective studies should be conducted in the future.

We have reported a technique for EUS-guided biliary drainage that does not use cauterization or balloon dilation. The technique was highly effective with a low complication rate, and has not been previously reported.

COMMENTS

Background

Endoscopic ultrasound-guided biliary drainage (EUS-BD) is a novel alternative treatment for the approximately 3%-10% of patients who fail standard endoscopic retrograde cholangio-pancreatography (ERCP). Techniques for biliary access and drainage were reported in recent years, however there was no data indicating which one is the best technique for minimizing complications and increasing the success.

Research frontiers

This was a retrospective study of 21 patients with advanced malignant bile duct obstruction who failed ERCP and underwent EUS-guided biliary drainage. The techniques used in this case series created the hepaticogastrostomy and choledochoduodenostomy tract in the absence of cauterization and balloon dilatation. Tapered tip Teflon catheters were used for dilation. The procedure had very high technical (95.2%) and clinical (90.5%) success, while maintaining lower complication rates (9.5%) when compared to other studies (15%-35%).

Innovations and breakthroughs

The goal of this study was to demonstrate another option for EUS-guided biliary drainage that used novel instruments and techniques resulting in an enhanced suc-

cess rate and a decreased complication rate.

Applications

This novel EUS-guided biliary drainage technique could be widely used as a safer and more effective alternative to cauterization or balloon dilation techniques that are widely used currently.

Terminology

EUS-BD is a novel alternative treatment for patients who suffered from biliary obstruction and failed endoscopic retrograde cholangiopancreatography. The most common procedures are EUS guided hepaticogastrostomy and EUS-guided choledochoduodenostomy. Regarded as a less invasive procedure when comparing to surgical bypass with a complication rate of 15%-35%, it becomes a preferable treatment option in some particular patients.

Peer review

This was an interesting case series that demonstrated alternative treatment options and techniques for endoscopic ultrasound guided-biliary drainage in patients with advanced malignant bile duct obstruction who failed conventional biliary drainage techniques.

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Quality of life after laparoscopic vs open sphincter-preserving resection for rectal cancer

Simon Siu-Man Ng, Wing-Wa Leung, Cherry Yee-Ni Wong, Sophie Sok-Fei Hon, Tony Wing-Chung Mak, Dennis Kwok-Yu Ngo, Janet Fung-Yee Lee

Simon Siu-Man Ng, Wing-Wa Leung, Cherry Yee-Ni Wong, Sophie Sok-Fei Hon, Tony Wing-Chung Mak, Dennis Kwok-Yu Ngo, Janet Fung-Yee Lee, Division for Colorectal Surgery, Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China

Author contributions: Ng SSM and Leung WW designed the research; Ng SSM, Hon SSF, Mak TWC, Ngo DKY, and Lee JFY performed the surgeries and followed up the patients; Leung WW administered the questionnaires; Wong CYN collected the data; Ng SSM and Leung WW analyzed the data; Ng SSM wrote the manuscript.

Correspondence to: Simon Siu-Man Ng, Professor, Division for Colorectal Surgery, Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Clinical Sciences Building, Room 64045, 4/F, Shatin, New Territories, Hong Kong, China. simonng@surgery.cuhk.edu.hk

Telephone: +86-852-26321495 Fax: +86-852-26377974

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Abstract

AIM: To compare quality of life (QoL) outcomes in Chinese patients after curative laparoscopic vs open surgery for rectal cancer.

METHODS: Eligible Chinese patients with rectal cancer undergoing curative laparoscopic or open sphincter-preserving resection between July 2006 and July 2008 were enrolled in this prospective study. The QoL outcomes were assessed longitudinally using the validated Chinese versions of the European Organization for Research and Treatment of Cancer QLQ-C30 and QLQ-CR38 questionnaires before surgery and at 4, 8, and 12 mo after surgery. The QoL scores at the different time points were compared between the laparoscopic and open groups. A higher score on a functional scale indicated better functioning, whereas a higher score on a symptom scale indicated a higher degree of symptoms.

RESULTS: Seventy-four patients (49 laparoscopic and 25 open) were enrolled. The two groups of patients were comparable in terms of sociodemographic data, types of surgery, tumor staging, and baseline mean QoL scores. There was no significant decrease from baseline in global QoL for the laparoscopic group at different time points, whereas the global QoL was worse compared to baseline beginning at 4 mo but returned to baseline by 12 mo for the open group ($P = 0.019$, Friedman test). Compared to the open group, the laparoscopic group had significantly better physical (89.9 ± 1.4 vs 79.2 ± 3.7 , $P = 0.016$), role (85.0 ± 3.4 vs 63.3 ± 6.9 , $P = 0.005$), and cognitive (73.5 ± 3.4 vs 50.7 ± 6.2 , $P = 0.002$) functioning at 8 mo, fewer micturition problems at 4-8 mo (4 mo: 32.3 ± 4.7 vs 54.7 ± 7.1 , $P = 0.011$; 8 mo: 22.8 ± 4.0 vs 40.7 ± 6.9 , $P = 0.020$), and fewer male sexual problems from 8 mo onward (20.0 ± 8.5 vs 76.7 ± 14.5 , $P = 0.013$). At 12 mo after surgery, no significant differences were observed in any functional or symptom scale between the two groups, with the exception of male sexual problems, which remained worse in the open group (29.2 ± 11.3 vs 80.0 ± 9.7 , $P = 0.026$).

CONCLUSION: Laparoscopic sphincter-preserving resection for rectal cancer is associated with better preservation of QoL and fewer male sexual problems when compared with open surgery in Chinese patients. These findings, however, should be interpreted with caution because of the small sample size of the study.

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Key words: Quality of life; Rectal cancer; Laparoscopic surgery; Sphincter-preserving surgery; European Organization for Research and Treatment of Cancer QLQ-C30; European Organization for Research and Treatment of Cancer QLQ-CR38

Core tip: This prospective nonrandomized study dem-

onstrates that laparoscopic sphincter-preserving resection for rectal cancer is associated with better preservation of quality of life (QoL) and fewer male sexual problems when compared with open surgery in Chinese patients in the first postoperative year. Our study has several strengths. First, our study only focused on Chinese patients undergoing curative sphincter-preserving rectal resection, thus minimizing the impact of other potential confounders on the QoL assessment. Second, all our questionnaires were administered by a single research assistant and were completed by the patients during clinic visits. Therefore, we achieved 100% compliance at different time points.

Ng SSM, Leung WW, Wong CYN, Hon SSF, Mak TWC, Ngo DKY, Lee JFY. Quality of life after laparoscopic vs open sphincter-preserving resection for rectal cancer. *World J Gastroenterol* 2013; 19(29): 4764-4773 Available from: URL: <http://www.wjg-net.com/1007-9327/full/v19/i29/4764.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4764>

INTRODUCTION

Accumulating evidence from recent randomized trials indicates that laparoscopic surgery for rectal cancer is associated with clear short-term benefits and similar tumor clearance when compared with open surgery^[1-5]. Researchers currently are particularly eager to know whether the long-term oncologic results are also comparable between the two approaches for rectal cancer^[6]. Indeed, long-term survival has always been regarded as the most important study endpoint in these clinical trials^[7]. However, functional outcomes and quality of life (QoL) must not be ignored in the quest for surgical and oncologic excellence.

Notably, up to 30% of rectal cancer survivors will develop urinary and sexual dysfunctions after surgery attributable to inadvertent injury of the pelvic autonomic nerves^[8]. Bowel dysfunction and fecal incontinence are also not uncommon after sphincter-preserving rectal surgery and radiotherapy^[9,10]. These functional hazards will have a significant negative impact on the patients' functioning and QoL for the remainder of his/her life^[11].

Therefore, in addition to traditional study endpoints such as postoperative recovery, morbidity, and survival, functional results and QoL have recently become important outcome parameters for defining surgical performance in clinical trials^[12]. Within the context of medical and healthcare research, QoL is the patient's subjective perception of the impact of his/her disease and its treatments on his/her physical, psychological, and social functioning and general well-being^[13]. Health-related QoL after cancer surgery can be assessed by standardized instruments such as the questionnaires developed by the European Organization for Research and Treatment of Cancer (EORTC), which contain multidimensional generic and disease-specific domains; the EORTC

QLQ-C30 and QLQ-CR38 are the most commonly used questionnaires in colorectal cancer trials^[14,15].

The magnified vision and less traumatic surgery offered by the laparoscopic approach may allow better preservation of the pelvic autonomic nerves^[4,5], and presumably, functional outcomes following laparoscopic surgery for rectal cancer may be better compared to open surgery. However, conflicting results have been reported in the literature^[2,16]; some studies have even reported a higher incidence of sexual dysfunction after laparoscopic rectal surgery^[17,18]. Furthermore, it is also unclear whether the short-term and long-term clinical benefits associated with the laparoscopic approach will translate into better QoL outcomes for patients with rectal cancer. To date, few studies have specifically compared QoL outcomes between laparoscopic and open surgery for rectal cancer^[19]. We therefore conducted this prospective study to compare QoL outcomes in Chinese patients after curative laparoscopic vs open sphincter-preserving surgery for rectal cancer. Changes in QoL over time were also longitudinally assessed and compared between the two groups.

MATERIALS AND METHODS

Between July 2006 and July 2008, eligible Chinese patients with rectal cancer undergoing curative laparoscopic or open sphincter-preserving resection at our hospital were enrolled in this prospective study. The study was approved ethically by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (CRE-2005.259). All patients provided written informed consent. We excluded the following patients: patients who presented with recurrent disease, patients who required multivisceral en bloc resections, patients who required conversion from laparoscopic to open surgery, patients with intestinal obstruction or perforation, and patients with known dementia or cognitive dysfunction.

The operative approach (laparoscopic or open resection) was decided by the operating surgeon after considering the tumor characteristics and the patient's preference. All operations were performed by surgeons experienced in both laparoscopic and open colorectal surgery. Our laparoscopic techniques for resection of rectal cancer were previously described^[20,21]. For mid and low rectal cancer located 5-12 cm from the anal verge, sphincter-preserving total mesorectal excision with protective loop ileostomy was performed. All patients in this study underwent ileostomy closure within 7 mo after the primary surgery. Adjuvant therapy was administered to patients with pathologic stage II or III disease. Clinical parameters including patient sociodemographic data, types of surgery, tumor staging, and short-term clinical outcomes were prospectively recorded.

After surgery, all patients were followed-up regularly at 4-mo intervals for clinical examination and carcinoembryonic antigen testing. All patients were free of recurrence during the study period.

Quality of life assessment

Patient QoL was assessed using the QLQ-C30 and QLQ-CR38 questionnaires developed by the EORTC^[14,15]. The clinical validity and reliability of the Chinese versions of both QLQ-C30 and QLQ-CR38 have been confirmed^[22-24]. QLQ-C30 is a generic questionnaire for the assessment of QoL in cancer patients^[14]. It includes 30 items, 24 of which are combined to form a global QoL scale, five functional scales (physical, role, emotional, cognitive, and social), and three symptom scales (fatigue, nausea/vomiting, and pain). The other six single items evaluate dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties. QLQ-CR38 is a specific questionnaire module specifically designed for assessment of QoL in patients with colorectal cancer^[15]. It consists of 38 items covering symptoms and side effects related to different colorectal cancer treatment modalities. The module contains four functional scales (body image, sexual functioning, sexual enjoyment, and future perspective) and eight symptom scales/items (micturition problems, chemotherapy side effects, gastrointestinal tract symptoms, male sexual problems, female sexual problems, defecation problems, stoma-related problems, and weight loss).

The questionnaires were scored according to the EORTC Scoring Manual^[25]. Each item has four response alternatives (scoring 1-4), “not at all”, “a little”, “quite a bit”, and “very much”, except for the global QoL scale, which has seven alternatives (scoring 1-7) from “very poor” to “excellent”. All questionnaire responses and scores were linearly transformed to a 0-100 scale. A higher score on the global QoL and functional scales represented a higher level of QoL and functioning, whereas a higher score on the symptom scales/items represented a higher degree of symptoms or dysfunction.

All questionnaires were administered by a single research assistant and completed by the patients before surgery and at 4, 8 and 12 mo after surgery (during clinic visits). Every effort was made to avoid missing data during questionnaire administration.

Statistical analysis

QoL scores were presented as the mean $\pm$ SD. For longitudinal assessment of changes of QoL scores over time, the Friedman test was used to identify overall significant differences between QoL scores at the four different time points (before surgery and at 4, 8 and 12 mo after surgery) for each variable. When the overall *P* value indicated statistical significance (*i.e.*, $P < 0.05$), a *post-hoc* Wilcoxon signed-rank test for paired data using a simplified Bonferroni correction was used to compare pairs of QoL scores (with $P < 0.0083$ considered significant for six pair-wise comparisons). Cross-sectionally, to test for differences in QoL scores between the laparoscopic and open groups at different time points, the Mann-Whitney *U* test was used. The baseline characteristics of the two groups of patients were compared using the χ^2 test (or Fisher's exact test when appropriate), Student's *t* test, and

the Mann-Whitney *U* test for categorical, parametric, and non-parametric data, respectively. A *P* value of less than 0.05 was considered statistically significant, whereas a difference in mean QoL scores of more than 10 points was regarded as clinically significant^[26]. Using a 5% significance level, a total sample size of 75 (50 laparoscopic and 25 open) would have a power of 80% to detect a minimum difference of 10 points in mean QoL scores between the two groups.

RESULTS

Between July 2006 and July 2008, 74 patients were enrolled in this study: 49 patients underwent laparoscopic surgery, and 25 patients underwent open surgery. The two groups of patients were comparable in terms of sociodemographic data, types of surgery, tumor staging, and the proportion of patients who received adjuvant therapy (Table 1). The overall short-term morbidity rates of the laparoscopic and open groups were 34.7% and 52%, respectively ($P = 0.152$, χ^2 test). Transient urinary retention and septic complications (including chest infection, wound infection, and urinary tract infection) occurred more frequently in the open group. No patient in this study required reoperation for postoperative complications. With the exception of higher baseline symptom scores for insomnia in the open group, there was no significant difference in baseline mean QoL scores for any of the functional or symptom scales between the two groups (Table 2).

EORTC QLQ-C30

There was no significant decrease from baseline in global QoL scores for the laparoscopic group at the evaluated time points; the statistically significant difference detected with the Friedman test ($P = 0.044$) was due to an increase in global QoL scores from 4 to 8 mo ($P = 0.031$, post-hoc Wilcoxon signed-rank test) (Figure 1A). For the open group, the global QoL was worse than at baseline from 4 mo onward but gradually returned to baseline by 12 mo ($P = 0.019$, Friedman test; a significant decrease occurred between baseline and 4 mo, $P = 0.004$, post-hoc Wilcoxon sign-ranked test) (Figure 1A). Both the laparoscopic and open groups showed a significant decrease in physical functioning from 4 to 12 mo postoperatively ($P < 0.001$, Friedman test) (Figure 1B). Role functioning and social functioning were significantly worse than at baseline from 4 to 12 mo for the open group but remained the same as at baseline for the laparoscopic group (Figure 1C and D). There was no change from baseline in emotional functioning for either group. Cognitive functioning fluctuated over time for the laparoscopic group ($P = 0.035$, Friedman test) but remained the same as at baseline for the open group.

There was no significant change from baseline in fatigue scores for the laparoscopic group; more fatigue was reported in the open group beginning at 4 mo, but it returned to the baseline level by 12 mo ($P = 0.003$, Fried-

Table 1 Sociodemographic and clinicopathologic data of the patients *n* (%)

	Laparoscopic group	Open group	<i>P</i> value
Number of patients	49	25	/
Age (yr, mean $\pm$ SD)	65.6 $\pm$ 11.3	66.7 $\pm$ 12.4	0.705 ¹
Sex (male/female)	30/19	15/10	0.919 ²
Body mass index (kg/m ² , mean $\pm$ SD)	22.4 $\pm$ 3.4	21.4 $\pm$ 3.5	0.210 ¹
Number of patients with comorbidities	27 (55.1)	13 (52)	0.800 ²
Marital status (married/divorced/widow/single)	36/5/6/2	18/3/4/0	0.742 ²
Education level (under primary/primary/secondary/tertiary or above)	10/19/16/4	8/10/6/1	0.623 ²
Tumor location in rectum (upper/middle/lower)	26/15/8	12/10/3	0.697 ²
Types of surgery (AR/LAR with TME)	24/25	12/13	0.936 ²
Number of patients with temporary ileostomy	25 (51)	13 (52)	0.936 ²
Number of patients with complications	17 (34.7)	13 (52)	0.152 ²
Subclinical anastomotic leak	0	1	
Anastomotic bleeding	1	0	
Chest infection	1	3	
Wound infection	2	5	
Urinary tract infection	2	5	
Urinary retention	4	5	
Prolonged ileus	7	3	
Others	1	3	
Reoperation	0	0	
AJCC staging (I / II / III)	3/26/20	2/13/10	0.955 ²
Adjuvant chemotherapy	20 (40.8)	10 (40)	0.946 ²
Adjuvant radiotherapy	11 (22.4)	7 (28)	0.599 ²

¹Student's *t* test; ² χ^2 test. AR: Anterior resection; LAR: Low anterior resection; TME: Total mesorectal excision; AJCC: American Joint Committee on Cancer.

man test; a significant increase occurred between baseline and 4 mo, $P = 0.004$, *post-hoc* Wilcoxon sign-ranked test) (Figure 2A). The 4-mo to 12-mo symptom scores remained similar to those at baseline for nausea/vomiting, pain, dyspnea, insomnia, appetite loss, constipation, and diarrhea for both groups. More financial difficulties were reported at 4 mo postoperatively, but this returned to baseline levels by 12 mo for both groups.

Compared to the open group, the laparoscopic group had significantly better global QoL at 4 and 8 mo, better physical, role, and cognitive functioning at 8 mo, less fatigue at 4 and 8 mo, and less nausea/vomiting, appetite loss, and financial difficulties at 8 mo (Table 2). However, at 12 mo after surgery, no significant differences were observed in any of the EORTC QLQ-C30 functional or symptom scales between the two groups.

EORTC QLQ-CR38

There was no significant change from baseline in body image for the open group; for the laparoscopic group, body image was significantly worse compared to baseline beginning at 4 mo but returned to baseline levels by 12 mo ($P = 0.002$, Friedman test). Sexual functioning remained the same as at baseline for both groups (Figure 1E), but the overall scores for sexual functioning were low (Table 2), indicating that the majority of patients were sexually inactive. There was a trend toward worsening of future perspective scores over time for the laparoscopic ($P = 0.074$, Friedman test) and open ($P = 0.094$, Friedman test) groups, but the change was statistically insignificant.

Improvement in micturition problems was noted

in the laparoscopic group ($P = 0.031$, Friedman test; a decrease in symptom scores primarily occurred between baseline and 8 mo, $P = 0.019$, *post-hoc* Wilcoxon sign-ranked test), but there was no significant change from baseline for the open group (Figure 2B). More problems with chemotherapy side effects were reported at 4 mo postoperatively, but returned to baseline levels by 12 mo for patients receiving chemotherapy in both groups (Figure 2C). There was no significant change from baseline in gastrointestinal tract symptoms for either group (Figure 2D). Defecation problems significantly decreased from 4 to 8 mo for patients without stoma in the laparoscopic group ($P = 0.003$, Friedman test; $P = 0.003$, *post-hoc* Wilcoxon sign-ranked test), but they remained the same as at baseline for the open group. A significant improvement in weight loss over time was observed in both groups (Figure 2E). Compared to the open group, the laparoscopic group had significantly fewer micturition problems at 4 and 8 mo and fewer gastrointestinal symptoms at 8 mo (Table 2).

Sexual enjoyment and sexual problems were not evaluated in the female patients in this study because they were all sexually inactive. Altogether, 19 male patients (14 in the laparoscopic group and 5 in the open group) who had been sexually active before surgery were assessed for changes in QoL related to sexual activities (Table 3). Sexual enjoyment and male sexual problems remained relatively stable at different time points for the laparoscopic group, but less sexual enjoyment and more sexual problems were reported from 4 to 12 mo for the open group (Figures 1F and 2F). Compared to the laparoscopic group, the open group had significantly more sexual

Table 2 Comparison of European Organization for Research and Treatment of Cancer QLQ-C30 and QLQ-CR38 scores between the laparoscopic and open groups at different time points

	Baseline			4 mo			8 mo			12 mo		
	Lap	Open	<i>P</i> value	Lap	Open	<i>P</i> value	Lap	Open	<i>P</i> value	Lap	Open	<i>P</i> value
EORTC QLQ-C30												
Functional scales												
Global QoL	72.4 (3.4)	68.3 (5.2)	0.443	65.8 (3.6)	47.3 (5.9)	0.009	73.6 (3.8)	52.0 (5.9)	0.003	71.1 (3.4)	61.0 (7.0)	0.371
Physical	94.7 (1.3)	91.5 (2.6)	0.255	86.4 (2.3)	77.6 (4.1)	0.056	89.9 (1.4)	79.2 (3.7)	0.016	87.1 (2.6)	81.3 (4.0)	0.149
Role	88.8 (3.0)	92.7 (3.1)	0.660	75.9 (4.5)	67.3 (6.6)	0.155	85.0 (3.4)	63.3 (6.9)	0.005	82.7 (3.9)	71.3 (7.1)	0.129
Emotional	71.1 (4.1)	66.0 (5.4)	0.404	76.0 (4.0)	82.0 (4.9)	0.401	79.8 (3.4)	71.3 (6.3)	0.379	79.1 (3.2)	71.0 (6.3)	0.579
Cognitive	71.1 (4.2)	66.7 (5.5)	0.415	67.7 (3.6)	59.3 (5.8)	0.284	73.5 (3.4)	50.7 (6.2)	0.002	61.9 (4.2)	60.0 (6.7)	0.940
Social	82.7 (3.2)	88.0 (4.4)	0.292	73.5 (3.1)	64.7 (5.7)	0.268	76.9 (3.8)	62.7 (7.1)	0.110	76.5 (4.0)	62.7 (7.4)	0.124
Symptom scales/items												
Fatigue	16.6 (2.8)	14.2 (2.9)	0.859	17.7 (2.6)	27.6 (4.3)	0.042	13.8 (2.7)	28.9 (5.9)	0.027	12.0 (2.2)	15.6 (4.0)	0.520
Nausea/vomiting	0 (0)	0.7 (0.7)	0.162	3.1 (1.3)	6.7 (3.7)	0.931	0 (0)	2.7 (2.1)	0.046	0 (0)	1.3 (1.3)	0.162
Pain	14.3 (3.2)	16.7 (4.5)	0.772	20.1 (2.7)	25.3 (5.2)	0.534	16.7 (2.7)	27.3 (5.2)	0.089	15.6 (2.9)	19.3 (5.4)	0.755
Dyspnea	4.1 (1.6)	2.7 (1.8)	0.581	7.5 (2.2)	16 (5.5)	0.208	2.7 (1.3)	12 (6.0)	0.252	4.1 (2.1)	12.0 (5.0)	0.065
Insomnia	33.3 (5.1)	58.7 (8.5)	0.011	32.0 (5.1)	46.7 (8.2)	0.141	27.2 (4.8)	45.3 (7.9)	0.052	30.6 (5.1)	46.7 (8.4)	0.129
Appetite loss	8.8 (2.7)	6.7 (4.3)	0.313	7.5 (2.2)	14.7 (6.4)	0.794	6.8 (2.2)	17.3 (4.8)	0.035	7.5 (3.0)	10.7 (4.2)	0.330
Constipation	18.4 (4.6)	29.3 (8.0)	0.292	11.6 (4.2)	12.0 (4.7)	0.525	15.0 (4.4)	6.7 (2.7)	0.508	18.4 (4.5)	10.7 (5.3)	0.205
Diarrhea	19.7 (4.4)	17.3 (6.1)	0.759	23.1 (4.9)	30.7 (7.2)	0.394	15.0 (4.2)	29.3 (8.2)	0.156	15.0 (4.1)	21.3 (6.6)	0.379
Financial difficulties	13.6 (4.0)	14.7 (4.7)	0.426	24.5 (4.3)	37.3 (6.5)	0.066	23.1 (5.2)	42.7 (7.1)	0.010	13.6 (3.6)	20.0 (6.7)	0.580
EORTC QLQ-CR38												
Functional scales												
Body image	93.9 (1.9)	93.3 (2.6)	0.945	81.0 (4.2)	82.7 (6.0)	0.699	81.2 (3.9)	85.3 (4.8)	0.834	87.8 (3.7)	84.4 (5.6)	0.526
Sexual functioning	18.7 (3.9)	14.0 (5.3)	0.268	18.7 (4.1)	8.7 (5.1)	0.069	19.0 (4.2)	16.0 (5.7)	0.630	19.0 (4.3)	17.3 (5.7)	0.807
Future perspective	54.4 (4.9)	64.0 (7.2)	0.272	54.4 (4.4)	45.3 (6.0)	0.309	44.2 (5.1)	42.7 (7.8)	0.779	44.2 (4.9)	44.0 (6.9)	0.995
Symptom scales/items												
Micturition problems	37.8 (4.4)	38.7 (6.9)	0.907	32.3 (4.7)	54.7 (7.1)	0.011	22.8 (4.0)	40.7 (6.9)	0.020	31.6 (4.5)	41.3 (6.9)	0.246
Chemotherapy side effects ¹	16.7 (4.1)	10.0 (2.6)	0.530	41.1 (7.3)	44.4 (11.1)	0.846	20.6 (4.7)	28.9 (7.8)	0.422	8.9 (3.3)	17.8 (6.0)	0.214
Gastrointestinal tract symptoms	20.7 (2.2)	19.5 (2.8)	0.817	16.6 (1.9)	18.1 (2.8)	0.656	15.9 (1.9)	24.8 (3.3)	0.022	18.0 (2.2)	16.3 (3.3)	0.546
Defecation problems ²	22.2 (2.3)	15.5 (3.5)	0.133	24.3 (3.6)	21.4 (3.0)	0.821	11.8 (2.5)	23.0 (4.9)	0.062	13.2 (2.9)	16.3 (3.3)	0.235
Weight loss	27.9 (4.7)	38.7 (8.3)	0.376	8.8 (3.2)	24.0 (7.6)	0.079	6.1 (2.1)	17.3 (6.1)	0.094	6.1 (2.1)	10.7 (4.2)	0.383

¹Only for patients who received chemotherapy, 20 in the laparoscopic group and 10 in the open group; ²Only for patients without temporary loop ileostomy, 24 in the laparoscopic group and 12 in the open group. Quality of life (QoL) scores are presented as the mean (standard error of mean). Scores ranged from 0 to 100. A higher score on a functional scale indicates better functioning, whereas a higher score on a symptom scale indicates a higher degree of symptoms. Scores in the laparoscopic and open groups were compared by the Mann-Whitney *U* test. EORTC: European Organization for Research and Treatment of Cancer.

problems at 8 mo ($P = 0.013$, Mann-Whitney *U* test) and 12 mo ($P = 0.026$, Mann-Whitney *U* test) postoperatively.

At 12 mo after surgery, no significant differences were observed in any of the EORTC QLQ-CR38 functional or symptom scales between the two groups, with the exception of male sexual enjoyment and sexual problems, which remained worse in the open group.

DISCUSSION

This prospective study was specifically designed to compare QoL outcomes in Chinese patients after curative laparoscopic *vs* open sphincter-preserving resection for rectal cancer. Our study has several strengths. First, although nonrandomized, the baseline characteristics and sociodemographic data of the two groups of patients were similar, and a fair comparison could therefore be made. The social backgrounds of patients, such as marital status and education level, which may impact QoL after surgery^[27], have seldom been provided by other studies comparing QoL after laparoscopic and open rectal surgery^[1-3,28,29]. Second, other studies have included meta-

static cases and abdominoperineal resection in their QoL analysis^[1,3,28], whereas our study only focused on Chinese patients undergoing curative sphincter-preserving rectal resection, thus minimizing the impact of other potential confounders on the QoL assessment. Third, all our questionnaires were administered by a single research assistant and were completed by the patients during clinic visits. Therefore, we achieved 100% compliance at different time points, a figure that was not achieved by other studies in which the questionnaires were collected by mail^[28,29]. Full compliance with the questionnaires is essential to ensure a precise longitudinal assessment of QoL changes.

Our results showed that laparoscopic sphincter-preserving resection for rectal cancer was associated with better preservation of QoL and fewer male sexual problems when compared with the open approach in the first year after surgery. Other benefits of the laparoscopic approach, such as better physical functioning and fewer micturition and gastrointestinal problems, were evident only in the short term. These findings are in accordance with those reported by Braga *et al*^[3], who found that QoL after laparoscopic surgery for rectal cancer was better

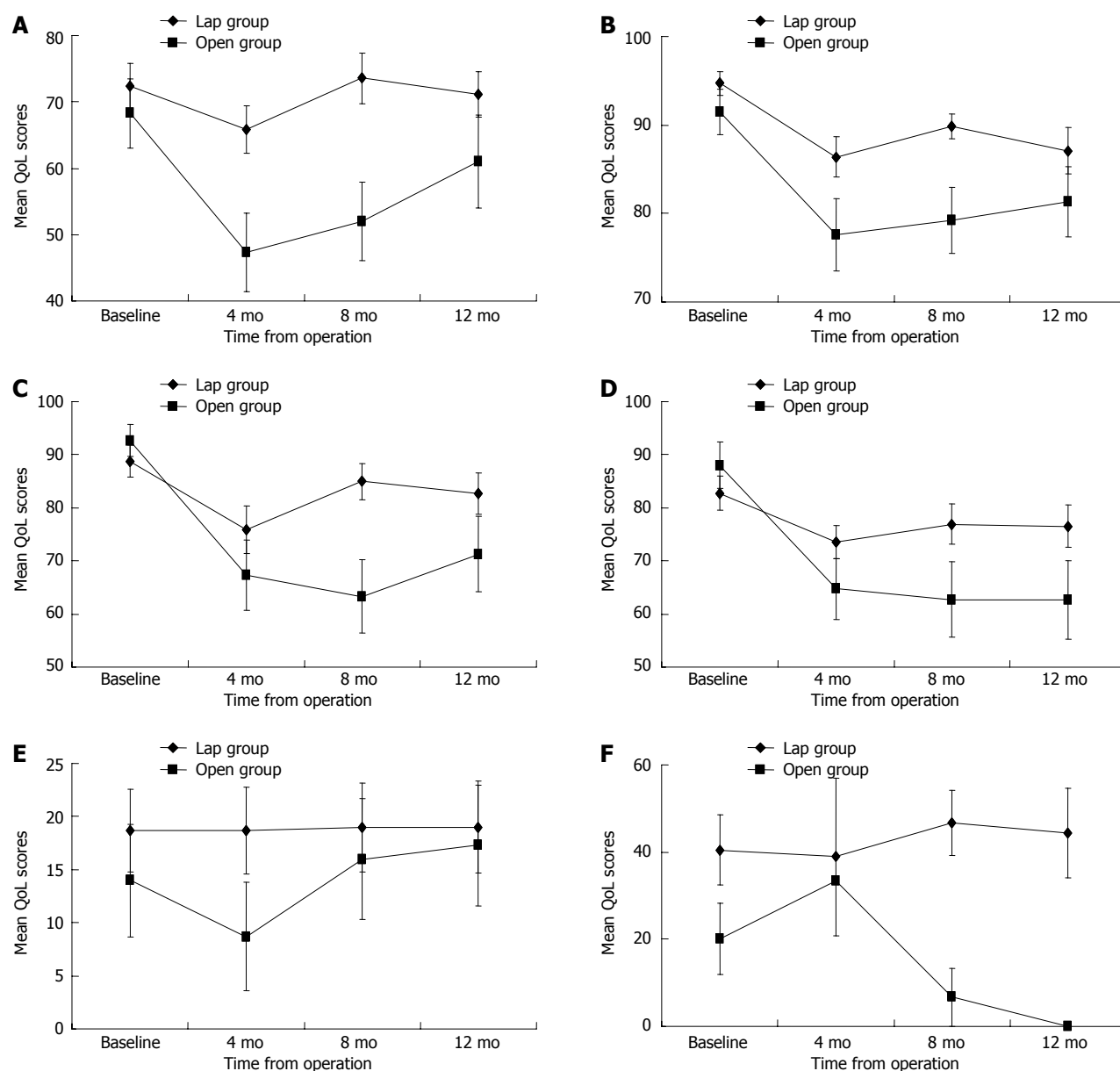


Figure 1 Longitudinal assessment of changes in quality of life scores over time for global quality of life and various functional scales. A: Global health status/quality of life (QoL); B: Physical functioning; C: Role functioning; D: Social functioning; E: Sexual functioning; F: Sexual enjoyment (only for men who have been sexually active). A higher score indicates better functioning. Error bars indicate the standard error of the mean. The Friedman test was not performed for sexual enjoyment, as the number of patients changed at different time points.

than the open approach only in the first year after surgery. Li *et al.*^[28] also reported transient QoL benefits in the early postoperative period after laparoscopic rectal surgery when compared with the open approach, but the overall QoL of the two groups was similar at a 1-year follow-up. Better QoL in the laparoscopic arm was also reported by the comparison of open *vs* laparoscopic surgery for mid and low rectal cancer after neoadjuvant chemoradiotherapy (COREAN) trial at 3 mo, but 1-year data were not provided^[2].

On longitudinal assessment, the QoL scores of most of the functional and symptom scales of the laparoscopic group in our study remained relatively stable at different time points, whereas most of the QoL scores in the open group predominantly showed deterioration at 4–8 mo but

gradually recovered by 1 year after surgery. This explains why significant differences in QoL scores between the laparoscopic and open groups in our study were primarily observed at 8 mo after surgery.

We have previously reported better short-term clinical outcomes and less long-term morbidity among patients undergoing laparoscopic surgery for rectal cancer when compared with the open approach^[7,20,21]; this may partly account for the better short-term QoL associated with the laparoscopic arm in our study. However, in addition to the healthcare experience, patients' expectations also play an important role in the determination of QoL. According to the dynamic model proposed by Carr *et al.*^[30], QoL is typically impacted when the health experience falls short of expectations. The better preservation of

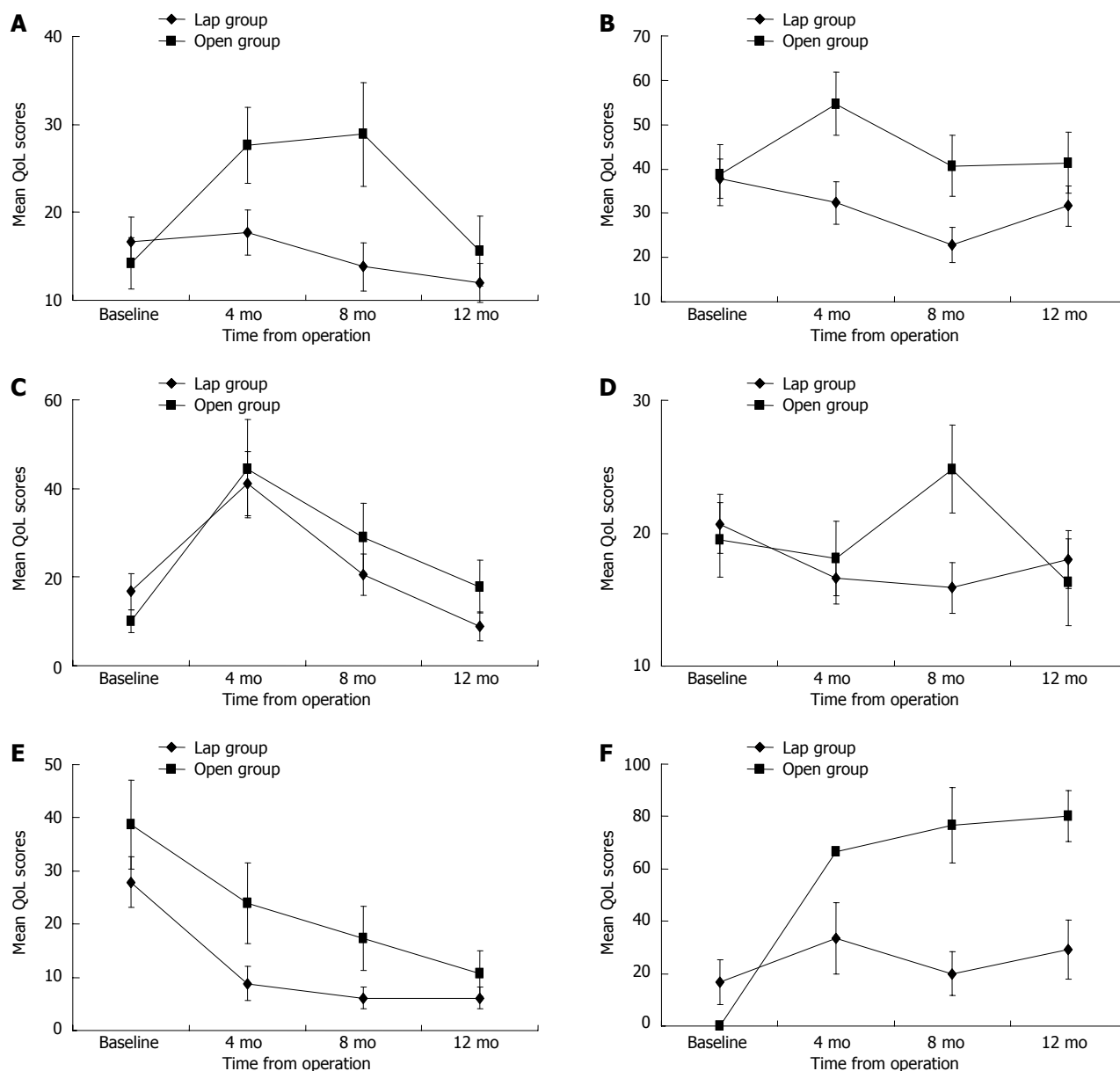


Figure 2 Longitudinal assessment of changes in quality of life scores over time for various symptom scales/items. A: Fatigue; B: Micturition problems; C: Chemotherapy side effects (only for patients who received chemotherapy); D: Gastrointestinal tract symptoms; E: Weight loss; F: Male sexual problems (only for men who have been sexually active). A higher score indicates a higher degree of symptoms. Error bars indicate the standard error of the mean. The Friedman test was not performed for male sexual problems, as the number of patients changed at different time points. QoL: Quality of life.

QoL in the laparoscopic group over time may imply that most of their initial positive expectations of laparoscopic surgery (the treatment that they had chosen) were met by their postoperative experience (the clinical benefits) and that the “expectation-experience homeostasis” remained unchanged throughout the entire assessment period. However, discrepancies between preoperative expectations and the postoperative experience may explain the initial deterioration of QoL in the open group, and a period of adaptation and alteration of expectations may have been needed to reestablish “homeostasis” by 1 year after surgery.

Interestingly, the same argument can also be used to explain the paradoxical finding of a worse functional scale of body image after laparoscopic surgery in our

study. Patients in the laparoscopic group might have had high expectations regarding the initial cosmetic results. However, when they realized that the final cosmetic outcome (a 5-cm incisional wound over the left iliac fossa and an ileostomy over the right iliac fossa) did not meet their preoperative expectations, a significant impact on QoL with respect to body image occurred. Conversely, patients in the open group who did not have high expectations regarding the cosmetic results might not have experienced a significant change in the functional scale of body image after surgery.

Urinary and sexual dysfunctions are recognized complications after rectal cancer surgery, which may have a negative impact on QoL^[11]. In our study, the laparoscopic group had significantly fewer micturition problems at 4-8

Table 3 Sexual enjoyment and sexual problems among men who have been sexually active: Laparoscopic *vs* open groups

	Baseline			4 mo			8 mo			12 mo		
	Lap	Open	<i>P</i> value	Lap	Open	<i>P</i> value	Lap	Open	<i>P</i> value	Lap	Open	<i>P</i> value
Number of men who have been sexually active	14	5	/	6	1	/	10	5	/	12	5	/
Sexual enjoyment (functional scale)	40.5 (8.0)	20.0 (8.2)	0.164	38.9 (18.1)	33.3 (/)	1.000	46.7 (7.4)	6.7 (6.7)	0.004	44.4 (10.3)	0 (0.0)	0.019
Male sexual problems (symptom scale)	16.7 (8.4)	0 (0)	0.194	33.3 (13.6)	66.7 (/)	0.309	20.0 (8.5)	76.7 (14.5)	0.013	29.2 (11.3)	80.0 (9.7)	0.026

Quality of life scores are presented as the mean (standard error of mean). Scores ranged from 0 to 100. A higher score on a functional scale indicates better functioning, whereas a higher score on a symptom scale indicates a higher degree of symptoms. Scores in the laparoscopic and open groups were compared by the Mann-Whitney *U* test.

mo after surgery when compared with the open group, but the benefit disappeared at 1 year. The COREAN trial also reported fewer micturition problems in the laparoscopic group when compared with the open group at 3 mo after surgery^[2]. This benefit of less urinary dysfunction is believed to be the result of better preservation of the autonomic nerves and less traumatic surgery, attributable to the magnified view provided by laparoscopic surgery^[2,4,5]. However, when the transient neuropraxia of the pelvic autonomic nerves in the open group has fully recovered, this benefit will disappear.

In our study, male sexual enjoyment and male sexual problems were the only two QoL scales that remained worse in the open group when compared with the laparoscopic group at 1 year after surgery. Yang *et al.*^[29] also reported fewer male sexual problems and better sexual functioning at 12–18 mo after laparoscopic total mesorectal excision for low rectal cancer when compared with open surgery; better sexual enjoyment in the laparoscopic group was even observed after 24 mo postoperatively. By contrast, a nonsignificant trend for worse sexual function in males after laparoscopic surgery for rectal cancer was reported by the United Kingdom Medical Research Council trial of conventional *vs* laparoscopic-assisted surgery in colorectal cancer (CLASICC)^[16]. Interestingly, the design of the CLASICC trial required that every participating surgeon had undertaken at least 20 laparoscopic resections, and most of the surgeons were likely still on their learning curve^[1,31]. Although the laparoscopic approach can provide a clear, magnified view in the deep pelvis, the risk of autonomic nerve injury will still be substantial if the rectal dissection is performed by an inexperienced surgeon.

Similar to the study by Yang *et al.*^[29], our study is limited by its nonrandomized design, and the risk of selection bias is inevitable. Furthermore, the number of sexually active men recruited and analyzed was small, and therefore, a very strong conclusion regarding sexual function after laparoscopic *vs* open surgery for rectal cancer could not be drawn. Nevertheless, based on our findings, we may still conclude that laparoscopic sphincter-preserving resection for rectal cancer is associated with better preservation of QoL and fewer male sexual problems when compared with the open approach in the first year after surgery. Further large-scale, multicenter, randomized trials, including the American College of Surgeons On-

cology Group Z6051 trial and the European COLOR II trial^[32,33], will more definitively evaluate whether laparoscopic surgery truly provides better QoL and reduces urosexual dysfunction in patients with rectal cancer.

In conclusion, this prospective nonrandomized study demonstrates that laparoscopic sphincter-preserving resection for rectal cancer is associated with better preservation of quality of life and fewer male sexual problems when compared with open surgery in Chinese patients. These findings, however, should be interpreted with caution because of the small sample size of the study.

COMMENTS

Background

Most colorectal surgeons are only concerned about the surgical and oncologic safety of laparoscopic surgery for rectal cancer in comparison with the open approach, and many have ignored the importance of functional outcomes and quality of life (QoL). Furthermore, few studies have evaluated QoL outcomes in Chinese patients after laparoscopic surgery for rectal cancer. Authors therefore conducted a prospective study to compare QoL outcomes in Chinese patients after curative laparoscopic *vs* open sphincter-preserving resection for rectal cancer.

Research frontiers

The magnified vision and less traumatic surgery offered by the laparoscopic approach may allow better preservation of the pelvic autonomic nerves, and presumably, functional outcomes following laparoscopic surgery for rectal cancer may be better compared to open surgery. However, conflicting results have been reported in the literature; some studies have even reported a higher incidence of sexual dysfunction after laparoscopic rectal surgery. Furthermore, it is also unclear whether the short-term and long-term clinical benefits associated with the laparoscopic approach will translate into better QoL outcomes for patients with rectal cancer.

Innovations and breakthroughs

This study has several strengths. First, although nonrandomized, the baseline characteristics and sociodemographic data of the two groups of patients were similar, and a fair comparison could therefore be made. Second, other studies have included metastatic cases and abdominoperineal resection in their QoL analysis, whereas the study only focused on Chinese patients undergoing curative sphincter-preserving rectal resection, thus minimizing the impact of other potential confounders on the QoL assessment. Third, all their questionnaires were administered by a single research assistant and were completed by the patients during clinic visits. As a result, authors achieved 100% compliance at different time points, a figure that was not achieved by other studies in which the questionnaires were collected by mail.

Applications

Their prospective nonrandomized study, albeit small in sample size, demonstrates that laparoscopic sphincter-preserving resection for rectal cancer is associated with better preservation of quality of life and fewer male sexual problems when compared with open surgery in Chinese patients. Further large-scale, multicenter, randomized trials, including the American College of Sur-

geons Oncology Group Z6051 trial and the European COLOR II trial, will more definitively evaluate whether laparoscopic surgery truly provides better QoL and reduces urosexual dysfunction in patients with rectal cancer.

Terminology

Within the context of medical and healthcare research, QoL is the patient's subjective perception of the impact of his/her disease and its treatments on his/her physical, psychological, and social functioning and general well-being. Health-related QoL after cancer surgery can be assessed by standardized instruments such as the questionnaires developed by the European Organization for Research and Treatment of Cancer (EORTC), which contain multidimensional generic and disease-specific domains; the EORTC QLQ-C30 and QLQ-CR38 are the most commonly used questionnaires in colorectal cancer trials.

Peer review

The external validity of this study is limited by the fact that only Chinese patients with low body mass index were included, and patients with stage IV disease were excluded.

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Sonographic evaluation of proximal gastric accommodation in patients with functional dyspepsia

Xiu-Ping Fan, Lin Wang, Qiang Zhu, Teng Ma, Chun-Xia Xia, Ya-Jing Zhou

Xiu-Ping Fan, Lin Wang, Qiang Zhu, Teng Ma, Chun-Xia Xia, Ya-Jing Zhou, Department of Diagnostic Ultrasound, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China

Author contributions: Zhu Q designed the research and revised the manuscript; Fan XP and Wang L performed the experiments and wrote the paper; Ma T, Xia CX and Zhou YJ analyzed the data.

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Correspondence to: Qiang Zhu, MD, PhD, Department of Diagnostic Ultrasound, Beijing Tongren Hospital, Capital Medical University, 1 Dong-Jiao-Min-Xiang, Dong Cheng District, Beijing 100730, China. qzhu_mail@126.com

Telephone: +86-10-58268051 Fax: +86-10-65131244

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Abstract

AIM: To assess the value of ultrasonography (US) in evaluation of proximal gastric accommodation disorder in patients with functional dyspepsia (FD).

METHODS: Between April 2011 and March 2012, 45 patients with FD and 27 healthy volunteers were enrolled in this study. Two-dimensional ultrasound (2DUS) and 3-dimensional ultrasound (3DUS) were performed sequentially to measure proximal gastric area (PGA), maximal proximal gastric diameter (MPGD), and proximal gastric volume (PGV). These values were measured separately in the two groups every other 5 min for a duration of 25 min after the beginning of ingestion of a test meal. Air pocket grading was done separately for images of 2DUS and blocks of 3DUS obtained at five scanning time points.

RESULTS: Both PGA and PGV of patients were significantly smaller than healthy controls ($P = 0.000$ and

0.002, respectively). Comparing the two parameters between the groups at each time point, the differences were also statistically significant ($P = 0.000-0.013$), except at 10 min for the PGV ($P = 0.077$). However, no overall difference was found between the groups in the MPGD measurements ($P = 0.114$), though it was statistically significant at a 20-minute examination point ($P = 0.026$). A total of 360 sets or blocks of images were obtained for both 2DUS and 3DUS. For the images analyzed by 2DUS, none were excluded because of gastric gas, and 50 (13.9%) and 310 (86.1%) sets were determined as air pockets grades 1 and 2, respectively. For the images analyzed by 3DUS, 23 (6.4%) blocks were excluded from the measurement due to presence of a large fundus air pocket (grade 3); fifty (13.9%) and 287 (79.7%) blocks were also graded as 1 and 2, respectively.

CONCLUSION: Measurement of both PGA and PGV by 2DUS and 3DUS could be useful for assessment of the proximal gastric accommodation.

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Key words: Functional dyspepsia; Gastric accommodation; Ultrasonography; Diagnosis; 2-dimensional ultrasound; 3-dimensional ultrasound

Core tip: We adopted 2-dimensional and 3-dimensional ultrasonography to measure area and volume of the proximal stomach in patients with functional dyspepsia; a condition whereby patients can experience impaired gastric accommodation. Area and volume could be used to assess accommodation impairment, because both area and volume of the patients were smaller than the controls ($P < 0.05$). Therefore, the ultrasound measurement of gastric area and volume could help predict the functional dyspepsia.

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graphic evaluation of proximal gastric accommodation in patients with functional dyspepsia. *World J Gastroenterol* 2013; 19(29): 4774-4780 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4774.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4774>

INTRODUCTION

Functional dyspepsia (FD) is the presence of symptoms thought to originate from the gastro-duodenal region, in the absence of organic, systemic, or metabolic disease that is likely to explain the symptoms^[1]. The prevalence of FD is 24.4% in Australia and 23.5% in China, based on the Rome II criteria^[2]. The pathogenesis of FD is still unknown, but several studies have indicated that the proximal stomach, which includes the fundus and the proximal one-third of the body, is the site of an accommodation disorder that is likely to substantially contribute to the pathogenesis of FD^[3-5]. Cannon *et al.*^[6] first described gastric accommodation in 1911. It is thought to be a vagally mediated reflex that occurs postprandially and results in reduction of tone, providing a reservoir for the meal^[7]. In patients with impaired gastric accommodation, the proximal stomach cannot relax and change its volume to the content following meal ingestion, and the subsequent increase of intragastric pressure contributes to postprandial discomfort^[8]. The impairment of proximal gastric accommodation has been found in 40% of patients with FD^[3]. Hence, it is likely that FD can be diagnosed through the recognition of impaired gastric accommodation.

There are two methods to measure proximal gastric accommodation. One method is the intragastric barostat technique, in which a polyethylene bag is directly placed into the proximal stomach *via* oral intubation. The intragastric barostat bag technique is regarded as the gold standard because it allows simultaneous acquisition of volume, pressure, and tone, and makes the user correlate these variables to sensory parameters^[9]. The disadvantages of the method are its interventional and time-consuming nature, leading to discomfort of patients^[10], and a likely interference with gastric physiology due to pressure caused by the bag^[11]. The second method is imaging, such as magnetic resonance (MR) imaging, single photon emission computed tomography (SPECT), and ultrasonography (US). MR imaging and SPECT can estimate volumetric change of the stomach directly and accurately^[12], but the equipment is not widely available and is costly, and the natural state of the stomach impacted by gravity is also neglected owing to the flat position of the examination. In addition, there is a problem of SPECT-associated radiation exposure^[13].

US is widely available, inexpensive, non-radioactive, and can be performed repeatedly, even at the bedside, making it a much more attractive option for the measurement of proximal gastric accommodation. Moreover, because gravity plays a role in the propulsion of gastric

contents, accommodation should be measured in a sitting or standing position that can be easily accomplished during the US examination^[12,14,15]. However, published studies reviewing the feasibility of this method are limited, presumably due to the complex procedure of scanning the stomach with US. Therefore, the aim of the current study was to investigate the usefulness of US, including 2-dimensional US imaging (2DUS) and 3-dimensional US imaging (3DUS), in the measurement of proximal gastric accommodation disorders in patients with FD compared to healthy controls.

MATERIALS AND METHODS

Subject characteristics

Between April 2011 and March 2012, 46 consecutive patients with FD underwent US scanning. One patient was excluded from the study because of nephroptosis, which obscured the left kidney as a landmark in obtaining a sagittal plane of the proximal stomach. Thus, 45 enrolled patients consisted of 17 men and 28 women, with an age range of 19-64 years (mean: 33.70 ± 9.86 years) and a body mass index (BMI) of 16.33 - 25.95 kg/m² (mean: 20.67 ± 2.34 kg/m²). None of the patients had a history of other abdominal diseases, abnormal hepatic function tests, organic changes on gastroendoscopy, and positive findings on routine abdominal US scanning.

The Rome III classification system was the basis for the diagnostic criteria for inclusion of patients with FD^[1]. According to these criteria, the patient must have one or more of the following symptoms: bothersome postprandial fullness, early satiation, epigastric pain, or epigastric burning. Further, the patient could have no evidence of structural gastrointestinal diseases on upper endoscopy likely to explain the symptoms, and the symptoms must have occurred 6 mo prior to diagnosis and be active for the last 3 mo. Of the 45 patients, 33 (73.3%), 20 (44.4%), 19 (42.2%), and 14 (31.1%) presented with postprandial fullness, epigastric pain, early satiation, and epigastric burning, respectively.

Twenty-seven healthy volunteers were examined by US. This sample included 14 men and 13 women with an age range of 19-75 years (mean: 38.07 ± 14.55 years) and a BMI of 18.02 - 24.21 kg/m² (mean: 21.10 ± 1.74 kg/m²). Healthy controls had no symptoms and physical signs of gastrointestinal diseases in the past six months, history of other abdominal diseases, abnormal hepatic function tests, and positive findings on routine abdominal US examination.

There were no statistically significant differences between the patients with FD and control groups with respect to age and BMI. Informed consent was obtained from all of the subjects.

Test meal

A 500 mL esculent liquid was used as the test meal, and was prepared by mixing 200 mL of nutrient emulsion (Enteral Nutritional Emulsion; Sino-Swed Pharmaceutical

Corp, Beijing, China) with 300 mL of warm water. The emulsion contained 15 g of protein, 11.6 g of fat, and 34 g of carbohydrate (300 kcal). To decrease the presence of small bubbles in the nutridrink, the meal was allowed to sit stationary on a table for approximately 10 min before consumption.

US equipment

A Voluson 730 expert system with a RAB 2-5 type probe with 3DUS imaging function was employed (GE Medical Systems, Milwaukee, WI, United States).

Examination protocol

To avoid increscent gas within the stomach, the examination was performed before 10:00 am after an overnight fasting of > 8 h. Administration of medication affecting gastrointestinal motility was discontinued for at least 48 h prior to US. Smoking was not allowed on the day of examination. All the patients were examined within 7 d following gastric endoscopy.

The subjects were scanned in a half-sitting position, leaning back at an angle of approximately 80° on an examining couch. The antrum was observed 2-3 min before nutridrink ingestion to avoid antral contractions and emptying into duodenum, in which the elevation of proximal stomach tone is induced by an enterogastric reflex occurring in phase III of the migrating motor complex^[16]. Thereafter, in the other phases without the contraction, a 500 mL meal was ingested with a straw within 4 min. The proximal stomach of each subject was scanned every other 5 min during 25 min after beginning ingestion.

Air pocket grading

To assess image quality, a grading system based on the amount of air pockets in the proximal stomach was established as follows: grade 1 (absence of visible air within the stomach); grade 2 (some air within the stomach, but the following measurements still being able to be proceed); and grade 3 (a great amount of gastric air so that the image would be excluded from the measurement). Grading was done separately using 2DUS and 3DUS using five examinations for each subject.

US scanning and measurement

Subjects were instructed not to move and to hold their breath at the end of expiration to permit diaphragmatic rising and restoration of the gastric configuration.

For 2DUS imaging, a scanning probe was placed longitudinally under the left subcostal margin and tilted cranially in the long axial direction to show the top of the gastric fundus. In this way, a sagittal section of the proximal stomach was visualized, in which the left renal sinus, the left lobe of the liver, and the tail of the pancreas served as anatomic landmarks (Figure 1). Then, the probe was rotated 90° and tilted cranially in the short axial direction to obtain a maximal transverse section of the proximal stomach, in which the left diaphragm

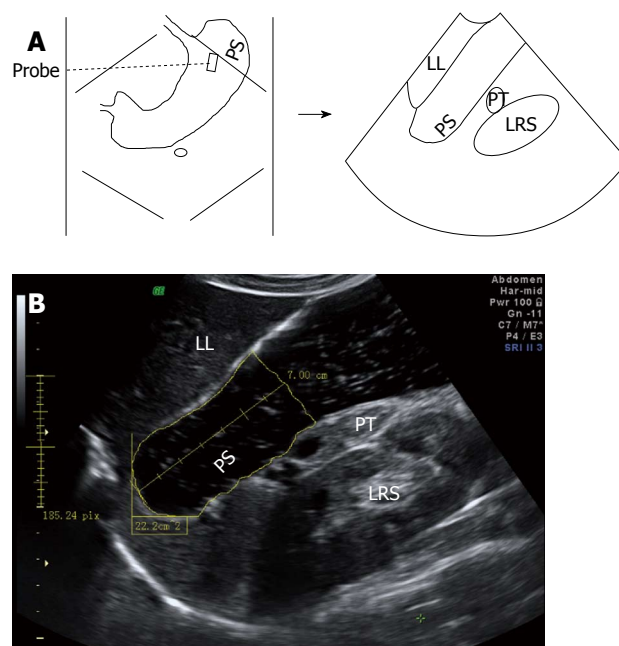


Figure 1 Sagittal section of the proximal stomach. A: To obtain the section, a probe is placed longitudinally under the left subcostal margin and tilted cranially in the long axial direction of proximal stomach (PS) to show the top of gastric fundus, in which left renal sinus (LRS), left liver (LL), and pancreatic tail (PT) are simultaneously displayed; B: Proximal gastric area (PGA) is measured by means of outlining along the echogenic mucosa surface of PS in the distance between the echoic inner surface of the fundus top down to 7 cm level (between cursors).

and the left liver were landmarks (Figure 2). Image post-processing was done using image-processing software (4D View, version 5.0; GE Medical Systems). On the sagittal section, the proximal gastric area (PGA) was outlined by tracing along the luminal echogenic surface corresponding to the interface between the liquid and mucosa of the gastric wall, from the top margin of the fundus to 7 cm level inferiorly (Figure 1). On the transverse section, a maximal proximal gastric diameter (MPGD) was measured between the inner echogenic surfaces of the lesser and greater curvatures (Figure 2).

For 3DUS analysis, volumetric image data was acquired immediately following 2DUS using similar placement of the probe to that of the above sagittal section. A sweeping angle of 85° was set. The proximal stomach was scanned *via* automated sweeping between the curvatures over 5-10 s. The block cut at a distance of 7 cm inferior to the top of the fundus was saved for further processing on the workstation. Using a virtual organ computer-aided analysis (VOCAL) technique of the 4D View, six sections of one block were separately outlined manually along the echoic interface, with each rotating 30° from the previous section. The proximal gastric volume (PGV) was automatically calculated from these six highlighted areas and displayed as a reconstructive volume (Figure 3).

One physician (Wang L), blinded to the subject (FD patient or healthy control), completed the measurement. The mean value was calculated after two measurements.

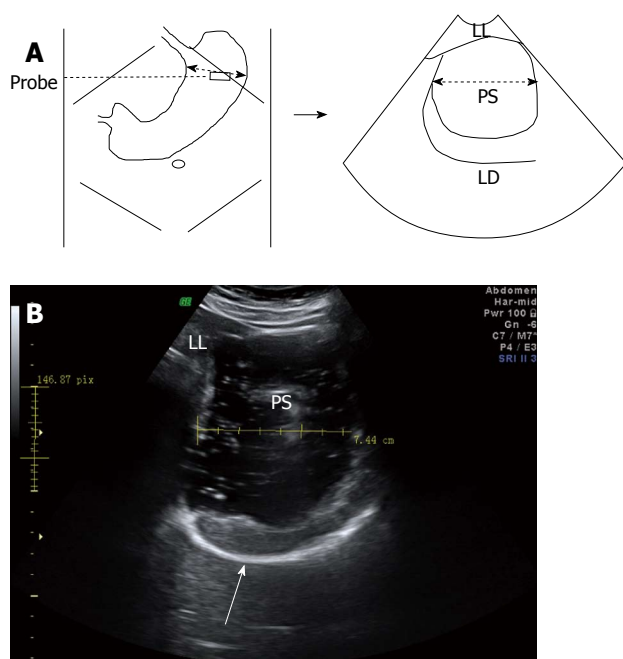


Figure 2 Maximal transverse section of the proximal stomach. A: After acquiring the previous sagittal section, the probe is rotated about 90° to show the maximal transverse diameter (dotted line with double arrow), in which the left diaphragm (LD) and left liver (LL) are simultaneously depicted; B: In measuring maximal proximal gastric diameter, the cursors are placed on the echogenic mucosal surfaces of the lesser and greater curvatures. LL and the arrow indicate left liver and left diaphragm, respectively. PS: Proximal stomach.

Statistical analysis

The measurement values are presented as the mean $\pm$ SD. With repeated measures analysis of variance (ANOVA), the values of PGA, MPGD, and PGV were compared between two groups, as a total and at each scanning time within 25 min. Statistical significance was accepted as $P < 0.05$.

RESULTS

Air pocket grading

Three-hundred-and-sixty sets of 2DUS images (one sagittal and one transverse section), and the same number of 3DUS blocks, were graded. Of these, 225 were obtained from patients and 135 from controls in five time examinations. The 2DUS imaging revealed 50 (13.9%) and 310 (86.1%) sets of the image were determined as grades 1 and 2, respectively, and none were excluded due to grade 3. In 3DUS, 50 (13.9%) and 287 (79.7%) blocks were graded as 1 and 2, and the other 23 (6.4%) were grade 3 and in turn excluded from the measurement. Of these excluded blocks, 13 (56.5%) appeared at 10 min, and the remaining four (17.4%), three (13.0%), one (4.3%) and two (8.7%) occurred at 5, 15, 20 and 25 min after the meal, respectively.

PGA, MPGD and PGV measurements

The PGA and PGV of patients were significantly smaller than those of healthy controls ($P = 0.000$ and 0.002 ,

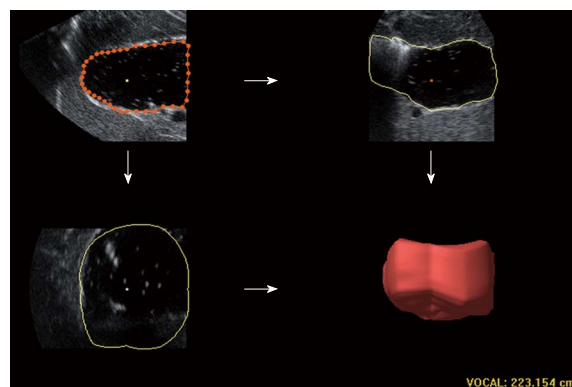


Figure 3 Three-dimensional ultrasound applied for measuring proximal gastric volume. The volume is measured similarly from the top inner margin of the fundus to 7 cm level inferiorly along the long axis of proximal stomach; six sections of the block from six 30° rotations are separately outlined along the echoic interface in the upper left view. A reconstructive volume is displayed in the lower right view.

respectively). When the two parameters were compared at each time point separately, the differences were also statistically significant between the two groups ($P = 0.000$ - 0.013), except at 10 min of the PGV.

The patients with FD revealed shorter MPGD than healthy controls postprandially; however, it was not statistically significant in the two groups, and the difference was significant ($P = 0.026$) only at 20 min when comparing each time point (Table 1).

DISCUSSION

Based on the theory that the impairment of proximal gastric accommodation is likely to lead to the pathogenesis of FD, both 2DUS and 3DUS imaging were utilized to measure the size of the proximal stomach. The data indicated that both PGA and PGV could help assess the proximal gastric accommodation.

US can provide an indirect evaluation of stomach relaxation and intragastric pressure by measuring the size of the stomach^[17]. The 2DUS method of assessing gastric accommodation was developed first by Gilja *et al.*^[18]. According to their study, 2DUS could offer a geometric estimation of proximal gastric size by measuring PGA and MPGD. They also found that the patients with FD exhibited a smaller PGA and MPGD than controls ($P = 0.018$ and 0.046 , respectively)^[19]. Having adopted a similar method, our study showed that the PGA was significantly different between FD patients and healthy controls, but the MPGD was not different overall, being significant only at 20 min ($P = 0.026$). The reason why PGA was superior to MPGD could be related to an irregular shape of the proximal stomach. When estimating the size of an organ that had an irregular contour, using an area was likely more accurate than a diameter. The other reason might be that the left renal sinus, which served as a key landmark in a sagittal section used for PGA measurement, had a relatively narrow distance in a transverse di-

Table 1 Ultrasonography measurement of postprandial size of proximal stomach (mean $\pm$ SD)

Time in min	Patients (n = 45)	Controls (n = 27)	t	P
PGA in cm ²				
5	22.78 $\pm$ 6.59	30.68 $\pm$ 6.97	4.819	0.000
10	23.13 $\pm$ 6.39	29.52 $\pm$ 7.46	3.853	0.000
15	22.32 $\pm$ 5.93	27.51 $\pm$ 7.13	3.332	0.001
20	21.34 $\pm$ 6.34	28.25 $\pm$ 7.76	4.110	0.000
25	21.51 $\pm$ 6.02	26.35 $\pm$ 7.23	3.062	0.003
F = 17.499 P = 0.000				
MPGD in cm				
5	6.77 $\pm$ 1.34	7.08 $\pm$ 1.10	1.035	0.304
10	6.92 $\pm$ 1.31	7.27 $\pm$ 1.00	1.185	0.240
15	6.66 $\pm$ 1.43	7.11 $\pm$ 1.03	1.432	0.157
20	6.33 $\pm$ 1.29	7.00 $\pm$ 1.06	2.275	0.026
25	6.31 $\pm$ 1.50	6.66 $\pm$ 1.24	1.032	0.305
F = 2.562 P = 0.114				
PGV in cm ³				
5	145.75 $\pm$ 60.40	185.08 $\pm$ 60.81	2.645	0.010
10	152.91 $\pm$ 52.10	177.13 $\pm$ 59.10	1.797	0.077
15	142.46 $\pm$ 49.50	184.16 $\pm$ 52.28	3.358	0.001
20	132.45 $\pm$ 46.70	169.12 $\pm$ 48.64	3.147	0.002
25	126.15 $\pm$ 50.23	157.46 $\pm$ 49.97	2.544	0.013
F = 10.319 P = 0.002				

PGA: Proximal gastric area; MPGD: Maximal proximal gastric diameter; PGV: Proximal gastric volume.

rection and a constant relationship to the proximal stomach. Therefore, the measurement of PGA might tend to be of less operator variability in various examination time points. Besides, when MPGD was transversely measured, the right cursor was placed on or near the gastric lesser curvature with less expansive function^[20], and hence the difference of the transverse dimension between the patient and the control would be decreased. This phenomenon also occurred in other previous studies^[21,22]. Thus, the application value of MPGD was limited in assessment of gastric accommodation.

The feasibility of assessing the proximal gastric accommodation with PGA on 2DUS images has been confirmed by several studies^[21-24]. The measuring of PGA was simple, with only one section being outlined in this process. It was less likely to be affected by gastric air, which was verified since no subject was excluded for this factor. However, it was difficult to find the landmarks in subjects with obese body types, nephroptosis, or renal ectopy. The volumetric estimation that was based on values of 2DUS, *i.e.*, $V = PGA \times MPGD$, and adopted by other studies before the advent of 3DUS could to some extent bring an error because of the irregular-shaped stomach^[18].

In general, 3DUS has advantages over 2DUS, which can measure a volume directly and needs no landmarks. Using this technique, Gilja *et al.*^[25] obtained a good correlation ($r = 0.997$, $P < 0.05$) between the estimated volume of porcine stomach filled with water *in vitro* and the actual quantity of water injected. Another study demonstrated that 3DUS had a moderate correlation ($r = 0.55$, $P = 0.002$) with the barostat in measurement of proximal gastric volumes^[26]. However, there were some drawbacks of

the freehand 3DUS technique used in these studies^[23,24]. An additional tracking device that consisted of a transmitter generating a pulse magnetic field and a position sensor attached to the probe was required. Therefore, the environment where the patient was examined requires magnetic shielding to avoid image distortion. The distortion also appeared while the data of both image and position could not be transformed simultaneously to a post-processing workstation. The time-consuming process of coordinating images with their spatial locations also restricted it. The previous method of 3DUS scanning was still limited by the complicated manipulation in which the operator had to scan several times to obtain an image with high quality, because the image was easily distorted in free-hand moving a probe on body surface at an appropriate speed. In the current study, a new type of 3DUS probe (RAB 2-5, Voluson 730) was used, in which a 1D transducer array moving mechanically through a designed trajectory was mounted together with integrated positioning system and sensor. Hence, the data acquisition was automatic rather than manual, could be done during a single breath-hold, and could be displayed on the monitor immediately after scanning. Using this type of transducer, PGV could be obtained in most (337/360, 93.6%; air pockets grading 1 and 2) of the 3DUS blocks at five examination time points. Consequently, the patients with FD showed smaller PGV than healthy controls ($P = 0.002$). Adopting the same transducer, Manini *et al.*^[27] measured the whole gastric volume accurately, with the results comparable to that from SPECT, establishing a reference standard for measuring gastric size.

An air grading system was designed to assess the image quality. Intragastric gas is a critical interference factor in US stomach examination, which may induce multiple reflection artifacts and limit the stomach outline. The accumulation of gas in the fundus was a gradually incremental process. Small bubbles could be seen in the entire stomach immediately after drinking a test meal, in addition to the usual existence of fundus air pockets, but these bubbles did not decline the image quality. Over time, they burst and mixed into the fundus air pockets, which to some degree obstructed the visualization of the gastric wall, especially the posterior wall. Within minutes, the gas reduced because of burping and the image quality was improved again. In our results, none of images were excluded from the 2DUS analysis but 23 (6.4%) 3DUS blocks could not be utilized due to the grade 3 air pockets. We hypothesize that the higher resolution of the single section 2DUS images^[28], combined with fact that 3DUS was more subject to the intragastric gas explains this phenomenon. We also found that most of the grade 3 (13/23, 56.5%) appeared at 10 min after the beginning of ingestion. The decrease of sample size after excluding the 13 3DUS blocks might cause no significant difference in PGV measurement between two groups this time. These results suggested that 3DUS testing at the 10 min time point or with the appearance of fundus air pockets should be avoided.

There were some limitations in the current study. The

limited sample size did not allow us to divide patients into two subgroups of postprandial distress syndrome and epigastric pain syndrome according to the Rome III criteria^[1]. After fasting overnight, ingesting a 500 mL test meal in a short time would make subjects uncomfortable, and consequently the smaller amount of the meal should be tested. The observation duration of 25 min might not be enough to investigate into the gastric accommodation, which lasts in the entire process of postprandial digestion^[29]. Because no reference method as the barostat procedure or SPECT was adopted in our study, the comparative study of 2DUS and 3DUS imaging could not be carried out to find out which one was more accurate.

In conclusion, we show that the impaired gastric accommodation to a test meal was present in patients with FD. Two parameters of PGA and PGV on 2DUS and 3DUS images could be used for assessing the proximal gastric accommodation in which 2DUS was simpler in manipulation and less likely to be degraded by gastric gas, and 3DUS had the merit of measuring volume directly, providing less gastric gas.

COMMENTS

Background

There is a high global incidence of functional dyspepsia (FD), with about half of the reported gastroenterological outpatients being in China. Several studies have indicated that impaired gastric accommodation is a major pathogenesis of the disease. Hence, assessment of proximal gastric accommodation disorder could help diagnose FD.

Research frontiers

Imaging can provide an indirect evaluation of gastric relaxation in accommodation reflex by measuring the size (*i.e.*, area and volume) of the proximal stomach. Ultrasonography is a preferable imaging method, which is accurate, easy to manipulate, non-invasive, and cost effective.

Innovations and breakthroughs

The proximal gastric accommodation was evaluated by measuring the proximal gastric area (PGA) and proximal gastric volume (PGV) in patients with FD using 2-dimensional and 3-dimensional ultrasound. PGA and PGV of the patients were significantly smaller than the controls. Therefore, the change of PGA or PGV could be pertinent to the impairment of gastric accommodation and utilized in predicting FD.

Applications

US is likely to be a convenient and accurate imaging modality to assess the gastric accommodation. It has the potential value of diagnosing FD and evaluating the effect of therapy.

Terminology

The proximal stomach is a compartment consisting of the fundus and the upper one-third of gastric body, which has the function of gastric accommodation. The boundary between the proximal and distal stomach has not been definitely determined anatomically and physiologically. Gastric accommodation is a vagally mediated reflex that occurs postprandially, results in reduction of gastric tone, and provides a reservoir for the meal.

Peer review

In this study, the authors found that both PGA and PGV on 2-dimensional ultrasound and 3-dimensional ultrasound images could be used in assessment of the proximal gastric accommodation. Very interesting study, it could give the readers some new information about the use of US in evaluation of proximal gastric accommodation disorder in patients with functional dyspepsia. The manuscript is well written.

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Influence of endoscopic submucosal dissection on esophageal motility

Bao-Guo Bu, En-Qiang Linghu, Hui-Kai Li, Xiao-Xiao Wang, Rong-Bin Guo, Li-Hua Peng

Bao-Guo Bu, En-Qiang Linghu, Hui-Kai Li, Xiao-Xiao Wang, Rong-Bin Guo, Li-Hua Peng, Department of Gastroenterology and Hepatology, PLA General Hospital, Beijing 100853, China

Author contributions: Linghu EQ designed the research; Bu BG performed the research and contributed to acquisition, analysis and interpretation of data, and wrote the article; Li HK, Wang XX, Guo RB and Peng LH contributed to acquisition of data.

Correspondence to: En-Qiang Linghu, Professor, Department of Gastroenterology and Hepatology, PLA General Hospital, 28 Fuxing Road, Haidian District, Beijing 100853, China. linghuenqiang@vip.sina.com

Telephone: +86-10-66936998 Fax: +86-10-68154653

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Abstract

AIM: To assess esophageal motility after esophageal endoscopic submucosal dissection (ESD).

METHODS: Twelve patients (6 men and 6 women) aged 53-64 years (mean age, 58 years) who underwent regular examination 3-12 mo after esophageal ESD for neoplasms of the esophageal body were included in this study. The ESD procedure was performed under deep sedation using a combination of propofol and fentanyl, and involved a submucosal injection to lift the lesion and use of a dual-knife and an insulated-tip knife to create a circumferential incision around the lesion extending into the submucosa. Esophageal motility was examined using a high-resolution manometry system. Dysphagia was graded using a five-point scale according to the Mellow and Pinkas scoring system. Patient symptoms and the results of esophageal manometry were then analyzed.

RESULTS: Of the 12 patients enrolled, 1 patient had

grade 2 dysphagia, 1 patient had grade 1 dysphagia, and 3 patients complained of sporadic dysphagia. Ineffective esophageal motility was observed in 5 of 6 patients with above semi-circumference of resection extension. Of these 5 patients, 1 patient complained of grade 2 dysphagia (with esophageal stricture), one patient complained of grade 1 dysphagia, and 3 patients complained of sporadic dysphagia. Normal esophageal body manometry was observed in all 6 patients with below semi-circumference of resection extension. The 6 patients with normal esophageal motility did not complain of dysphagia.

CONCLUSION: Extensive esophageal ESD may cause esophageal dysmotility in some patients, and might also have an influence on dysphagia although without esophageal stricture.

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Key words: Esophageal neoplasm; Endoscopic submucosal dissection; Dysphagia; Ineffective esophageal motility; Esophageal manometry

Core tip: Endoscopic submucosal dissection (ESD) is widely used to treat esophageal epithelial neoplasms. ESD has the advantage over esophagectomy of being less invasive and having lower postoperative morbidity. ESD also has the advantage over endoscopic mucosal resection of enabling the removal of larger epithelial neoplasms in an *en bloc* manner for complete resection. It is not known whether esophageal ESD affects esophageal motility. Therefore, the present study aimed to evaluate the effects of ESD on esophageal motility.

Bu BG, Linghu EQ, Li HK, Wang XX, Guo RB, Peng LH. Influence of endoscopic submucosal dissection on esophageal motility. *World J Gastroenterol* 2013; 19(29): 4781-4785 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4781.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4781>

INTRODUCTION

Endoscopic submucosal dissection (ESD) is widely used to treat esophageal epithelial neoplasms^[1-3]. ESD has the advantage over esophagectomy of being less invasive and having lower postoperative morbidity^[4-6]. ESD also has the advantage over endoscopic mucosal resection (EMR) of enabling the removal of larger epithelial neoplasms in an *en bloc* manner for complete resection^[7-9]. Some postoperative complications such as bleeding and perforation may occur, but can be minimized by the development of the endoscopist's skill and the use of advanced equipment^[10,11]. Postoperative stricture is a major complication of ESD due to the increase in the scope of ESD resection, which can result in dysphagia and thus affect patients' quality of life. Some studies have found that more than 3/4 of the circumference of esophageal ESD has a high risk of esophageal stricture^[12,13]. Post-ESD stricture can be prevented with repeated endoscopic balloon dilation and oral prednisolone^[14,15].

In addition, dysphagia is not always related to esophageal stricture and motility abnormalities also play an important role^[16-19]. Some patients undergoing esophageal ESD at our center complained of sporadic dysphagia without postoperative esophageal stricture, especially when swallowing a large mass of food. This observation has raised concerns that sporadic dysphagia in these patients may be due to postoperative esophageal dysmotility. Moreover, the healing of an ESD-induced iatrogenic esophageal ulcer is also involved in destruction and fibrosis of the muscularis propria^[20]. It is not known whether esophageal ESD affects esophageal motility. Therefore, the present study aimed to evaluate the effects of ESD on esophageal motility.

MATERIALS AND METHOD

Patients

Twelve patients (6 men, 6 women; mean age, 58 years; range, 53-64 years) examined between 3 and 12 mo after ESD for neoplasms of the esophageal body at the Chinese PLA General Hospital were included in this study. Clinicopathological data of the patients are shown in Table 1. After written informed consent was obtained, data on dysphagia were collected by questionnaire and all patients underwent esophageal manometry.

ESD procedure

The ESD procedure was performed in a standardized way under deep sedation using a combination of propofol and fentanyl. Patients were continuously monitored by electrocardiography. The margins of the lesion were marked by electrocautery (30 W soft coagulation) to determine the resection border. A submucosal injection was then performed to lift the lesion. When the lesion was lifted sufficiently, a dual-knife and insulated-tip knife were used to create a circumferential incision around the lesion extending into the submucosa and a submucosal

dissection was performed to remove the lesion in an *en bloc* fashion.

Grading of dysphagia

Dysphagia was graded on a five-point scale according to Mellow and Pinkas^[21]: 0 = no dysphagia, 1 = dysphagia to normal solids, 2 = dysphagia to soft solids, 3 = dysphagia to solids and liquids, 4 = complete dysphagia, even to saliva.

Esophageal manometry

Assessment of esophageal motility was performed using a high-resolution manometry system with 36 channels spaced at 1 cm intervals (outer diameter 4.2 mm) (Sierra Scientific Instruments, Los Angeles, CA, United States). This system can automatically capture esophageal motor function from the pharynx to the stomach with a single placement of the catheter.

Esophageal manometry was performed after fasting for at least 6 h, and the high-resolution catheter was passed transnasally, positioned 2 cm below the lower esophageal sphincter (LES) and then fixed in place by taping it to the nose. The upper esophageal sphincter and LES were located using a real-time pressure monitor. The protocol included a 5-min period to assess basal sphincter pressure and then ten swallows of water (5 mL each swallow) in the supine position^[22]. All swallows and pressure measurements were analyzed by Manoview Analysis Software (Sierra Scientific Instruments, United States).

RESULTS

Grading of dysphagia

Of the 12 patients, one patient complained of grade 2 dysphagia, one complained of grade 1 dysphagia and three patients complained of sporadic dysphagia (especially when swallowing a large mass of food) (Table 1).

Esophageal manometry

Esophageal manometry showed that of the 6 patients with above semi-circumference of resection extension, 5 patients had low esophageal body pressure, including four patients with low mean esophageal body pressure and one with low pressure at an interval of 3 cm below the LES (Table 1). Of these 5 patients, 1 patient (with post-ESD esophageal stricture) complained of grade 2 dysphagia, 1 patient (without post-ESD esophageal stricture) complained of grade 1 dysphagia and 3 patients complained of sporadic dysphagia. Segmental simultaneous wave was observed in ESD scar site in 4 of these 5 patients, and wave amplitude was significantly decreased (Figure 1).

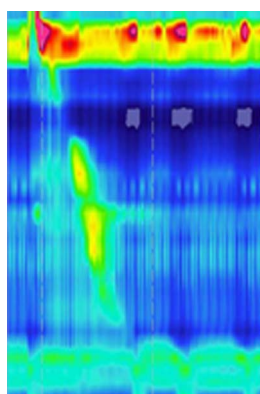
DISCUSSION

ESD is a feasible method for treating early esophageal epithelial neoplasms, and was developed for *en bloc* and

Table 1 Clinicopathological features and esophageal manometry of 12 patients with esophageal epithelial neoplasms treated by endoscopic submucosal dissection

Number of patient	Sex	Age (yr)	Post-ESD pathology	Circumferential extension	Grade of dysphagia	Pressure of esophageal body (mmHg)			Non-peristaltic contractions of esophageal body in ten water swallows
						7.0 cm above LES	3.0 cm above LES	mean	
1	F	64	T1 cancer	2/4-3/4	0	129.8	108.7	119.2	0%
2	M	64	LGIN	1/4-2/4	0	99.9	75.3	87.6	0%
3	M	53	HGIN	2/4-3/4	2	26.1	45.2	35.6	40%
4	F	55	HGIN	2/4-3/4	Sporadic	31.8	41.2	36.5	50%
5	M	55	HGIN/Tis	1/4-2/4	0	139.1	125.9	132.5	0%
6	M	55	LGIN	2/4-3/4	Sporadic	90.1	31.9	61.0	20%
7	M	58	T1 cancer	> 3/4	Sporadic	34.1	21.7	27.9	20%
8	F	64	HGIN/Tis	1/4-2/4	0	58.6	76.6	67.6	0%
9	F	55	LGIN	1/4-2/4	0	99.0	109.2	104.1	0%
10	F	57	HGIN	> 3/4	1	34.3	52.5	43.4	50%
11	F	58	HGIN	1/4-2/4	0	96.2	109.2	102.7	0%
12	M	57	LGIN	1/4-2/4	0	90.6	76.2	83.4	0%

HGIN: High grade intraepithelial neoplasia; LGIN: Low grade intraepithelial neoplasia; LES: Lower esophageal sphincter; ESD: Endoscopic submucosal dissection; Tis: Cancer *in situ*; M: Male; F: Female.

**Figure 1** Segmental simultaneous wave and decreased amplitude in endoscopic submucosal dissection scar site.

complete resection of large esophageal epithelial neoplasms, to allow precise histological assessment of specimens excised in one piece with tumor-free lateral/basal margins and to reduce residual disease and local recurrence^[1-3]. With the increase in the scope of esophageal ESD resection, postoperative esophageal stricture and dysphagia are major complications during long-term follow-up, and require repetitive endoscopic balloon dilatation which severely affects patient quality of life. More than 3/4 of the circumference of esophageal ESD is a significant cause of postoperative esophageal stricture^[12,13]. Ono *et al.*^[10] reported that post-ESD stricture with dysphagia was successfully managed with repeated endoscopic balloon dilation in a median of 2 sessions.

Honda *et al.*^[23] found that in a dog model, mucosal defects after EMR were associated with inflammation and although the muscularis propria appeared not to have been damaged by the EMR procedure, myofiber atrophy was found to develop after the first postoperative week, eventually leading to fibrosis. Ota *et al.*^[20] found that an artificial ulcer after esophageal EMR/ESD in humans had a similar healing time to that in dogs. Kahrilas *et al.*^[24] reported an increased proportion of failed primary peristalsis and

a reduction in peristalsis amplitude in peptic esophagitis. Singh *et al.*^[25] found a reduction in peristaltic amplitude after healing of esophagitis. Malhi-Chowla *et al.*^[19] found that photodynamic therapy may worsen esophageal motility in some patients with esophageal adenocarcinoma and Barrett's esophagus or Barrett's esophagus with high-grade dysplasia. Esophageal dysmotility resulting from photodynamic therapy or caustic esophageal burns is due to muscularis propria destruction. Bautista *et al.*^[16] found that caustic esophageal burns can also result in esophageal dysmotility without stricture. McDougall *et al.*^[26] found that there was no improvement in esophageal motility after healing of esophagitis, and they suggested that there was either a primary motility problem, or that reflux damage caused irreversible impairment of motility which failed to recover when the mucosa healed. Healing of post-ESD esophageal ulcer is a similar process to that in caustic esophageal burns and photodynamic therapy. It is not known whether esophageal ESD affects esophageal motility.

In the present study, ineffective esophageal body motility was observed in 5 of 6 patients with above semi-circumference of resection extension. Segmental simultaneous wave was observed in ESD scar site in 4 of these 5 patients, and wave amplitude was significant decreased. These results showed that extensive esophageal ESD might have an effect on esophageal motility. These were some observed phenomenon, and further research was needed to confirm.

Malhi-Chowla *et al.*^[19] found that some patients complained of dysphagia after photodynamic therapy without esophageal stricture, while esophageal dysmotility was observed in these patients. Therefore, they considered that dysphagia may be related to underlying esophageal dysmotility and may not always be caused by stricture or underlying carcinoma.

In the present study, 2 patients complained of dysphagia, and 3 patients complained of sporadic dysphagia. Of these 5 patients, only one had esophageal stricture.

However, ineffective esophageal motility was observed in all five patients. These results showed that besides esophageal stricture, ineffective esophageal motility might play an important role in dysphagia after esophageal ESD.

In summary, extensive esophageal ESD may cause ineffective esophageal motility, and ineffective esophageal motility may be a cause of dysphagia after esophageal ESD. Further studies should be encouraged to compare esophageal motility in patients before and after they undergo esophageal ESD.

COMMENTS

Background

Endoscopic submucosal dissection (ESD) is used to resect the esophageal epithelial neoplasms and has the advantage over endoscopic mucosal resection of enabling the removal of the larger epithelial neoplasms in an *en bloc* manner for complete resection. The previous study showed that esophageal dysmotility after caustic burn may cause dysphagia. However, esophageal dysmotility after esophageal ESD has not been studied. The present study therefore aimed to assess esophageal motility after esophageal ESD.

Research frontiers

The study involved testing the esophageal motility after esophageal ESD and analyzing the relation of esophageal dysmotility and extension of ESD.

Innovations and breakthroughs

Esophageal dysmotility after esophageal ESD has not been reported. In this study, the authors found that low esophageal body pressure was observed in 5 patients of 6 patients with above semi-circumference of resection extension, whereas normal esophageal body manometry was observed in all 6 patients with below semi-circumference of resection extension. The 5 patients with low esophageal body pressure complained of various degree of dysphagia.

Applications

The results suggest that extensive esophageal ESD may cause ineffective esophageal motility, and ineffective esophageal motility may be a cause of dysphagia after esophageal ESD.

Peer review

ESD is widely used to treat esophageal epithelial neoplasms. The authors evaluated the effects of ESD on esophageal motility. The authors suggest that extensive esophageal ESD may cause ineffective esophageal motility, and ineffective esophageal motility may be a cause of dysphagia after esophageal ESD. The data in this study is very important and informative.

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Learning curve of transumbilical suture-suspension single-incision laparoscopic cholecystectomy

Ming-Xin Pan, Zhi-Wei Liang, Yuan Cheng, Ze-Sheng Jiang, Xiao-Ping Xu, Kang-Hua Wang, Hai-Yan Liu, Yi Gao

Ming-Xin Pan, Yuan Cheng, Ze-Sheng Jiang, Xiao-Ping Xu, Kang-Hua Wang, Hai-Yan Liu, Yi Gao, Second Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, Guangdong Province, China
Zhi-Wei Liang, Department of Hepatobiliary Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450000, Henan Province, China

Author contributions: Pan MX, Liang ZW, and Cheng Y contributed equally to this work; Gao Y and Pan MX designed the trial; Wang KH collected and analyzed these data; Liu HY, Jiang ZS, Xu XP and Liang ZW performed the research; Liang ZW and Cheng Y revised the paper.

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Correspondence to: Yi Gao, MD, Second Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University, No. 253, Gongye Avenue, Haizhu District, Guangzhou 510282, Guangdong Province, China. gaoyi6146@163.com

Telephone: +86-20-62743207 Fax: +86-20-62783586

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Abstract

AIM: To investigate the learning curve of transumbilical suture-suspension single-incision laparoscopic cholecystectomy (SILC).

METHODS: The clinical data of 180 consecutive transumbilical suture-suspension SILCs performed by a team in our department during the period from August 2009 to March 2011 were retrospectively analyzed. Patients were divided into nine groups according to operation dates, and each group included 20 patients operated on consecutively in each time period. The surgical outcome was assessed by comparing operation time, blood loss during operation, and complications between groups in order to evaluate the improvement in technique.

RESULTS: A total of 180 SILCs were successfully performed by five doctors. The average operation time was 53.58 ± 30.08 min (range: 20.00-160.00 min) and average blood loss was 12.70 ± 11.60 mL (range: 0.00-100.00 mL). None of the patients were converted to laparotomy or multi-port laparoscopic cholecystectomy. There were no major complications such as hemorrhage or biliary system injury during surgery. Eight postoperative complications occurred mainly in the first three groups ($n = 6$), and included ecchymosis around the umbilical incision ($n = 7$) which resolved without special treatment, and one case of delayed bile leakage in group 8, which was treated by ultrasound-guided puncture and drainage. There were no differences in intraoperative blood loss, postoperative complications and length of postoperative hospital stay among the groups. Bonferroni's test showed that the operation time in group 1 was significantly longer than that in the other groups ($F = 7.257$, $P = 0.000$). The majority of patients in each group were discharged within 2 d, with an average postoperative hospital stay of 1.9 ± 1.2 d.

CONCLUSION: Following scientific principles and standard procedures, a team experienced in multi-port laparoscopic cholecystectomy can master the technique of SILC after 20 cases.

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Key words: Single incision laparoscopic surgery; Cholecystectomy; Learning curve; Suture-suspension

Core tip: As a new technology, single-incision laparoscopic cholecystectomy (SILC) is more difficult to perform than multi-port laparoscopic cholecystectomy with higher technical demands. Thus, SILC may have a specific learning curve. The present study retrospectively analyzed the surgical outcomes of transumbilical suture-suspension SILC performed by the same team in our department to investigate the learning curve of this technology, thereby guiding the surgeons to pass the

initial learning period smoothly, safely, and quickly.

Pan MX, Liang ZW, Cheng Y, Jiang ZS, Xu XP, Wang KH, Liu HY, Gao Y. Learning curve of transumbilical suture-suspension single-incision laparoscopic cholecystectomy. *World J Gastroenterol* 2013; 19(29): 4786-4790 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4786.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4786>

INTRODUCTION

Laparoscopic cholecystectomy has become widely accepted and is considered the gold standard for the treatment of benign gallbladder diseases. With continuous development in techniques and devices, laparoscopy has moved in the direction of minimally invasive surgery in order to meet patients' cosmetic requirements, *i.e.*, from a four-port conventional laparoscope to three-port, two-port, and the present transumbilical single-port. As a new technology, single-incision laparoscopic cholecystectomy (SILC) is more difficult to perform than multi-port laparoscopic cholecystectomy with higher technical demands. Thus, SILC may have a specific learning curve. The present study retrospectively analyzed the surgical outcomes of transumbilical suture-suspension SILC performed by the same team in our department to investigate the learning curve of this technology, thereby guiding the surgeons to pass the initial learning period smoothly, safely, and quickly.

MATERIALS AND METHODS

General information

During the period from August 2009 to March 2011, 180 patients received transumbilical SILCs in our department, including 78 males and 102 females, with an average age of 48.7 ± 13.0 years (range: 18.0-79.0 years). All patients had agreed to receive SILC, and for this study the records were retrospectively analyzed. Patients were eligible for SILC if they met the following criteria: (1) clinical diagnosis of symptomatic gallstones, gallbladder polyps, with acute cholecystitis (less than 72 h); and (2) ability to tolerate the procedure without a functional failure in important organs including the lungs, heart, liver and kidneys. Patients were considered ineligible if they had an American Society of Anesthesiologists score > 3 or were unable to tolerate general anesthesia due to other reasons, suspected gallbladder malignancy, and a history of recent upper abdominal surgery.

All operations were completed by the same surgical team. The principal surgeons were both hepatobiliary surgeons with 10 or more years of clinical experience, and had independently performed more than 500 consecutive cases of multiple-port laparoscopic cholecystectomy. The assistants were senior residents who had laparoscopic training on virtual or animal models in the training centre.

The 180 patients were divided evenly into 9 groups according to the time period they received surgery (20 patients in each period). Clinical data collected included patient name, gender, age, primary diagnosis, and previous abdominal surgery history. The difference in operation time, the amount of blood loss during the operation and postoperative complications in all groups were analyzed and compared.

Surgical procedure

The procedure has been described previously^[1]. In summary, a single, curved 1.5-cm incision was made below the umbilicus and a Veress needle was inserted followed by the creation of a pneumoperitoneum which was maintained at 13 mmHg. An 11-mm trocar was inserted as the port for a 30° laparoscope at the left side of the incision. A 5-mm trocar was inserted as a manual port at the right side of the incision. The tissues between the two ports were kept to prevent gas leakage. Two size 7-0 silk threads were placed through the tissues on the bottom of the gallbladder and the muscular layer of the ampulla, respectively, and then pulled out through the abdominal wall in order to fully expose the cystic duct and the gallbladder triangle. After the gallbladder triangle was dissected using an ultrasonic scalpel, the cystic duct was clipped with titanium clips and excised. Next, the whole gallbladder was isolated, dissected, and extracted through the umbilical incision. Careful control of homeostasis and bile leakage was achieved. The abdominal cavity was then rinsed, the pneumoperitoneum was released and the incision was closed.

Statistical analysis

Quantitative data were expressed as mean $\pm$ SD. Comparisons between groups were performed using one-way ANOVA followed by Bonferroni's test, and quantitative data was analyzed by the χ^2 test. SPSS 15.0 software was used for statistical analysis, and a value of $P < 0.05$ was considered statistically significant.

RESULTS

Demographic data

Of the 180 patients enrolled, 78 were men and 102 were women. The average age was 48.7 ± 13.0 years (range: 18.0-79.0 years). The mean body mass index was 23.31 ± 2.83 kg/m² (range: 16.35-29.36 kg/m²). Among these 180 patients, 17 had a history of abdominal surgery, 11 of whom had undergone open procedures, including appendectomy ($n = 5$), cesarean section ($n = 4$), total hysterectomy ($n = 1$) and bilateral tubal ligation ($n = 1$); the other six candidates had undergone laparoscopic appendectomy ($n = 4$) and laparoscopic herniorrhaphy ($n = 2$) (Table 1). The nine groups were similar with respect to sex, age, body mass index and previous history of abdominal surgery (Table 2)

Clinical outcomes

All patients underwent successful SILC, and no patient

Table 1 General data

SILCs (<i>n</i> = 180)		
Gender		
Male		78
Female		102
Age (yr)		48.7 ± 13.0
BMI (kg/m ²)		23.31 ± 2.83
Previous history of abdominal surgery		
Yes	5 appendectomy	4 laparoscopic appendectomy
	4 cesarean sections	2 laparoscopic herniorrhaphy
	1 total hysterectomy	
	1 bilateral tubal ligation	
No		163

SILC: Single-incision laparoscopic cholecystectomy; BMI: Body mass index.

required conversion to standard LC or open surgery. The average operative time was 53.58 ± 23.44 min (range: 20-160 min) and the average blood loss during surgery was 12.7 ± 11.6 mL (range: 0-100 mL). There was no intraoperative abdominal organ damage or complications such as hemorrhage or biliary system injury during surgery. Eight postoperative complications occurred, mainly in the first three groups ($n = 6$), and included ecchymosis around the umbilical incision ($n = 7$) which resolved without special treatment, and one case of delayed bile leakage in group 8, which was treated by ultrasound-guided puncture and drainage. The majority of patients in each group were discharged within 2 d, with an average postoperative hospital stay of 1.9 ± 1.2 d. There were no differences in intraoperative blood loss and postoperative complications among the groups (Table 3).

Bonferroni's test showed that the operation time in group 1 was significantly longer than that in the other groups ($F = 7.257$, $P = 0.000$). No differences were found following comparisons within the other groups ($P \geq 0.05$). The standard deviation of operative time was 30.1 min in group 1, and gradually declined and stabilized from group 2 to group 9 (Figure 1). The blood loss in group 1 was 16.65 ± 9.96 mL, which was greater than that in the other groups, however, the difference was not statistically significant ($F = 2.130$, $P = 0.082$, Table 3).

DISCUSSION

Single-incision laparoscopy is a new technique, which has evolved due to the recent development of minimally invasive surgery. This technique has been applied in hepatobiliary surgery^[1-8], general surgery^[9-12], urological surgery^[13-17], gynecology and obstetrics^[18,19], and other areas. Of these, SILC is the most widely used and sophisticated technique. Some researchers believe that it has the potential to replace multi-port laparoscopic techniques and become the gold standard for cholecystectomy^[20-22]. Compared with multi-port laparoscopy, single-incision laparoscopic surgery has unique features. First, it requires the operator to have a comprehensive knowledge of *in*

vivo micro-anatomy and three-dimensional sensibility in order to compensate for limited surgical fields. Second, it requires skillful techniques and clear procedure understanding to replace the sense of hand touch. Finally, it requires good team work and appropriate surgical devices to compensate for interference between instruments. For these reasons, operators in the early stage of training will inevitably experience a process of exploration and skill acquisition, and this process is the learning curve of single-port laparoscopic cholecystectomy.

A learning curve is usually evaluated by the number of surgeries required to progress from an initial to a skilled state. After completing a certain number of operations, a surgeon's skill will be improved significantly and they will be competent in the surgical procedure and able to manage complications skillfully. At this stage, the learning curve is complete and the learning plateau is reached^[23]. The criteria for evaluating the skillfulness of performing SILC are operative time, blood loss during the operation, and complications including bile leakage and wound infection.

The change in operation time is the most direct and accurate indicator reflecting the learning curve, because it reflects a surgeon's mastery of the technique. Operation time is influenced by four main factors, including operator skill, surgical concepts, the cooperation of the surgical team, and the choice of surgical devices. In order to use operation time as an indicator of skill, we chose a team with experience in conventional laparoscopic surgery and used the same set of surgical devices to complete the surgeries in this study. Therefore, the impact of different teams and surgical devices on operation time were minimized as much as possible.

Single-port laparoscopic cholecystectomy has been incorporated into national standards for the quality control of single disease, and the procedures are standardized. Once patients were recruited into this study, the operation was performed using a standard procedure, and the difference resulting from variation in surgical concepts was excluded. In this study, one-way ANOVA followed by Bonferroni's test were performed for multiple statistical comparisons. The advantage of Bonferroni's test is that it is a simple and conservative method to control type I error. We found that the decrease in operation time was significant from group 1 to group 2, and was relatively stable from group 2 to group 9; thus, the number of cases in group 1 reflects the learning curve. The fluctuation in operation time was greatest in group 1, indicating that in the first 20 cases the surgical technique was not standardized and the stability of technique was poor. As shown in Figure 1, the operation time continuously decreased and the shortest operation time was 20 min, suggesting that after continuous learning and training it is possible to improve the technique.

Patients recruited for SILC in the early period were carefully selected. Even so, blood loss in group 1 was greater than that in the other groups, but without statistical significance. After the first 20 cases, the average blood loss decreased rapidly and gradually stabilized. There

Table 2 Patient characteristics

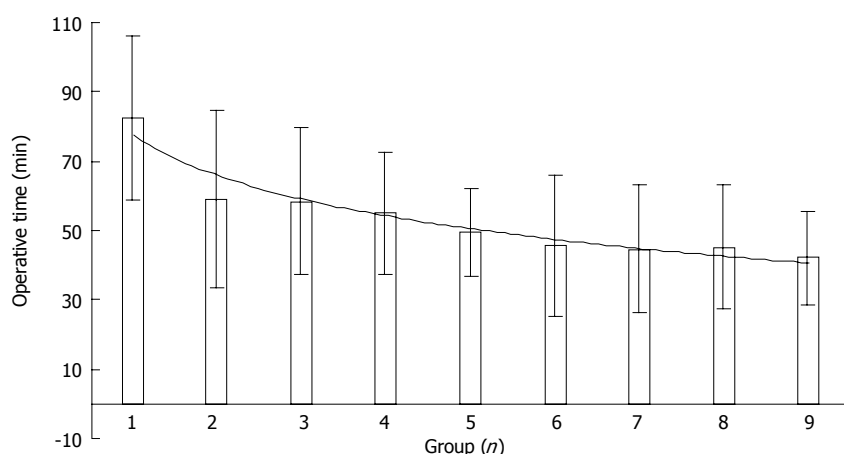
Groups	1	2	3	4	5	6	7	8	9	F	P
Age (yr) ^{1,2}	47.7 ± 10.8	45.1 ± 13.8	50.4 ± 14.1	50.0 ± 11.7	49.4 ± 13.8	52.7 ± 14.5	52.9 ± 12.0	46.9 ± 14.3	43.7 ± 11.2	1.2082	0.297
Gender (M/F) ³	11/9	7/13	9/11	6/14	5/15	11/9	9/11	7/13	13/7	11.343	0.183
Previous history of abdominal surgery ³	0	2	1	2	1	2	3	2	4	6.333	0.610
BMI (kg/m ²) ^{1,2}	23.11 ± 2.86	23.96 ± 3.49	22.31 ± 2.99	23.83 ± 3.28	23.75 ± 2.28	22.96 ± 2.41	24.26 ± 2.76	22.80 ± 2.16	22.85 ± 2.89	1.0752	0.383

¹Values are mean ± SD; ²One-way ANOVA; ³ χ^2 test. BMI: Body mass index; M/F: Male/female.

Table 3 Surgical data

	Overall	Group 1 ⁵	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Surgery time ^{1,4}	53.58 ± 30.08	82.45 ± 23.44 ²	59.00 ± 25.47	58.50 ± 21.16	55.00 ± 17.77	49.50 ± 12.45	45.50 ± 20.22	44.75 ± 18.67	45.25 ± 17.95	42.25 ± 13.52
Blood loss ¹	12.70 ± 11.60	16.65 ± 9.96	12.55 ± 13.45	14.05 ± 14.55	13.70 ± 9.76	12.90 ± 12.01	11.60 ± 12.31	12.70 ± 12.23	11.60 ± 11.10	10.55 ± 9.83
Complications ³	8	3	1	2	0	1	0	0	1	0

¹One-way ANOVA; ²Values are mean ± SD; ³ χ^2 test; ⁴Comparisons between groups were performed using ANOVA followed by Bonferroni's test; ⁵The operation time in group 1 was significantly longer than that in the other groups ($F = 7.257$, $P = 0.000$).

**Figure 1 The changes in operation time.**

were no significant differences between the groups in terms of complications and duration of hospitalization, suggesting that in the early period the operation was carried out very carefully and successfully. In all 180 patients, the outcomes were satisfactory and no patients required conversion to laparotomy or multi-port laparoscopy, and no patients required additional surgery. These results indicate that SILC is safe and feasible when performed by surgeons who are experienced in multi-port laparoscopic cholecystectomy.

The circumstances are different in different medical centers and endoscopy teams, thus, the surgical experience and concepts would also be different. As a result, the learning curves for SILC would be different^[23-26]. Our results showed a rapid decline in operation time in the early period, and a slow stabilizing, declining curve in the late period, demonstrating that the learning curve was approximately 20 cases. That is, surgeons who are experienced in multi-port laparoscopic cholecystectomy are likely to pass the learning curve smoothly and safely after performing 20 cases of SILC.

COMMENTS

Background

The potential benefits of single-incision laparoscopic technology include reduced postoperative pain, improved cosmetic result and earlier return to normal life. Some investigators have predicted that single-incision laparoscopic cholecystectomy (SILC) may become an alternative standard approach for benign gallbladder diseases. However, there is still controversy with regard to its safety and reproducibility as SILC is more difficult to perform than multi-port laparoscopic cholecystectomy with higher technical demands.

Research frontiers

SILC is a rapidly advancing technique in laparoscopic surgery. However, there is limited evidence on the learning curve and practicality of performing this procedure. The present study retrospectively analyzed the surgical outcomes of transumbilical suture-suspension SILC performed by the same team in our department to investigate the learning curve of this technology, thereby guiding the surgeons to pass the initial learning period smoothly, safely, and quickly.

Innovations and breakthroughs

This is a large-scale retrospective study to explore the safety and reproducibility of SILC for the management of benign gallbladder diseases in selected patients.

Applications

The results confirmed that SILC was a safe and effective method for treating benign gallbladder diseases. There was a rapid decline in operation time in the early period, and a slow stabilizing, declining curve in the late period, demon-

strating that the learning curve was approximately 20 cases. That is, surgeons who are experienced in multi-port laparoscopic cholecystectomy are likely to pass the learning curve smoothly and safely after performing 20 cases of SILC.

Terminology

SILC is a minimally invasive surgical procedure in which cholecystectomy is accomplished exclusively through a single 15-25 mm incision in the patient's navel. Unlike the traditional multi-port laparoscopic approach, SILC leaves only a single small scar in the navel.

Peer review

This is an interesting article, which presents a series of SILC procedures. The study does demonstrate that there is learning curve for SILC.

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Stepwise sedation for elderly patients with mild/moderate COPD during upper gastrointestinal endoscopy

Can-Xia Xu, Xiong Chen, Yan Jia, Ding-Hua Xiao, Hui-Fang Zou, Qin Guo, Fen Wang, Xiao-Yan Wang, Shou-Rong Shen, Ling-Ling Tong, Ke Cao, Xiao-Ming Liu

Can-Xia Xu, Xiong Chen, Yan Jia, Ding-Hua Xiao, Hui-Fang Zou, Qin Guo, Fen Wang, Xiao-Yan Wang, Shou-Rong Shen, Xiao-Ming Liu, Department of Gastroenterology, Third Xiangya Hospital of Central South University, Changsha 410013, Hunan Province, China

Ling-Ling Tong, Department of Health Statistics, University of South China, Hengyang 421001, Hunan Province, China

Ke Cao, Department of Oncology, Third Xiangya Hospital of Central South University, Changsha 410013, Hunan Province, China

Author contributions: Xu CX designed the study, performed the endoscopic procedures, analyzed the data, and wrote the manuscript; Chen X, Jia Y, Xiao DH, Zou HF, Guo Q, Wang F, Wang XY and Shen SR performed the endoscopic procedures; Tong LL, Cao K, and Liu XM analyzed the data.

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Correspondence to: Can-Xia Xu, MD, Professor, Department of Gastroenterology, Third Xiangya Hospital of Central South University, 138 Tongzipo Street, Changsha 410013, Hunan Province, China. xucanxia2000@hotmail.com

Telephone: +86-731-88618631 Fax: +86-731-88618012

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[COPD with stepwise sedation (group Cs), and non-COPD with stepwise sedation (group Ns)] or continuous sedation involving continuous administration of propofol combined with midazolam [COPD with continuous sedation (group Cc), and non-COPD with continuous sedation (group Nc)]. Saturation of peripheral oxygen (SpO₂), blood pressure, and pulse rate were monitored, and patient discomfort, adverse events, drugs dosage, and recovery time were recorded.

RESULTS: All endoscopies were completed successfully. The occurrences of hypoxemia in groups Cs, Cc, Ns, and Nc were 4 (9.3%), 12 (27.9%), 3 (7.3%), and 5 (12.2%), respectively. The occurrence of hypoxemia in group Cs was significantly lower than that in group Cc ($P < 0.05$). The average decreases in value of SpO₂, systolic blood pressure, and diastolic blood pressure in group Cs were significantly lower than those in group Cc. Additionally, propofol dosage and overall rate of adverse events in group Cs were lower than those in group Cc. Finally, the recovery time in group Cs was significantly shorter than that in group Cc, and that in group Ns was significantly shorter than that in group Nc ($P < 0.001$).

CONCLUSION: The stepwise sedation method is effective and safer than the continuous sedation method for elderly patients with mild/moderate COPD during upper GI endoscopy.

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Key words: Upper gastrointestinal endoscopy; Adverse events; Sedation; Monitoring; Chronic obstructive pulmonary disease

Core tip: Many patients with chronic obstructive pulmonary disease (COPD) have to undergo upper gastroin-

Abstract

AIM: To investigate stepwise sedation for elderly patients with mild/moderate chronic obstructive pulmonary disease (COPD) during upper gastrointestinal (GI) endoscopy.

METHODS: Eighty-six elderly patients with mild/moderate COPD and 82 elderly patients without COPD scheduled for upper GI endoscopy were randomly assigned to receive one of the following two sedation methods: stepwise sedation involving three-stage administration of propofol combined with midazolam

testinal (GI) endoscopy because of digestive symptoms. A sedation method specially designed for elderly patients with COPD is urgently needed for use in clinical practice. In this study, we designed a new stepwise sedation method. Eighty-six elderly patients with COPD and 82 elderly patients without COPD scheduled for upper GI endoscopy were randomly assigned stepwise sedation or continuous sedation. The results indicate that the stepwise sedation method is effective and safer than the continuous sedation method for elderly patients with mild/moderate COPD during upper GI endoscopy.

Xu CX, Chen X, Jia Y, Xiao DH, Zou HF, Guo Q, Wang F, Wang XY, Shen SR, Tong LL, Cao K, Liu XM. Stepwise sedation for elderly patients with mild/moderate COPD during upper gastrointestinal endoscopy. *World J Gastroenterol* 2013; 19(29): 4791-4798 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4791.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4791>

INTRODUCTION

Upper gastrointestinal (GI) endoscopy is a commonly used interventional examination method for reliable diagnosis of upper GI diseases. The incidence of chronic obstructive pulmonary disease (COPD) in Chinese urban populations over 40 years old is 8.2%^[1]. Due to tobacco smoking, solid-fuel use, and other reasons, an estimated 65 million people will die of COPD in China between 2003 and 2033^[2]. Many patients with COPD have to undergo upper GI endoscopy because of digestive symptoms. Due to inadequate experience with the application of sedation technology during endoscopy in past decades, patients typically underwent routine endoscopic procedures without sedatives, receiving only local pharyngeal lidocaine anesthesia before endoscopy. However, this methodology can lead to various adverse effects, including fear, nausea and vomiting, elevated blood pressure, angina, myocardial infarction, and even death. Moreover, the risk of adverse effects is even greater in elderly patients with cardiopulmonary diseases^[3-6]. These issues give rise to patient reluctance to be examined and delay of diagnosis and treatment of alimentary system diseases.

In recent years endoscopy with sedation has become a popular option for both patients and gastroenterologists. Midazolam and propofol are generally used as sedatives during endoscopic procedures^[7-10]. However, the use of sedatives during endoscopy in elderly patients with COPD remains controversial because of safety concerns^[11]. Compared with younger patients, elderly patients exhibited a significant increase in risk of oxygen saturation below 90% and oxygen saturation decrease more than 5%^[12]. Moreover, propofol is a potent depressant of airway reflexes at hypnotic concentrations^[13]. Elderly patients with COPD usually have cough, phlegm and respiratory insufficiency, and are more likely to experience decreased saturation of peripheral oxygen (SpO₂) because

of the dysfunction of the cough reflex and respiratory track blockage by phlegm during an endoscopic procedure with sedation. These symptoms commonly lead to a higher risk of adverse events during GI endoscopy. Therefore, a sedation method specially designed for elderly patients with COPD is urgently needed in clinical practice. Most episodes of hypoxemia under sedation occur in the five minute interval following medication administration and/or intubation, and less frequent administration of medications or diligent monitoring during this period might decrease hypoxemia^[14].

In this study, we designed a stepwise sedation method that involves three-stage administration of propofol combined with midazolam so that the sedation depth is approached gradually. Through analysis of SpO₂, blood pressure, pulse rate, patient discomfort, adverse events, midazolam dosage, propofol dosage, and recovery time; we compared the efficacy and safety of this new method with the continuous sedation method during upper GI endoscopy in elderly patients with and without COPD.

MATERIALS AND METHODS

Patients

Eighty-six elderly patients with mild/moderate COPD and 82 elderly patients without COPD (> 70 years old) who underwent diagnostic upper GI endoscopic procedures using sedation at the endoscopic unit of Third Xiangya Hospital of Central South University between March 2011 and December 2012 were included in this study. COPD was diagnosed based on the guidelines for the diagnosis and treatment of COPD^[15]. Two hundred sixty-one other patients were excluded because they did not consent to participate ($n = 35$) or because they had other diseases or excluding conditions ($n = 226$). These included hypertension (> 140/90 mmHg), hypotension (< 90/60 mmHg), sick sinus syndrome, neurologic or psychiatric disease, metabolic disease, liver/renal insufficiency, severe cough and sputum, SpO₂ < 90%, COPD class III/IV, American Society of Anesthesiology (ASA) class IV/V, and drug allergy. Thus, our study focused exclusively on mild/moderate COPD patients.

The eligible participants with and without COPD were randomized into the two treatment groups in equal numbers. Computer-generated randomization blocks were utilized. Sealed envelopes containing the treatment protocol were opened in the procedure room after enrollment in the study.

Figure 1 shows the flow of participants through the trial, and Table 1 lists data and clinical characteristics of the study population. The differences in COPD and ASA classification between COPD with stepwise sedation (group Cs) and COPD with continuous sedation (group Cc) were not significant, and ASA classification did not differ significantly between non-COPD with stepwise sedation (group Ns) and non-COPD with continuous sedation (group Nc). The differences in other factors among the four groups were not significant. Gastroenterologists

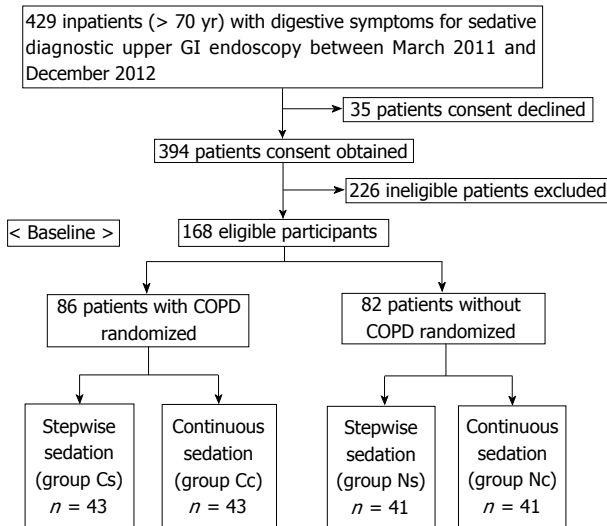


Figure 1 Flow of participants throughout the trial. GI: Gastrointestinal; COPD: Chronic obstructive pulmonary disease; Group Cs: COPD with stepwise sedation; Group Cc: COPD with continuous sedation; Group Ns: Non-COPD with stepwise sedation; Group Nc: Non-COPD with continuous sedation.

Table 1 Demographic data and clinical characteristics of the study population

Parameter	Group			
	Cs (n = 43)	Cc (n = 43)	Ns (n = 41)	Nc (n = 41)
Sex: male/female	35/8	34/9	30/11	30/11
Age: yr, mean $\pm$ SD (range)	74.5 $\pm$ 2.5 (71-80)	74.2 $\pm$ 2.3 (71-80)	77.6 $\pm$ 4.6 (71-88)	76.7 $\pm$ 4.6 (71-90)
Weight: kg, mean $\pm$ SD (range)	56.3 $\pm$ 6.4 (45-70)	56.8 $\pm$ 6.6 (48-71)	56.5 $\pm$ 7.0 (46-73)	57.2 $\pm$ 6.5 (47-71)
Alcohol consumption: Y/N	7/36	5/38	6/35	4/37
Smoking: Y/N	28/15	26/17	24/17	22/19
COPD classification				
I	28	30		
II	15	13		
ASA classification				
I	0	0	35	37
II	11	13	6	4
III	32	30	0	0
Major endoscopic findings				
Chronic superficial gastritis	14	11	13	15
Chronic atrophic gastritis	5	6	4	5
Gastric ulcer	6	5	7	4
Duodenal ulcer	8	7	9	6
Gastric cancer	3	4	2	3
Gastric polyp	2	3	2	4
Esophagus cancer	2	3	1	2
Reflux esophagitis	3	4	3	2

Group Cs: COPD with stepwise sedation; Group Cc: COPD with continuous sedation; Group Ns: Non-COPD with stepwise sedation; Group Nc: Non-COPD with continuous sedation; COPD: Chronic obstructive pulmonary disease; ASA: American Society of Anesthesiology; Y/N: Yes/no.

with specific expertise in GI endoscopy performed endoscopic procedures, and an anesthetist administered the sedatives. Before the procedure, the participants signed an informed consent form. Our institution's ethics committee approved this study.

Sedation procedure

All patients were given lidocaine *via* throat spray before the endoscopic procedure and received nasal oxygen insufflations at a rate of 2 L/min during the endoscopy. Both stepwise and continuous methods of sedation were used.

For the stepwise sedation (groups Cs and Ns), the following step-up sedation method was used: Step 1: Initial administration of an intravenous injection of midazolam (Nhwa Pharmaceutical Group Co. Ltd., Xu-zhou, China; technical concentration of 5 g/L diluted to 0.25 g/L with normal saline) at 0.015 mg/kg (with a maximum dose of 1.0 mg). The Ramsay Sedation Scale score here was 1, and after a 3-5 min interval, a mouth-piece was placed in the patient's mouth for the start of step 2. Step 2: Administration of 15-40 mg propofol (AstraZeneca, Shanghai, China; technical concentration of 10 g/L diluted to 5 g/L with normal saline) *via* intravenous injection until the Ramsay Sedation Scale score was 2-3. The endoscope then was passed through the patient's throat. Step 3: Administration of an additional intravenous injection of propofol at 1.0 mg/kg per minute until the Ramsay Sedation Scale score was 5-6 (when retardation or loss of eyelash reflex was achieved)^[16]. At this point, the endoscopic procedure was carried out.

For continuous sedation (groups Cc and Nc), the following technique was used: The patient was initially given an intravenous injection of midazolam at 0.015 mg/kg (with a maximum dose of 1.0 mg). Next, a mouth-piece was placed in the patient's mouth, and he/she received an intravenous injection of propofol at 1.0 mg/kg per minute that did not stop until the Ramsay Sedation Scale score reached 5-6, when the endoscopic procedure was carried out. If necessary, propofol was administered again to prevent the patient from experiencing discomfort during long-lasting endoscopic procedures.

Patient age, sex, weight, alcohol and cigarette consumption, COPD and ASA classification, major endoscopic findings, SpO₂, blood pressure, pulse rate, adverse events, dosage of midazolam and propofol, and recovery time were recorded. Recovery time was defined as the interval between the moment when propofol injection was stopped and when the patient could open his/her eyes in response to the doctor's call and answer questions.

Monitoring

Degree of pharyngeal malaise during the endoscopic procedure: The same anesthetist and endoscopist, each with more than 5 years of experience, and a registered nurse who had worked for 10 years in an endoscopy room, independently evaluated the degree of pharyngeal

Table 2 Pharyngeal malaise when passing the endoscope through the throat *n* (%)

Group	<i>n</i>	Scores of degree of pharyngeal malaise			
		0	1	2	3
Cs	43	33 (76.7) ^a	7 (16.3)	3 (7.0)	0
Cc	43	40 (93.0)	2 (4.7)	1 (2.3)	0
Ns	41	31 (75.6) ^a	6 (14.6)	4 (9.8)	0
Nc	41	38 (92.7)	2 (4.9)	1 (2.4)	0

Group Cs: Chronic obstructive pulmonary disease (COPD) with stepwise sedation; Group Cc: COPD with continuous sedation; Group Ns: Non-COPD with stepwise sedation; Group Nc: Non-COPD with continuous sedation. ^a*P* < 0.05 between Group Cs *vs* Group Cc and Group Ns *vs* Group Nc.

Table 3 SpO₂, blood pressure, and pulse rate during the endoscopy procedure (mean ± SD)

Group	Time	SpO ₂ (%)	SBP (mmHg)	DBP (mmHg)	P (bpm)
Cs (<i>n</i> = 43)	Before	98.1 ± 1.6	126.0 ± 11.2	80.7 ± 6.7	74.4 ± 13.7
	During	95.8 ± 6.0 ^b	115.6 ± 11.9 ^b	72.8 ± 6.6 ^b	73.8 ± 14.0
	After	98.0 ± 1.6	124.9 ± 11.3 ^b	79.1 ± 6.5 ^b	74.2 ± 14.1
Cc (<i>n</i> = 43)	Before	98.0 ± 1.8	128.5 ± 11.0	80.5 ± 5.6	77.2 ± 14.3
	During	90.7 ± 13.6 ^b	113.5 ± 9.4 ^b	71.0 ± 4.9 ^b	75.0 ± 14.3 ^b
	After	97.3 ± 2.6 ^b	126.3 ± 11.0 ^b	78.5 ± 5.8 ^b	76.6 ± 14.5
Ns (<i>n</i> = 41)	Before	98.2 ± 1.8	130.2 ± 5.0	82.0 ± 4.1	73.7 ± 13.7
	During	96.5 ± 5.0 ^a	120.0 ± 5.2 ^b	74.3 ± 4.4 ^b	73.0 ± 13.7
	After	98.1 ± 1.7	127.9 ± 5.0 ^b	80.0 ± 4.3 ^b	73.4 ± 13.6
Nc (<i>n</i> = 41)	Before	98.2 ± 1.8	127.8 ± 9.3	81.4 ± 2.6	70.4 ± 12.7
	During	95.6 ± 6.7 ^b	117.1 ± 8.7 ^b	73.5 ± 2.9 ^b	69.0 ± 12.3 ^b
	After	98.1 ± 1.7	125.5 ± 8.9 ^b	79.0 ± 2.7 ^b	70.4 ± 12.5

Group Cs: Chronic obstructive pulmonary disease (COPD) with stepwise sedation; Group Cc: COPD with continuous sedation; Group Ns: Non-COPD with stepwise sedation; Group Nc: Non-COPD with continuous sedation; SpO₂: Saturation of peripheral oxygen; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; P: Pulse rate. ^a*P* < 0.05, ^b*P* < 0.01 *vs* before the endoscopy.

malaise. Pharyngeal malaise was scored according to observations of the patient's discomfort and the effect of passing the endoscope through the throat, as follows: obvious nausea and vomiting, difficulty continuing the endoscopic procedure (3 points); nausea and vomiting, able to continue the endoscopic procedure (2 points); slight nausea and vomiting, no effect on the endoscopic procedure (1 point); no nausea or vomiting, easy to finish the endoscopic procedure (0 points).

SpO₂, blood pressure, and pulse rate: All patients were continuously monitored for SpO₂, blood pressure, and pulse rate using a multi-functional monitor. The SpO₂, blood pressure, and pulse rate were recorded before the procedure (1-2 min before the use of sedatives; this value, measured when the patient was resting on his/her side, was used as the base value), during the procedure (to identify the minimum value throughout the procedure), and after the procedure (1-2 min after the endoscopic procedure was completed). After the endoscopy, patients were continuously monitored in the recovery unit until mental ability and walking gait recovered to the normal level (usu-

ally within 30-60 min). Occurrences of adverse events were observed.

Statistical analysis

Data are presented as mean ± SD. Statistical analyses were performed using SPSS software (version 17.0 for Windows; SPSS Inc., Chicago, IL, United States). The measurement data were compared using the two-sample *t*-test for normally distributed data and the Mann-Whitney test for non-normally distributed data. The enumeration data were expressed as *n* (%) and compared using the χ^2 test. A two sided *P*-value < 0.05 was considered statistically significant.

RESULTS

Degree of pharyngeal malaise during the endoscopic procedure

All endoscopies were completed successfully. Degrees of pharyngeal malaise evaluated by the anesthetist, endoscopist, and nurse did not differ significantly. The degree of pharyngeal malaise when the endoscope was passing through the throat in group Cs was greater than that in group Cc, and that in group Ns also was greater than group Nc (*P* < 0.05) (Table 2). However, the discomfort disappeared after the endoscope passed through the throat and propofol was administered again. All patients had no pharyngeal malaise and no memory of this discomfort after endoscopy.

SpO₂, blood pressure, and pulse rate

The average SpO₂, systolic blood pressure (SBP), and diastolic blood pressure (DBP) in all four groups decreased during the endoscopic procedure, but the blood pressure in almost all patients remained within the normal range. Only one patient in group Cc exhibited hypotension, but it was transient, did not require any treatment, and returned to normal rapidly after the endoscopic procedure. The pulse rate changed significantly during the procedure in group Cc and group Nc (Table 3).

Adverse events

Table 4 lists the adverse events in the four treatment groups. The overall rate of adverse events in group Cs was lower than that in group Cc (*P* < 0.01), but the difference between group Ns and group Nc was not statistically significant.

Hypoxemia

Twenty-four patients (14.3%) in all four groups exhibited hypoxemia. The occurrence of hypoxemia in group Cs was significantly lower than that in group Cc (9.3% and 27.9%, respectively, *P* < 0.05). The average decrease in value of SpO₂ during the procedure in group Cs was significantly lower than that in group Cc (*P* < 0.05). For 22 patients with slight hypoxemia (SpO₂ ≥ 60%), SpO₂ quickly returned to normal after holding the mandible with two hands, patting him/her on the back, and increasing oxygen flow through the nasal catheter. For

Table 4 Adverse events in the four treatment groups *n* (%)

Adverse events	Group			
	Cs (<i>n</i> = 43)	Cc (<i>n</i> = 43)	Ns (<i>n</i> = 41)	Nc (<i>n</i> = 41)
Hypoxemia (SpO ₂ < 90% for ≥ 15 s)	4 (9.3) ^a	12 (27.9)	3 (7.3)	5 (12.2)
SpO ₂ : 89%-80%	2 (4.7)	5 (11.2)	2 (4.9)	3 (7.3)
SpO ₂ : 79%-60%	2 (4.7)	5 (11.2)	1 (2.4)	2 (4.9)
SpO ₂ : 59%-40%	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
SpO ₂ < 40%	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
Average decrease in value of SpO ₂	2.3 ± 5.4 ^a	7.3 ± 12.6	1.7 ± 4.3	2.6 ± 5.8
Hypotension (SBP < 90 mmHg or DBP < 60 mmHg)	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
Extent of SBP decrease				
≤ 10	28 (65.1)	17 (39.5)	34 (82.9)	28 (68.3)
11-20	15 (34.9) ^a	26 (60.5)	7 (17.1)	13 (31.7)
> 20	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Average decrease in value of SBP	10.4 ± 3.3 ^b	15.1 ± 6.8	10.2 ± 3.3	10.6 ± 4.5
Extent of DBP decrease				
≤ 10	40 (93.0)	34 (79.1)	37 (90.2)	38 (92.7)
11-20	3 (7.0)	9 (20.9)	4 (9.8)	3 (7.3)
> 20	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Average decrease in value of DBP	7.9 ± 2.2 ^b	9.5 ± 1.8	7.6 ± 2.6	8.0 ± 1.7
Tachycardia (> 100 bpm)	3 (7.0)	2 (4.7)	2 (4.9)	1 (2.4)
Bradycardia (< 60 bpm)	3 (7.0)	6 (14.0)	3 (7.3)	4 (9.8)
Decrease of pulse rate	33 (76.7)	36 (83.7)	29 (70.7)	28 (68.3)
Increase of pulse rate	8 (18.6)	6 (14.0)	8 (19.5)	8 (19.5)
No change of pulse rate	2 (4.7)	1 (2.3)	4 (9.8)	5 (12.2)
Average change in value of pulse rate	0.6 ± 2.8 ^a	2.2 ± 2.8	0.7 ± 2.4	1.4 ± 2.5
Arrhythmias	2 (4.7)	1 (2.3)	1 (2.4)	0 (0.0)
Other adverse events	3 (7.0)	5 (11.6)	2 (4.9)	3 (7.3)
Extrapyramidal reactions	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
Somnolence	1 (2.3)	2 (4.7)	1 (2.4)	1 (2.4)
Dizziness	2 (4.7)	2 (4.7)	1 (2.4)	2 (4.9)
Overall rate of adverse events	15 (34.9) ^b	27 (62.8)	11 (26.8)	13 (31.7)

Group Cs: Chronic obstructive pulmonary disease (COPD) with stepwise sedation; Group Cc: COPD with continuous sedation; Group Ns: Non-COPD with stepwise sedation; Group Nc: Non-COPD with continuous sedation; SpO₂: Saturation of peripheral oxygen; SBP: Systolic blood pressure; DBP: Diastolic blood pressure. Overall rate of adverse events included hypoxemia, hypotension, tachycardia, bradycardia, arrhythmias and other adverse events. ^a*P* < 0.05, ^b*P* < 0.01 *vs* Group Cc.

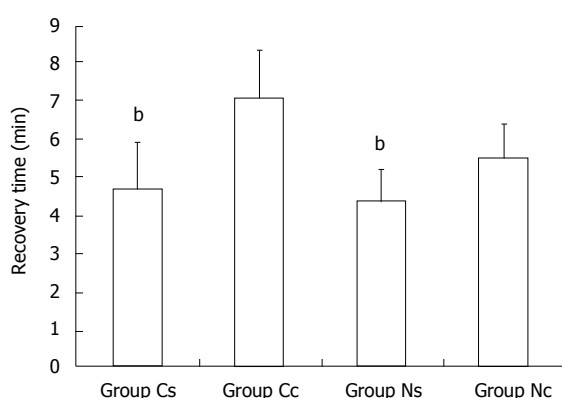


Figure 2 Recovery times of patients in the four groups. Group Cs: Chronic obstructive pulmonary disease (COPD) with stepwise sedation; Group Cc: COPD with continuous sedation; Group Ns: Non-COPD with stepwise sedation; Group Nc: Non-COPD with continuous sedation. ^a*P* < 0.01 between Group Cs *vs* Group Cc and Group Ns *vs* Group Nc.

these patients, the endoscopic procedure was continued and completed. For two patients with serious hypoxemia (SpO₂ < 60%), SpO₂ returned to normal after removing the endoscope, holding the mandible with two hands, pressing the chest, sucking out sputum, giving oxygen by mask, and intravenously injecting the benzodiazepine an-

tagonist flumazenil (0.5 mg). The endoscopic procedure in these patients was restarted and completed after awakening, and with no obvious signs of discomfort during the endoscopic procedure.

Hypotension

Only one patient in group Cc had hypotension (88/60 mmHg). The average decreases in values of SBP and DBP in group Cs were significantly lower than those in group Cc (*P* < 0.001 for SBP and *P* < 0.005 for DBP).

Change of pulse rates

The pulse rates of patients in the four groups showed a slight increase (1-6 bpm), no change, or a slight decrease (1-7 bpm) during the procedure. The average change in value of pulse rate in group Cs was lower than that in group Cc (*P* < 0.05).

Other adverse events

During the injection with propofol, one patient in group Cc exhibited extrapyramidal signs (abnormal involuntary movement of limbs and opisthotonus), which disappeared after 30-60 s. Incidence of somnolence and dizziness did not differ significantly among the four groups,

and disappeared within 10-50 min after the endoscopic procedure ended.

Dosage of midazolam and propofol

Midazolam dosages in groups Cs, Cc, Ns and Nc were 0.6-1.0 mg (0.90 ± 0.12 mg, 0.90 ± 0.11 mg, 0.90 ± 0.11 mg, and 0.92 ± 0.10 mg, respectively), and differences in dosages were not statistically significant among the four groups. Propofol dosages in groups Cs, Cc, Ns and Nc were 53.3 ± 9.4 mg (30-80 mg), 58.9 ± 11.6 mg (40-80 mg), 64.8 ± 10.1 mg (40-80 mg), and 70.7 ± 9.6 mg (50-88 mg), respectively. Propofol dosage in group Cs was significantly lower than that in group Cc ($P < 0.05$), and dosage in group Ns also was significantly lower than group Nc ($P < 0.01$).

Recovery time

The recovery times in groups Cs, Cc, Ns and Nc were 4.7 ± 1.2 min (2.5-8.0 min), 7.1 ± 1.2 min (3.5-10.0 min), 4.4 ± 0.8 min (2.5-6.0 min), and 5.5 ± 0.9 min (3.0-7.0 min), respectively. The recovery time in group Cs was significantly shorter than that in group Cc, and that in group Ns also was significantly shorter than group Nc ($P < 0.001$) (Figure 2). All patients were fully conscious and able to answer questions accurately within 10 min after endoscopy. They were able to walk normally when they left the endoscopic unit 30-60 min after endoscopy. The majority of patients reported no discomfort; only 12 patients had slight dizziness and somnolence, which disappeared within 10-50 min after the endoscopic procedure ended.

DISCUSSION

Both the number and complexity of endoscopic procedures have increased considerably due to the wide availability and application of sedation, but the best methods for sedation during endoscopy are still being debated^[17]. Providing an adequate regimen of sedation is an art, as it influences the quality of the examination and patient and physician satisfaction with the sedation process^[18]. Conscious sedation, a type of sedation in which the individual can respond to verbal directions, is used for GI endoscopy. Even with this sedation, patients can experience discomforts such as nausea and vomiting, which in some cases precludes completing the endoscopic procedure. Deep sedation may be preferred for procedures in which it is important for patients to remain immobile^[19]. However, selection of the most suitable drug or combination of drugs for use and the safety of the sedation method for special patient groups, such as elderly individuals and patients with co-morbidities, are important issues that need to be resolved^[11,20-22].

Martínez *et al.*^[23] reported that continuous propofol sedation during endoscopic procedures is as safe in elderly patients > 80 years as it is in younger patients. Our results also showed that differences in hypoxemia, hypotension, and all adverse events in non-COPD patients with continuous sedation were not statistically dif-

ferent from those in non-COPD patients with stepwise sedation. We have used continuous propofol combined with midazolam for deep sedation during upper GI endoscopy since 1999^[24], but sedation for patients with respiratory diseases accompanied by cough, phlegm, and snoring has been limited, as these patients are prone to experience respiratory track blockage by phlegm and lingual root fall back under sedation, resulting in decreased SpO₂ and respiratory inhibition. Most episodes of hypoxemia during sedation for gastrointestinal endoscopy occur within 5 min of the administration of medication and/or intubation, and less frequent administration of medications or diligent monitoring during this period might decrease hypoxemia^[14]. For this class of patients, we proposed using a new sedation method that would allow patients to gradually reach the appropriate sedation depth *via* administration of propofol combined with midazolam in three stages. When the Ramsay Sedation scale was 2-3, the endoscope was passed through the patient's throat. At this moment, the patients were conscious, and the procedure usually did not lead to cough reflex disappearance, lingual root fall back, or respiratory track blockage by phlegm. Therefore, this method reduced the decline of SpO₂ during the endoscopy.

In this study, the patients who underwent the stepwise sedation method also exhibited a smaller decrease in SBP, DBP, and pulse rate than patients who received continuous sedation. The new method, moreover, reduced the propofol dosage and the overall rate of adverse events. Elderly patients with COPD have much greater risk of experiencing cardiopulmonary abnormality during upper GI endoscopic procedures than elderly patients without COPD, thus use of the stepwise sedation method is safer for these patients. This method will contribute to the wider use of upper GI endoscopy in diagnosis and treatment of alimentary system diseases.

Midazolam and propofol are commonly used in sedative endoscopic procedures at doses of 0.05-0.1 mg/kg and 1-3 mg/kg, respectively. In our study, propofol combined with midazolam was used, and the midazolam dosage was decreased to less than 0.015 mg/kg (the range was 0.6-1.0 mg). Moreover, the required propofol dosage in the stepwise sedation was significantly lower than that in the continuous sedation (53.3 ± 9.4 mg and 58.9 ± 11.6 mg, respectively, for elderly patients with COPD). In addition, the concentration of midazolam was diluted 20 times and that of propofol was diluted two times, which resulted in reduced drug dosage, consistent with previous reports^[25]. Drug dosage (which allows for control of degree of sedation) is one of the key factors for a successful upper GI endoscopy procedure. Generally, drug dosage is proportional to its adverse effects. In our study, the dosage of sedative was lower and the incidence of hypoxemia and the extent of decreased blood pressure were lower than those reported in the literature^[26]. The overall rate of adverse events when the stepwise sedation was used for elderly patients with mild/moderate COPD was significantly lower than that when the continuous sedation was

used. The recovery time also was shorter for the stepwise sedation than the continuous sedation. There was no mortality in our study. Agostoni *et al*^[27] reported that the endoscopic procedure resulted in 3 deaths in a total of 17999 patients (mortality rate = 0.017%).

In conclusion, the findings of this study showed that the stepwise sedation method reduced the propofol dosage and the extent of the drop in SpO₂ and blood pressure, and also decreased the incidence of hypoxemia and the overall rate of adverse events. Thus, this method was shown to be safer than the continuous sedation method in elderly patients with mild/moderate COPD during upper GI endoscopy.

COMMENTS

Background

The incidence of chronic obstructive pulmonary disease (COPD) in Chinese urban populations over 40 years old is 8.2%. Many patients with COPD have to undergo upper gastrointestinal (GI) endoscopy because of digestive symptoms. However, elderly patients with COPD usually have cough, phlegm, and respiratory insufficiency, and are more likely to experience decreased saturation of peripheral oxygen (SpO₂) because of dysfunction of the cough reflex and respiratory track blockage by phlegm during an endoscopic procedure with sedation. This oftentimes leads to a higher risk of adverse events during routine GI endoscopy in these patients. Therefore, a sedation method specially designed for elderly patients with COPD is urgently needed for use in clinical practice.

Research frontiers

In recent years endoscopy with sedation has become a popular option for both patients and gastroenterologists. Midazolam and propofol are generally used as sedatives during endoscopic procedures. However, propofol is a potent depressant of airway reflexes at hypnotic concentrations. It is critical to properly determine how to improve the safety of GI endoscopy by lowering the doses of sedatives.

Innovations and breakthroughs

Due to inadequate experience with the application of sedation technology during endoscopy in past decades, patients typically underwent routine endoscopic procedures without sedatives. This methodology can lead to various adverse effects, including fear, nausea and vomiting, elevated blood pressure, angina, myocardial infarction, and even death. These issues give rise to patient reluctance to be examined and delay of diagnosis and treatment of alimentary system diseases. We took the lead in using sedatives during endoscopic procedures in China in 1998. In the current study, we designed a new stepwise sedation method that involves three-stage administration of propofol combined with midazolam so that sedation depth is approached gradually. The results indicate that the stepwise sedation method is effective and safer than the continuous sedation method for elderly patients with mild/moderate COPD during upper GI endoscopy.

Applications

This stepwise sedation method can reduce drug dosage and the overall rate of adverse events in elderly patients with mild/moderate COPD during upper GI endoscopy with sedation. This will contribute to the wider use of upper GI endoscopy in diagnosis and treatment of alimentary system diseases.

Peer review

This is an interesting study and deserves attention, as the results are relevant to specific patients with COPD who need to undergo endoscopy examination. This manuscript presents new concepts and ideas. The authors analyzed the efficacy and safety of stepwise sedation for elderly patients with mild/moderate COPD during GI endoscopy. The results suggest that stepwise sedation is an effective and safe sedation method that can be used for elderly patients with mild/moderate COPD.

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Meta-analysis of stapled hemorrhoidopexy vs LigaSure hemorrhoidectomy

Jun Yang, Pei-Jing Cui, Hua-Zhong Han, Da-Nian Tong

Jun Yang, Hua-Zhong Han, Da-Nian Tong, Department of Surgery, Sixth People's Hospital, Shanghai Jiao Tong University, Shanghai 200233, China

Pei-Jing Cui, Department of Geriatrics, Ruijin Hospital, Shanghai Jiao Tong University, Shanghai 200233, China

Author contributions: Yang J and Cui PJ contributed equally to this work; Tong DN designed research; Yang J and Cui PJ performed the data search and meta-analysis; Han HZ and Tong DN wrote the paper.

Correspondence to: Da-Nian Tong, Chief Physician, Department of Surgery, Sixth People's Hospital, Shanghai Jiao Tong University, No. 600 Yishan Road. Shanghai 200233, China. tongdanian@126.com

Telephone: +86-21-64369181 Fax: +86-21-64368920

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Abstract

AIM: To compare outcome of stapled hemorrhoidopexy (SH) vs LigaSure hemorrhoidectomy (LH) by a meta-analysis of available randomized controlled trials (RCTs).

METHODS: Databases, including PubMed, EMBASE, the Cochrane Library, and the Science Citation Index updated to December 2012, were searched. The main outcomes measured were operating time, early postoperative pain, postoperative urinary retention and bleeding, wound problems, gas or fecal incontinence, anal stenosis, length of hospital stay, residual skin tags, prolapse, and recurrence. The meta-analysis was performed using the free software Review Manager. Differences observed between the two groups were expressed as the odds ratio (OR) with 95%CI. A fixed-effects model was used to pool data when statistical heterogeneity was not present. If statistical heterogeneity was present ($P < 0.05$), a random-effects model was used.

RESULTS: The initial search identified 10 publica-

tions. After screening, five RCTs published as full articles were included in this meta-analysis. Among the five studies, all described a comparison of the patient baseline characteristics and showed that there was no statistically significant difference between the two groups. Although most of the analyzed outcomes were similar between the two operative techniques, the operating time for SH was significantly longer than for LH ($P < 0.00001$; OR= -6.39, 95%CI: -7.68 - -5.10). The incidence of residual skin tags and prolapse was significantly lower in the LH group than in the SH group [2/111 (1.8%) vs 16/105 (15.2%); $P = 0.0004$; OR= 0.17, 95%CI: 0.06-0.45). The incidence of recurrence after the procedures was significantly lower in the LH group than in the SH group [2/173 (1.2%) vs 13/174 (7.5%); $P = 0.003$; OR= 0.21, 95%CI: 0.07-0.59].

CONCLUSION: Both SH and LH are probably equally valuable techniques in modern hemorrhoid surgery. However, LigaSure might have slightly favorable immediate postoperative results and technical advantages.

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Key words: Stapled hemorrhoidopexy; Ligasure hemorrhoidectomy; Hemorrhoids; Meta-analysis

Core tip: Stapled hemorrhoidopexy (SH) and Ligasure hemorrhoidectomy are probably equally valuable techniques in modern hemorrhoid surgery. However, appropriate surgical techniques are important in SH, especially the placement of the purse-string suture. Its misplacement may cause operative and postoperative complications.

Yang J, Cui PJ, Han HZ, Tong DN. Meta-analysis of stapled hemorrhoidopexy vs LigaSure hemorrhoidectomy. *World J Gastroenterol* 2013; 19(29): 4799-4807 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4799.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4799>

INTRODUCTION

Around 5% of the general population has hemorrhoidal disease to some extent, especially those aged > 40 years^[1,2]. There is a vast number of available therapeutic methods, and hemorrhoidectomy is well established as the most effective and definitive treatment for grades 3 and 4 symptomatic hemorrhoidal disease^[3]. Two well-established methods of hemorrhoidectomy, the open (Milligan-Morgan)^[4] and closed (Ferguson)^[5] techniques are especially popular. However, despite the relatively minor surgical trauma of these two methods, the intra-operative pain and protracted postoperative course are major concerns^[6]. Thus, continuing efforts have been made to develop new techniques and modifications that promise a less painful course and faster recovery. Stapled hemorrhoidopexy [SH, also known as procedure for prolapse and hemorrhoids (PPH)] was introduced by Longo in 1998, and uses a specially designed circular stapling instrument to excise a ring of redundant rectal mucosa or expanded internal hemorrhoids^[7]. Although some SH-related complications have been reported^[8], its advantages, such as shorter operating time, less postoperative pain, and a quicker return to normal activity have been confirmed by several controlled studies^[9-11]. Another new method, LigaSure hemorrhoidectomy (LH), uses the LigaSure vessel sealing system, which consists of a bipolar electrothermal hemostatic device that allows complete coagulation of vessels up to 7 mm in diameter with minimal surrounding thermal spread and limited tissue charring. The advantages of this method include simple and easy learning, excellent hemostatic control, minimal tissue trauma, lower postoperative pain, and shorter wound healing time^[12-14].

Although meta-analysis of clinical trials has shown that SH and LH have some advantages over conventional hemorrhoidectomy^[15], there is still a lack of evidence about the operative and postoperative outcomes of SH and LH. Therefore, we performed a meta-analysis of randomized controlled trials (RCTs) that compared the efficiency of SH and LH in treating hemorrhoidal disease.

MATERIALS AND METHODS

Literature search

Electronic databases, including PubMed (1966 to December 2012), EMBASE (1980 to December 2012), the Cochrane library (Issue 12, December 2012) and Science Citation Index (1975 to December 2012), were searched. Literature reference lists were hand-searched for the same time period. The search terms used were “Stapled hemorrhoidopexy or PPH and LigaSure hemorrhoidectomy”.

Study selection

The initial inclusion criteria were as follows: (1) all originally published RCTs; (2) the treatment group underwent SH for hemorrhoidal disease; and (3) a parallel control group underwent LH for hemorrhoidal disease. Studies that met the initial inclusion criteria were then further

Table 1 Quality analysis of included trials

Ref.	Randomization method	Allocation concealment	Blinding	Withdraws	Jadad score
Arslani <i>et al</i> ^[11]	Not mentioned	Adequate	No	Described	4
Basdanis <i>et al</i> ^[18]	Not mentioned	Adequate	No	Not mentioned	3
Chen <i>et al</i> ^[19]	Not mentioned	Adequate	No	Not mentioned	3
Kraemer <i>et al</i> ^[20]	Computer-generated	Adequate	No	Described	5
Sakr <i>et al</i> ^[21]	Computer-generated	Adequate	Single-blind	Described	5

examined. Those with duplicate publications, unbalanced matching procedures or incomplete data were excluded, in addition to abstracts without accompanying full texts.

Data extraction

Data were extracted independently by two reviewers (Yang J and Cui PJ) according to the prescribed selection criteria. Any disagreements were resolved by discussion between the two reviewers. The following data were extracted: baseline trial data (*e.g.*, sample size, mean age, gender, study protocol, grade of hemorrhoids, and follow-up time); operative and postoperative outcomes (operating time, early postoperative pain, postoperative urinary retention and bleeding, wound problems, gas or fecal incontinence, anal stenosis, length of hospital stay, residual skin tags, prolapse, and recurrence). When necessary, the corresponding authors were contacted to obtain supplementary information.

Study quality

The quality of the included trials was assessed using the Jadad composite scale^[16] in addition to a description of an adequate method for allocation concealment^[17]. Study quality was assessed independently by two authors (Yang J and Cui PJ), and any discrepancies in interpretation were resolved by consensus (Table 1).

Statistical analysis

The meta-analysis was performed using the free software Review Manager (Version 4.2.10, Cochrane Collaboration, Oxford, United Kingdom). Differences observed between the two groups were expressed as the OR with the 95%CI. A fixed-effects model was used to pool data when statistical heterogeneity was not present. If statistical heterogeneity was present ($P < 0.05$), a random-effects model was used.

RESULTS

The initial search identified 10 publications (Figure 1). After screening, seven RCTs were identified. Consequently, two trials were excluded from the pooled meta-analysis. We compared the conventional Ferguson technique with SH and LH and the other study was a duplicate publi-

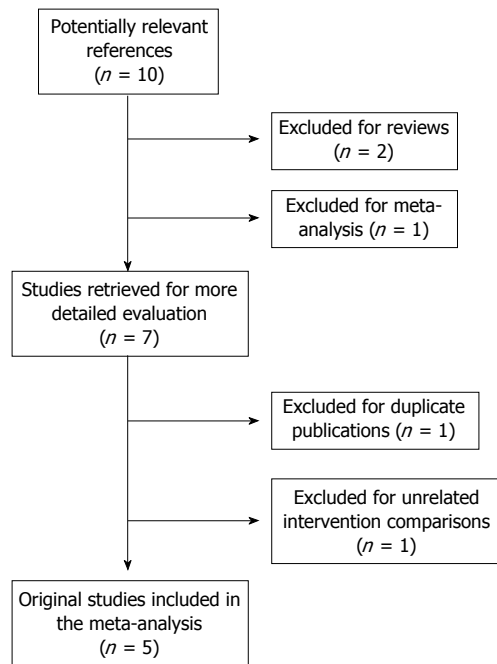


Figure 1 Search protocol for the meta-analysis.

cation. Five RCTs^[1,18-21] published as full articles were included in this meta-analysis. All five studies described a comparison of the patient baseline characteristics and showed that there was no statistically significant difference between the two groups. The principal characteristics of the included studies are shown in Tables 2 and 3. The outcomes were measured as follows.

Operating time

Four trials reported the operating time during hemorrhoidectomy^[18-21]. However, two of them only reported the average operating time^[18,20]. The combined data showed that the operating time of SH was significantly longer than that of LH ($P < 0.00001$; OR = -6.39, 95%CI: -7.68 - -5.10) (Figure 2A).

Early postoperative pain

All five trials reported early postoperative pain at varied time points after hemorrhoidectomy^[1,18-21] with a Visual Analog scale (VAS) score (0 indicating no pain and 10 severe pain). Two trials reported average VAS scores^[1,18] and only one showed the trend in postoperative VAS scores^[20]. Combined data from the other two trials showed that there was no difference between LH and SH ($P = 0.23$; OR = 1.24, 95%CI: -0.78 - -3.26) (Figure 2B).

Postoperative urinary retention

Four trials reported urinary retention^[1,18,20,21] after the procedure and there was no significant difference between the LH and SH groups [11/156 (7.1%) *vs* 13/155 (8.4%); $P = 0.74$; OR = 0.87, 95%CI: 0.37-2.01] (Figure 2C).

Postoperative bleeding

All five trials reported postoperative bleeding^[1,18-21]. There

was no significant difference between the LH and SH groups [5/198 (2.5%) *vs* 12/199 (6%); $P = 0.08$; OR = 0.42, 95%CI: 0.16-1.11] (Figure 2D).

Wound problems

Four trials reported procedure-related wound problems, including irritation, itching and moisture^[18-21]. There was no significant difference between the LH and SH groups [46/146 (31.5%) *vs* 12/153 (7.8%); $P = 0.3$; OR = 3.49, 95%CI: 0.33-37.32] (Figure 2E).

Postoperative gas or fecal incontinence

Four trials reported the incidence of postoperative gas or fecal incontinence^[1,18,20,21]. There was no significant difference between the LH and SH groups [5/156 (3.2%) *vs* 7/155 (4.5%); $P = 0.55$; OR = 0.70, 95%CI: 0.22-2.24] (Figure 2F).

Postoperative anal stenosis

Three trials reported postoperative anal stenosis^[1,20,21]. There was no significant difference between the LH and SH groups [3/111 (2.7%) *vs* 4/105 (3.8%); $P = 0.65$; OR = 0.71, 95%CI: 0.16-3.17] (Figure 2G).

Hospitalization

Four trials reported the length of hospital stay after hemorrhoidectomy^[18-21]. However, two of them only reported the average time^[18,20]. Combined data from the other two trials showed that there was no difference between LH and SH ($P = 0.44$; OR = 0.82, 95%CI: -1.27-2.91) (Figure 2H).

Residual skin tags and prolapse

Three trials reported residual skin tags and prolapse^[1,20,21]. The data showed that the incidence of residual skin tags and prolapse was significantly lower in the LH group than in the SH group [2/111 (1.8%) *vs* 16/105 (15.2%); $P = 0.0004$; OR = 0.17, 95%CI: 0.06-0.45] (Figure 2I).

Recurrence

Four trials reported the incidence of recurrence after the procedures^[1,18,19,21]. The data showed that the incidence of recurrence was significantly lower in the LH group than in the SH group [2/173 (1.2%) *vs* 13/174 (7.5%); $P = 0.003$; OR = 0.21, 95%CI: 0.07-0.59] (Figure 2J).

DISCUSSION

Hemorrhoid is one of the most common anorectal disorders^[2]. Although accepted as the gold standard for surgical treatment of hemorrhoids, conventional hemorrhoidectomy has some unavoidable drawbacks. Two recent techniques, SH and LH, provide some advantages over conventional hemorrhoidectomy. However, there is still a lack of evidence focusing on outcomes of SH and LH.

Our meta-analysis showed that LH took significantly less time to complete compared with SH. For SH, a

Table 2 Baseline characteristics of included trials in the meta-analysis

Ref.	Year	Technique (n)	Study protocol	Mean age (yr)	Sex (M/F)	Grade of hemorrhoids	Follow-up time (mo)
Arsalani <i>et al</i> ^[11]	2012	SH (46)	RUT	52 (17-72)	21/25	3	24
Basdanis <i>et al</i> ^[18]	2005	LH (52)	RUT	50 (18-78)	23/29	3 and 4	6-clinical, 18 (12-24)- telephone
		SH (50)		46 (25-72)	29/21		
Chen <i>et al</i> ^[19]	2007	LH (45)	RUT	44 (22-69)	25/20	3	6
		SH (44)		25-81 (48)	26/18		
Kraemer <i>et al</i> ^[20]	2005	LH (42)	RUT	23-85 (46)	24/18	3 and 4	1.5
		SH (25)		58 (40-71)	14/11		
Sakr <i>et al</i> ^[21]	2010	LH (25)	RBT	48 (28-82)	13/12	3 and 4	18
		SH (34)		43.7 ± 4.66 (29-56)	21/13		
		LH (34)		39.3 ± 4.68 (33-52)	19/15		

RUT: Randomized unblinded trial; RBT: Randomized blinded trial; SH: Stapled hemorrhoidopexy; LH: LigaSure hemorrhoidectomy.

special anal dilator was used to set an interrupted purse-string suture above the dentate line. Then the suture was tightened around the anvil of the circular stapler. In some patients with significant prolapse of the anal mucosa, two circular interrupted sutures were used. After removal of the stapler, interrupted stitches were usually inserted to control bleeding points. With regard to LH, the procedure was more convenient. The LigaSure instrument was used to grasp the base of the hemorrhoid and activated. After coagulation, the hemorrhoid skin was excised with scissors. The reduced operating time was related to better hemostatic control and lack of any need to ligate the pedicles. Our meta-analysis was in accordance with the results of a study showing that LH was comparatively simple and easy to learn^[20]. However, the median value and standard deviation (SD) were reported only in two studies, so this variable should be investigated in further studies.

Another significant difference between the SH and LH groups in our meta-analysis was a higher frequency of postoperative residual skin tags, prolapse and recurrence with SH. This might have been because SH does not excise the hemorrhoids but rather a circumferential column of mucosa and submucosa 2-3 cm above the dentate line and then staples the defect. Besides, it does not deal with external hemorrhoids or associated anal canal problems^[22-24]. However, patients with the third or fourth degree hemorrhoids usually present with large unequally sized prolapsing piles or circumferential hemorrhoids. Chen *et al*^[25] proposed one modified method with one to four additional traction sutures placed at sites about 1 cm below the level of the purse-string suture for those prominent hemorrhoidal positions. This helped to incorporate more distal components of internal hemorrhoids into the “stapler housing” and facilitated further resection. It was also able to pull the external components or skin tags into the anal canal and made the anal surface smoother. An alternative is to remove the residual prolapsing hemorrhoidal tissue or skin tags during the operation or at the postoperative stage.

Long-term risk of recurrence, which is usually defined as recurring symptoms or new prolapse (but not residual prolapse or skin tags)^[11], is the main concern of patients and surgeons. Some studies found that the resid-

ual prolapsed piles could cause recurrent symptoms^[26,27], so it is understandable that recurrence was higher in the SH group. Our meta-analysis was in accordance with the findings of several studies that reported a high recurrence rate of 10%-53%^[11,28,29]. SH is therefore considered by some authors to be unsuitable for grade 4 hemorrhoids^[22,29]. On the contrary, LH is more appropriate for treating anatomical deformities such as skin tags and prolapse. Using LH, concomitant external hemorrhoid components and skin tags can be addressed, ensuring complete removal of the hemorrhoid tissues^[30]. When severe external piles are dominant or large skin tags accompany hemorrhoid prolapse, LH will be a good choice^[12-14]. Considering that the surgical principle in LH is more similar to that of conventional hemorrhoidectomy, it would be expected that LH would have lower recurrence rates^[30]. However, the follow-up time did not exceed two years in our included trials, therefore, further studies with longer follow-up are needed.

One study showed that SH caused severe postoperative pain^[31]. However, the results were challenged by several other studies^[26,32,33]. To the best of our knowledge, the rectal wall is innervated by the sympathetic and parasympathetic nerves, thus, excising the rectal mucosa should be painless. So, it is inexplicable why pain is a common immediate complication of SH. Pain is usually caused by anal dilatation, which leads to internal sphincter fragmentation in some patients^[34], and the inclusion of smooth muscle in the doughnut^[31]. It is conceivable that SH is more technically demanding and operator dependent. If the purse-string suture is not at an inadequate level or depth, serious postoperative pain may be avoided^[28]. VAS scores in the SH group were always lower in patients with no fibers included in the excised piles and doughnuts^[18]. Considering the surgical similarity between LH and conventional hemorrhoidectomy, it would be expected that patients receiving LH would present with greater postoperative pain compared with SH, as is found with conventional hemorrhoidectomy. However in our meta-analysis, there was no difference between LH and SH regarding average VAS scores. The low level of postoperative pain with LH may result from the fact that LH has no need for anal dilatation, which reduces the possibility of anal spasm^[35] and temporary third degree burn

Table 3 Characteristics of randomized comparisons of stapled hemorrhoidopexy and LigaSure hemorrhoidectomy reported in the literature

Ref.	Technique	Operation time (min)	Hospitalization (d)	Postoperative pain (Visual Analog score)	Parenteral analgesic use	Postoperative urinary retention	Postoperative bleeding	Return to normal activity or work	Incontinence for gas or stool after the operation	Postoperative anal stenosis	Residual skin tags and prolapse	Wound Problems	Recurrence
Arsilani <i>et al</i> ^[1]	SH	NR	NR	3 (1-5)	36	1	3	3-4 wk	2	2	6	NR	5
	LH			3 (1-6)	41	2	1	2-4 wk	1	1	0		1
Basdanis <i>et al</i> ^[18]	SH	15 (8-17)	4 (2-10)	3 (1-6)	1	7	0	NR	1	NR	NR	6	3
	LH	13 (9.2-16.1)	5 (2-10)	6 (3-7)	0	5	1		2			39	0
Chen <i>et al</i> ^[19]	SH	19.0 ± 6.4	3.3 ± 1.1	3.1 ± 1.3	23	NR	4	NR	NR	NR	NR	4	1
	LH	12.0 ± 4.1	5.2 ± 1.4	5.4 ± 2.4	35		1					3	0
Kraemer <i>et al</i> ^[20]	SH	21 (6-54)	1.6 (1-2)	Only showed the trend	3.8 (2-12)	4	3	6.3 (1.5) d	0	0	0	2	NR
	LH	26 (10-80)	2.1 (2-3)	Only showed the trend	3.2 (1-8)	2	1	9.8 (1.9) d	0	1	0	1	
Sakr <i>et al</i> ^[21]	SH	26.9 ± 3.26	2.44 ± 0.504	5.29 ± 0.914	5.7 ± 0.855	1	2	8.65 ± 0.485 d	4	2	8	0	4
	LH	20.8 ± 3.35	2.21 ± 0.410	5.53 ± 1.02	5.0 ± 0.776	2	1	7.68 ± 0.638 d	2	1	2	1	1

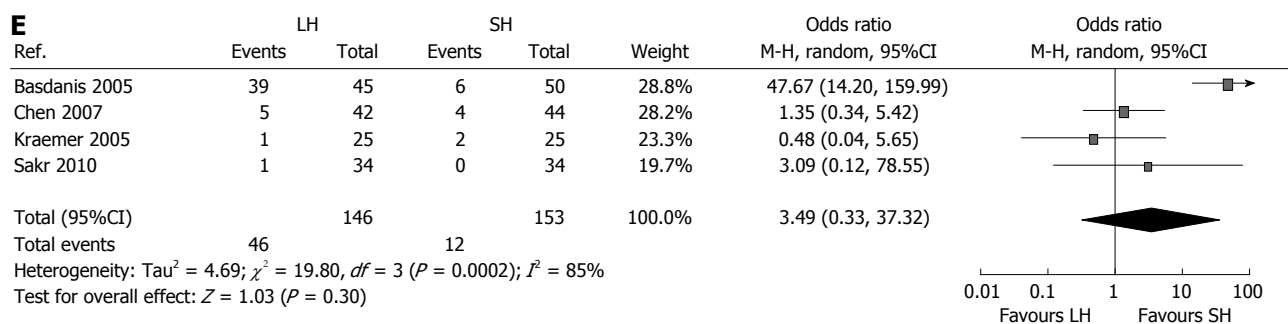
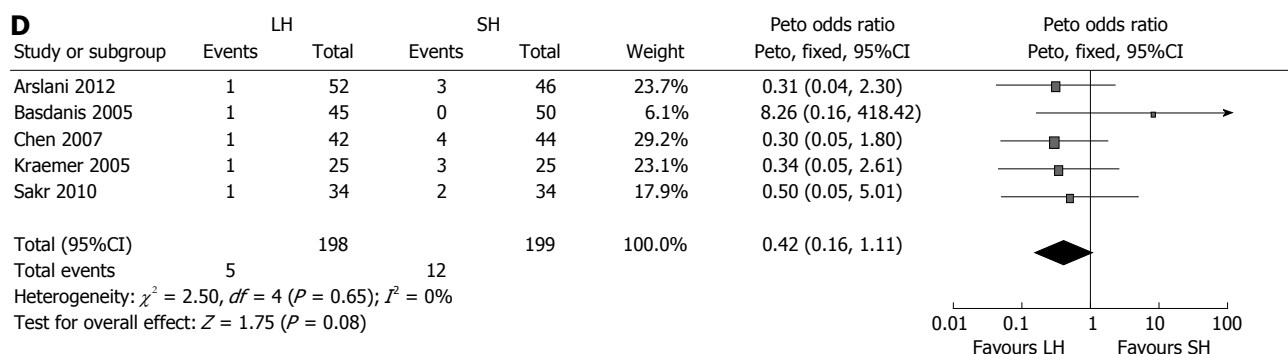
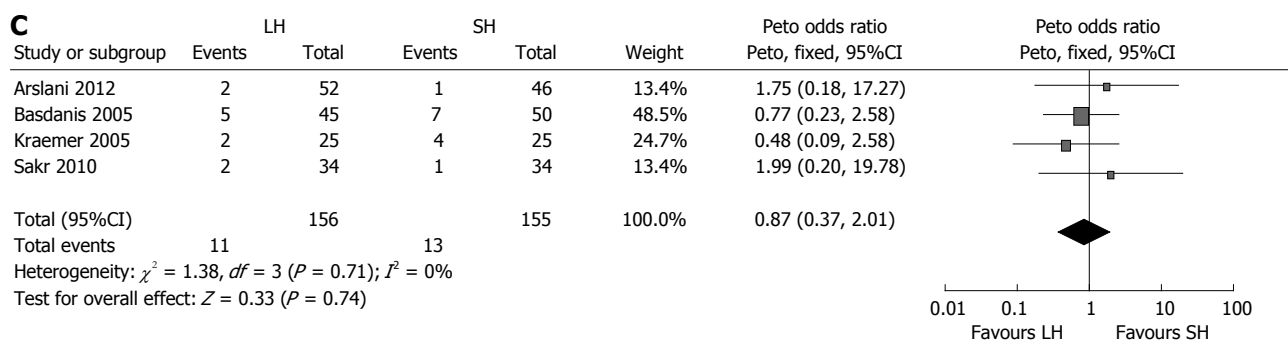
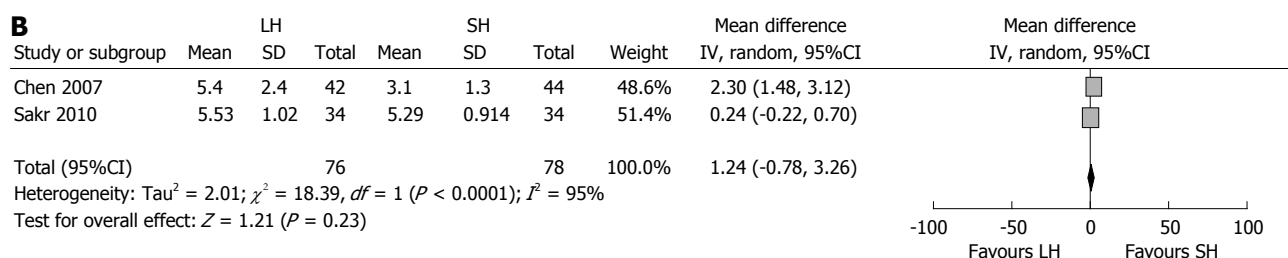
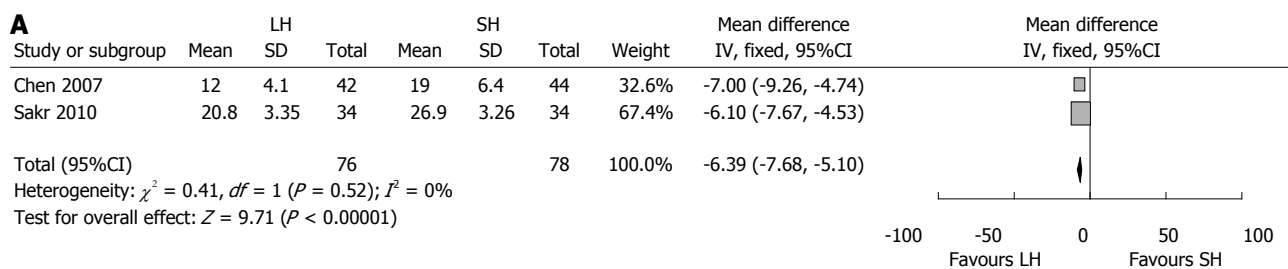
NR: Not reported; LH: LigaSure hemorrhoidectomy SH: Stapled hemorrhoidopexy.

injury to nerve endings at the site of the wound^[36]. However, there were some limitations to our data. The median value and SD were only reported in two studies, and oral and parenteral analgesia requirements were reported too inconsistently for quantitative analysis.

In our meta-analysis the occurrence of postoperative bleeding was equivalent in the two groups. LigaSure is a diathermy system and allows complete coagulation of blood vessels up to 7 mm in diameter, using a precise amount of bipolar energy and pressure that permanently changes collagen and elastin within the vessel wall. However, for SH, the expected frequency of bleeding was at least 50%^[37]. In some circumstances, too much folded mucosa in the stapled line will increase the occurrence of inefficient hemostasis^[38]. Therefore, interrupted stitches were needed to control bleeding points after removal of the stapler in almost all patients.

Early postoperative partial incontinence may be explained by pain that hinders voluntary sphincter contraction^[39]. However potential anal sphincter injury may cause impairment of fecal continence. During SH, intraoperative sphincter stretching may play a role in postoperative fecal incontinence. The procedure requires insertion of a relatively large anal dilator, usually 33 mm, and placement of the circular stapler can lead to further sphincter injury^[37], especially when excessive mucosal prolapse hampers the instrument encompassing all of the redundant tissue. Thus, in patients with pre-existing sphincter injury or with a narrow anal canal, modified techniques introduced by Ho *et al*^[40] may minimize the risk of stretching the internal anal sphincter. They used the smaller Eisenheimer anal retractor instead of the circular anal dilator. Furthermore, if the deeper layers of the rectal wall are not included into the purse-string, making a mucosal instead of an all wall rectal layer anastomosis may reduce the incidence of SH-related postoperative incontinence and stenosis^[38]. Theoretically, intraoperative sphincter stretching was minimized when using the LigaSure system^[41]. The system also had an effect on preservation of internal sphincter pressure^[42]. However, in our meta-analysis, five cases of temporary gas incontinence and three of anal stenosis were encountered after LH, and one patient remained incontinent to gas for 1 mo^[18]. Ramcharan *et al*^[35] reported that after LH, the perianal skin, including the skin bridges, appeared scalded. At 3 mo follow-up, there was some mild circumferential fibrosis in the skin of the anal margin, which produced symptomatic anal stenosis that required once daily anal dilation with a 12-15-mm dilator for 3 mo. Therefore, occurrence of incontinence and stenosis with LH may be related to the device and technique. The LigaSure clamp may grasp the internal anal sphincter as well as the hemorrhoidal tissue above it. Thermal energy causes scalding that can contribute to anal stenosis. Similar mechanisms may result in injury to the anal sphincter, which may account for the fecal incontinence. To avoid this phenomenon, it is important: (1) to cut the anorectal margin with the cold knife before hemorrhoidectomy; (2) on the mucosal margin rather than on the cutaneous margin; and (3) to retract the cutaneous margin from bipolar blades before the sealing cycle begins^[30/43]. When stenosis occurs, an early conservative approach with dilators will successfully treat this condition^[43].

The prolonged hospital stays and delayed recovery usually related to the postoperative pain and wound problems. Our previous data showed that SH and LH have an equal effect in reducing postoperative pain. Although Basdanis *et al*^[18] reported high occurrence of pruritus with LH immediately after the operation, our meta-analysis showed that



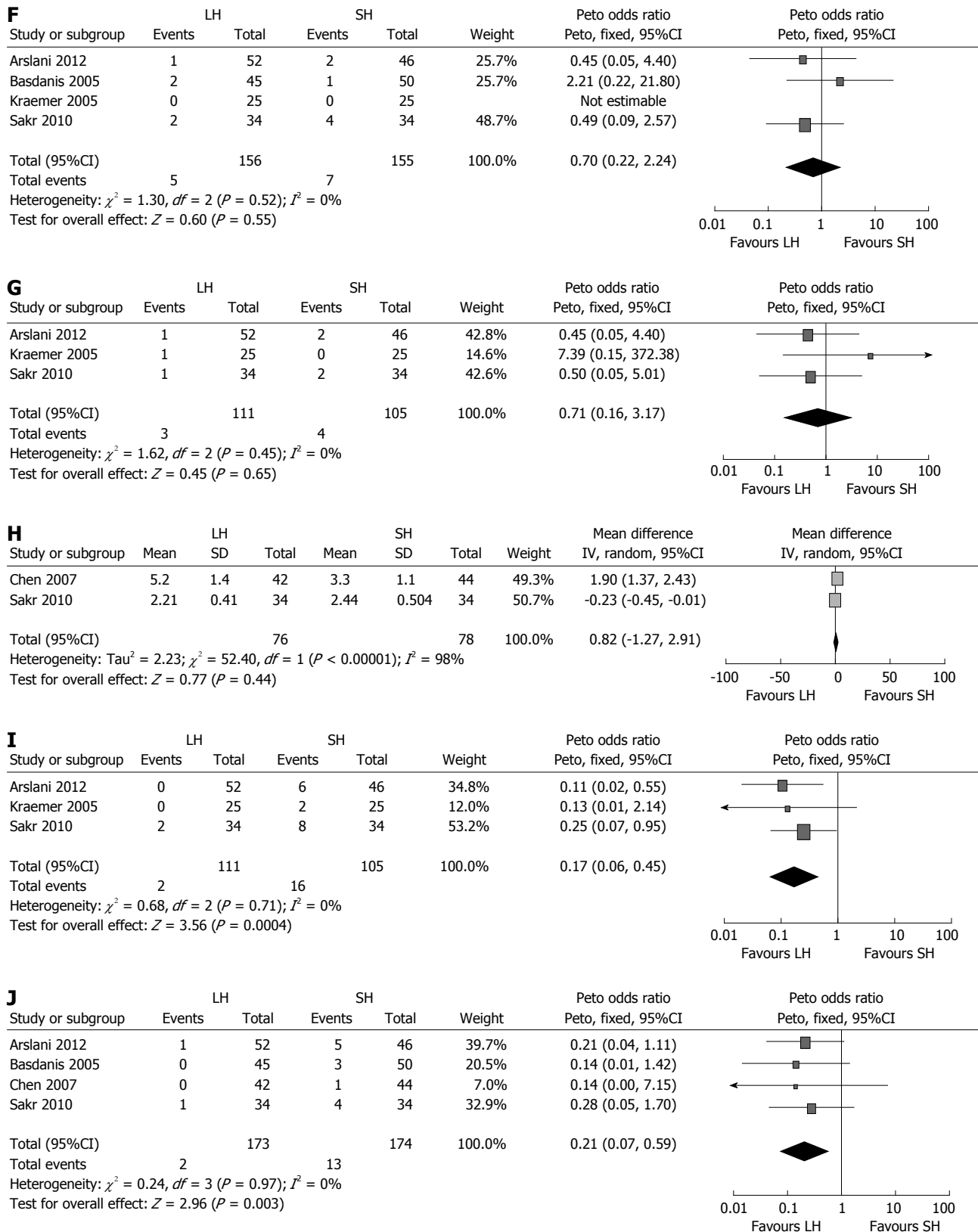


Figure 2 Comparison of outcome between LigaSure hemorrhoidectomy and stapled hemorrhoidopexy. A: Operating time; B: Early postoperative pain; C: Postoperative urinary; D: Postoperative bleeding; E: Wound problems; F: Postoperative gas or fecal incontinence; G: Postoperative anal stenosis; H: Hospitalization; I: Residual skin tags and prolapse; J: Recurrence. LH: LigaSure hemorrhoidectomy SH: Stapled hemorrhoidopexy.

wound problems did not differ significantly between the LH and SH groups. Therefore, as a consequence of reduced postoperative pain and tissue injury, it is understandable that there is no significant difference regarding the length of hospital stay between the two procedures. However, statistical heterogeneity was present, which may be a reflection of differences in hospital discharge protocols and the way in which the length of hospital stay was determined in these studies.

Our meta-analysis had several limitations. The small number of studies and the restricted sample size of most trials implied that the quantitative analysis was not very powerful. Moreover, the limited follow-up time of the included studies and different outcome measures considered may also have led to biased results. Large multicenter studies based on commonly accepted endpoints with long-term follow-up are warranted to compare better the results of these two different techniques of hemorrhoidectomy.

In conclusion, our meta-analysis supports that both SH and LH are probably equally valuable techniques in modern hemorrhoid surgery. However, LH might have slightly favorable immediate postoperative results and technical advantages.

COMMENTS

Background

Many clinical trials have shown that stapled hemorrhoidopexy (SH) and LigaSure hemorrhoidectomy (LH) have some advantages over conventional hemorrhoidectomy. However, there is still a lack of evidence comparing the clinical outcomes between SH and LH.

Research frontiers

Around 5% of the general population has hemorrhoidal disease to some extent, especially those > 40 years of age. There is a vast number of available therapeutic methods, but hemorrhoidectomy is well established as the most effective and definitive treatment for grades 3 and 4 symptomatic hemorrhoidal disease. SH and LH are new techniques that promise a less painful course and faster recovery.

Innovations and breakthroughs

Meta-analyses of clinical trials have shown that SH and LH have some advantages over conventional hemorrhoidectomy. There is still a lack of evidence focusing on the operative and postoperative outcomes of SH and LH. The present meta-analysis suggested that the operating time of SH was significantly longer than that of LH. The incidence of residual skin tags, prolapse and recurrence were significantly lower in LH than in SH.

Applications

The present meta-analysis showed that LH was more favorable than SH for patients with concomitant external hemorrhoid components and skin tags due to its slightly favorable technical advantages and immediate postoperative results, such as shorter operating time and lower occurrence of residual skin tags, prolapse and postoperative recurrence.

Terminology

SH (also known as PPH) was introduced by Longo in 1998, and uses a specially designed circular stapling instrument to excise a ring of redundant rectal mucosa or expanded internal hemorrhoids. LH uses the LigaSure vessel sealing system that consists of a bipolar electrothermal hemostatic device that allows complete coagulation of vessels up to 7 mm in diameter with minimal surrounding thermal spread and limited tissue charring.

Peer review

this study is very important meta-analysis for recently invented methods of treatment of hemorrhoid. This report is worthy for publication.

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FDG-PET in diagnosis, staging and prognosis of pancreatic carcinoma: A meta-analysis

Zhen Wang, Jun-Qiang Chen, Jin-Lu Liu, Xin-Gan Qin, Yuan Huang

Zhen Wang, Jun-Qiang Chen, Jin-Lu Liu, Xin-Gan Qin, Yuan Huang, Department of Gastrointestinal Surgery, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Author contributions: Wang Z, Chen JQ designed this study; Liu JL and Qin XG performed literature search; Liu JL and Huang Y extracted the data; Wang Z and Huang Y analyzed the data; Chen JQ and Qin XG interpreted the results; Wang Z drafted the manuscript; Chen JQ, Liu JL and Qin XG revised the paper; all authors read and approved the final manuscript to be published.

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Correspondence to: Jun-Qiang Chen, MD, PhD, Professor, Department of Gastrointestinal Surgery, the First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning 530021, Guangxi Zhuang Autonomous Region, China. gxhans@163.com

Telephone: +86-771-5356701 Fax: +86-771-5350031.

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Abstract

AIM: To investigate the potential role of positron emission tomography (PET) in the diagnosis, staging and prognosis predicting of pancreatic carcinoma (PC).

METHODS: A systematic review of relevant literatures in PubMed, Embase and Cochrane Library was performed. The sensitivity and specificity of diagnostic and staging studies, and HRs for prognosis predicting studies were pooled. The bivariate model was used for diagnostic studies and the random-effect model for prognostic studies. Heterogeneity between included studies was tested using χ^2 test, and subgroup analysis was performed to explain the heterogeneities. All of the calculations were performed using Stata version 11.0.

RESULTS: A total of 39 studies were included. The pooled sensitivity of PET in diagnosing PC (30 studies, 1582 patients), evaluating N staging (4 studies, 101 patients) and liver metastasis (7 studies, 316 patients) were 0.91 (95%CI: 0.88-0.93), 0.64 (95%CI: 0.50-0.76), and 0.67 (95%CI: 0.52-0.79), respectively; and the corresponding specificity was 0.81 (95%CI: 0.75-0.85), 0.81 (95%CI: 0.25-0.85), and 0.96 (95%CI: 0.89-0.98), respectively. In prognosis analysis (6 studies, 198 patients), significant difference of overall survival was observed between high and low standardized uptake value groups (HR = 2.39, 95%CI: 1.57-3.63). Subgroup analysis showed that PET/CT was more sensitive than PET alone in evaluating liver metastasis of PC, 0.82 (95%CI: 0.48-0.98) and 0.67 (95%CI: 0.52-0.79), respectively.

CONCLUSION: PET can be used as a valuable diagnostic and predictive tool for PC, but its effect in the staging of PC remains indeterminate.

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Key words: Pancreatic carcinoma; Positron emission tomography; Diagnosis; Staging; Prognosis; Meta-analysis

Core tip: Positron emission tomography (PET) is an important tool for the diagnosis, staging and prognosis predicting of tumors. However, no consensus has been reached with regard to the role of PET in pancreatic carcinoma (PC) diagnosis. We performed meta-analysis of 39 included studies. The pooled results showed that PET can be used as a valuable diagnostic and predictive tool for PC, but its effect in the staging remains indeterminate. New tracers and PET scanning technology, as well as other parameters besides of standardized uptake value should be noticed in order to improve the diagnostic and predictive accuracy of PET in PC.

Wang Z, Chen JQ, Liu JL, Qin XG, Huang Y. FDG-PET in diagnosis, staging and prognosis of pancreatic carcinoma: A meta-analysis. *World J Gastroenterol* 2013; 19(29): 4808-4817 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4808.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4808>

INTRODUCTION

Pancreatic carcinoma (PC) is one of the leading causes of cancer death worldwide and is steadily increasing in incidence in most countries^[1]. In industrialized countries, the incidence of PC ranks second after colorectal cancer among all gastrointestinal malignancies^[1]. Despite recent significant advances in cancer diagnosis and treatment, the prognosis of PC remains extremely unfavorable with a reported 5-year survival rate of only 1%-10%^[2,3]. For PC, surgery remains the only curative treatment, and the success depends on the stage of disease at diagnosis, but not the histological type^[4]. Unfortunately, only 10%-15% of cancers are found to be resectable at the time of diagnosis for the late onset of the symptoms^[5]. Therefore, to choose the most appropriate treatment and to avoid unnecessary surgical risk, timely diagnosis and staging is essential in the evaluation of patients with PC.

Although significant advances have been achieved in diagnostic technologies such as computed tomography (CT), endoscopic ultrasonography (EUS) and magnetic resonance imaging (MRI), the preoperative diagnosis and staging of PC remains suboptimal, which restricts the treatment planning of this disease^[5]. The discrimination between inflammatory processes and PC, and the assessment of local resectability and distant metastases of the PC are still challenging with different imaging modalities^[6]. Over the years, positron emission tomography (PET) has played an important role in oncology, especially for diagnosis, staging, and for evaluating the response to treatment and the prognosis of tumors^[7]. However, there has been no consensus with regard to the role of PET in PC now. Some researchers held that PET could be used as a valuable measure in the diagnosis, staging and prognosis predicting of PC^[8]; but others did not find enough evidences to justify the use of PET in PC^[9]. Therefore, a systemic review aimed to evaluate the effect of PET in the diagnosis, staging and prognosis predicting of PC is urgently needed.

In this study, we assessed the pertinent literatures and conducted a meta-analysis to further investigate the potential role of PET in PC.

MATERIALS AND METHODS

Literature search

A systematic literature search was performed to identify studies assessing the effect of PET in the diagnosis, staging and prognosis predicting of PC. The PubMed, Embase and Cochrane Library databases were searched

with the MeSH headings ("pancreatic neoplasms" and "tomography emission computed") and keywords ("pancreas or pancreatic neoplasms" or "pancreatic tumor/tumour" or "pancreatic cancer" or "PC" or "cancer of the pancreas") and (PET or "diffusion" or "weighted imaging"). The upper limit of search date was not limited, and the lower limit was December 2012. The language was not limited. In addition, reference lists from the included studies were hand searched.

Inclusion and exclusion criteria

Inclusion criteria for this meta-analysis were: (1) Studies assessing the effect of PET in the diagnosis, staging and prognosis predicting of PC. The participants were clinically suspected of PC, and diagnosed with PC by histology or follow-up exceeding 6 mo; (2) For diagnosis and staging, the results were judged with histopathology or clinical follow-up exceeding 6 mo; (3) For diagnosis and staging, true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) results of imaging methods could be calculated for per-patient; for prognosis, HRs and their 95% CIs for overall survival (OS) data were available or able to be calculated from original articles; (4) For eligible studies with data published more than once, we only included the articles with the largest sample size of patients; and (5) PET was performed with intravenous administration of ¹⁸F-FDG.

Exclusion criteria for this meta-analysis were: (1) studies included patients with non-primary PC in staging or prognosis analysis (*e.g.*, metastatic cancer); (2) primary data were confounding and not able to be analyzed; (3) for staging, studies included patients who received radiotherapy or chemotherapy preoperatively, which may cause downstaging because neo-adjuvant protocols can lead to tumor downstaging and affect the diagnostic accuracy of imaging; (4) vitro studies and animal experiments; (5) Studies with a sample size less than 10; and (6) papers were not original research in type (*e.g.*, review articles).

Data extraction and quality assessment

Two authors extracted data using pre-defined tables, which included the following items: authors and publication time, country, study design, participants, sample size, quality score, and outcomes (TP, FP, TN and FN for diagnosis and staging analysis; HRs and their 95% CIs of OS for prognosis analysis). Follow-up period was recorded for prognosis analysis.

For diagnosis and staging, nine items of QUADAS closely related to this study were used to assess the methodological quality of eligible studies (the other five items of QUADAS were not related to this test)^[10]. For prognosis, four items (closely related to this study) from previous literatures were selected as the quality standard^[11]. Each item was described as Yes (high quality), Unclear, or No (low quality).

Statistical analysis

For diagnosis and staging analysis, the calculation was

based on max standardized uptake value (SUV), and pooled estimates of sensitivity and specificity of PET (with corresponding 95% CIs) were analyzed using the bivariate model^[12], which was considered as a more valid statistical model for diagnostic meta-analysis^[13,14]. The bivariate model uses a random effect approach for both sensitivity and specificity, which allows for heterogeneity beyond chance as a result of clinical and methodological differences between studies. To graphically present the results, we plotted the hierarchical summary receiver operating characteristic (HSROC) curves^[13]. As a concern for meta-analysis of diagnostic trials, publication bias was tested using the funnel plot and Deeks test^[15], which was conducted by a regression of diagnostic log OR against $1/\sqrt{\text{effective sample size}}$, weighting by effective sample size, with $P < 0.1$ for the slope coefficient indicating significant asymmetry.

For prognosis analysis, HRs and their CIs for OS were retrieved from each primary study. In case they were not directly reported in primary literatures, we derived them from the survival curves using published method^[16,17]. Kaplan-Meier curves of included studies were read by Engauge Digitizer version 2.11 (free software downloaded from <http://sourceforge.net>). HR calculation spreadsheet was freely downloaded from <http://www.trialsjournal.com/content/supplementary/1745-6215-8-16-s1.xls>. HRs for OS were pooled using a random-effect model.

Heterogeneity between included studies was tested using χ^2 test ($P < 0.1$ was considered significant). If heterogeneities were present, subgroup analysis was attempted to explain them.

All of the calculations were performed using Stata version 11.0. All P values were two-sided and all CIs had a two-sided probability coverage of 95%.

RESULTS

Study selection and description

According to the search strategy, a total of 629 papers were selected: 362 in PubMed, 216 in EMBASE, 37 in Cochrane Library and 14 by hand search. After browsing the titles and abstracts, we found that many studies were irrelevant and some were identified in more than one database; and 103 articles remained for potential inclusion and full texts were obtained. After screening the full text, 64 articles were excluded. The main reasons for exclusion were: nonclinical trials (such as review articles), repetitive publication, incomplete data, and inappropriate reference standard. At last, 39 studies were eligible for inclusion^[18-56]. The number of studies evaluating primary tumor diagnosis, N staging, liver metastasis and prognosis was 30^[18-21,25,32-56], 4^[18-21], 7^[19-25] and 6^[26-31], respectively. The study selection process is summarized in Figure 1. The characteristics of included studies are listed in Tables 1-4. And the quality of included studies is shown in Figure 2.

Meta-analysis

In the diagnosis of primary tumors, 30 studies (1582 patients) were eligible for meta-analysis^[18-21,25,32-56]. The

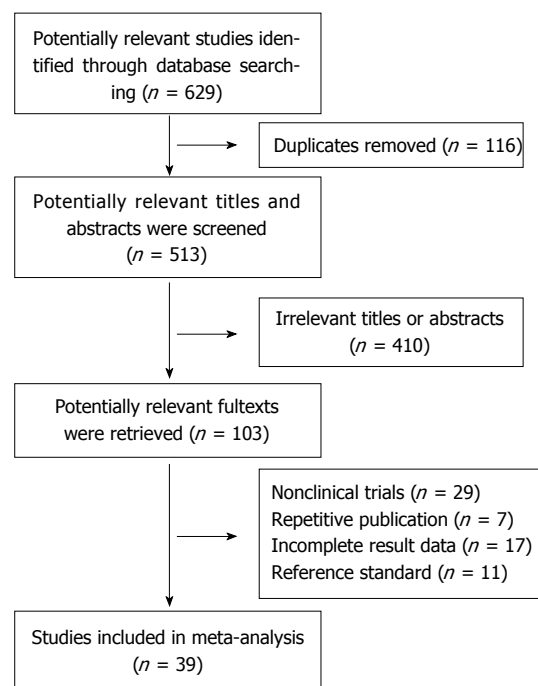


Figure 1 QUORUM flow chart for studies.

pooled sensitivity and specificity of PET in the diagnosis of PC were 0.91 (95%CI: 0.88-0.93) and 0.81 (95%CI: 0.75-0.85), respectively.

For lymph node metastasis, 4 studies (101 patients) were eligible for meta-analysis^[18-21]. The pooled sensitivity, specificity and negative predictive value of PET in the diagnosis of N staging were 0.64 (95%CI: 0.50-0.76), 0.81 (95%CI: 0.25-0.85), and 0.65 (95%CI: 0.28-0.90), respectively.

For liver metastasis, 7 studies (316 patients) were eligible for meta-analysis^[19-25]. The pooled sensitivity and specificity of PET in the diagnosis of liver metastasis were 0.67 (95%CI: 0.52-0.79) and 0.96 (95%CI: 0.89-0.98), respectively.

For predicting the prognosis, 6 studies (198 patients) were eligible for meta-analysis^[26-31]. In the study by Nakata *et al.*^[29], the data about resectable and unresectable tumors were reported separately. The pooled HR for OS was 2.39 (95%CI: 1.57-3.63), which suggested that patients in low SUV group had a significant longer OS than patients in high SUV group (Figure 3).

Subgroup analysis

The P values of heterogeneity test for the meta-analysis were all less than 0.1. Considering that the results might be influenced by the study design and imaging method, we performed subgroup analysis according to the design and imaging method of included studies. The results of subgroup analysis are listed in Table 5.

HSROC curves

We plotted HSROC curves to graphically present the results of diagnosis and staging (Figure 4). In HSROC curves, the index test's sensitivity (TP rate) was plotted on the y axis against 1-specificity (FN rate) on the x axis.

Table 1 Characteristics of included studies for diagnosis

Ref.	Study design	Imaging	Population	n (M/F)	Results			
					TP	FP	FN	TN
Stollfuss <i>et al</i> ^[32]	NR	PET	Suspected PC or CP	73 (54/19)	41	3	2	27
Wang <i>et al</i> ^[33]	NR	PET	Pancreatic mass	40 (27/13)	26	3	1	10
Rose <i>et al</i> ^[34]	R	PET	Suspected PC	65 (NR)	48	2	4	11
Kauhanen <i>et al</i> ^[18]	P	PET	Suspected PC	38 (19/19)	17	3	1	17
Herrmann <i>et al</i> ^[35]	P	PET	Suspected PC or CP	41 (27/14)	30	4	3	4
		PET/CT		31 (NR)	24	5	1	1
Nakamoto <i>et al</i> ^[36]	P	PET	Suspected PC	47 (31/16)	22	3	5	17
Friess <i>et al</i> ^[37]	P	PET	Suspected PC or CP	74 (57/17)	41	4	1	28
Keogan <i>et al</i> ^[38]	P	PET	Suspected PC	37 (22/15)	22	2	3	10
Koyama <i>et al</i> ^[39]	NR	PET	Suspected PC	86 (50/36)	53	4	12	17
Nishiyama <i>et al</i> ^[19]	NR	PET	Suspected PC	86 (64/22)	49	11	6	20
Inokuma <i>et al</i> ^[40]	P	PET	Suspected PC	46 (25/21)	33	2	2	9
Bares <i>et al</i> ^[20]	P	PET	Suspected PC	40 (25/15)	25	2	2	11
Van <i>et al</i> ^[41]	NR	PET	Suspected PC or CP	109 (65/44)	29	10	3	67
Zimny <i>et al</i> ^[42]	P	PET	Suspected PC	106 (NR)	63	5	11	27
Kato <i>et al</i> ^[43]	NR	PET	Patients with PC or CP	24 (20/4)	14	2	1	7
Ruf <i>et al</i> ^[21]	R	PET	Suspected PC	32 (20/12)	14	10	1	7
Rasmussen <i>et al</i> ^[44]	P	PET	Suspected PC	20 (12/8)	9	1	3	7
Delbeke <i>et al</i> ^[45]	R	PET	Suspected PC	65 (33/32)	52	3	0	10
Farma <i>et al</i> ^[46]	R	PET/CT	Suspected PC	82 (43/39)	58	2	7	15
Borbath ^[25]	R	PET	Suspected PC	59 (29/30)	42	5	6	6
Sendler <i>et al</i> ^[47]	P	PET	Suspected PC	42 (21/21)	22	4	9	7
Bang <i>et al</i> ^[48]	NR	PET	Suspected PC	102 (76/26)	90	2	3	7
Papós <i>et al</i> ^[49]	NR	PET	Suspected PC	22 (13/9)	6	2	0	14
Rajput <i>et al</i> ^[50]	R	PET	Suspected PC	11 (NR)	8	0	1	2
Ho <i>et al</i> ^[51]	NR	PET	Suspected PC	14 (7/7)	8	2	0	4
Mertz <i>et al</i> ^[52]	P	PET	Suspected PC	35 (NR)	27	2	4	2
Takanami <i>et al</i> ^[53]	R	PET/CT	Suspected PC	16 (13/3)	7	0	2	7
Sperti <i>et al</i> ^[54]	P	PET	Suspected PC	64 (33/31)	24	1	2	37
Tann <i>et al</i> ^[55]	R	PET	Suspected PC	30 (16/14)	4	8	3	15
		PET/CT		30 (16/14)	6	2	1	21
Bares <i>et al</i> ^[56]	NR	PET	Suspected PC	15 (11/4)	12	0	1	2

M: Male; F: Female; NR: Not report; R: Retrospective study; P: Prospective; PC: Pancreatic carcinoma; CP: Chronic pancreatitis; TP: True-positive; FP: False-positive; FN: False-negative; TN: True-negative; PET: Positron emission tomography; CT: Computed tomography.

Table 2 Characteristics of included studies for N staging

Ref.	Study design	Imaging method	Population	n (M/F)	Results			
					TP	FP	FN	TN
Kauhanen <i>et al</i> ^[18]	P	PET	Histologically proved PC	8 (NR)	2	0	5	1
Nishiyama <i>et al</i> ^[19]	NR	PET	PC diagnosed by histology or follow-up	55 (NR)	14	1	6	34
Bares <i>et al</i> ^[20]	P	PET	Histologically proved PC	23 (NR)	10	2	3	8
Ruf <i>et al</i> ^[21]	R	PET	PC diagnosed by histology or follow-up	15 (9/6)	8	2	5	0

M: Male; F: Female; NR: Not report; R: Retrospective study; P: Prospective; PC: Pancreatic carcinoma; TP: True-positive; FP: False-positive; FN: False-negative; TN: True-negative; PET: Positron emission tomography.

Additionally, the 95%CI and a 95% prediction region around the pooled estimates were plotted to illustrate the precision with which the pooled values were estimated (confidence ellipse of a mean) and to show the between-study variation (prediction ellipse; the likely range of values for a new study)^[13].

Publication bias

Because the included studies for staging and prognosis were too few (less than 10), we explored publication bias

using the data of PET/CT in the diagnosis of primary tumors, which included 30 studies. As a result, the funnel plot seemed symmetrical with a *P* value of 0.11, which suggested a low risk of publication bias (Figure 5).

DISCUSSION

In recent years, PET imaging has been increasingly used to identify and stage PC, and also utilized as a prognostic indicator. However, the value of PET in the management

Table 3 Characteristics of included studies for liver metastasis

Ref.	Study design	Imaging	Population	n (M/F)	Results			
					TP	FP	FN	TN
Strobel <i>et al</i> ^[22]	R	PET	Histologically proved PC	50 (25/25)	5	0	6	39
		PET/CT		50 (25/25)	9	1	2	38
Nakamoto <i>et al</i> ^[23]	NR	PET	Histologically proved PC	34 (22/12)	11	2	1	20
Nishiyama <i>et al</i> ^[24]	NR	PET	Histologically proved PC	42 (26/16)	10	3	3	26
Nishiyama <i>et al</i> ^[19]	NR	PET	PC diagnosed by histology or follow-up	55 (NR)	11	0	7	37
Bares <i>et al</i> ^[20]	P	PET	Histologically proved PC	23 (NR)	4	1	3	15
Ruf <i>et al</i> ^[21]	R	PET	PC diagnosed by histology or follow-up	15 (9/6)	3	2	5	5
Borbath <i>et al</i> ^[25]	R	PET	PC diagnosed by histology or follow-up	47 (NR)	10	1	2	34

M: Male; F: Female; NR: Not report; R: Retrospective study; P: Prospective; PC: Pancreatic carcinoma; TP: True-positive; FP: False-positive; FN: False-negative; TN: True-negative; PET: Positron emission tomography.

Table 4 Characteristics of included studies for prognosis

Ref.	Study design	Imaging method	Population	n (M/F)	Follow-up period	HR (95%CI)
Sperti <i>et al</i> ^[26]	R	PET	Histologically proved PC	60 (34/26)	NR	3.96 (1.92-8.17)
Maisey <i>et al</i> ^[27]	P	PET	Histologically proved PC	11 (7/4)	NR	3.4 (2.01-5.73)
Zimny <i>et al</i> ^[28]	NR	PET	Histologically proved PC	52 (33/19)	NR	2.27 (1.69-3.05)
Nakata <i>et al</i> ^[29]	NR	PET	Histologically proved PC	37 (21/16)	NR	0.93 (0.70, 1.25) ¹ 4.9 (1.19-20.2) ²
Maemura <i>et al</i> ^[30]	NR	PET	PC diagnosed by histology or follow-up	24 (NR)	NR	2.1 (1.5-2.92)
Nakata <i>et al</i> ^[31]	NR	PET	Histologically proved PC	14 (NR)	6-17 mo	2.99 (2.25-3.97)

¹Patients received operation; ²Patients did not receive operation. M: Male; F: Female; NR: Not report; R: Retrospective study; P: Prospective; PC: Pancreatic carcinoma; PET: Positron emission tomography.

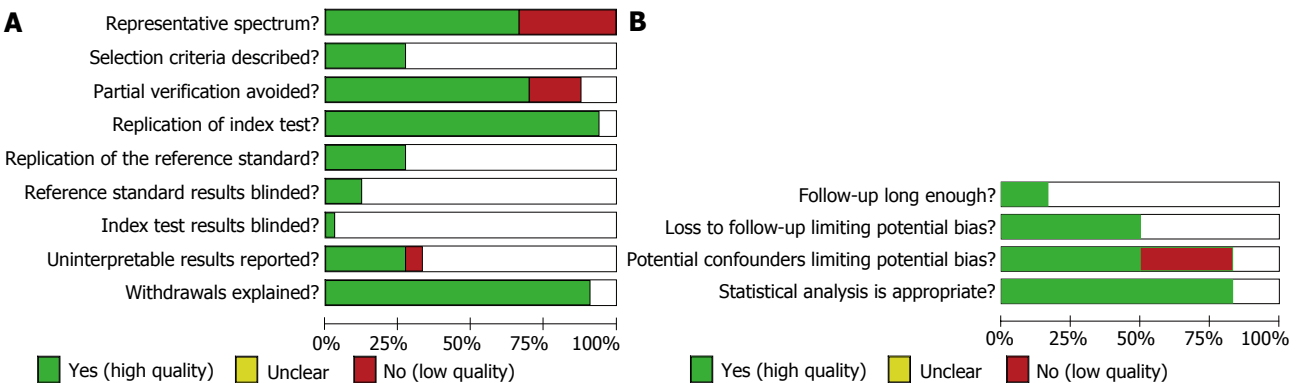


Figure 2 Methodological quality graph. A: Diagnosis and staging studies; B: Prognosis studies. Authors' judgments about each methodological quality item presented as percentages across all included studies.

of PC remains indeterminate. In our study, we collected existing data to assess the value of PET in the diagnosis, staging and prognosis predicting of PC. We found that PET could be used as a valuable diagnostic and predictive tool for PC; but for staging, PET has a moderate sensitivity and a relatively high specificity (Table 5).

Clinically, the diagnostic pathway for detection and staging of PC usually starts with abdominal ultrasound (US) followed by CT or MRI of the upper abdomen. However, even combined diagnostic approaches are limited by a low sensitivity for the detection of small lesions (a diameter of less than 2 cm) and for differentia-

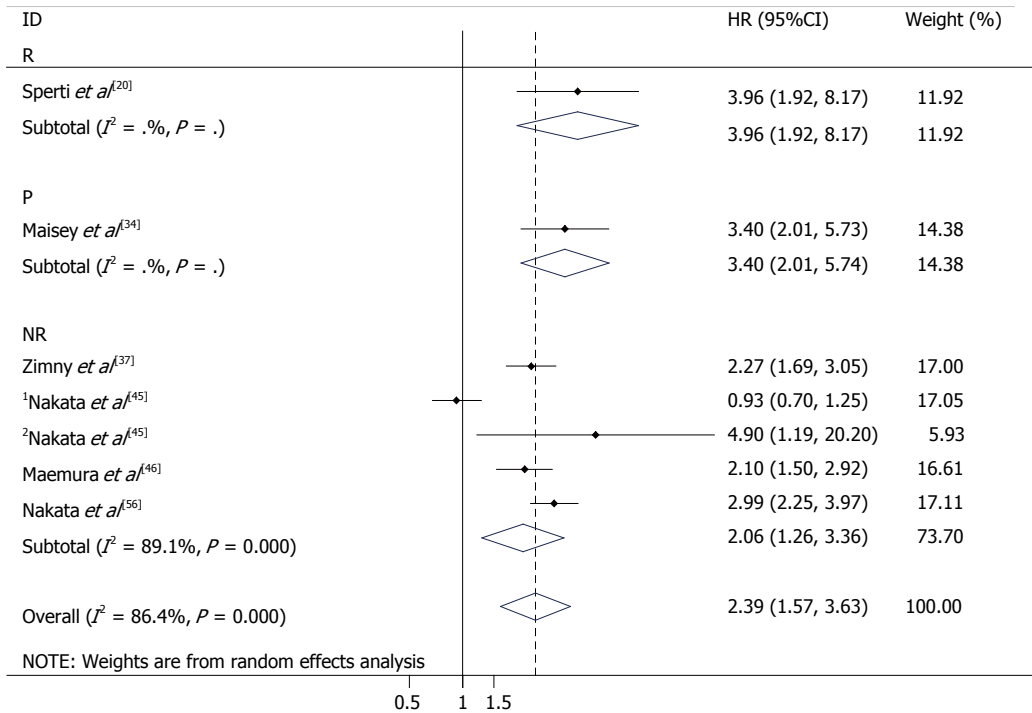


Figure 3 Forrest plot for the prognosis of pancreatic carcinoma. ¹Patients received operation; ²Patients did not receive operation.

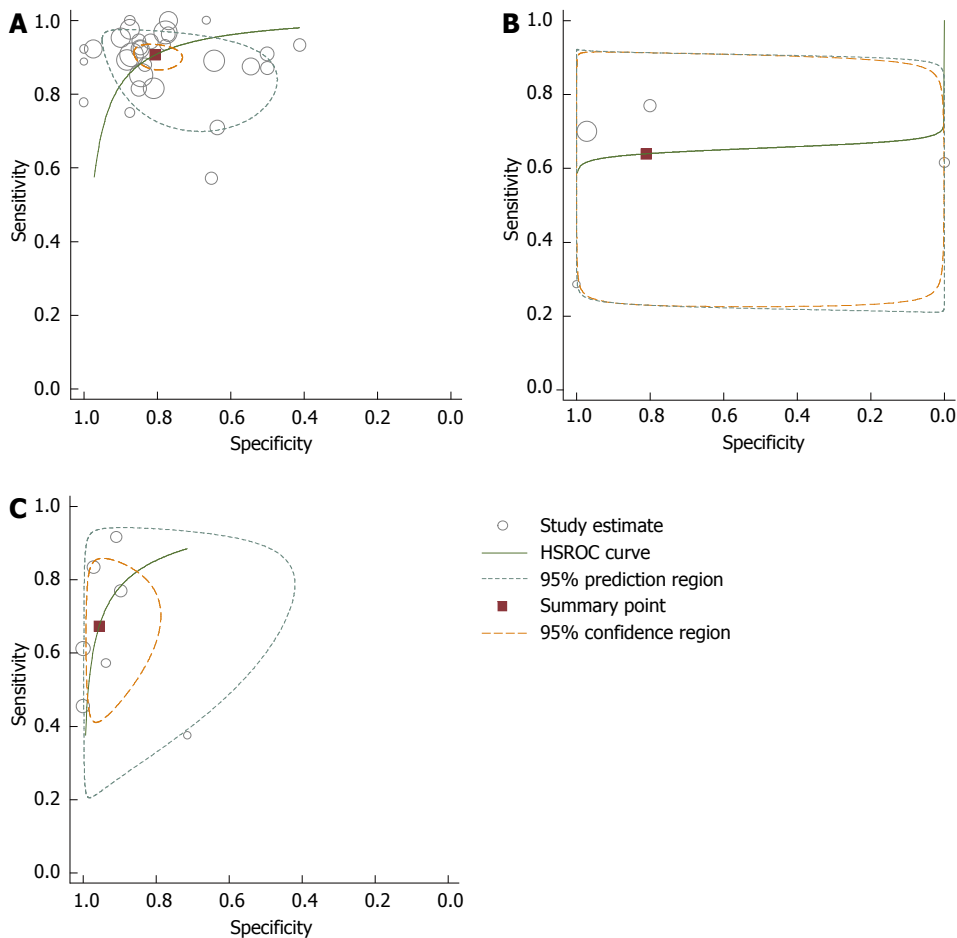


Figure 4 Hierarchical summary receiver operating characteristic curve. A: For the diagnosis of pancreatic carcinoma; B: For N staging of pancreatic carcinoma; C: For liver metastasis of pancreatic carcinoma. HSROC: Hierarchical summary receiver operating characteristic.

Table 5 Results of meta-analysis

Groups	Diagnosis		N staging			Liver metastasis		Prognosis HR (95%CI)
	Sen (95%CI)	Spe (95%CI)	Sen (95%CI)	Spe (95%CI)	Pv- (95%CI)	Sen (95%CI)	Spe (95%CI)	
Overall	0.91 (0.88-0.93)	0.81 (0.75-0.85)	0.64 (0.50-0.76)	0.81 (0.25-0.85)	0.65 (0.28-0.90)	0.67 (0.52-0.79)	0.96 (0.89-0.98)	
P subgroup	0.89 (0.84-0.92)	0.84 (0.76-0.89)	0.56 (0.15-0.90)	0.79 (0.48-0.94)	-	0.57 (0.21-0.88)	0.94 (0.68-0.99)	2.39 (1.57-3.63)
R subgroup	0.90 (0.83-0.95)	0.75 (0.58-0.87)	0.61 (0.32-0.85)	0.17 (0.04-0.81)	-	0.56 (0.28-0.81)	0.94 (0.65-0.99)	3.40 (2.01-5.74)
NR subgroup	0.93 (0.88-0.96)	0.82 (0.74-0.87)	0.70 (0.46-0.87)	0.97 (0.84-0.99)	-	0.74 (0.52-0.88)	0.92 (0.83-0.96)	3.96 (1.92-8.17)
PET subgroup	0.91 (0.88-0.93)	0.80 (0.74-0.85)	-	-	-	0.67 (0.52-0.79)	0.96 (0.89-0.98)	2.06 (1.26-3.36)
PET/CT subgroup	0.90 (0.79-0.95)	0.85 (0.38-0.98)	-	-	-	0.82 (0.48-0.98)	0.97 (0.87-1.00)	-

N: Lymph node; P: Prospective; R: Retrospective; NR: Not reporting; Sen: Sensitivity; Spe: Specificity; Pv-: Negative predictive value; PET: Positron emission tomography; CT: Computed tomography.

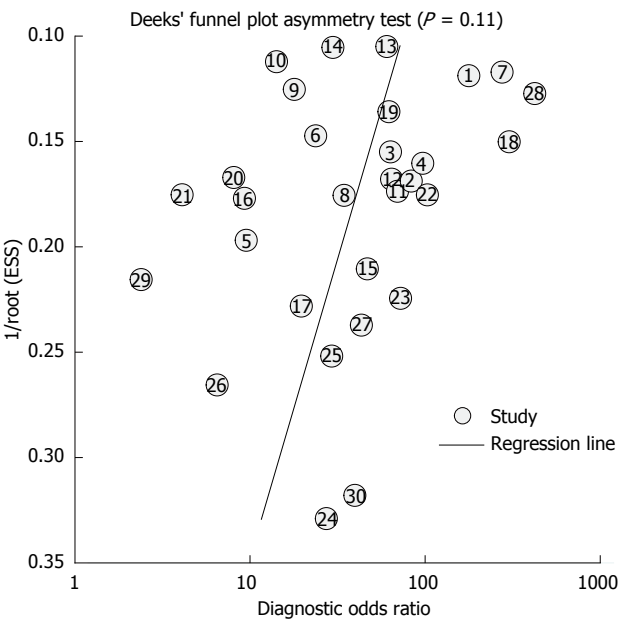


Figure 5 Funnel plot based on the data of positron emission tomography/computed tomography for the diagnosis of pancreatic carcinoma.

tion between malignant and benign lesions^[57]. Recently, promising results in the diagnostic value of PET as a diagnostic and staging tool in PC have been reported^[19-21]. In our review, we found PET had an acceptable sensitivity and specificity [sensitivity: 0.91 (95%CI: 0.88-0.93); specificity: 0.81 (95%CI: 0.75-0.85)] in the diagnosis of PC, which demonstrated that PET was valuable in the diagnosis of PC. This result was consistent with previous reports^[18,22,32,33]. Considering that diagnostic accuracy might be influenced by study design and the usage of CT, we conducted subgroup analysis. However, the other confounding factors (such as tumor diameter, serum glucose and C-reactive protein levels) were not considered because of incomplete data in included studies. This reduced the reliability of our results to some extent, although the impact of these factors on diagnostic accuracy is indeterminate^[19,34]. Because the only curative treatment for PC is surgery, accurate staging is necessary to properly select patients (surgical resection benefits only those patients with localized disease). Previous studies reported that both PET and CT were poor for N staging, although the diagnostic

accuracy of PET was a little higher than CT^[58]. This is consistent with our study (Table 5). As for liver metastasis, the value of PET is still controversial^[7]. In our study, we found a sensitivity of 0.67 (95%CI: 0.52-0.79) for PET in detecting liver metastasis of PC. This suggests that the value of PET in assessing liver metastasis of PC remains indeterminate, although it has a relatively high specificity [0.96 (95%CI: 0.89-0.98)]. Recently, studies found that combined PET/CT could improve detection rates in the staging of PC^[3]. In our study, we found that combined PET/CT was more sensitive than PET alone in assessing liver metastasis (82% *vs* 67%, Table 5), this confirmed the previous findings. Efforts have been made to improve the diagnostic accuracy of PET in PC. It has been found that delayed PET scanning helped differentiate malignant lesions from benign ones, and new tracers such as ¹⁸F fluorothymidine (FLT) could improve the diagnostic accuracy^[19,59]. However, these findings need to be further validated. Patients with PC usually have extremely poor prognosis among gastrointestinal malignancies. With conventional imaging modalities, it is often difficult to predict the prognosis of patients with PC preoperatively. Recently, studies found that the metabolic activity of the pancreas tumor, measured by PET usually through SUV, seemed to be useful in evaluating the prognosis of PC^[29]. This result was consistent with ours, which suggested that patients with a higher SUV were associated with worse prognosis (HR = 2.39, 95%CI: 1.57-3.63). Additionally, the result did not change in the subgroup analysis (Table 5). This demonstrated that what we found was reliable. However, some researchers considered that the usage of SUV for prognostic assessment had some serious limitations (besides tumor characteristics, absolute value of SUV can also be influenced by several institution-dependent factors)^[60]. And they found that SUVmax difference (between pre- and post-treatment scans) or the usage of relative values (such as the retention index) allowed more accurate prognostic evaluation^[60,61]. Of course, more studies are needed to confirm these findings in the future. In this study, we designed a systematic search strategy, selected studies according to the strict inclusion criteria, assessed the methodological quality using uniform criteria, and performed subgroup analysis in the presence of heterogeneity. Thirty-nine studies were included. These increased the reliability of the results to some extent.

However, several concerns must also be addressed when interpreting the pooled results. First, clinical follow-up was used as the reference standard in most of the included studies. Although the follow-up period was long enough, it might not correctly classify the target condition in some cases, which would affect the accuracy of the results. Second, some parameters (such as tumor diameter, glucose and C-reactive protein levels) which would affect the accuracy of the results were not considered in our study because of incomplete data, we failed to perform subgroup analysis or meta-regression, which might find out other possible causes of heterogeneity. Finally, publication bias was not tested because the few number of included studies in evaluating the staging and prognosis of PC may induce potential bias.

In conclusion, PET can be used as a valuable diagnostic and predictive tool for PC, but its effect in the staging of PC remains unclear. New tracers and PET scanning technology, as well as other parameters of PET besides SUV, should be noticed in order to improve the diagnostic and predictive accuracy of PET in PC.

COMMENTS

Background

Pancreatic carcinoma (PC) is one of the leading causes of cancer death worldwide and is steadily increasing in incidence in most countries. In industrialized countries, the incidence of PC ranks second after colorectal cancer among all gastrointestinal malignancies. Although significant advances have been achieved in diagnostic technologies, the preoperative diagnosis and staging of PC remains suboptimal.

Research frontiers

Over the years, positron emission tomography (PET) has played an important role in oncology, especially in the diagnosis, staging and prognosis prediction of tumors. However, there is no consensus with regard to the role of PET in PC now.

Innovations and breakthroughs

PET had an acceptable sensitivity and specificity [sensitivity: 0.91 (95%CI: 0.88-0.93); specificity: 0.81 (95%CI: 0.75-0.85)] in the diagnosis of PC. And higher standard uptake value measured by PET was associated with worse prognosis of PC patients (HR = 2.39, 95%CI: 1.57-3.63). However, the accuracy of PET in evaluating N staging and liver metastasis of PC was unsatisfied. This article gives them a comprehensive update based on previous studies.

Applications

PET can be used as a valuable diagnostic and predictive tool for PC, but its effect in the staging of PC remains indeterminate.

Peer review

Based on previous studies, this study evaluated the comprehensive role of PET in PC, including the diagnosis, staging and prognosis prediction. The authors found that PET can be used as a valuable diagnostic and predictive tool for PC, but its effect in the staging of PC remains indeterminate. The study is well designed, methodologically correct, elaborately prepared and full of significance in the field.

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Intrahepatic endometriosis as differential diagnosis: Case report and literature review

Georg Fluegen, Frank Jankowiak, Luisa Zacarias Foehrding, Feride Kroepil, Wolfram T Knoefel, Stefan A Topp

Georg Fluegen, Luisa Zacarias Foehrding, Feride Kroepil, Wolfram T Knoefel, Stefan A Topp, Department of Surgery (A), Heinrich-Heine-University and University Hospital Duesseldorf, 40225 Duesseldorf, Germany

Frank Jankowiak, Institute of Pathology, Heinrich-Heine-University and University Hospital Duesseldorf, 40225 Duesseldorf, Germany

Author contributions: Fluegen G designed the report and analyzed and interpreted the data; Fluegen G, Kroepil F, Knoefel WT and Topp SA were attending doctors of the patient; Topp SA performed the operation; Jankowiak F performed pathological examinations; Zacarias Foehrding L, Knoefel WT and Topp SA critically revised the report and gave important intellectual input.

Correspondence to: Dr. Stefan A Topp, Professor, Department of Surgery (A), Heinrich-Heine-University and University Hospital Duesseldorf, Moorenstr 5, 40225 Duesseldorf, Germany. stefan.topp@uni-duesseldorf.de

Telephone: +49-211-8117349 Fax: +49-211-8119205

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intrahepatic endometriosis presented with cyclical pain in the upper right abdominal quadrant accompanying menstruation. This lack of a "typical" clinic makes it challenging to diagnose extragonadal endometriosis without histopathology. A previous history of endometriosis was described in only twelve cases, thus the diagnosis of this condition should not be limited to patients with a known history of endometriosis. Six of 18 patients were postmenopausal, demonstrating this condition is not limited to women of reproductive age. A preoperative diagnosis was only reached in seven of the previously described cases, highlighting the importance of preoperative biopsies. Yet due to the potential adverse effects, a transhepatic biopsy must be discussed individually. Although rare, intrahepatic endometriosis should always be considered as a differential diagnosis in women with recurrent hepatic cysts, regardless of age or previous medical history. In such cases, histology is essential and a pericystectomy should be performed as standard of care.

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Abstract

Intrahepatic endometriosis is one of the rarest forms of atypical endometriosis; only eighteen cases have been reported in the English literature. We describe the case of a 32-year-old woman, who presented with persistent, non-cyclical upper right quadrant abdominal pain, a central liver cyst, and no history of endometriosis. Three years previous, she was diagnosed with an intrahepatic cyst. The lesion progressed and two laparoscopic deroofting-operations were performed, yet the diagnosis of intrahepatic endometriosis was never reached. She presented in our clinic with further progress of the cyst as well as obstruction of the intrahepatic biliary system. The magnetic resonance imaging showed a 9.5 cm × 12 cm, lobulated intrahepatic cyst. We performed an ultrasonic pericystectomy. Immunostaining confirmed intrahepatic endometriosis. Only one of the previously described eighteen patients with

Key words: Intrahepatic endometriosis; Atypical endometriosis; Extrapelvic endometriosis; Hepatic cysts

Core tip: We describe the case of a 32-year-old woman who presented with non-cyclical upper right quadrant abdominal pain and a central liver cyst. Upon ultrasonic pericystectomy, the patient was diagnosed with intrahepatic endometriosis. The lack of "typical" clinical symptoms makes it challenging to diagnose extragonadal endometriosis without histopathology. Only eighteen cases of intrahepatic endometriosis have been reported in the literature, with only one reporting cyclical pain. Six of the eighteen patients were postmenopausal, and in twelve cases a previous history of endometriosis was described. Intrahepatic endometriosis should always be considered as a differential diagnosis in women of any age.

Fluegen G, Jankowiak F, Zacarias Foehrding L, Kroepil F, Knoefel WT, Topp SA. Intrahepatic endometriosis as differential diagnosis: Case report and literature review. *World J Gastroenterol* 2013; 19(29): 4818-4822 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4818.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4818>

INTRODUCTION

Endometriosis, a common clinical condition most commonly noted in the pelvis, is found in approximately 6%-10% of women of reproductive age^[1] and approximately 2.5% of postmenopausal women^[2]. Atypical endometriosis, when the condition is found in extrapelvic regions^[3], is rare. Endometrial tissue deposits have been described in almost all organs of the human body, and even very rarely in males^[4]. Intrahepatic endometriosis is one of the rarest forms of atypical endometriosis. Since the first description of intrahepatic endometriosis in 1986^[5], only 18 cases have been reported in the English literature^[5-20]. We describe the case of a 32-year-old woman, who presented with persistent upper right quadrant abdominal pain and a central liver cyst. We discuss the occurrence of intrahepatic endometriosis in review of the previously published eighteen cases and summarize the proposed pathogenesis of this rare condition.

CASE REPORT

A 32-year-old woman, nulligravida, nullipara, consulted our hospital with constant right upper quadrant abdominal tenderness. She had no history of endometriosis. Three years previous, she was diagnosed with an intrahepatic cyst in segment IV. The lesion was closely monitored and upon progression two laparoscopic deroofing-operations, one in combination with a cholecystectomy, were performed. Due to further progression of the cyst with obstruction of the intrahepatic biliary system and resulting jaundice, multiple endoscopic retrograde cholangio pancreaticography were performed and on three occasions transhepatic drains were applied. As the patient presented at our clinic, the preoperative magnetic resonance imaging showed a 9.5 cm × 12 cm, lobulated cyst in segments IV, V and VIII (Figure 1). The serology for echinococcal disease and the tumor markers (carcinoembryonic antigen, carbohydrate antigen 19-9, α -fetoprotein) were normal, as was the routine lab work. We performed an ultrasonic pericystectomy and were able to remove the cyst with minimal damage to the surrounding liver tissue. We did not detect any other abnormalities during the operation. The patient was discharged on the eleventh postoperative day.

Histopathology analysis revealed a lobulated cyst, filled with old blood and detritus. While the surrounding liver tissue was normal, immunostaining of the cyst showed strong coloring for estrogen and progesterone

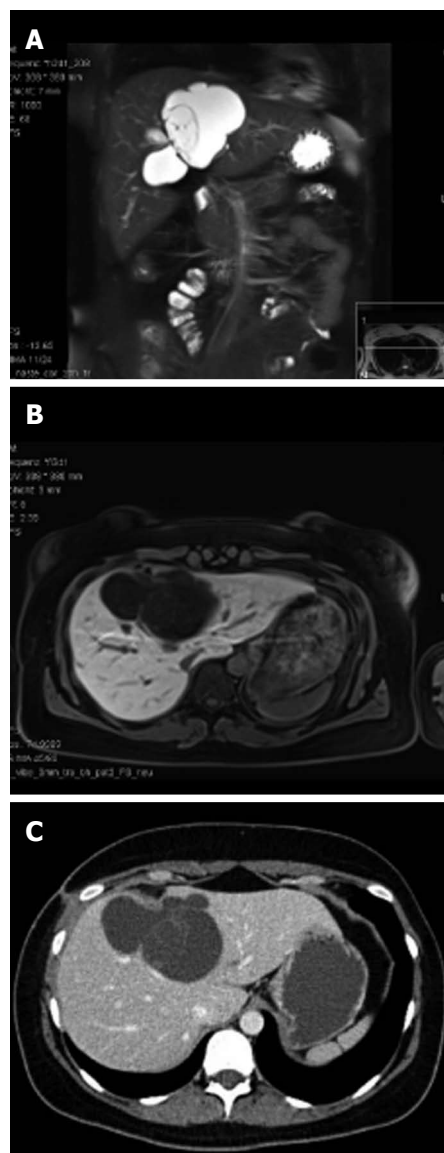


Figure 1 Radiographic images of the central liver cyst. Magnetic resonance imaging (MRI) demonstrated a well-defined lobulated cystic lesion without enhancement after administration of liver-specific contrast material (gadoteric acid) in the arterial, venous or delayed phase. A: MRI, T2, coronary reconstruction; B: MRI, T1, transversal; C: Contrast-enhanced computed tomography scan, transversal.

receptor as well as CK7, which proved the epithelial origin of the cyst. Since no atypical cells were detected, the diagnosis of a benign intrahepatic endometriosis was confirmed (Figure 2).

DISCUSSION

Only 18 cases of this rare form of endometriosis are reported in the English literature (Table 1). Further, only one (5.5%) of these patients presented with cyclical pain in the upper right abdominal quadrant accompanying menstruation. In all other cases, patients presented with tenderness or pain, even jaundice, but no obvious connection to the menstrual cycle. This lack of a “typical” clinic makes it difficult to diagnose extragonadal endo-

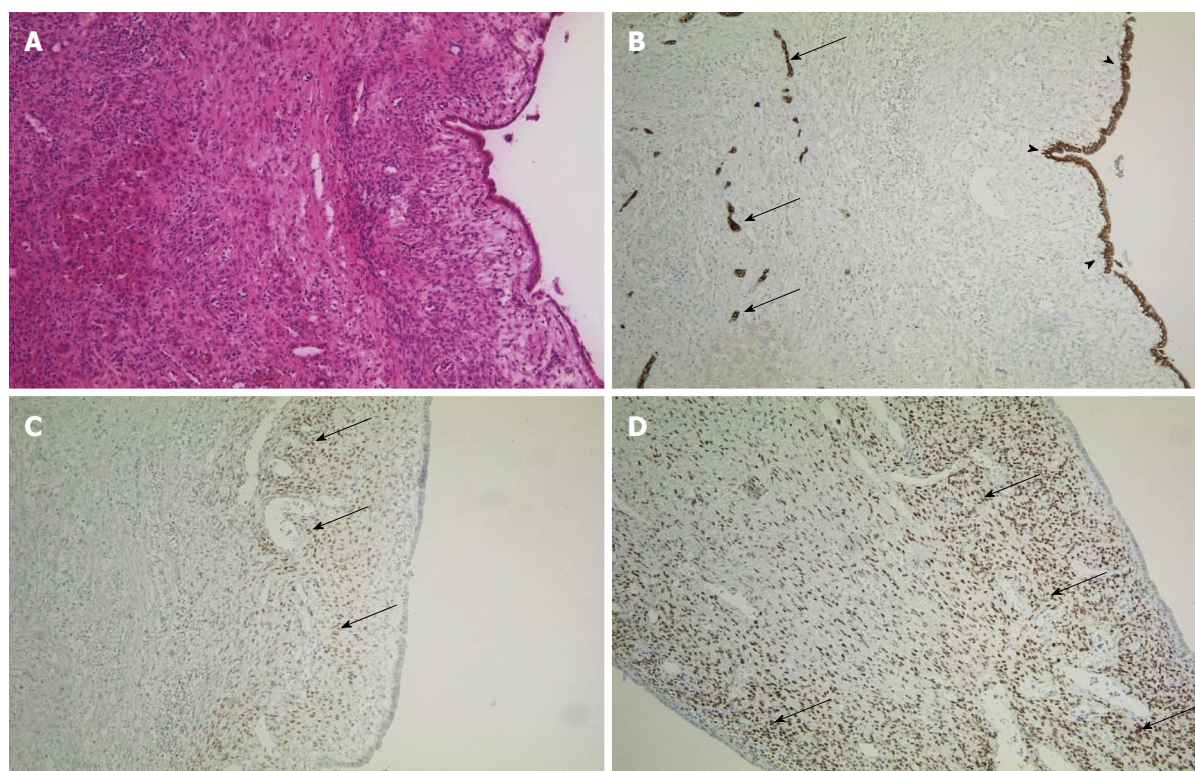


Figure 2 Immunohistochemistry of the cyst. A: Cyst and normal liver tissue in HE staining, $\times 100$; B: Immunohistochemistry for CK7, an epithelial marker, $\times 100$. The intrahepatic vessels (arrows, endothelium) and the cystic wall (arrowheads) are indicated; C and D: Estrogen receptor and progesterone receptor staining of the cystic wall (respectively), $\times 100$. Arrows indicate positive cells.

metriosis without histopathology. Twelve cases (67%) reported a previous history of endometriosis, mostly in a typical pelvic location, while 6 patients (33%) had no history of endometriosis at all. Thus the diagnosis of this condition should not be limited to patients with a known history of endometriosis. Also, 6 of 18 patients (33%) were postmenopausal, thus showing this condition is not limited to women of reproductive age. Due to the difficulty of diagnosing this condition by preoperative radiography^[6], only seven (39%) of the previously described eighteen cases were diagnosed preoperatively. In these cases, a transhepatic biopsy was obtained and the diagnosis verified histologically. Although our patient underwent several invasive procedures previous to the operation at our hospital, unfortunately no histological sample was obtained. This prolonged the suffering of the patient and highlights the importance of both histological and microbiological samples in any recurrent cystic formation.

The cause of endometriosis, first described by Rokitsky in 1860 in the pelvis, is still largely uncertain. Two major theories involve either the implantation of endometrial cells (implantation theory)^[21] or the metaplasia of the peritoneal epithelium (coelomic metaplasia theory)^[22] in the region of occurrence. Each theory, individually, fails to explain all cases of endometriosis. The frequent occurrence of retrograde menstruation is a strong argument for the implantation theory^[23]. Yet how these endometrial cells may reach atypical locations such as the brain, the heart^[24,25] or, and in our case, the parenchyma

of the liver, is hard to explain with this theory. The transport of endometrial cells *via* blood and lymph vessels, similar to the metastasis of cancerous cells, may be a possible explanation for these atypical locations^[9,21,26]. Keichel *et al*^[26] found endometriosis-like cells in lymphatic vessels as well as endometriosis in locoregional lymph nodes in patients with deep infiltrating endometriosis.

The metaplasia of (peritoneal) epithelium due to chronic inflammation or an unknown signaling cascade^[22] may be better suited to explain the occurrence of endometriosis in obscure locations, such as in the heart or even the male. Yet the observation that atypical endometriosis usually occurs in only one location in each individual is hard to resolve. If, as postulated by this theory, some signaling cascade prompts coelomic cells to develop into endometrial tissue, why does this usually happen in only one atypical location in a prone individual? Also, the high number of extragonadal, pelvic cases of endometriosis seems to favor a model in which proximity to the uterus may play an important role.

In conclusion, intrahepatic endometriosis is a rare condition in women. Due to the difficulties of radiographic diagnosis and the “atypical” clinic (no cyclical pain and lack of dysmenorrhea), the diagnosis may only be reached preoperatively by transhepatic biopsy^[6]. Due to the potential adverse effects (risk of bleeding, possible dissemination of cells, biliary leakage) this procedure should be discussed individually. The operation of problematic cysts is a proven cure of the tenderness and

Table 1 Case reports of intrahepatic endometriosis

Ref.	Age (yr)	Meno pause	Cyclical pain	History of endo metriosis	Pre-operation diagnosis	Previous operations
Finkel <i>et al</i> ^[5]	21	No	No	No	No	Yes
Grabb <i>et al</i> ^[10]	21	No	No	No	No	Yes
Rovati <i>et al</i> ^[17]	37	No	No	Yes	Yes	No
Cravello <i>et al</i> ^[8]	34	No	Yes	Yes	Yes	No
Verbeke <i>et al</i> ^[19]	62	Yes	No	No	No	Yes
Verbeke <i>et al</i> ^[19]	34	No	No	No	No	No
Chung <i>et al</i> ^[7]	40	No	No	Yes	No	Yes
Weinfeld <i>et al</i> ^[20]	60	Yes	No	Yes	No	Yes
Inal <i>et al</i> ^[12]	25	No	No	Yes	No	No
N'Senda <i>et al</i> ^[15]	54	Yes	No	No	Yes	Yes
Huang <i>et al</i> ^[11]	56	Yes	No	Yes	No	Yes
Jeanes <i>et al</i> ^[13]	31	No	No	Yes	Yes	Yes
Khan <i>et al</i> ^[14]	31	No	No	Yes	Yes	Yes
Khan <i>et al</i> ^[14]	59	Yes	No	Yes	Yes	Yes
Tuech <i>et al</i> ^[18]	42	No	No	No	No	No
Reid <i>et al</i> ^[16]	46	No	No	Yes	No	Yes
Goldsmith <i>et al</i> ^[9]	48	No	No	Yes	No	Yes
Asran <i>et al</i> ^[6]	61	Yes	No	Yes	Yes	Yes
Fluegen <i>et al</i> , this study	32	No	No	No	No	Yes

pain mostly reported with this condition^[27]. A selective operation should be considered top priority, even if a malignant progression is very rare^[28]. Lifelong hormone therapy may also reduce the symptoms, as described in the case of Inal *et al*^[12], but carries the risk of side effects and long-term dependence on medication. Rare conditions like intrahepatic endometriosis should always be considered as a differential diagnosis in women with recurrent hepatic cysts after surgical deroofing, regardless of age or previous medical history. In such cases, histology is essential and a pericystectomy should be performed.

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Bleeding Dieulafoy's-like lesions of the gut identified by capsule endoscopy

Lidia Ciobanu, Oliviu Pascu, Brindusa Diaconu, Daniela Matei, Cristina Pojoga, Marcel Tanțău

Lidia Ciobanu, Oliviu Pascu, Brindusa Diaconu, Daniela Matei, Cristina Pojoga, Marcel Tanțău, Regional Institute of Gastroenterology and Hepatology, University of Medicine and Pharmacy, 400162 Cluj, Romania

Author contributions: Ciobanu L analyzed the capsule endoscopy records and wrote the paper; Pascu O analyzed the capsule endoscopy records; Matei D, Diaconu B and Pojoga C attended the patients; Tanțău M performed the enteroscopies.

Correspondence to: Lidia Ciobanu, MD, Assistant Professor, Regional Institute of Gastroenterology and Hepatology, University of Medicine and Pharmacy, 19-21 Croitorilor Street, Cluj-Napoca, 400162 Cluj, Romania. ciobanulidia@yahoo.com

Telephone: +40-745-042155 Fax: +40-284-455995

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Core tip: This case series emphasizes the role of capsule endoscopy in active obscure gastrointestinal bleeding, even in hemodynamically unstable patients, because it is able to identify the site of bleeding and to guide therapeutic procedures. Emergency capsule endoscopy reduces the number of diagnostic enteroscopies in Dieulafoy's-like lesions of the small bowel and colon.

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Abstract

Dieulafoy's-like lesions (DLs-like) represent a cause of obscure gastrointestinal bleeding, enteroscopy being the main diagnostic and therapeutic procedure. Frequently, more than one enteroscopy is needed to identify the bleeding vessel. In our practice, video capsule endoscopy (VCE) identified and guided therapy in four cases of DLs-like; three of them were localized on the small bowel. We report, for the first time, a diagnosis of colonic DL-like performed by colon capsule endoscopy. Two patients presented with severe cardiovascular disorders, being hemodynamically unstable during VCE examination. Based on the VCE findings, only one invasive therapeutic procedure per patient was necessary to achieve hemostasis. VCE and enteroscopy may be regarded as complementary procedures in patients with gut DLs-like.

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Key words: Obscure gastrointestinal bleeding; Dieulafoy's-like lesion; Video capsule endoscopy; Enteroscopy; Small bowel

INTRODUCTION

Dieulafoy's lesion (DL) is characterized by a small mucosal erosion involving an unusually large submucosal artery in an otherwise normal mucosa^[1]. Even a predilection for proximal stomach was considered previously; no region of the gut is immune^[1-3]. Characteristic histological lesions consist of a normal artery with an abnormally large diameter, maintaining a constant width of 1-3 mm, that runs a tortuous course within the submucosa and protrudes through a small mucosal defect, varying from 2 to 5 mm, which fibrinoid necrosis at its base^[4]. The term DL-like is used to describe bleeding when angiographic, endoscopic ultrasound or pathological data are unavailable, the diagnosis being based on clinical and endoscopic features^[4]. DLs are responsible for 3.5% of mid gastrointestinal bleeding^[2]. The diagnosis is challenging, not only because of the small size of the lesion and the normal surrounding mucosa, but also because of the intermittent nature of bleeding; frequently more than one enteroscopic procedure being required for small bowel DLs-like.

In recent years, the extensive use of video capsule endoscopy (VCE) proved to be a useful procedure with high diagnostic yield in obscure gastrointestinal bleeding (OGIB)^[5]. Recent reports also document the utility of this procedure in active, severe OGIB^[5-8]. Some case reports describe DLs-like diagnosis on capsule endoscopy^[9-11], but none of the larger series gives specific details on this topic. Colon capsule endoscopy, initially designed as a non-invasive procedure for colorectal cancer screening^[12], with many technical improvements, can assess the entire length of the gut to identify sources of bleeding.

During 2006 to 2012, in our capsule endoscopy unit, 227 VCE procedures were performed, 70 patients being investigated for overt OGIB. Four DLs-like were diagnosed by capsule endoscopy (three localized on the small bowel and one in the colon) in active gastrointestinal bleeding and successful guided therapeutic procedures were performed. Capsule endoscopy findings suggestive of DLs-like were: active blood streaming from a minute mucosa defect or through surrounding mucosa, visualization of a protruding vessel or the appearance of a fresh, densely adherent clot with a narrow point of attachment to a minute mucosal defect or to normal appearing mucosa, as described in conventional endoscopy^[1].

CASE REPORT

Case 1

A 69-year-old female, receiving long-term warfarin therapy for a prosthetic mitral valve was referred to our department for intermittent melena in the last four weeks. No bleeding source was identified after two upper and lower gastrointestinal endoscopies. She did not use nonsteroidal anti-inflammatory drugs (NSAIDs). At the time of VCE, her blood pressure was 90/60 mmHg, her heart rate was 110 beats/min and her hemoglobin level was 6.8 g/dL. Capsule endoscopy revealed significant amounts of fresh blood in the first jejunal loop (Figure 1A), without an identifiable lesion, highly suggestive of DL-like. On the same day, an emergency push enteroscopy was performed, with therapeutic hemocclipping of jejunal bleeding mucosal break. Her clinical status significantly improved, and she was discharged a few days later, with a hemoglobin level of 10 g/dL. She did not experience any gastrointestinal bleeding during a 5-year follow-up period.

Case 2

A 58-year-old female who experienced four episodes of melena in the last five years, without an identifiable source of bleeding at multiple upper and lower endoscopies. At the time of VCE she had melena and a hemoglobin level of 5.8 g/dL. Capsule endoscopy revealed a very small mucosal break suggestive of DL-like (Figure 1B), with a significant amount of fresh blood in the jejunum. Based on these findings, 12 h later, an emergency enteroscopy was performed that was able to stop the bleeding by hemocclipping. She remained in excellent clinical condition 1 year later.

Case 3

A 68-year-old female presented two severe episodes of melena and hematochezia with hemorrhagic shock in the last five months. She was not using NSAIDs and no other symptoms were present. She was investigated with upper and lower gastrointestinal endoscopies on the first episode without an identifiable source of bleeding. On the second episode of bleeding, another gastroscopy did not find any bleeding source, and she was referred for capsule endoscopy. After a standard preparation we used a colon capsule endoscopy to examine the entire gut mucosa. Capsule endoscopy found fresh blood only in the colon with a mucosal break (Figure 1C). Colonoscopy was performed the same night: a bleeding vessel was found on the transverse colon and hemoclips were placed. The patient was released from hospital five days later in good clinical condition. She did not experience other episodes of gastrointestinal bleeding during a 2 years follow up period.

Case 4

Case 4 was an 80-year-old female patient with known cardiovascular disorders that required both antiagregants (low doses of aspirin) and anticoagulants (warfarin). She was admitted for a severe episode of hematochezia, with hemorrhagic shock, with a 5.8-g/dL hemoglobin level. Upper and lower endoscopies did not find the source of the bleeding; therefore, a capsule endoscopy was performed. The patient being closely monitored because she was hemodynamically unstable. VCE detected active bleeding in the proximal jejunum and a small mucosal break (Figure 1D). An emergency push enteroscopy was performed the next morning, which was able to place metal clips on the bleeding jejunum mucosal vessel. Her clinical condition improved and she left the hospital five days later. During a 6-mo follow up period she did not experience another episode of gastrointestinal bleeding.

DISCUSSION

DLs account for 1%-2% of all gastrointestinal bleeding episodes^[4]. From 1884, when it was first described by Gallard^[13], until today, the management of DLs-like has represented a challenge. The patients present with acute onset of bleeding, frequently without identifiable risk factors, sometimes life-threatening. Identifying the site of bleeding by endoscopy procedures may be difficult, as the dimension of the mucosal break is minute and is not easy to visualize, in the context of large amounts of blood or if the bleeding stops^[4]. These aspects are even more relevant for small bowel localization, as the bleeding lesions are more difficult to reach. Small bowel DLs-like account for 3.5% of OGIB, as assessed recently by Dulic-Lakovic *et al*^[2] in a large retrospective study based on enteroscopy findings in patients with OGIB. Enteroscopy, even if it is invasive, requires sedation and sometimes may be technically difficult, has proved to

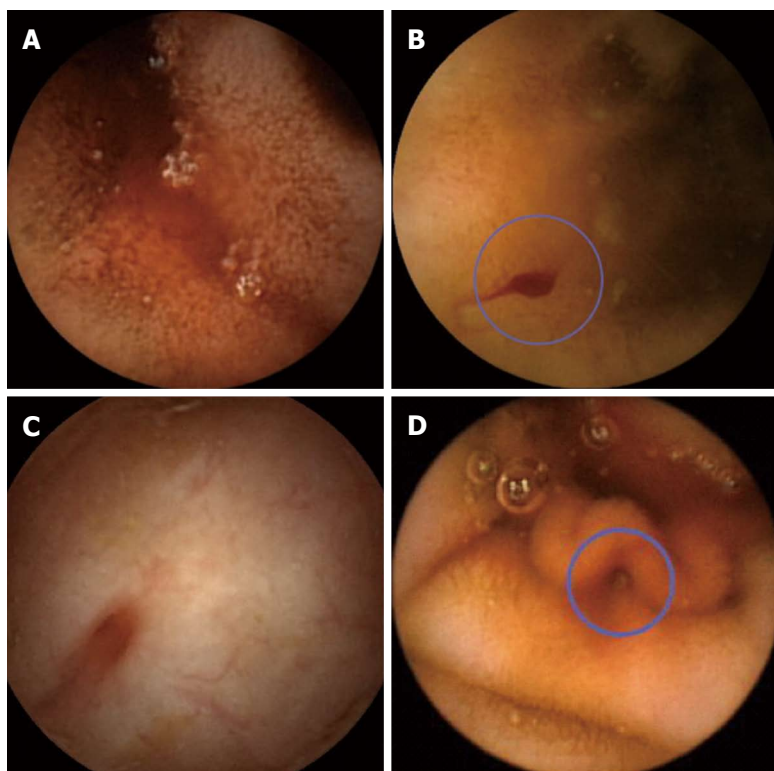


Figure 1 Capsule endoscopy. A: Significant amounts of fresh blood in the first jejunal loop; B: A very small mucosal break suggestive of Dieulafoy's lesion in the jejunum (circle); C: A bleeding mucosal break on the colonic mucosa; D: A small mucosal break in the proximal jejunum (circle).

be a very efficient diagnostic and therapeutic procedure for these lesions. Patients with small bowel DLs-like frequently require more than one enteroscopy until the source is identified and treated; a mean of 1.5 enteroscopies per case being reported^[2].

Previous case reports document a correct diagnosis of DLs-like achieved by VCE, with subsequently successful guided therapeutic procedures^[9-11]. All our cases also argue for the good performance of VCE in identifying and guiding therapy, even in hemodynamically unstable patients (cases 1 and 4). An important issue to consider in these patients is the timing of both VCE and enteroscopy. In active bleeding patients, with hemodynamically instability, VCE is not routinely used, as it may be considered time consuming; however, recent studies report a good diagnostic yield in identifying the site of acute bleeding in 75% of patients and the source of bleeding in 67% of cases^[6-8]. Based on these findings, successful guided therapy was performed in 73%-76% of patients^[7,8]. In these studies, no specific results or discussion are presented for DLs-like. Our patients presented clinical signs of active bleeding at the time of VCE, which substantially increased the chance for detecting blood or lesions. Capsule endoscopy images were interpreted immediately after the procedure and were followed by emergency endoscopy in the next few hours.

To the best of our knowledge, no previous report describes colon DL-like diagnosed with colon VCE. Previous studies reported missing bleeding lesions localized in the stomach or cecum, by conventional endoscopies and identified on capsule endoscopy^[14,15]. In case 3, previous colonoscopy did not find fresh blood in the colon, meaning that DL was inactive. A second colonoscopy guided

by findings on VCE enabled the correct identification of the lesion and treatment.

In our patients, after correct localization of the bleeding active site by VCE, only one interventional endoscopy per patient was needed for treatment (3 push enteroscopies and 1 colonoscopy), hemostasis being achieved by hemoclippping. VCE findings provided a very good selection for subsequent therapeutic procedures, reducing the number of interventional diagnostic maneuvers. Hemoclippping is a highly effective therapy, very well documented for gastric DLs-like^[16]. Angiography can identify and treat DLs by embolization, being used in difficult to treat cases^[3]. Surgery could be regarded as an alternative treatment only if the bleeding is not stopped by endoscopic or angiographic procedures^[3].

No pathogenetic association between NSAIDs and DLs-like has been identified in the literature^[17,18]. However, as DLs-like seem to be more frequent in older patients with significant comorbidities^[4] that may require NSAIDs and/or anticoagulants, it is assumed that concomitant administration of these agents prolongs and increases the severity of bleeding, as was observed in cases 1 and 4. In addition, differential diagnosis may be challenging, as NSAIDs enteropathy can display a wide spectrum of features from mucosal breaks to large bleeding ulcers^[19]. In actively bleeding NSAIDs enteropathy, multiple erosions or ulcers could be documented as necro-inflammation, representing the background pathology induced by these drugs^[20].

The limitations of this report are related to the retrospective analyses of cases and inclusions of patients with active OGIB, highly suspicious of DLs-like. Despite these limitations, this case series emphasizes the role of

capsule endoscopy in reducing the number of diagnostic enteroscopies in DLs-like.

In conclusion, VCE should be considered in active OGIB, even in hemodynamically unstable patients, because it is able to identify the site of bleeding and to guide therapeutic procedures.

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Endoscopic appearance of AIDS-related gastrointestinal lymphoma with *c-MYC* rearrangements: Case report and literature review

Shohei Tanaka, Naoyoshi Nagata, Sohtaro Mine, Toru Igari, Taiichi Kobayashi, Jun Sugihara, Haruhito Honda, Katsuji Teruya, Yoshimi Kikuchi, Shinichi Oka, Naomi Uemura

Shohei Tanaka, Naoyoshi Nagata, Department of Gastroenterology and Hepatology, National Center for Global Health and Medicine, Tokyo 162-8655, Japan

Sohtaro Mine, Toru Igari, Clinical Laboratory and Pathology, National Center for Global Health and Medicine, Tokyo 162-8655, Japan

Taiichi Kobayashi, Jun Sugihara, Haruhito Honda, Katsuji Teruya, Yoshimi Kikuchi, Shinichi Oka, Division of AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo 162-8655, Japan

Naomi Uemura, Department of Gastroenterology and Hepatology, National Center for Global Health and Medicine, Kohnodai Hospital, Chiba 272-0827, Japan

Author contributions: Tanaka S and Nagata N contributed equally to this work; Nagata N, Kobayashi T, Sugihara J, Honda H, Teruya K, Kikuchi Y, Oka S and Uemura N helped to edit the manuscript; Mine S and Igari T provided discussion of the pathological features; Tanaka S and Nagata N wrote of the paper.

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Correspondence to: Dr. Naoyoshi Nagata, MD, Department of Gastroenterology and Hepatology, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. nnagata_ncgm@yahoo.co.jp

Telephone: +81-3-32027181 Fax: +81-3-32071038

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Abstract

Acquired immune deficiency syndrome (AIDS)-related lymphoma (ARL) remains the main cause of AIDS-related deaths in the highly active anti-retroviral therapy (HAART) era. Recently, rearrangement of *MYC* is associated with poor prognosis in patients with diffuse large B-cell lymphoma. Here, we report a rare case of gastrointestinal (GI)-ARL with *MYC* rearrangements and coinfecting with Epstein-Barr virus (EBV) infection

presenting with various endoscopic findings. A 38-year-old homosexual man who presented with anemia and was diagnosed with an human immunodeficiency virus infection for the first time. GI endoscopy revealed multiple dish-like lesions, ulcerations, bloody spots, nodular masses with active bleeding in the stomach, erythematous flat lesions in the duodenum, and multiple nodular masses in the colon and rectum. Magnified endoscopy with narrow band imaging showed a honeycomb-like pattern without irregular microvessels in the dish-like lesions of the stomach. Biopsy specimens from the stomach, duodenum, colon, and rectum revealed diffuse large B-cell lymphoma concomitant with EBV infection that was detected by high tissue EBV-polymerase chain reaction levels and Epstein-Barr virus small RNAs *in situ* hybridization. Fluorescence *in situ* hybridization analysis revealed a fusion between the immunoglobulin heavy chain (IgH) and *c-MYC* genes, but not between the IgH and BCL2 loci. After 1-mo of treatment with HAART and R-CHOP, endoscopic appearance improved remarkably, and the histological features of the biopsy specimens revealed no evidence of lymphoma. However, he died from multiple organ failure on the 139th day after diagnosis. The cause of his poor outcome may be related to *MYC* rearrangement. The GI tract involvement in ARL is rarely reported, and its endoscopic findings are various and may be different from those in non-AIDS GI lymphoma; thus, we also conducted a literature review of GI-ARL cases.

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Key words: Acquired immune deficiency syndrome-related lymphoma; Non-Hodgkin-lymphoma; Epstein-Barr virus infection; *c-MYC* rearrangement; Endoscopic appearance

Core tip: Endoscopic findings in gastrointestinal-ac-

quired immune deficiency syndrome (GI-AIDS) related lymphoma (ARL) are miscellaneous and may be different from non-AIDS GI lymphoma. We report a rare case of GI-ARL with *MYC* rearrangements and coinfecting with Epstein-Barr virus infection, and there are multiple findings involving stomach, duodenum, and colon and rectum. Magnified endoscopy with narrow band imaging showed a honeycomb-like pattern without irregular microvessels in the dish-like lesions of the stomach. Moreover we conducted literature review of GI-ARL. To our knowledge, this is the first report of GI-ARL with *MYC* arrangements and presenting an atypical endoscopic appearances.

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INTRODUCTION

Non-Hodgkin-lymphoma (NHL) occurs in 5%-10% of individuals with an human immunodeficiency virus (HIV) infection. The early diagnosis of acquired immunodeficiency syndrome (AIDS)-related lymphoma (ARL) is highly important because patients with ARL tend to exhibit an aggressive clinical course, short survival, and poor treatment response. Chromosomal translocations of 8q24, encoding the *c-myc* oncogene, are considered to be associated with NHL oncogenesis, and are normally seen in patients with Burkitt lymphoma^[1]. Recently, *MYC* rearrangements have been seen occasionally in diffuse large B-cell lymphoma (DLBCL) and are associated with a poor prognosis^[2]. Here, we report a rare case of gastrointestinal (GI)-ARL with *MYC* rearrangements and an Epstein-Barr virus (EBV) infection presenting with various endoscopic findings. As the endoscopic findings in ARL are variable and may be different from those of non-AIDS GI lymphoma, we conducted a literature review of GI-ARL cases.

CASE REPORT

A 38-year-old homosexual man was admitted to our hospital with shortness of breath and multiple lymphadenopathy. He was diagnosed with an HIV infection for the first time. Physical examination showed slight upper abdominal tenderness, hepatomegaly, and splenomegaly without watery or bloody stools. Blood sample tests showed a low CD4 lymphocyte count (240 cells/ μ L), high quantity of HIV RNA (2.9×10^7 copies/mL), anemia (hemoglobin, 93 g/L), high lactate dehydrogenase (4.882 U/L), low serum albumin (24 g/L), and high EBV-PCR levels (9.0×10^5 copies/ μ g DNA). The patient was

Helicobacter pylori (*H. pylori*) negative with the titer of the *H. pylori*-antibody under 3.0 U/mL. To diagnose anemia, we performed upper and lower GI endoscopy and revealed multiple dish-like elevated lesions (Figure 1A and B), bloody spots (Figure 1C), and ulcers in the body of the stomach (Figure 1D). Magnification endoscopy with narrow band imaging (NBI) showed a honeycomb-like pattern at the edge of the elevated lesions (Figure 1E), but there was no irregular microvascular pattern in the ulcers (Figure 1F). We also found erythematous flat lesions in the duodenum (Figure 1G). Lower GI endoscopy showed multiple nodular masses in the colon and rectum (Figure 1H). Biopsy from these lesions revealed pleomorphic, atypical lymphoid cells with eosinophilic cytoplasm, marked nucleoli, and vesicular nuclei with hematoxylin and eosin staining (Figure 2). Immunohistochemistry showed positive staining for CD20, CD79a, CD38, MUM-1, BCL2, CD30, EMA, and latent membrane protein 1 and no staining for CD138, BCL6, and CD10 (Figure 2). Biopsy specimens from the upper and lower GI tract also revealed positive EBER *in situ* hybridization and high EBV-PCR levels (100000 copies/ μ g DNA). We also conducted a biopsy from the right inguinal lymph node. Fluorescence *in situ* hybridization analysis revealed fusion between the immunoglobulin heavy chain (IgH) and *c-MYC* genes, but not between the IgH and BCL2 loci. Computed tomography showed splenomegaly, slight hepatomegaly, and lymphadenopathy. Positron emission tomography detected radioisotope uptake within the bone marrow, lymph nodes, spleen, and gallbladder. The final diagnosis was DLBCL clinical stage 4B, according to the Ann Arbor Staging Classification for Lymphomas, and concomitant with an EBV infection. The patient was administered oral highly active anti-retroviral therapy (HAART) and R-CHOP chemotherapy. After 1 mo of treatment, the endoscopic appearance of the elevated lesions, blood spots, and ulcers had improved. The histological features of the biopsy specimens revealed no evidence of NHL. However, after 7 cycles of R-CHOP chemotherapy, blood sample tests showed high levels of lactate dehydrogenase (2568 U/L), hyperferritinemia (31810 ng/mL), and cytomegalovirus (CMV)-PCR (200 copies/ μ g DNA). Bone marrow aspiration revealed infiltration by activated histiocytes and hemosiderin-filled macrophages. The patient showed CMV viremia, tumor lysis syndrome, and hemophagocytic syndrome. He died of multiple organ failure on the 139th day after diagnosis.

DISCUSSION

The incidence of ARL has not decreased over time despite the widespread use of chemotherapy and the improved management of long-term HAART^[3]. Furthermore, ARL remains the most frequently observed AIDS-defining event leading to death^[3]. Therefore, the early diagnosis of ARL is very important. In 5%-10% of cases with DLBCL with typical morphology, a *MYC* rearrangement (*MYC*+) is also observed, and these cases are considered a sub-category of DLBCL^[2]. The presence of

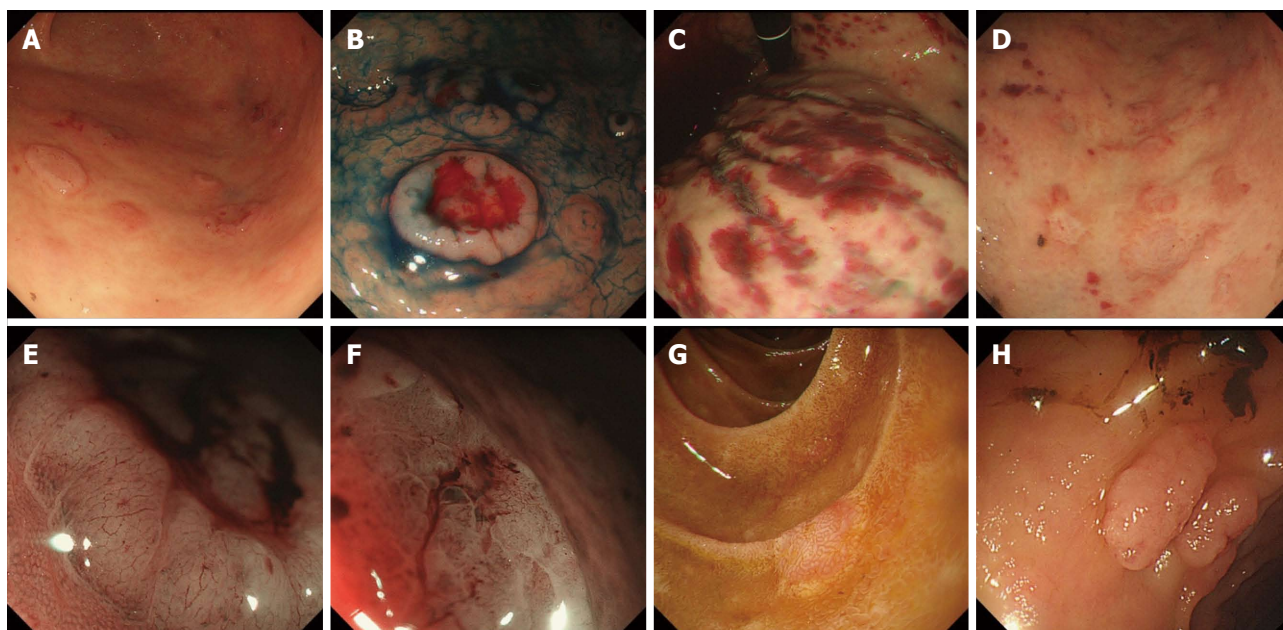


Figure 1 Upper and lower gastrointestinal endoscopic findings. A: Multiple elevated lesions in the body of the stomach; B: Multiple dish-like lesions with bleeding dyed with indigo carmine; C: Bloody spots in the body of the stomach; D: Ulceration with bleeding in the upper body of the stomach; E: Narrow band imaging (NBI) with magnification showing a honeycomb-like pattern at the edge of the elevated lesion; F: Irregular microsurface pattern in ulceration with NBI; G: Erythematous flat lesions in the duodenum; H: Multiple nodular masses in the colon and rectum.

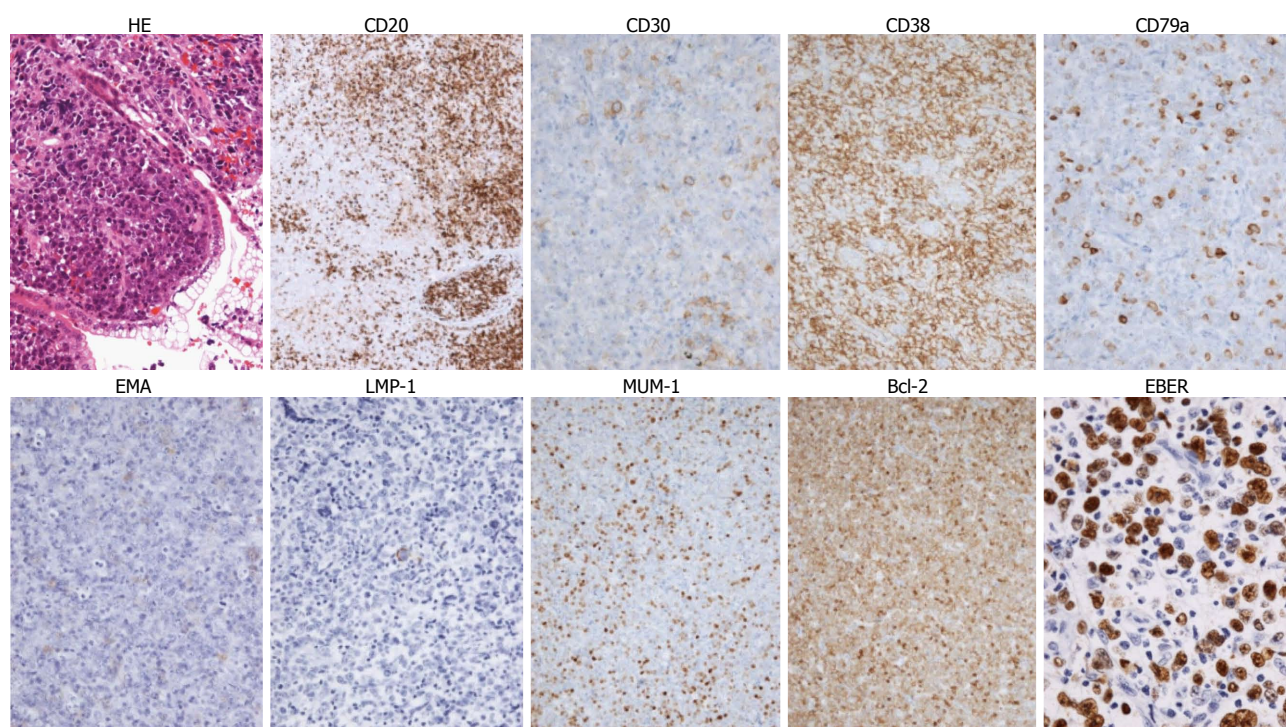


Figure 2 Histological findings and immunostaining of the biopsy specimen. Pleomorphic, atypical lymphoid cells with eosinophilic cytoplasm, marked nucleoli, and vesicular nuclei with hematoxylin and eosin staining ($\times 10$). Immunohistochemistry shows positive staining for CD20 ($\times 4$), CD30 ($\times 20$), CD38 ($\times 10$), CD79a ($\times 20$), EMA ($\times 10$), LMP-1 ($\times 10$), MUM-1 ($\times 10$), and BCL2 ($\times 10$). Biopsy specimens revealed positive EBER-*in situ* hybridization ($\times 40$). LMP-1: Latent membrane protein 1; MUM-1: Multiple myeloma oncogene 1; EMA: Epithelial membrane antigen.

a *MYC* rearrangement is associated with inferior overall survival and there is a trend for a reduction in event-free survival^[4]. Our case had a poor prognosis in spite of their temporary clinical and endoscopic improvement after chemotherapy. We suggest that the aggressive course of

his illness was related to *MYC* rearrangement.

Endoscopy with biopsy is essential to make a definite diagnosis of GI-ARL; however, it is usually indicated for patients with GI symptoms. In our case, he had only slight tenderness, but we performed endoscopy to inves-

Table 1 Literature review of the endoscopic findings in acquired immune deficiency syndrome-related lymphoma patients

Ref.	Patients	Involved GI tract	Endoscopic findings
Friedman ^[9]	NHL (<i>n</i> = 12)	Esophagus, stomach, duodenum, small bowel, colon, rectum	Multiple bulky tumor masses, small polypoid lesions
Heise <i>et al</i> ^[10]	NHL (<i>n</i> = 48)	Esophagus, stomach, duodenum, small bowel, colon, rectum	Bulky tumor mass, polypoid lesions, deep well-defined ulceration, necrotic abscesses
Corti <i>et al</i> ^[11]	Burkitt lymphoma (45-yr man)	Duodenum	Pseudo-polypoid masses
Andrews <i>et al</i> ^[12]	NHL (<i>n</i> = 30)	Stomach, small bowel, colon	Tumor mass, ulceration, nodular lesions
Mani <i>et al</i> ^[13]	Plasmablastic lymphoma (40-yr man)	Esophagus, stomach	Tumor mass, ulceration
Mahmoudi <i>et al</i> ^[14]	Lymphoma (38-yr woman)	Stomach	Ulceration, non-specific inflammation
Chow <i>et al</i> ^[15]	41-yr woman	Esophagus	A flat and solitary ulcer
Cappell <i>et al</i> ^[16]	GI lymphoma in AIDS patients (<i>n</i> = 6)	Esophagus, stomach, duodenum	Multiple volcano-like masses, ulceration, stricture with ulceration, nodular lesions, tumor mass, large sessile polypoid lesions
Yehya <i>et al</i> ^[17]	44-yr man	Colon	Mass with ulceration
Rezende <i>et al</i> ^[18]	NHL in AIDS patients (<i>n</i> = 6)	Stomach	Polypoid lesions, ulceration
Fujita <i>et al</i> ^[19]	41-yr man	Stomach	Multiple submucosal tumors with ulceration
Nakazuru <i>et al</i> ^[20]	68-yr man	Duodenum	Well-defined ulceration, auricle-like shaped mass with scattered tiny white spots

NHL: Non-Hodgkin-lymphoma; GI: Gastrointestinal.

tigate the cause of his anemia, which enabled us to make a diagnosis. The knowledge of the distinctive endoscopic appearance of GI-ARL also leads to an early diagnosis, but its detailed characteristics remain unknown. Therefore, we reviewed the English literature in the MEDLINE database by searching with the key words “HIV”, “lymphoma”, and “endoscopy” (Table 1). We identified 65 reports, but there was no description of endoscopic findings in 53; finally, we selected 12 reports.

The most frequently involved GI sites are the stomach (24%), small bowel (10%), and colon/rectum (7%). The most common site of involvement in the colon is the cecum (45%-75%), followed by the rectum^[5]. How-

ever, in immunocompetent patients, GI lymphomas are found in the stomach in 50%-80% of all cases and rarely in the colon, small bowel, or perianal region^[5]. There are a wide range of endoscopic findings in ARL. Findings of multiple, polypoid lesions, nodular masses, bulky tumor mass, or ulcerations are commonly found. Our search of the literature revealed no findings of multiple bloody spots with exudates or flat erythematous lesions in GI lymphoma. Previous study has shown that *H. pylori*-induced T-cell plays an important role in gastric mucosa-associated lymphoid tissue lymphoma development^[6]. However, *H. pylori* was negative in this case, thus other mechanisms have been suggested. We believed that EBV activation caused by the immunosuppression in AIDS patients plays a role in the pathogenesis of the various and unique endoscopic findings, such as multiple nodular masses, bloody spots, and ulcers, because high EBV DNA blood levels are considered to be associated with the development of NHL^[7].

In this case, we also perform magnified endoscopy using NBI. NBI endoscopy for diagnosing superficial gastric cancer is accurate for the diagnosis of cancer when the diagnostic triad of the disappearance of fine mucosal structures, microvascular dilation, and heterogeneity is used^[8]. Our case did not satisfy this triad, suggesting that NBI endoscopy is a useful technique for the differential diagnosis of epithelial tumors and non-epithelial tumors.

Endoscopic biopsy is highly valuable to diagnose GI-ARL. We need to re-realize that GI-ARL can present with various endoscopic findings and involve the entire GI tract, which may be different from non-AIDS GI lymphoma due to differences in the immune status of patients with these conditions. NBI endoscopy may be used to differentiate GI-ARL from gastric cancer.

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Laparoscopic cholecystectomy in patients with anesthetic problems

Bum-Soo Kim, Sun-Hyung Joo, Jin-Hyun Joh, Jae-Woo Yi

Bum-Soo Kim, Sun-Hyung Joo, Jin-Hyun Joh, Jae-Woo Yi, Department of Surgery, School of Medicine, Kyung Hee University, Seoul 134-727, South Korea

Jae-Woo Yi, Departments of Surgery and Anesthesiology and Pain Medicine, School of Medicine, Kyung Hee University, Seoul 134-727, South Korea

Author contributions: Yi JW performed the anesthesia; Kim BS and Joo SH performed the operations; Kim BS wrote the manuscript; Joh JH provided advice during manuscript preparation.

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Correspondence to: Sun-Hyung Joo, MD, PhD, Department of Surgery, School of Medicine, Kyung Hee University, 149 Sangil-dong, Gangdong-gu, Seoul 134-727, South Korea. sunhyung@chol.com

Telephone: +82-2-4406135 Fax: +82-2-4406295

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considered as a valid option for patients with gallbladder disease who are poor candidates for general anesthesia due to cardiopulmonary problems. We report three cases of laparoscopic cholecystectomy successfully performed in patients with anesthetic problems that included cardiopulmonary disease, severe kyphosis, and ankylosing spondylitis.

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Key words: Laparoscopic cholecystectomy; Kyphosis; Ankylosing spondylitis; Anesthetic problems

Core tip: This paper reported three cases of laparoscopic cholecystectomy successfully performed in patients with anesthetic problems that included cardiopulmonary disease, severe kyphosis, and ankylosing spondylitis. This study demonstrates that regional anesthesia can be considered as a valid option for patients with gallbladder disease who are poor candidates for general anesthesia due to cardiopulmonary problems.

Kim BS, Joo SH, Joh JH, Yi JW. Laparoscopic cholecystectomy in patients with anesthetic problems. *World J Gastroenterol* 2013; 19(29): 4832-4835 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v19/i29/4832.htm> DOI: <http://dx.doi.org/10.3748/wjg.v19.i29.4832>

Abstract

Laparoscopic cholecystectomy is a standard operation for benign gallbladder disease. As experience with laparoscopic cholecystectomy has increased, the procedure has become possible in patients with anesthetic problems. Patients with ankylosing spondylitis or severe kyphosis represent a challenging group to anesthesiologists and laparoscopic surgeons since these diseases are associated with difficult intubation, restrictive ventilatory defects, and cardiac problems. The relatively new approach of awake fiberoptic intubation is considered to be the safest option for patients with anticipated airway difficulties. Laparoscopic cholecystectomy is usually performed under general anesthesia but considerable difficulties in anesthetic management are encountered during laparoscopic surgery; for example, hemodynamic instability may develop in patients with cardiopulmonary dysfunction due to pneumoperitoneum and position changes during the operation. Nonetheless, regional anesthesia can be

INTRODUCTION

Laparoscopic cholecystectomy is a standard procedure for the management of gallbladder disease. As experience with laparoscopic cholecystectomy has increased, laparoscopic cholecystectomy in patients with anesthetic problems has become possible. Ankylosing spondylitis is a challenge to the anesthesiologist and to the laparoscopic surgeon because it is associated with difficult intuba-

tion, restrictive ventilatory defects, and cardiac problems. Kyphotic patients may also suffer considerable perioperative morbidity such as cardiac problems and respiratory insufficiency. While laparoscopic cholecystectomy is classically performed under general anesthesia, regional anesthesia must be considered as a valid option for patients with gallbladder disease who are poor candidates for general anesthesia. We report three cases of laparoscopic cholecystectomy successfully performed in patients with anesthetic problems that included cardiopulmonary disease, severe kyphosis, and ankylosing spondylitis. All patients were informed about the operative and anesthetic procedures and provided consent.

CASE REPORT

Case 1

A 71-year-old, American Society of Anesthesiology (ASA) physical status III male patient (168 cm, 56 kg) underwent laparoscopic cholecystectomy due to acute cholecystitis with cholelithiasis. The patient had undergone a right pneumonectomy nine years previous to this procedure (Figure 1). A standard pulmonary function test revealed a moderate obstructive and restrictive pattern. Hypokinesia of the apical anterior and septum segments was observed on echocardiography. Pulmonology consultation reported a moderate postoperative risk that could be fatal if postoperative pneumonia developed. Cardiology consultation reported a postoperative risk of 1%-5% based on modified Goldman cardiac risk criteria^[1]. Because severe postoperative complications were anticipated, segmental spinal anesthesia was performed and CO₂ inflation to an intra-abdominal pressure of 8 mmHg. Laparoscopic cholecystectomy was performed; the procedure was smooth and uneventful. The patient was discharged 3 d after surgery.

Case 2

A 42-year-old male patient (173.6 cm, 68.9 kg) suffering from ankylosing spondylitis was admitted to our hospital presenting with right upper quadrant pain. Abdominal ultrasound revealed a 2 cm sized cholelithiasis for which he was scheduled to undergo laparoscopic cholecystectomy. The patient was ASA III and Mallampati grade IV on airway examination. The patient had fixed rigidity of the cervical spine and could not lie supine (Figure 2A) and his mouth opening was restricted. Accordingly, he was placed in a supine position with three pillows under his head. The anesthesiology team decided to perform fiberoptic intubation through the nasotracheal tree while the subject was conscious (Figure 2B). The patient was informed of an anticipated difficult orotracheal intubation. Laparoscopic cholecystectomy was performed without any complication. There were no perioperative complications related to airway management, and the patient was discharged 3 d after surgery.



Figure 1 Chest posterior to anterior of case 1. The patient had undergone a right pneumonectomy.



Figure 2 A 42-year-old male patient suffering from ankylosing spondylitis was admitted to our hospital presenting with right upper quadrant pain. A: Operating position used for case 2; B: Awake fiberoptic intubation through the nasotracheal tree used for case 2.

Case 3

A 54-year-old female patient (140 cm, 45.6 kg) with ASA physical status III suffering from severe thoracolumbar kyphosis (Figure 3) presented with right upper quadrant pain. The patient was diagnosed with acute cholecystitis on abdominal ultrasound and was scheduled to undergo laparoscopic cholecystectomy. Moderate restrictive pattern was found following a pulmonary function test. The patient could not lie supine and needed thick pillows beneath her head for support. Her thoracic cage was tilted anteriorly resulting in a low-ly-

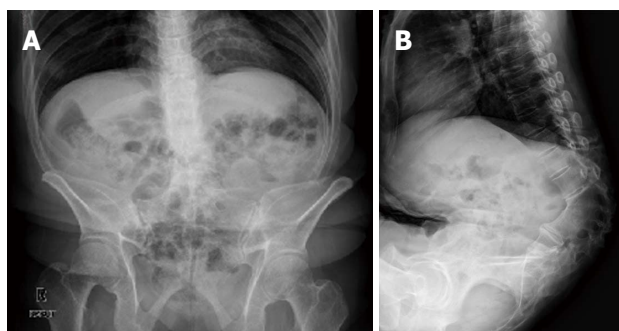


Figure 3 Thoracolumbar spine image for case 3. The anteroposterior (A) and lateral (B) views are shown.

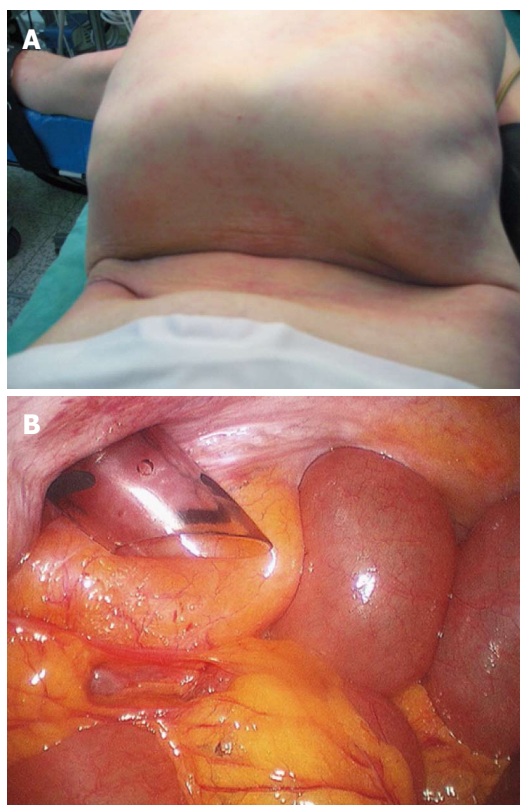


Figure 4 Insertion of the trocar in case 3. A: The narrow abdomen provided a challenge for trocar insertion; B: Internal view of trocar placement in the narrow abdomen.

ing costal margin and narrow abdomen. It was difficult to insert trocars because of narrow abdomen (Figure 4). CO₂ was used to inflate her intra-abdominal pressure to 8 mmHg. The patient underwent laparoscopic cholecystectomy successfully and discharged from hospital at 5 d after surgery.

DISCUSSION

Considerable difficulties in anesthetic management are encountered during laparoscopic surgery. Hemodynamic instability in patients with cardiopulmonary dysfunction may develop due to pneumoperitoneum and position changes during the operation. While laparoscopic

cholecystectomy is usually performed under general anesthesia, regional anesthesia has been used in special high-risk cases^[2]. However, laparoscopic cholecystectomy under general anesthesia has been reported as safe and feasible even in high-risk patients^[3]. Here, we show that carbon dioxide pneumoperitoneum with an intra-abdominal pressure of 8–12 mmHg for high-risk patients can be safe if the patients are well monitored and hemodynamic instability is actively treated. However, the patient in case 1 was at risk of post-operative death if pneumonia developed in the remnant lung, and was scheduled for the regional anesthesia. Due to the harmful effects of high intra-abdominal pressure, it is recommended that the lowest pressure possible that allows adequate exposure of the operative field be used^[4]. A laparoscopic cholecystectomy was successfully carried out by maintaining CO₂ pressure < 8 mmHg during the operation with the active participation of the attending anesthesiologists.

Ankylosing spondylitis is a chronic and progressive inflammatory disease involving articulations of the spine and adjacent tissue. Oral intubation is difficult in ankylosing spondylitis patients with cervical spine rigidity. Recently, awake fiberoptic intubation has been increasingly used, and considered to be the safest option for patients with anticipated airway difficulties^[5,6]. To secure the airway, we used awake fiberoptic intubation in case 2 with written informed consent of the patient. In addition to airway management, anesthesiologists need to be concerned with restrictive ventilator defects and airway problems associated with ankylosing spondylitis. Therefore, preoperative examination for cardiovascular and pulmonary function is required^[7].

Kyphosis is a deformity of the spine. Anesthesia for kyphosis is common with ankylosing spondylitis is often challenging. There is usually secondary involvement of the respiratory, cardiovascular, and neurologic systems. A detailed pre-anesthetic assessment and optimization of the respiratory and cardiovascular systems should be done^[8]. It is desirable to maintain the lowest intra-abdominal pressure to prevent the adverse hemodynamic effect of pneumoperitoneum in restrictive lung disease. Another concern for the laparoscopic surgeon for a patient with kyphosis is positioning of the patient and surgical approach to the abdomen. Increasing numbers of older patients are presenting with benign gallbladder disease with senile kyphosis caused by aging or osteoporosis. Laparoscopic surgery is minimally invasive, and in severely kyphotic patients the procedure may prove difficult in approaching the narrow abdomen. In the third case, we performed this difficult surgery because the chest of the patient was tilted anteriorly and the abdominal cavity was narrowed by low-lying costal margin. Abnormalities of the spine can alter the contour of the pelvic vessels. Kyphosis, scoliosis, and the other skeletal abnormalities can affect vascular anatomy and must be considered during trocar insertion^[9]. Severe senile kyphosis is not a contraindication for laparoscopic surgery^[10].

Patients with spinal deformity such as ankylosing spondylitis and kyphosis form a challenging group to anesthesiologists and laparoscopic surgeons because of difficulties in intubation, positioning, and abdominal approaches. Careful preoperative anesthetic assessment for respiratory dysfunction, cardiac impairment, difficult airway, and associated specific problems in patients with ankylosing spondylitis or kyphosis is imperative. In patients with severely compromised respiratory function the final surgical plan should be a joint decision between surgeon and anesthesiologist. The patient and family should be warned about the possibility of postoperative ventilation and prolonged respiratory weaning and support.

As newer and more extensive laparoscopic procedures have been implemented, close cooperation between the laparoscopic surgeon and the anesthesiologist is required. We have performed laparoscopic cholecystectomy in seven patients with kyphosis. No perioperative complications occurred. While several studies^[11] about obstetrics and gynecology and spine surgery in patients with ankylosing spondylitis and kyphosis have been reported, only one report has addressed laparoscopic cholecystectomy^[12,13].

In conclusion, we successfully performed three cases of laparoscopic cholecystectomy in patients with anesthetic problems including cardiopulmonary disease, severe kyphosis, and ankylosing spondylitis.

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Idiopathic chronic ulcerative enteritis with perforation and recurrent bleeding: A case report

Xia Gao, Zhen-Jun Wang

Xia Gao, Department of General Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China
Zhen-Jun Wang, Department of General Surgery, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

Author contributions: Gao X and Wang ZJ designed the case report; Gao X wrote the paper; Wang ZJ revised the paper.

Correspondence to: Zhen-Jun Wang, MD, Department of General Surgery, Beijing Chaoyang Hospital, Capital Medical University, 8 Gongtinan Road, Chaoyang District, Beijing 100020, China. wang3zj@yahoo.com.cn

Telephone: +86-10-58268538 Fax: +86-10-65131244

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Abstract

Idiopathic chronic ulcerative enteritis (ICUE) is a distinct entity without a defined etiology and is rarely seen in the clinic. Patients with ICUE mainly present with insidious abdominal symptoms such as chronic abdominal pain and intermittent gastrointestinal hemorrhage and symptoms of malnourishment in the early stages of the disease. ICUE is always difficult to diagnose. However, as the disease progresses, patients have a variety of acute abdominal complications such as hemorrhage, perforation, or ileus. Surgical intervention is always needed, and the condition can recur and require repeat laparotomy. When diffuse ulceration of the small bowel is present in the absence of recognizable causes, it is classified as nonspecific or idiopathic. The histological examination always demonstrates an acute, chronic inflammatory infiltration without giant cells, granulomas, or villous atrophy. The etiology of ICUE has not been identified, and its pathogenesis is poorly understood; therefore, radical surgical resection is considered the best available treatment. Here, we report a case of ICUE characterized by nonspecific, multiple, small intestinal ulcers resulting in perforation and recurrent bleeding. The differential diagnosis and the treatment

are also discussed.

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Key words: Idiopathic ulcerative enteritis; Small intestinal ulcer; Perforation; Gastrointestinal hemorrhage; Bleeding.

Core tip: This is a case report of idiopathic chronic ulcerative enteritis (ICUE) with perforation and recurrent bleeding. Complete clinical material of the patient is presented in this case report. We also provide several images, including colonoscopy, radiography, computed tomography, and pathology. All of this material reflected the clinical characteristics of ICUE. When patients present with multiple, nonspecific, small intestinal ulcers without a defined etiology, a diagnosis of ICUE should be considered. This case report will help diagnose and treat patients with similar clinical symptoms.

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INTRODUCTION

Idiopathic chronic ulcerative enteritis (ICUE) is uncommon. It was first described by Nyman in 1949 as a kind of ulcerous jejunoileitis^[1]. Since then, numerous descriptive terms have been used, including ulcerative jejunitis^[2], chronic ulcerative jejunoileitis^[3], nongranulomatous ulcerative jejunoileitis^[4], and chronic nonspecific ulcerative duodenojejunoileitis^[5]. These terms all attempt to describe a type of small bowel ulcerative disorder in the absence of a recognizable cause. Patients with ICUE present with hemorrhage, perforation, or obstruction and often need

emergency surgical intervention. The pathogenesis of ICUE is not clear, and it is associated with a high mortality rate^[6], therefore, surgical treatment remains a major challenge in clinical practice. Thus far, radical surgical resection is considered the best available treatment^[7].

Here, we report a rare case of ICUE characterized by nonspecific, multiple, small intestinal ulcers resulting in perforation and recurrent bleeding, and discuss the differential diagnostic considerations and treatment.

CASE REPORT

A 78-year-old man was admitted to the hospital with intermittent dull pain in the upper quadrant of his abdomen of > 2 mo duration. The pain occurred irregularly and had no obvious relationship with meals. He reported experiencing nausea, vomiting, heartburn, diarrhea, melena, anorexia, fatigue, and weight loss of 12.5 kg. No hematemesis, hematochezia, tenesmus, or stools with mucus or pus were reported. The patient had a history of intestinal resection after trauma 10 years before. His mother had died of bladder cancer. Gastroscopy showed reflux esophagitis, duodenitis, and superficial gastritis throughout the stomach. No abnormality was noted on colonoscopy. On admission, his temperature (37 °C) and pulse (78 beats/min and regular) were normal. However, his blood pressure was a little lower than average (90/45 mmHg). Physical examination revealed tenderness in the right upper quadrant of the abdomen, without rebound tenderness or guarding. The bowel sounds were normal. Several enlarged lymph nodes could be palpated bilaterally in the neck and axilla. The lymph nodes were hard, stationary, and painless.

After admission, the patient had intermittent diarrhea, recurrent subxiphoid pain, abdominal distension, and anorexia. Screening for markers of viral hepatitis (A-E), cytomegalovirus, Epstein-Barr virus, herpes simplex virus, and varicella zoster virus was negative. Stool cultures for the pathogens *Shigella*, *Salmonella*, *Vibrio cholerae*, *Mycobacterium tuberculosis* and *Clostridium difficile* (*C. difficile*), and *C. difficile* toxin A were negative. Stool culture was positive for intestinal *Escherichia coli* and enterococci, which accounted for 90% and 10% of normal intestinal flora, respectively. Multiple ulcers with a segmented pattern were noted in the terminal ileum on review of the colonoscopy (Figure 1). From the transverse to the sigmoid colon, the mucosa was rough and had erosive patches. The histopathology of biopsy specimens demonstrated acute and chronic nonspecific inflammation in the mucosa and submucosa, with a large quantity of plasma cell infiltration in the wall of the ileum, forming focal erosion and ulcers. The vasculature of the lamina propria was dilated and edematous. Radiographs of the whole digestive tract showed thickening of the terminal ileum mucosa, and peristalsis was poor (Figure 2), but the emptying function was normal. Abdominal computed tomography (CT) showed mild intestinal dilatation and wall thickening in the terminal ileum, and moderate ascites in the ab-

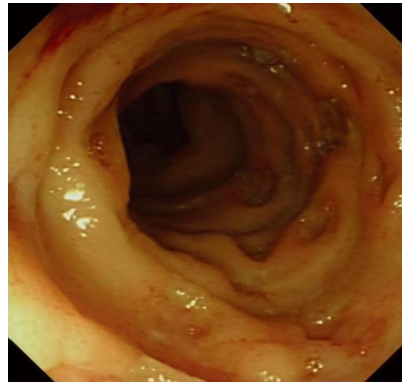


Figure 1 Colonoscopy demonstrated multiple ulcers with a segmented pattern in the terminal ileum.

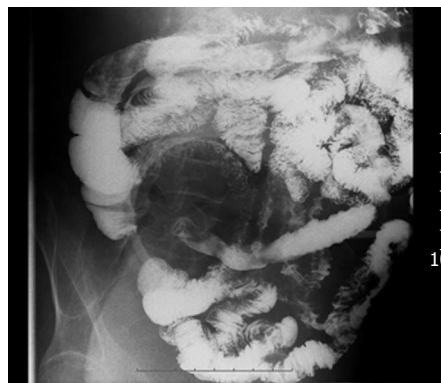


Figure 2 Radiography of the whole digestive tract showed that the terminal ileal mucosa was thickened and peristalsis was poor.

dominal cavity (Figure 3). The mesenteric vasculature was normal, and the mesenteric lymph nodes were considered to have reactive hyperplasia. The proton pump inhibitor omeprazole was given to suppress acid production and protect the gastric mucosa, alleviating symptoms caused by the gastrointestinal tract ulceration. Meanwhile, the patient was given nutritional support to improve his nutritional status, probiotics to regulate intestinal flora, and an infusion of albumin combined with diuretic therapy to relieve edema. One month later, hemafecia occurred and worsened. Moreover, the patient's general condition worsened, with anemia and hypoalbuminemia. On day 36 of hospitalization, his intermittent transient abdominal pain suddenly worsened. Symptoms of peritoneal irritation and severe distention of the entire abdomen emerged, with a rapid pulse and low blood pressure. The patient was diagnosed with diffuse peritonitis and septic shock. Emergency laparotomy and intraoperative endoscopy revealed multiple bleeding ulcers scattered in the ileum and perforation at the side of mesenteric margin. About 120 cm of the ileum had multiple bleeding ulcers, and the ulcerative perforation was resected. Although it had some superficial ulcers, the preserved proximal small intestine was not bleeding. Management was aimed at avoiding postoperative short bowel syndrome. The edematous small intestinal wall and poor nutritional sta-

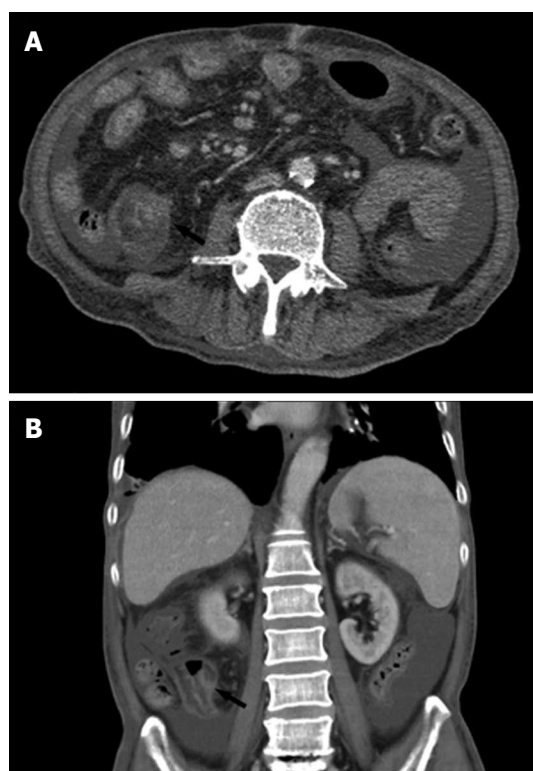


Figure 3 Abdominal computed tomography showed mild intestinal dilatation and wall thickening in the terminal ileum (marked with black arrow), and moderate ascites in the abdominal cavity. A: Horizontal slice; B: Coronal slice.



Figure 4 Multiple intestinal ulcers in the longitudinal section of the resected terminal ileum.

tus could have led to anastomotic leakage, therefore, a proximal and distal ileum double-lumen enterostomy was performed in the right lower quadrant of the abdomen.

Gross pathological examination was performed after the intestinal specimen was cut longitudinally. The intestinal ulcers were round or oval with a diameter of 0.3-1.5 cm. In some ulcers, the long axis was parallel to the intestinal long axis (Figure 4). A perforated ulcer could be seen at the side of the mesenteric margin. The mesentery was thick and edematous, with enlarged mesenteric lymph nodes. Postoperative histological examination demonstrated partial intestinal villous atrophy and multiple nonspecific ulcers in the terminal ileum (Figure

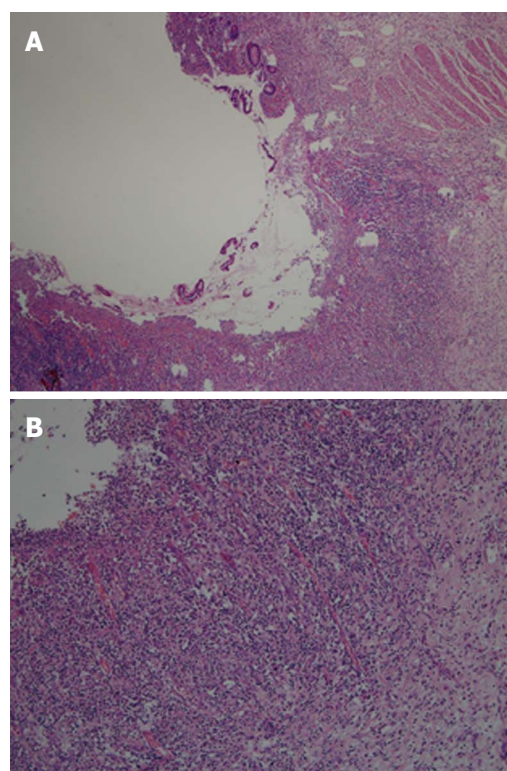


Figure 5 Histological examination. A: Glands of the small intestinal mucosa were necrotic and exfoliated, with intestinal villous atrophy and ulcer formation [hematoxylin and eosin (HE) stain, × 40]; B: Extensive small lymphocytes and plasma cell infiltration in the lamina propria, submucosa, and serosa of the terminal ileum (HE stain, × 100).

5A). The examination also showed chronic inflammatory infiltration, with extensive small lymphocytes and plasma cells in the lamina propria, submucosa, and serosa (Figure 5B). The mesenteric lymph nodes had histiocytes, which contained lymphocytes and plasma cells in the dilated sinuses. Phagocytosis similar to that of Rosai-Dorfman disease was noted. After surgery, the patient's vital signs were normal. However, on day 4, a large amount of bloody fluid began to leak from the ileal stoma. A blood transfusion and intravenous infusion did not alleviate symptoms of hemorrhagic shock. Bedside enteroscopy through the stoma showed multiple intestinal hemorrhages. Old and new ulcers were bleeding along 30 cm of the proximal intestine. Iced saline with norepinephrine and thrombin were used alternatively to irrigate the surface of the ulcers under enteroscopic guidance. The hemorrhage of the intestinal ulcers was controlled, but it recurred a few days later. Conservative treatment was continued. The patient's general condition gradually deteriorated due to recurrent bleeding. Ultimately, the patient died of pneumonia and respiratory functional failure.

DISCUSSION

A variety of clinical conditions can lead to small intestinal ulcerations. Common causes include ischemia, trauma, nutritional disturbance, immune disorders, infection, drugs, and hormones. However, these causes can be eas-

ily excluded through medical history and laboratory test results. Therefore, most patients with small bowel ulcers have recognizable pathology. After a wide differential diagnosis, only those without an underlying disorder can be diagnosed as having ICUE.

The symptoms of small intestinal ulcers are nonspecific and insidious in the early stages. Patients with small intestinal ulcers usually present with chronic abdominal pain, diarrhea, a positive fecal occult blood test, intermittent hemafecia or melena, and a variety of types of malnutrition, including iron deficiency anemia, hypoalbuminemia, and weight loss. The diagnosis is often delayed or overlooked because the small intestine is the part of the alimentary canal most remote from both the mouth and the anus. Consequently, it is the most difficult to investigate. Patients typically experience exacerbations and complications such as intestinal hemorrhage, perforation, or obstruction, which always require emergency surgical intervention. With development of medical techniques such as gastrointestinal radiography, double-balloon enteroscopy^[8], and capsule endoscopy^[9], small intestinal ulcers can be diagnosed more accurately before surgery.

The patient complained chiefly of chronic abdominal pain for 2 mo, with many nonspecific gastrointestinal symptoms and increasing malnutrition. Later, symptoms of small intestinal ulcer bleeding and perforation emerged. We ruled out infective, ischemic, and tumor-related diseases. According to the laboratory results, we first excluded bacterial infection. With no history of atherosclerosis, hyperlipidemia, congestive heart failure, or arrhythmia, combined with normal findings on enhanced CT and ultrasonography of mesenteric vessels, we also ruled out mesenteric ischemia. Although the superficial and abdominal lymph nodes were enlarged, we obtained biopsy specimens from multiple sites during endoscopy and bone marrow biopsy and conducted a marrow chromosome examination to rule out lymphoma.

We needed to differentiate ICUE from diseases such as Crohn's disease, Behcet's disease, nonsteroidal anti-inflammatory drug (NSAID)-induced enteropathy, and cryptogenic multifocal ulcerous stenosing enteritis (CMUSE) because they also can cause multiple small intestinal ulcers.

During surgery, we found that the multiple intestinal ulcers mainly diffused in the terminal ileum. The ulcers were sharply demarcated with a round or oval shape, and the intervening mucosa was normal. The diameter of the ulcers ranged from 0.3 to 1.5 cm, with varying depths. The superficial ulcers were limited to mucosa or submucosa, while the deep ones can reach the serosa and even form a transmural perforation. The lesions were different from those seen in Crohn's disease, which are generally longitudinal, with a cobblestone appearance and fistulas.

ICUE can also be differentiated to the intestinal involvement of Behcet's disease. In Behcet's disease, ulcers are usually found from terminal ileum to the cecum, with transmural inflammation extending to the serous membrane and crater-shaped ulcer margins. In addition, Behcet's disease usually has a triad of symptoms consisting

of aphthous stomatitis, genital ulcers, and ocular symptoms, which this patient did not have.

Since 1960, it has been recognized that NSAIDs can cause small intestinal ulcers. The macroscopic lesions of NSAID-induced enteropathy are characterized by multiple circumferential ulcers with severe concentric stenosis, referred to as "diaphragm disease"^[10]. The different pathological characteristics in the patient and no history of NSAID use excluded NSAID-induced enteropathy.

CMUSE also can cause nonspecific multiple intestinal ulcers. Perlemuter *et al*^[11] summarized the clinicopathological features of CMUSE as unexplained small intestinal strictures, superficial ulceration of the mucosa and submucosa, no biological signs of systemic inflammatory reaction, a chronic or recurrent clinical course even after surgery, and a positive response to use of corticosteroids. For our patient, the syndrome was characterized by recurrent intestinal bleeding and perforation, ulcers that were not limited to the submucosa, and no ulcerative stenosis or obstruction. Therefore, we excluded the diagnosis of CMUSE.

The etiology of ICUE is unknown, and symptoms of the disease are nonspecific and insidious. In the early stages, patients usually present with chronic abdominal pain and symptoms of malabsorption that do not respond to conservative treatments such as a gluten-free diet or adrenocorticosteroids. The clinical course could be severe and rapidly fatal due to complications such as hemorrhage, perforation, sepsis, and cachexia^[12]. Surgical treatment is always required, after which the condition may recur and require repeat laparotomy. In many reports, ICUE was diagnosed after several operations and exclusion of other underlying disorders. The diagnosis was confirmed by histological features characterized by the diffuse nongranulomatous ulcerations called multiple nonspecific small intestinal ulcers. Some patients survive after radical, aggressive surgical resection^[7]. In the present case, considering the advanced age and poor nutritional status of the patient and the risk of short bowel syndrome, part of the affected segment was preserved. Nevertheless, the conserved superficial ulcers deteriorated, and multiple new ulcers emerged; both were bleeding after surgery. Corticosteroid therapy is not effective in ICUE^[13], and it is never used in patients with gastrointestinal hemorrhage, therefore, we avoided corticosteroid therapy. The ulcers were deep and transmural, thus, we believed that multiple intestinal perforations would emerge as the disease progressed. Postoperative aggravation of the ulcerative injury in the small intestine precluded reoperation and led to patient's debility and death.

In conclusion, early diagnosis and treatment are important in ICUE. Radical surgical resection is considered the best available treatment for patients presenting with abdominal ulcerative complications such as hemorrhage, perforation, and obstruction, although the true etiology of ICUE is unknown. When patients present with multiple, nonspecific, small intestinal ulcers in the absence

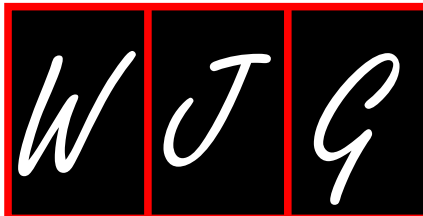
of well-documented causes, a diagnosis of ICUE should be considered. However, patients of advanced age with compromised nutritional status and severe complications cannot undergo aggressive surgery. In these patients, the prognosis is usually guarded.

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Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

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Editorial office

Jin-Lei Wang, Director

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World Journal of Gastroenterology

Room 903, Building D, Ocean International Center,
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Fax: +86-10-85381893

E-mail: wjg@wjgnet.com

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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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- 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]

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- 15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

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- 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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