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ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Dr. Naoki Hashimoto was awarded his medical degree from Kobe University in 1975 and his PhD from Hyogo Medical College in 1984. Over the last 10 years, his scientific interest has remained focused on topics related to reflux of duodenal contents inducing esophageal carcinogenesis, with his research efforts including both experimental and clinical approaches. His practical expertise encompasses biomedical imaging, surgical treatment and chemoradiotherapy for advanced esophageal cancer, and he practices in the Kindai University's Department of Surgery. He is the recipient of many academic honors, from such esteemed groups as European Conference on General Thoracic Surgery in 2012 and World Organization for Specialized Studies of Diseases of the Esophagus (OESO) in 2013 and 2015. His academic career embodies a continual pursuit towards conducting more innovative, translational and enduring research.

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Regulation of the intestinal microbiota: An emerging therapeutic strategy for inflammatory bowel disease

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Abstract

The rapid development of metagenomics, metabolomics, and metatranscriptomics provides novel insights into the intestinal microbiota factors linked to inflammatory bowel disease (IBD). Multiple microorganisms play a role in intestinal health; these include bacteria, fungi, and viruses that exist in a dynamic balance to maintain mucosal homeostasis. Perturbations in the intestinal microbiota disrupt mucosal homeostasis and are closely related to IBD in humans and colitis in mice. Therefore, preventing or correcting the imbalance of microbiota may serve as a novel prevention or treatment strategy for IBD. We review the most recent evidence for direct or indirect interventions targeting intestinal microbiota for treatment of IBD in order to overcome the current limitations of IBD therapies and shed light on personalized treatment options.

Key words: Inflammatory bowel disease; Pro/Prebiotics; Fecal microbiota transplantation; Herbal medicines; Clinical application

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Core tip: In this review, we explore therapies targeting intestinal microbiota, such as fecal bacteria transplantation, pro/prebiotics, and herbal medicinal products, that represent effective therapeutic options to control and slow the progression of inflammatory bowel disease (IBD). We also discuss some challenges and controversies in relation to these emerging therapeutic strategies. This has direct inspiration for researchers to overcome the current limitations of IBD therapies and shed light on personalized treatment options.

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INTRODUCTION

Inflammatory bowel disease (IBD), which has been listed by the World Health Organization as one of the most refractory diseases, includes ulcerative colitis (UC) and Crohn's disease (CD) and shows a continually increasing incidence^[1]. Although genetic, epigenetics, immunological, microbial, and environmental factors are involved in the etiology of IBD, none have been identified as the explicit and direct cause of IBD^[2,3]. A generally accepted perspective is that the gut microbiota is affected by environmental factors (*e.g.*, diet, medications, smoking, and contaminants) that further impact the host immune response, contributing to the occurrence and development of IBD^[4]. It is thus clear that gut microbiota represent a link between environmental factors and the immune response^[5]. Recent studies have found that lack of intestinal microorganisms during early childhood influences the maturation and tolerance of the intestinal immune system, thus increasing IBD risk in adulthood^[6]. In addition, the defects of several pattern recognition receptors genes, such as toll-like receptors and nod-like receptors genes, lead to disturbances of innate immunity, which can ultimately reduce the host tolerance against intestinal microorganisms^[7]. Therefore, healthy gut microbiota are vital for intestinal health.

It has been confirmed that the intestine has rich microbial abundance, which includes enteric bacteria (99.1% of the gut microflora), archaea (the majority of the remainder), as well as only 0.1% of fungi and viruses^[8,9]. The total number of microorganisms present is more than 10 times the total number of human cells^[10]. The intestinal microbiota is dominated by *Firmicutes* (49%-76%) and *Bacteroidetes* (16%-23%) phyla, while others are less abundant bacterial phyla. The main fungal microbiota in intestinal tract are *Ascomycota* and *Basidiomycota* phyla^[11]. The enteric virome includes all nucleic acids (DNA and/or RNA) that mapped to viral genomes from fecal samples or virus-like particles rooted in fecal samples^[12]. With regard to the enteric virome, eukaryotic viruses, bacteriophages, and pathogenic viruses are present in the gastrointestinal tract^[13]. However, in recent years, increasing evidence suggests that the intestinal microbial composition is significantly altered in IBD patients compared with that in healthy subjects^[14]. Therefore, regulation of the disturbed intestinal microbiota may represent a new therapeutic strategy for IBD.

Classical therapeutic approaches for IBD are varied, and include anti-inflammatory, immunosuppressive, and biologic therapies, largely applied and developed in clinical practice^[15]. IBD is a persistent and recurrent disease and requires long-term treatment, which often results in drug-induced side effects. Furthermore, numerous IBD patients do not respond to clinically approved drugs^[16], necessitating the development of novel therapies or complementary and alternative medicine for IBD. It is worth mentioning that, with the rapid advancement in metagenomics, complementary and alternative therapies for IBD based on modulation of gut microbiota have developed rapidly and preliminary achievements have been reported^[17]. Pro/prebiotics, herbal medicinal products, and fecal bacteria transplantation (FMT) are emerging therapeutic strategies for IBD that target intestinal microbiota in a direct or indirect way, thus benefiting intestinal health^[18].

In this review, we explore therapies targeting intestinal microbiota, such as FMT, pro/prebiotics, and herbal medicinal products, that may represent effective therapeutic options to control and slow the progression of IBD. We also discuss some clinical applications and where to place more focus on these emerging therapeutic strategies (Figure 1).

THERAPEUTIC STRATEGIES TARGETING INTESTINAL MICROBIOTA

Probiotics: Live bacterial biotherapeutics

Probiotics were first proposed in 1908 by Nobel laureate Eile Metchnikoff, who also defined the first probiotic agents, lactic acid bacteria, which exert the physiological

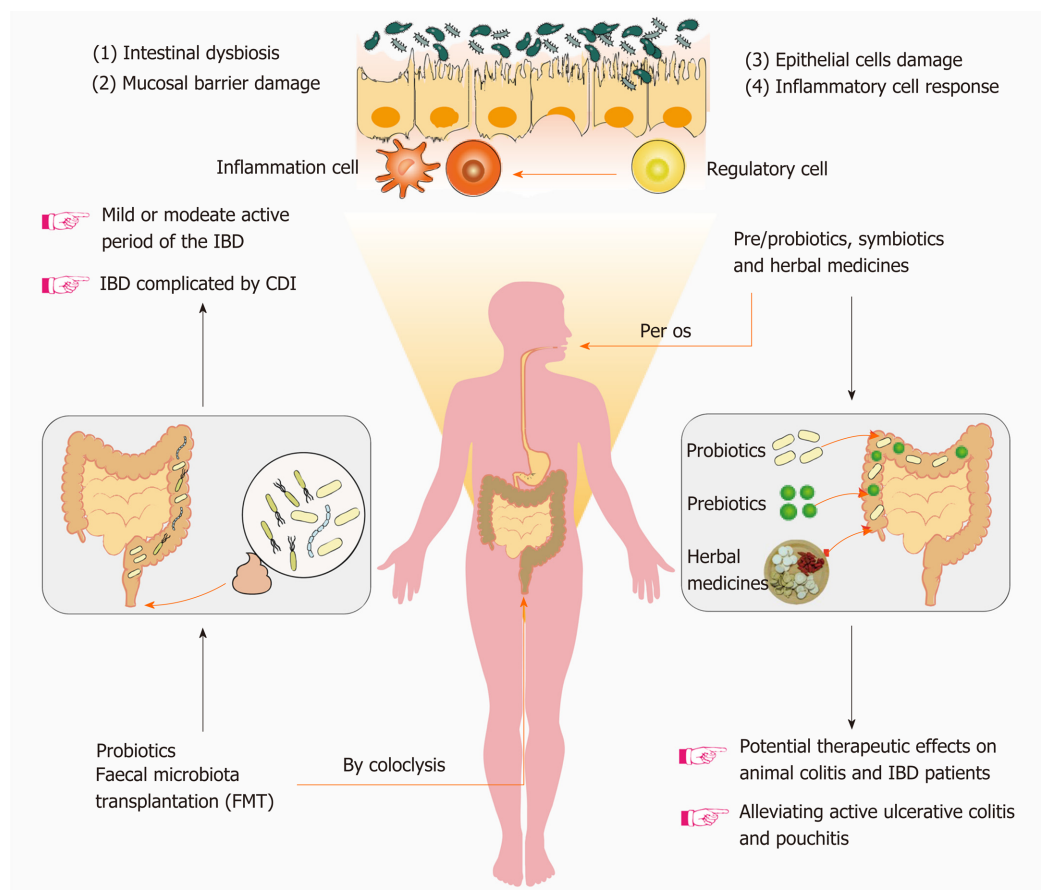


Figure 1 Regulation of intestinal microbiota as a therapeutic strategy for inflammatory bowel disease. Microbe-based therapies for inflammatory bowel disease (IBD) can be divided into two categories, namely: Direct regulation of microbiota [probiotics and fecal bacteria transplantation (FMT)] and indirect regulation (prebiotics and herbal medicines). Intestinal dysbiosis, mucosal barrier damage, epithelial cell damage and inflammatory cell response often coexist in patients with IBD. However, FMT has now been used to test the treatment of mild or moderate active period of IBD as well as IBD patients complicated by *Clostridium difficile* infection. Pre/probiotics, symbiotics and herbal medicines display potential therapeutic effects in animal colitis as well as certain IBD patients, especially for active ulcerative colitis. Hence, it is necessary to screen and design personalized microbiota-based therapies in order to enhance the specificity and selectivity of the therapeutic strategy targeting intestinal microbiota. IBD: Inflammatory bowel disease; FMT: Fecal bacteria transplantation; CDI: *Clostridium difficile* infection.

effects of inhibiting “intestinal autotoxicity”, delaying intestinal aging, and eliciting beneficial effects on human health^[19]. Since then, the concept of intestinal probiotics has been continuously developing, and new probiotic strains are still being identified. The latest scientific definition of probiotics, *i.e.* “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” was advanced in 2014^[20]. Probiotic strains discovered to date mostly belong to the phylum *Firmicutes* and include the genera *Aerococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Carnobacterium*, *Tetragenococcus*, *Vagococcus*, and *Weissella*; as well as *Bifidobacterium* genera attributed to *Actinobacteria*, and *Saccharomyces* belonging to *Eumycota*^[21]. *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* strains are probiotics that have a long history of application and have attracted much interest. Furthermore, with the evolution and innovations in sequencing technology, researchers have discovered novel probiotic strains referred to as the “next-generation of probiotics”, of which include *Akkermansia muciniphila*, *Propionibacterium* spp., and *Roseburia* spp., with promising applications^[22,23].

However, the clinical application of probiotic preparations is still very limited, and their scope of application and effectiveness are still being investigated^[24]. As a mixture of high-concentration probiotic preparations, VSL#3 comprises 8 live lyophilized bacterial strains, namely *Streptococcus thermophilus*, 3 strains of *Bifidobacterium* (*B. longum*, *B. breve*, and *B. infantis*) and 4 strains of *Lactobacilli* (*L. paracasei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii* subspecies *bulgaricus*)^[25]. VSL#3 has long been used in clinical settings for the treatment and remission of IBD. A study confirmed that VSL#3 achieved remission in patients with mild-to-moderately active UC, with high safety and efficacy^[26]. Moreover, in a recent systematic meta-analysis, VSL#3 also demonstrated efficacy in alleviating active UC and pouchitis, and could effectively

protect against its recurrence in a static period of disease; however, its potential utility in CD patients has not been demonstrated^[27,28]. The precise effects of probiotics in the intestinal tract are still unclear.

Elucidation of the mechanisms by which probiotic bacteria exert protective effects in IBD are crucial for identifying optimal treatment strategies. The potential effects of probiotics on the intestine may be classified into 4 categories: (1) Probiotics regulate immune responses and inhibit inflammatory reactions by mediating several signal transduction pathways, such as the Toll-like receptor signaling pathway^[29,30]; (2) Probiotics inhibit or directly eliminate enteropathogenic microorganisms by competing for nutrients and intestinal-epithelium adhesion sites, and secreting antimicrobial substances^[30,31]; (3) Probiotics help maintain intestinal epithelial homeostasis by promoting tight junction (TJ) formation, boosting mucus production, and anti-epithelial cell apoptosis^[31]; and (4) Probiotics can directly impact the metabolic profile of intestinal microbiota and the host, thus promoting the regulation of colonic cell proliferation and the clearance of hazardous substances from the intestinal tract^[32]. Diverse antimicrobial mechanisms and substances are involved, *e.g.*, lactic acid can disrupt enteropathogenic-microorganism metabolism by decreasing luminal pH, bacteriocins can damage cytoplasmic membrane formation, and microcins disturb the macromolecular synthetic pathways^[33]. An antimicrobial protease encoded by *Lactobacillus paracasei* *partP*, lactocepin, which can selectively degrade pro-inflammatory chemokine IP-10 level^[34].

Prebiotics: Nourishing probiotic preparations

Nondigestible oligosaccharides, in particular fructo-oligosaccharides, have been used to promote health for a long time. Prebiotics were first defined as a “non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon” in 1995^[35]. Since then and particularly between 2001 and 2014, the concept and meaning of prebiotics has been extended; the latest and most widely accepted definition is “a substrate that is selectively utilized by host microorganisms conferring a health benefit”^[36]. A large category of prebiotics are oligosaccharides, which include cereal-derived arabinoxylans and arabinoxylan, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), glucans, gluco/xylo-oligosaccharides, isomalto-oligosaccharides, poly-dextrose, soya bean oligosaccharides, and *trans*-galacto-oligosaccharides. Others include inulin and lactulose, classified as non-digestible carbohydrates^[21,37,38]. A third type of emerging, and increasingly popular, prebiotic class is represented by plant polyphenols, ellagitannins, and proanthocyanidins; 90%-95% of these cannot be absorbed or utilized in the small intestine. However, in the colon, they can undergo a process of biotransformation by intestinal microorganisms and produce ingredients that are beneficial to health^[39,40]. Among these, the most widely studied prebiotics, whose biological functions are best understood, are inulin, FOS, GOS, and lactulose.

The effect of prebiotics is to stimulate several microbial groups, and to increase not only the abundance of commensal *Lactobacillus* and/or *Bifidobacterium*, but also other beneficial taxa, such as *Roseburia*, *Eubacterium* and *Faecalibacterium* spp^[19,36]. A recent study found that inulin causes a shift in the intestinal microecology, manifesting in an increased abundance of *Bifidobacterium* and *Anaerostipes*, and a decreased abundance of *Bilophila*^[41]. Several studies have attempted to explain how prebiotics alter the intestinal microbial composition: Prebiotics are crucial for the regulation of physiological activities and can be used as carbon or energy sources for intestinal probiotics; however, they cannot be directly absorbed and utilized by the host^[38]. In brief, prebiotics promote the propagation and growth of probiotics, whose metabolites confer health benefits to the host^[42,43]. Some organic acids, for instance, are major metabolites generated by the metabolism of prebiotics by host microorganisms. The main organic acids generated are short-chain fatty acids (SCFAs) (*e.g.*, acetate, butyrate, and propionate), which directly decrease the colonic intraluminal pH; additionally, SCFAs can mediate multiple signaling pathways for maintaining gut homeostasis and immune system balance^[36,44,45]. Moreover, bile salt hydrolases, which are crucial hydrolytic enzymes, are also generated by enteric microorganisms. These hydrolases mediate transformation and/or metabolism of bile acids and possess resistance to the harsh acidic environment in the intestine, as well as confer host health benefits^[46,47]. Interestingly, a study showed that bacterial deconjugation of taurine from primary bile acids was enhanced after consumption of prebiotic inulin, which is consistent with the increased enzyme activity of bile salt hydrolases^[48].

Several studies evaluating the potential therapeutic effects of prebiotics on animal colitis models and IBD patients have demonstrated beneficial effects^[49-53]. HLA-B27

transgenic rats, as an effective rodent model of IBD, have been used to evaluate the potential therapeutic efficacy of inulin and FOS against intestinal inflammation. It was shown that both inulin and FOS decreased chronic intestinal inflammation by regulating the composition of gut microbiota, and increasing the abundance of probiotics *Bacteroides-Prevotella-Porphyromonas* and *Bifidobacteria*^[54]. Moreover, a recent experimental study of prebiotics in IBD models demonstrated that these agents play a strong beneficial role in relieving 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, which was correlated with increased abundance of probiotics (*Lactobacillus* and *Bifidobacterium*), as well as increased production of SCFAs^[55]. Interestingly, the preventive effects of prebiotic fiber against microbiota-mediated colonic mucus deterioration were revealed in another study; this effect may serve as a novel complementary mechanism by which prebiotics alleviate intestinal inflammation^[56]. However, recently, researchers have found that prebiotics, including fermentable fibers and inulin, can shift the normal microbiota composition to cause gut dysbiosis and overproduction of colonic butyrate, contradicting previous research outcomes^[57]. In comparison with animal studies of prebiotic applications, studies of prebiotics in IBD are very limited and remain controversial^[49]. In brief, based on the current results for prebiotic interventions, we cannot conclude that prebiotics ameliorate IBD symptoms^[58]. Further research is therefore necessary to confirm the potential of prebiotics to relieve IBD.

FMT

FMT is the transfer of fecal microorganisms from healthy donors to individuals with certain diseases, *via* technical approaches such as enemas, nasogastric or nasojejunal tubes, and oral capsules^[59]. FMT was first used to remedy pseudomembranous enterocolitis (PMC) in 1958, by Eiseman *et al.*^[60]. Later studies have suggested that PMC is caused by infection with the anaerobic bacterium *C. difficile*, which can induce gut dysbiosis^[61]. FMT has since been gradually extended from the preliminary development and testing phase to being used as an approved therapeutic modality for *C. difficile* infection (CDI) in the clinic, with a success rate of near 92%, thereby representing an effective treatment compared with broad-spectrum antibiotics^[62]. Subsequently, FMT has been used to treat IBD complicated by CDI, and finally extended to treat patients suffering only from IBD^[63]. As a treatment strategy for IBD, FMT has been proposed for over 25 years; however, it has only attracted research interest in the context of IBD in recent years^[64,65]. Several clinical investigations have demonstrated promising treatment outcomes for patients in the mild or moderate active period of the disease.

A systematic review in 2012 showed that, among 41 cases of FMT therapy in IBD patients, symptoms were relieved in 76% of patients, medication could be terminated in 76% of patients, and 63% of patients showed disease alleviation^[66]. However, subsequently, a larger-scale meta-analysis (307 adult patients) did not show as high a rate of effectiveness, reporting that FMT only mitigated the clinical symptoms of 36% of UC patients and 50.5% of CD patients^[67]. According to a small double-blind randomized trial, which was conducted to evaluate the safety and efficacy of FMT for UC patients, 41.2% of patients (7/17) achieved the primary endpoint compared with 25.0% of controls (5/20)^[68]. Similar results were also obtained in a recent multicenter, double-blind, randomized, placebo-controlled clinical trial, which demonstrated the validity of FMT in only 11 (27%) of 41 UC patients, with adverse reactions in 32 (78%) cases; however, most of the adverse events were self-limiting gastrointestinal complaints^[69]. Furthermore, based on 16S rDNA sequence analysis, FMT-induced clinical remission and endoscopic improvement are correlated with the regulation of intestinal microbiota in active UC^[69].

Data on FMT-induced CD alleviation are less abundant than in UC, and randomized controlled trials in CD are inadequate; only several small uncontrolled cohort studies have been carried out, producing conflicting results. A prospective open-label study showed that FMT from healthy donors to active CD individuals relieved symptoms in 58% (11/19) of CD patients, all of whom exhibited an increase in microbial diversity^[70]. In another uncontrolled study in 10 subjects to evaluate FMT, 3 of 10 CD patients responded to the intervention; however, 2 recipients showed serious adverse events, necessitating larger controlled trials to confirm the safety and efficacy of FMT^[71]. However, the results of a long-term multiple fresh FMT trial conducted to evaluate the maintenance effect of symptom relief in CD complicated by an intraabdominal inflammatory mass revealed that the clinical symptom alleviation rates were 48.0% (12/25), 32.0% (8/25), and 22.7% (5/22), respectively, at 6 mo, 12 mo, and 18 mo; fresh FMT was repeated every 3 mo^[72]. Furthermore, the long-term clinical effects of varied frequency of FMT for CD were explored: An interval of treatment of

less than 4 mo was shown to effectively maintain the clinical benefits obtained by the first FMT^[73]. However, the dynamic gut-microbiota shifts and molecular interactions between donors and recipients during FMT remain poorly understood. In addition, further studies are required to determine the optimal FMT treatment intensity and match the optimal donor-recipient types based on microbial profiles.

Herbal compounds and prescriptions

There are some safety concerns with the long-term use of conventional medications (e.g., anti-inflammatory, immunosuppressive, and biologic therapies), which has increased interest in traditional medicines for the treatment of IBD^[15]. Hence, an increasing number of researchers have shifted their attention to traditional medicine in order to identify potentially therapeutic compounds in Chinese herbal medicine and/or traditional prescriptions. So far, various potent compounds have been found, some of which exhibit the effects of relieving intestinal inflammation, at least in part by regulating the intestinal microbiota^[17]. However, paucity of data can actually reflect the therapeutic effect in human clinical trials^[74]. Numerous types of natural compounds are derived from herbs, including herbal polysaccharides, polyphenols, flavonoids, saponins, and alkaloids^[75]. Moreover, herbal polysaccharides and polyphenols, which are present in various Chinese herbs and mostly only absorbed in the colon, are yet to be included in the category of prebiotics^[76,77]. Herbs containing polysaccharides include some Chinese medicines such as *American ginseng* and *wolfberry*, which both show the ability to correct intestinal dysbiosis and mitigate intestinal inflammation in mice^[78,79]. Polyphenols in herbal medicines include anthocyanin, catechinic acid, ellagic acid, and gallic acid, which can be converted into bioactive metabolites by intestinal microorganisms. Therefore, modulation of the microbial community structure benefits the intestinal tract^[74].

Other non-prebiotic natural ingredients also exhibit the ability to attenuate intestinal inflammation in mice with colitis by selectively altering the gut microbiota; however, it has not been proven whether these compounds are involved in bacterial metabolism. Several natural alkaloids, such as berberine, palmatine, and evodiamine, have been shown to ameliorate experimental colitis in an IBD model by improving the relative abundance of gut microbiota, as well as increasing the abundance of *Bacteroidetes* and *Firmicutes* and reducing *Proteobacteria* abundance, thus maintaining the homeostasis of intestinal microbiota^[80-82]. A natural limonoid compound, obacunone (100 mg/kg/day *via* oral gavage in mice) abundantly distributed in *Phellodendron chinense* and *Tetradium ruticarpum*, exhibits a modulating effect on the disordered gut microbiota of IBD mice^[83]. Others, such as *Indigo naturalis* (200 mg/kg/day *via* oral gavage in mice) and *salvianolic acid* (8 mg/kg/day by tail vein injection in rats), also target the intestinal microbiota, with beneficial effects on gut health^[84,85]. Moreover, recent studies have also demonstrated the efficiency of several traditional Chinese prescriptions: As a traditional compound, Bawei Xileisan (200 or 400 mg/kg/day *via* oral gavage) consists of 8 Chinese medicines, includes watermelonfrost, calcite, cowgallstone, pearl powder, borax, *Dryobalanops aromatica* Gaertn. f., ammonium chloride, and *Indigo naturalis*, and has been shown to relieve colitis in the mouse model of UC mainly by restoring Th17/Treg imbalance and improving *Lactobacillus* abundance^[86]. Rhubarb Peony decoction is another Chinese prescription that increasing *Butyricicoccus pullicaecorum* abundance and SCFA levels, thus alleviating pathological changes in colitis mice^[87]. Recently, Pyungwi-san (669.1 mg/kg/day *via* oral gavage) was found to protect against DSS and *C. difficile*-induced colitis mice, and the mechanism was related to restoration of a balance in gut microbial communities^[88].

POTENTIAL THERAPEUTIC MECHANISMS BY WHICH INTESTINAL MICROBIOTA ARE TARGETED

The above-mentioned emerging treatment strategies targeting intestinal microbiota, which share a common direct initiation mechanism, show varying efficacy in terms of regulating dysbiosis (including the inhibition of pathogenic microorganisms and promoting the entire gut microbiota community). Furthermore, gut dysbiosis is often concomitant with the reduction in beneficial metabolites, impairment of intestinal barrier function, and imbalance of immunity homeostasis^[89]. Therefore, potential therapeutic mechanisms by which intestinal microbiota are targeted may involve regulating microbial metabolism, enhancing the epithelial barrier, and maintaining intestinal immune homeostasis.

Regulating microbial metabolism

The hallmark of dysbiosis is the reduction in the abundance of commensals and the increase in pathogenic microbes. Commensal intestinal microbes play a crucial biological role in the host by producing bioactive metabolites such as SCFAs, trimethylamine, trimethylamine *N*-oxide, and tryptophan metabolites^[90]. Among them, SCFAs represent a significant proportion of microbial metabolites, whose peak concentrations can reach 130 mM in the proximal colon^[91]. The biosynthetic pathways of SCFAs were briefly reviewed by Zhang *et al*^[90]. Acetate, propionate, and butyrate are the most abundant SCFAs, and are used as energy substrates for absorption and utilization by the intestinal epithelium, promoting intestinal health and reducing inflammation^[92]. Studies have found that butyrate-producing bacteria, *Roseburia hominis* and *F. prausnitzii* belonging to *Firmicutes*, are dramatically decreased in UC patients compared with levels in healthy individuals^[93]. Interestingly, the effective utilization of probiotics and prebiotics increases the generation of SCFAs by promoting the proliferation of commensal bacteria, mainly SCFA-producing bacteria (*e.g.*, *Ruminococcus* and *Faecalibacterium*)^[94,95]. Furthermore, FMT also exhibits the biofunctionality of enhancing SCFA production^[96].

As an essential amino acid, tryptophan is found naturally in many foods such as fish, eggs, and red meat. Altered levels of tryptophan and tryptophan metabolites have been revealed in IBD patients by metabolomics analysis, and the expression of the aryl hydrocarbon receptor (AhR) in inflammatory intestinal tissues is reduced compared with that in healthy individuals^[91,97]. Moreover, the ability of several microorganisms to metabolize tryptophan to serotonin (5-hydroxytryptamine), kynurenine (Kyn), indole and indole derivatives (*e.g.*, indole-3-aldehyde; I3A) has been reported; the first-discovered tryptophan-degrading bacteria are *Escherichia coli* and *Vibrio cholera*^[98,99]. Both indole and I3A are ligands of the AhR; these bind AhR and thus regulate Th17/Treg immune homeostasis, maintaining the balance of mucosal reactivity^[100]. Several probiotics have been shown to affect the levels of tryptophan metabolites. For example, *Lactobacillus* present in the intestine, which spontaneously generates AhR agonists and protect against colitis with dysbiosis in gene-deficient mice (*Card^{-/-}*), has potential therapeutic effects involving the regulation of tryptophan metabolism^[101]. Moreover, as an important probiotic, *Lactobacillus reuteri* strains can reduce intestinal inflammation by inducing tryptophan-derived indole production, thus activating the AhR and promoting gut intraepithelial Treg cell differentiation^[102]. Two natural substances, patchouli alcohol and palmitate, derived from *Pogostemon cablin* and *Golden thread*, respectively, have been shown to relieve DSS-induced experimental colitis, at least partly by suppressing tryptophan catabolism^[81,103]. However, the relationship between microbial metabolism and intestinal health remains poorly understood, and the construction of microbial metabolism regulatory networks may be a promising research avenue to help clarify the orchestrated therapeutic mechanisms by which intestinal microbiota are targeted.

Protecting and enhancing the epithelial barrier

The integrity of the intestinal epithelial barrier is a prerequisite for intestinal mucosal immune homeostasis; the mucosa is an indispensable protective layer against chemical and pathogenic challenges from the colonic lumen^[104]. Studies also describe the therapeutic potential of protecting and enhancing the epithelial barrier in IBD treatment^[105]. Several probiotic strains possess the ability to protect or enhance the epithelial barrier, as shown by several *in vitro* studies, animal IBD models, and clinical trials^[106-108]. An earlier *in vitro* study found that probiotic strains, including those of *Streptococcus* and *Lactobacillus*, protected against intestinal epithelial barrier lesions caused by enteroinvasive *Escherichia coli*^[109]. Subsequent studies have shown that probiotics compete with pathogenic bacteria for adherence to mucosal sites, reflecting the anti-adherence function of probiotics and therefore supporting the mechanism of epithelial barrier protection by probiotics^[110,111]. For example, *Lactobacillus plantarum*, a well-known probiotic, can competitively prevent enteropathogenic *Escherichia coli* and mannose adhesion-dependent enteric pathogens (*e.g.*, *S. typhimurium*) from adhering to intestinal epithelial cells^[112,113]. In addition to these direct anti-adherence functions of probiotics, other mechanisms involving the suppression of toxin secretion by pathogenic microorganisms may also protect the intestinal barrier. *Bifidobacterium breve* strain Yakult, for instance, was found to inhibit the production of Shiga toxin derived from *Escherichia coli* O157:H7 *in vitro* as well as in a lethal mouse *Escherichia coli* infection model^[114]. Interestingly, a more precise probiotic mechanism has been reported in that the probiotic yeast protease, secreted by *Saccharomyces boulardii*, degrades toxin A produced by *C. difficile*^[115].

In addition to the indirect protective effect on the epithelial barrier, probiotics can enhance intestinal epithelial barrier function directly^[116]. It has been widely confirmed that probiotics can strengthen the intestinal barrier by increasing the expression levels of TJ proteins both *in vitro* and *in vivo*^[117]. The *Lactobacillus rhamnosus* GG-derived protein, p40, promotes intestinal epithelial proliferation, differentiation and the formation of TJ proteins^[118]. In addition, the expression levels of intestinal TJ proteins, such as claudin, occludin, and zonula occludens 1 (ZO-1) were significantly increased in newborn piglets after the administration of *Lactobacillus reuteri*^[119]. Moreover, the ability of *Lactobacillus plantarum* to recruit occludins and ZO-1 to the TJ region has been reported in a clinical trial^[117]. Furthermore, probiotic *Bifidobacteria* show similar effects to *Lactobacillus*, in terms of increased expression of ZO-1 and occludin by promoting the activation of extracellular signal regulated kinases and the p38 signaling pathway in human epithelial cells^[120]. In addition, a recent study revealed that TNBS-induced gut barrier dysfunction was improved noticeably after *Bifidobacterium longum* treatment owing to suppression of high mobility group box 1 protein release^[106]. Except for the single probiotic, several probiotic mixtures present a similar efficiency. Bifico, for example, which is a probiotic mixture comprising *Bifidobacterium*, *Lactobacillus acidophilus*, and *Enterococcus*, was shown to upregulate the expression of TJs in IL10^{-/-}/TNBS-induced models^[121]. However, similar evidence in clinical settings is scarce, and further study is warranted (Figure 2).

CLINICAL APPLICATIONS IN IBD TREATMENT

Compared with IBD, probiotics and prebiotics have been extensively applied to treat clinical gastrointestinal disease. Mild-to-moderate IBD or IBD accompanied by *C. difficile* infection are the main types of IBD that fit the scope of treatment with pro/prebiotics^[23]. The British Society of Gastroenterology consensus guidelines published in 2019 point out that pre/probiotics, symbiotics, FMT, and herbal treatments are all complementary and alternative therapies for IBD in adults^[18]. Although there is insufficient evidence to conclude that probiotic therapy induces remission of IBD, it may improve symptoms, at least to some extent, in mild-to-moderate UC^[122]. A subsequent study revealed that the evidence for maintaining remission is insufficient, and the only data demonstrating benefits are from patients with UC^[123,124]. The effects of probiotics on CD are controversial: A nonblind clinical trial demonstrated the safety and efficacy of probiotics to treat CD; however, a meta-analysis indicated that CD symptoms cannot be mitigated by probiotic treatment^[125,126]. The evidence for prebiotics is relatively scarce compared with that for probiotics, and the data in humans remain ambiguous. As a prebiotic, lactulose has shown certain benefits in UC and CD patients when administered at a daily dosage of 20 g^[127]. In comparison, the other two prebiotics, inulin and FOS, have shown mixed results in terms of clinical outcomes, demonstrating bioavailability in one small open-label study but no effects in a much larger study^[128,129]. Moreover, the use of probiotics and prebiotics in the treatment of IBD usually in conjunction with conventional medications provides limited evidence.

Four randomized placebo-controlled trials have been conducted on FMT to date; among these, three have shown a significant symptom reduction compared with placebo^[18]. An open-label study revealed that FMT is more applicable to UC than CD patients^[130]. Nevertheless, unified standards for the route and frequency of FMT administration in published trials are not available, which may be a potential reason for the discrepancies between them^[131]. Establishment of the optimal route and frequency of FMT administration, therefore, may provide a strong theoretical foundation and practical guidance for the clinical application of FMT. Quality control of donor feces is also critical for clinical application and to increase the stability and security of FMT^[132]. Recently, the U.S. Food and Drug Administration notified the potential risk of serious or life-threatening infections with the use of fecal microbiota for FMT, and claimed that bacterial infections are caused by multi-drug resistant organisms^[133]. Thus, the potential risk of FMT reminds researchers again to focus more on how to increase the stability and security of FMT. The proposed implementation of stool banks is a promising step toward the establishment of unified standards for donor feces^[134]. The Netherlands Donor Feces Bank was the first stool bank, established in 2015, aiming to provide a standard product for treating recurrent CDI in the Netherlands^[132]. Subsequently, FMT experts held an international consensus conference on stool banking, which confirmed the feasibility and maneuverability of stool banking to accelerate FMT application in clinical settings^[135]. In general, it is a

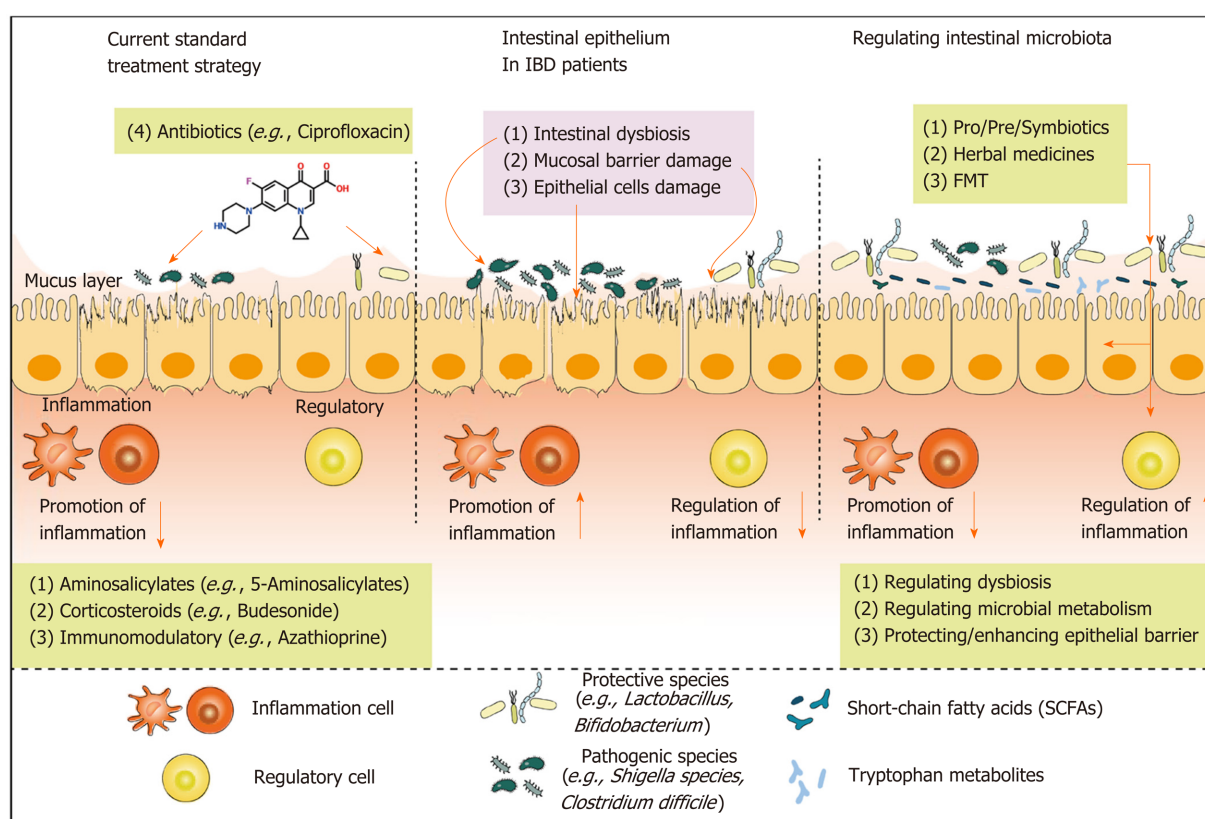


Figure 2 Primary mechanisms of standard treatment strategy and regulating intestinal microbiota strategy for inflammatory bowel disease. Current standard therapeutic medications for inflammatory bowel disease (IBD) are antibiotics (e.g., ciprofloxacin), aminosalicylates (e.g., 5-aminosalicylates), corticosteroids (e.g., budesonide) and immunomodulatory agents (e.g., azathioprine), and the mechanism mainly involves inhibiting the development of inflammation in the intestine. Intestinal dysbiosis is often found in IBD patients, which is manifested in higher abundance of pathogenic species (e.g., *Shigella species* and *C. difficile*) and less abundance of protective species (e.g., *Lactobacillus* and *Bifidobacterium*). However, interventions targeting intestinal microbiota, such as probiotics, prebiotics, symbiotics, herbal medicines and fecal bacteria transplantation exert therapeutic action primarily through the mechanism of correcting dysbiosis. Furthermore, the treatment strategy of regulating intestinal microbiota are also involved in regulating microbial metabolisms (e.g., short-chain fatty acids and tryptophan metabolites), and protecting/enhancing the intestinal epithelial barrier. IBD: Inflammatory bowel disease; FMT: Fecal bacteria transplantation.

solid foundation for the extensive exploration and promotion of FMT that ensure recipients receive security, reliable, timely and equitable donor feces.

Although some herbal medicines have already been used in clinical settings as complementary and alternative medicine for IBD, their underlying pharmacologic modes of action remain obscure^[136]. The mechanisms by which herbal compounds and prescriptions target intestinal microbiota have been described in experimental IBD models. These findings may represent only the tip of the iceberg in regard to the potential therapeutic mechanisms of herbal therapies.

CONCLUSION

Microbe-based therapies for IBD discussed in this review may be separated into two categories, namely: Those that directly target microbiota (probiotics and FMT) and those whose mechanisms involve indirect regulation (prebiotics and herbal medicines). IBD is a complex disease that correlates with immune, microbial, and environmental factors. Current treatment methods suffer limitations and offer low effectiveness with the rapid rise in IBD incidence. However, the emergence of microbe-based therapies affords an avenue in the pursuit of more effective and personalized treatment plans for IBD patients. Oka A and Sartor RB proposed that concomitant companion diagnostic tests should be carried out to profile an individual's microbiota for guiding optimal personalized microbial therapies^[137]. It is well known that the development of new therapies often accompanies the innovation of new methods and techniques. Therefore, the development and improvement of microbe-based therapies require multi-disciplinary approaches (such as genomics, microbiology, and

metabolomics), to obtain a deeper and more comprehensive understanding of the co-regulatory networks between microbiota, bacterial metabolites, and host immunity.

Probiotic strains that are known and applied, to date, have been derived from bacteria or fungi, but not viruses^[24,138]. For example, the *Bifidobacterium* and *Lactobacillus* genera have been commercialized worldwide, and next-generation probiotics (*Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, and *Eubacterium hallii*) are emerging^[138]. However, high-throughput sequencing shows that the dominant viruses that inhabit the intestines are *bacteriophages* (e.g., *prophages*), which can shape and influence the bacterial community structure by parasitizing or lysing bacterial cells^[139,140]. Therefore, future studies of probiotics should endeavor to focus on intestinal bacteriophages in order to elucidate the mechanisms underlying the relationships between bacteriophages and bacteria, with a view to identifying novel virus-based probiotics. Furthermore, the use of probiotics to find effective small-molecule chemicals (metabolites) or structural proteins may represent additional promising research directions.

Complementary therapies targeting the intestinal microbiota are indistinguishable, which do not follow the individual therapeutic scheme. Nevertheless, the intestinal microbial composition in different patients are highly individualized. Hence, it is necessary to screen the microflora and conduct follow-up investigations for different IBD patients in order to monitor individual differences in microbiota and design personalized microbiota-based therapies in order to enhance the specificity and selectivity of the therapeutic strategy targeting intestinal microbiota.

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Role of minimally invasive surgery for rectal cancer

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Abstract

Rectal cancer is one of the most common malignancies worldwide. Surgical resection for rectal cancer usually requires a proctectomy with respective lymphadenectomy (total mesorectal excision). This has traditionally been performed transabdominally through an open incision. Over the last thirty years, minimally invasive surgery platforms have rapidly evolved with the goal to accomplish the same quality rectal resection through a less invasive approach. There are currently three resective modalities that complement the traditional open operation: (1) Laparoscopic surgery; (2) Robotic surgery; and (3) Transanal total mesorectal excision. In addition, there are several platforms to carry out transluminal local excisions (without lymphadenectomy). Evidence on the various modalities is of mixed to moderate quality. It is unreasonable to expect a randomized comparison of all options in a single trial. This review aims at reviewing in detail the various techniques in regard to intra-/perioperative benchmarks, recovery and complications, oncological and functional outcomes.

Key words: Rectal cancer; Minimally invasive surgery; Laparoscopic surgery; Robotic surgery; Transanal total mesorectal excision; Transanal minimally invasive surgery

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Core tip: Rectal cancer is one of the most complex diseases as it combines oncological, anatomical, and functional challenges with a variety of technical and multimodality treatment options. While open surgery was long considered the surgical gold standard, less invasive approaches have evolved. These newer technologies have attractive advantages, however their overall benefit and risk analysis in the short and long run and their specific role for rectal cancer remain controversial and a matter of further research.

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INTRODUCTION

In the United States, there are approximately 44000 new cases of rectal cancer each year. Paired with colon cancer, colorectal cancer represents the third most common cancer both in incidence and in mortality^[1], hence representing a significant healthcare burden. Several factors contribute to the higher complexity of rectal cancer as compared to colon cancer. The importance of proper surgical techniques for improved outcomes has been documented on multiple occasions and nearly all circumstances. Innovation in the treatment of rectal cancer has been unstoppable which on one hand continues to be desperately needed and represents progress but on the other hand renders structured research and long-term comparisons difficult. Advances were seen in the radiation and chemotherapeutic fields, diagnostics, as well as the realm of surgery. The constantly changing landscape with multiple variables adds additional complexity to rectal cancer's intrinsic difficulty when treatment is to be measured not only by oncological outcome parameters but also functional and quality of life aspects (Table 1).

Until about 30 years ago, open surgical techniques represented the only modality available to remove a rectal tumor. Propagation of a specimen-oriented anatomical dissection technique, aka total mesorectal excision (TME), became a turning point in reducing local recurrence rates and became the gold standard^[2]. Since then, there has not only been a technological revolution with development of several new and less invasive platforms but also an overall paradigm shift in the management of rectal cancer. Multimodality treatment has become the standard for all but the very early rectal cancers. Laparoscopic surgery was introduced to colorectal surgery in the 1990s, but initially excluded the rectum. Increased familiarity with the technique and development of more sophisticated minimally invasive surgery tools allowed for an increasingly robust advantage that triggered a slow but steady market penetration. Independent of that, the implementation of a major management shift happened with introduction of enhanced recovery protocols (ERAS), which themselves led to a measurable reduction of the length of stay and complication rates.

In 2020, there are four major platforms to perform an oncological resection and hybrid versions thereof. In addition, endoluminal surgeries have evolved from simple transanal local excisions to more sophisticated technology-dependent interventions (Table 2). This review aims at highlighting the role of these various minimally invasive platforms for rectal cancer as opposed to the conventional open resection.

BACKGROUND

Evaluation and adoption of minimally invasive surgery for colorectal cancer in general and specifically for rectal cancer has been a comparably slow process spanning the last three decades. In contrast to gallbladder and appendiceal surgeries, colorectal surgery for cancer was slower due to a combination of the more complex surgery as such spanning multiple quadrants and not corroborated early concerns about a higher incidence of port site recurrences. Prior to rectal cancer trials, the appropriateness of the laparoscopic technique was evaluated for colon cancer by numerous nonrandomized observational studies before properly designed trials were published in the mid-2000's. It is important to note that rectal cancer was most commonly excluded. These randomized controlled trials (RCT) of laparoscopic *vs* open surgery for colon cancer included the Barcelona, COST, COLOR, and CLASICC trials^[3-6]. The results were largely similar and primarily showed at least equivalent oncologic outcomes, no difference in complications, and at best a modest reduction in length of stay and return of bowel function after laparoscopic surgery. Yet, as surgical history demonstrates, laparoscopic surgery continued to penetrate as routine resulted in more measurable benefits.

These landmark trials set the framework to expand research to laparoscopic rectal cancer surgery. The seminal clinical trials for minimally invasive rectal cancer surgery are listed in Table 3.

The first laparoscopic *vs* open rectal cancer randomized controlled trial was part of

Table 1 Goals of care in rectal cancer patients

Goals	Parameters
Oncological	Overall survival, disease-free survival, local recurrence rate, cure
Ostomy	Avoidance of permanent ostomy, stoma-free survival
Anatomical	Organ preservation Sphincter preservation
Functional	Preservation of QoL Anorectal and defacatory function Sexual and urinary function
Peri-/postoperative morbidity	Low intraoperative complication rate Avoidance of collateral injuries Low postoperative complication rates (leak, SSI, and any Clavien-Dindo complication > 2)

QoL: Quality of life.

Table 2 Surgical platforms for rectal neoplasias

	Oncological resection	Endoluminal local excision
Sphincter preserving/restorative surgery	Open TME Laparoscopic TME Robotic TME Transanal TME	Colonoscopic EMR/ESD Transanal excision Transanal endoscopic microsurgery Transanal minimally invasive surgery (L-TAMIS) Robotic TAMIS
Nonrestorative surgery	Open APR Laparoscopic APR Robotic APR Transanal APR	

TME: Total mesorectal excision; APR: Abdominal perineal resection; EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection; TAMIS: Transanal minimally invasive surgery.

the MRC CLASICC trial which included both colon and rectal cancer patients^[6]. The rectal arm found a non-significant increase in positive circumferential resection margins (CRM), but the authors argued against the use of laparoscopic surgery for rectal cancer at that time. It took another five years for another major randomized trial to finish. The COREAN trial out of South Korea found similar short-term outcomes between the two modalities^[7]. The COLOR II trial out of Europe found a quicker return of bowel function and significantly shorter lengths of stay in laparoscopic patients while the primary outcome of locoregional recurrence was identical with 5% in each group^[8]. In 2015, two parallel major RCTs of equal design carried out in the USA and Australia, respectively, compared the immediate pathological outcomes after laparoscopic *vs* open rectal surgery: The Z6051 and ALaCaRT trial^[9,10]. Both studies, based on a composite of oncologic margins, failed to demonstrate noninferiority of the laparoscopic approach. While the long-term oncological outcomes were then not yet reported, these preliminary results were cause to significant concerns among surgeons as there were suddenly three published high-quality studies with trends toward inferior pathologic margins after laparoscopic rectal cancer surgery when compared with the standard open approach. Since the initial commotion, both trials have published their intermediate-term oncological outcomes spanning two years of follow-up: There were no differences in overall or disease-free survival which cast doubt on the relevance of the initial CRM composite index^[11,12].

These studies mainly reported on conventional multi-port laparoscopic surgery

Table 3 Major randomized controlled trials in minimally invasive surgery for rectal cancer

Trial	Year	Comparison	Enrollment	Primary end point	Findings
MRC CLASICC ^[6]	2005	Lap <i>vs</i> Open	253 <i>vs</i> 128	Clear margins/mortality	Colon and rectal surgery compared, 12% <i>vs</i> 6% positive CRM
MRC CLASICC FU ^[81]	2013				No differences seen OS, DFS, LR at 62 mo
COREAN ^[7]	2010	Lap <i>vs</i> Open	170 <i>vs</i> 170		No differences seen in multiple short-term outcomes
COREAN FU ^[82]	2014			3-yr disease free survival	Noninferiority of laparoscopic approach was met, 79% <i>vs</i> 72% DFS
COLOR II ^[32]	2013	Lap <i>vs</i> Open	699 <i>vs</i> 345		Similar safety and margins, laparoscopic surgery had quicker recovery
COLOR II FU ^[8]	2015			3-yr locoregional recurrence	Similar recurrence rates at 5% for each group
ACOSOG Z6051 ^[9]	2015	Lap <i>vs</i> Open	240 <i>vs</i> 222	Clear margins	Noninferiority study not able to reach boundary of 6%
ACOSOG Z6051 FU ^[11]	2019				No differences seen in long term oncologic outcomes
ALaCaRT ^[10]	2015	Lap <i>vs</i> Open	238 <i>vs</i> 237	Clear margins	Noninferiority study not able to reach boundary of 8%
ALaCaRT FU ^[12]	2019				No differences seen in long term oncologic outcomes
ROLARR ^[15]	2017	Lap <i>vs</i> Robot	234 <i>vs</i> 237	Conversion to open surgery	No differences seen in conversion rate, 12% <i>vs</i> 8%
Kim <i>et al</i> ^[16]	2018	Lap <i>vs</i> Robot	73 <i>vs</i> 66	Completeness of TME	Similar TME specimens, 78% <i>vs</i> 80%
Bordeaux ^[17]	2014	Lap <i>vs</i> TaTME	50 <i>vs</i> 50	Quality of oncologic surgery	Significant decrease in CRM positivity for TaTME, 4% <i>vs</i> 18%
Bordeaux FU ^[54]	2018				No differences seen in long term oncologic outcomes at 60 months

FU: Follow up study; TME: Total mesorectal excision; TaTME: Transanal TME; CRM: Circumferential resection margin; OS: Overall survival; DFS: Disease-free survival.

with an abdominal extraction site. However, in the mid-2010's some surgeons focused on experimenting with alternative approaches. Single-port surgery relies on one larger port where the camera and all working instruments are inserted. This can later be used for the extraction of the specimen. Numerous feasibility studies and small comparative studies did show promise^[13], but no definitive advantage. Since that time, the use of single-port laparoscopic surgery has decreased - likely due to its technical challenges and the evolution of robotic surgery^[14]. While strict natural orifice transluminal endoscopic surgery (NOTES) never reached full feasibility, natural orifice specimen extraction through the vagina or the open rectal stump was investigated and advocated by some.

In the early 2000s, in the shadow of proving the value of laparoscopy and executing the above-mentioned trials, a third technical modality entered the arena: Robotic rectal cancer surgery. Robotic surgery rapidly gained traction, but similar arguments were voiced against the platform as had in the past at the onset of laparoscopy: Longer operative times, oncological inferiority, and higher cost. Again, evidence was first observational until in 2017, when the first prospective randomized multicenter trial was published. This ROLARR trial was a worldwide effort with a primary end point of conversion rates^[15]. Disappointingly, there were - except in subgroup analysis - no significant differences between the two modalities. A subsequent smaller RCT out of Korea focusing on the quality of the TME specimen as primary endpoint again found similar results between the 2 groups^[16].

Meanwhile, local transanal excision had taken its own its own technical evolution (see later) but was never considered equivalent to an oncological resection as it was found to be associated with unacceptably high local recurrence rates. However, as an offspring from natural orifice transluminal endoscopic surgery (NOTES), a transanal total mesorectal excision (TaTME) evolved as a bottom-up approach to carry out an oncological total mesorectal resection. This technique has been popularized since 2010 but the approach has remained under intense investigation and scrutiny. The major RCTs are still enrolling patients; but in 2014, an early single-center RCT, the Bordeaux trial, reported a lower CRM positivity rate for TaTME as compared to laparoscopic

TME^[17]. The TaTME was developed and marketed as an alternative to the transabdominal laparoscopic or robotic approaches but in reality required an abdominal support approach. The primary goal was to facilitate the most distal dissection in a narrow pelvis, especially in an increasingly obese patient population. In 2019, three large trials reported their initial outcomes from TaTME. The first detailed the nationwide Dutch experience with TaTME over a three-year period and compared it to laparoscopic TME^[18]. The primary outcome of CRM positive rates was identical at 4% in both groups. The second trial was a worldwide matched comparison of TaTME and robotic TME. The primary outcome in this study was a composite score of pathologic margins. There were no differences overall, however, the distal resection margin positivity was higher in TaTME specimens (1.8% *vs* 0.3%, $P = 0.051$)^[19]. Finally, the Norway experience of TaTME compared to all other modalities showed higher anastomotic leak and local recurrences rates^[20]. This resulted in a suspension of the procedure in that country^[21].

Just as with the laparoscopic rectal cancer trials, these initial reports with TaTME uncovered relevant concerns. However, judgement is being held until two large RCTs on TaTME are completed and more information is available about complication and functional outcomes.

BENCHMARKS OF SURGICAL TECHNIQUE

The specimen-oriented TME has evolved as the standard of care in rectal cancer surgery since it was initially reported in the 1980s^[22]. A complete TME with an with preservation of an intact mesorectal envelope on the specimen has been shown to be vital to minimizing local recurrences even without addition of radiation^[2]. A major goal and benchmark for any alternative, such as a minimally invasive technique, is hence to preserve the quality of the TME. The other major goal is to minimize the surgical trauma and to achieve a quicker recovery and optimized functional outcome.

The main disadvantage of open rectal surgery lies in the required large incision with a possible extension into the epigastrium for mobilization of a challenging splenic flexure. In contrast, laparoscopic rectal surgery eliminates this incision and bowel exposure to the room air as it excels in its plasticity with flexible placement and number of trocars as needed. A supportive hand port is optional and may be used to facilitate the dissection and serve as extraction site for the specimen. The position of the operating table can be adapted to momentary needs to take full advantage of gravity, and the surgeon can move easily around the table. These aspects are relevant in a multi-quadrant operation that ranges from the primary target in the pelvis, the ligation of the mesenteric vessels, to the mobilization of the splenic flexure. The steps are further facilitated by the magnification of the laparoscope. However, laparoscopic rectal surgery is a labor-intensive procedure with a significant learning curve that is estimated to be around 40-90 cases^[23]. The critical portion of the TME as such requires a high level of expertise. A challenge for laparoscopic instruments is that they are straight and may have difficulty at the pelvic inlet to navigate around the sacral promontory and reach the pelvic floor, particularly in a narrow and obese pelvis.

The robotic approach with stabilized 3D vision and higher degree of freedom for instrument motion and maneuverability was engineered to address some of those specific problems of laparoscopic surgery. The robotic TME has been standardized over time^[24,25]. The instruments are touted as having seven ranges of motion and behave more like a normal human wrist. Many studies found the learning curve to be quicker at around 15-44 cases^[23]. Furthermore, the robot offers an ergonomic advantage as the surgeon sits down for most of the procedure. Limitations for the robot are that the surgeon is not at the bedside which may impair the teaching of trainees and could prolong the time to execute an acute conversion to an open procedure. Maneuvering through other quadrants is more challenging than with laparoscopic surgery and on occasion may require to re-target or redock the robot prior to proceeding. While the standard table cannot be rotated without undocking the robot, newer models are available that are integrated with the robot to allow for continuous adjustment of the position^[26].

Particularly in the current era of epidemic obesity, any of the previously mentioned approaches may encounter limitations to achieve an optimal exposure and reach the pelvic floor. Merging the concepts of NOTES and TAMIS, the TaTME technique was developed to address this concern^[27]. The two main advantages of this approach are that (1) the bottom-up technique may proceed even in presence of substantial visceral obesity; and (2) that the distal margin can be visually chosen at the beginning of the

dissection. With the other modalities, the distal margin is often approximated based on feel, tattoo, or experience; when a stapler is fired, there is always the potential for encroaching on the distal margin. There are several limitations with this technique as well. In contrast to the other techniques which pursue the replication of the open method, the TaTME is a radically different approach that may lead to disorientation, incorrect dissection planes with unusual complications, as well as a stretch injury of the sphincter complex. The learning curve and training are cumbersome and require 45-51 cases before achieving proficiency^[28]. While this surgery can be performed completely *via* the transanal approach, most centers will work with two teams from the abdomen and transanally, duplicating the use of resources and team members at the same charge.

QUALITY OF DATA/ADOPTION

Due to the challenges, adoption of minimally invasive surgery techniques for rectal cancer has been slower than for colon resections. In 2005, about 90% of the proctectomies were done open^[29]. Since then, there was a slow overall increase in laparoscopic and robotic techniques to 52% by 2016. Notably, however, robotic rectal surgery rose faster than laparoscopic rectal surgery with a 3.8-fold *vs* 1.7-fold increase from 2010-2014, respectively^[30]. The gain in popularity of these surgeries allowed for better quality of the related research. Publications on each of the minimally invasive surgery platforms have followed the natural investigative pattern starting with the initial pilot series of feasibility, subsequent single center observational studies, and ultimately multicenter RCTs.

Correlating with the time since introduction to rectal cancer, laparoscopic surgery with currently five nationwide RCTs has the highest number of publications (Table 3 and 4), all of which meanwhile have follow-up reports on long-term outcomes. Following about a decade behind, robotic surgery has only one large multicenter RCT, but at least several single-center RCTs along with a plethora of matched observational studies from national databases and single centers (Table 5). Most researchers focused on comparing laparoscopic and robotic surgery while there is only a small number of studies comparing open to robotic surgery. Even though strictly speaking not permissible, there was the biased assumption that earlier findings from the laparoscopic *vs* open trials could be extrapolated onto robotic surgery since both were minimally invasive techniques. The body of evidence for TaTME as the newest surgical technique is still limited with only one modest sized RCT (Table 6). There are a several observational single-center studies starting in 2015 with a hybrid series of 140 patients^[31], as well as retrospective studies comparing TaTME to laparoscopic or robotic surgery, respectively.

Looking at these four modalities of achieving an oncological resection as well as additional hybrid variations, the total number of possible individual comparisons becomes exhaustive, and a randomized 4-arm is highly unlikely. In the following section, surgical outcomes are presented based on the highest available level of evidence for each modality.

SHORT TERM OUTCOMES: OPERATIVE CHARACTERISTICS

Operative times

Intraoperative findings that define quality in surgery include operative time, blood loss, and rate of conversions to an open procedure. Laparoscopic times, with an average of 215 min, have been consistently and on average 34 min longer than open surgery times (Table 4). The clinical significance of this remains uncertain, particularly since this applied to several of the earlier trials when minimally invasive surgery was not yet routine. The difference was most pronounced in the COREAN and COLOR II trial where the operating time was 47 and 52 min longer for laparoscopic surgery, respectively^[7,32].

Robotic times in most studies tend to be longer than laparoscopic times (Table 5), on average 250 min, *i.e.*, 15 min longer. Reflected in this is also the extra time and expertise to dock the robot which initially inflated times. The difference between laparoscopic and robotic arms was most pronounced in the Kim *et al*^[16]'s RCT with 227 min *vs* 339 min, whereas the ROLARR trial did not find a significant difference with 261 min *vs* 298 min, respectively^[15].

Table 4 Selected laparoscopic vs open rectal cancer surgery studies

Ref.	Yr	Trial	Surgery	n	OR Time (min)	EBL (mL)	CVR (%)	LOS (d)	Comp (%)	Mort (%)	DRM+ (%)	CRM+ (%)	LN	C-TME (%)	DFS (%)	OS (%)	LRR (%)
Guillou <i>et al</i> ^[6]	2005	MC RCT	Lap	253				11	59	4		12					
			Open	128				13	50	5		6					
Lujan <i>et al</i> ^[83]	2009	SC RCT	Lap	101	193	127	17	8	33		0	4	13		85	72	4.8
			Open	103	172	234		10	33		0	3	11		81	75	5.4
Kang <i>et al</i> ^[7]	2010	MC RCT	Lap	170	244	200	1	8	21			4	17	75			
			Open	170	197	217		9	23			3	18	72			
Liang <i>et al</i> ^[43]	2011	SC RCT	Lap	169	138				20				7			76	
			Open	174	118				19				7			83	
Lujan <i>et al</i> ^[84]	2013	MC PR	Lap	1387	217			8	38	1	1	10	14	82			
			Open	3018	186			11	45	4	1	16	14	75			
Van Der Pas <i>et al</i> ^[32]	2013	MC RCT	Lap	739	240	200	17	8	40	1		10	13	88			
			Open	364	188	400		9	37	2		10	14	92			
Jeong <i>et al</i> ^[82]	2014	MC RCT	Lap	170											72	88	2.6
			Open	170											79	85	4.9
Bonjer <i>et al</i> ^[8]	2015	MC RCT	Lap	699											75	87	5
			Open	345											71	84	5
Stevenson <i>et al</i> ^[10]	2015	MC RCT	Lap	238	210	100	9	8	19		1	7		87			
			Open	235	190	150		8	25		1	3		92			
Stevenson <i>et al</i> ^[12]	2019	MC RCT	Lap	225											80	94	5.4
			Open	225											82	93	3.1
Fleshman <i>et al</i> ^[9]	2015	MC RCT	Lap	240	266	256	11	7	57	1	2	12	18	92			
			Open	222	220	318		7	58	1	2	8	17	95			
Fleshman <i>et al</i> ^[11]	2019	MC RCT	Lap	240											80	85	4.6
			Open	222											83	86	4.5

CVR: Conversion rate; Comp: Complications; Mort: Mortality; CRM/DRM: Circumferential/distal resection margin; C-TME: Complete total mesorectal excision; sMC: Multicenter; RCT: Randomized controlled trial; SC: Single center; PR: Prospective review; OS: Overall survival; DFS: Disease-free survival; LRR: Local recurrence rates.

Table 5 Selected laparoscopic vs robotic rectal cancer surgery studies

Ref.	Yr	Trial	Surgery	n	OR time (min)	EBL (mL)	CVR (%)	LOS (d)	Comp (%)	Mort (%)	DRM+ (%)	CRM+ (%)	LN	C-TME (%)	DFS (%)	OS (%)	LRR (%)
Patriti <i>et al</i> ^[36]	2009	SC RR	Lap	37	208	127	20	10	32			0	11				
			Robot	29	202	137	0	12	26			0	10				
Cho <i>et al</i> ^[85]	2015	SC PSM	Lap	278	272	147	1	10	23		1	4.7	16		79	93	3.9
			Robot	278	361	179	1	10	25		0	5	15		81	92	5.9
Jayne <i>et al</i> ^[15]	2017	MC RCT	Lap	234	261		12	8	31	1		6.3	24	75			
			Robot	237	298		8	8	33	1		5.1	23	75			
Wang <i>et al</i> ^[59]	2017	SC RCT	Lap	66	207								16				
			Robot	71	246								16				
Kim <i>et al</i> ^[53]	2017	SC PSM	Lap	224	249		1	14	24	1		4.9	21		68	78	
			Robot	224	285		0	13	32	1		4	20		72	90	
Law <i>et al</i> ^[49]	2017	SC PR	Lap	171	225	100	4	6	22	1		8.2	12		80	74	5
			Robot	200	260	100	1	6	19	1		4.1	14		82	71	5
Rouanet <i>et al</i> ^[37]	2018	SC RR	Lap	200	232	100	10	11	24			11	19	90		88	
			Robot	200	243	200	2	10	25			8	15	85		84	
Sammour <i>et al</i> ^[44]	2018	SC O	Robot	276	100		2	4	34			2.5	22	76	82	87	2.4
Chang <i>et al</i> ^[86]	2019	SC O	Robot	1145	166	73	6	6	16	1		1.3	17	90			2.3

CVR: Conversion rate; Comp: Complications; Mort: Mortality; CRM/DRM: Circumferential/distal resection margin; MC: Multicenter; RCT: Randomized controlled trial; SC: Single center; PR: Prospective review; RR: Retrospective review; PSM: Propensity score matched; O: Observational study; OS: Overall survival; DFS: Disease-free survival; LRR: Local recurrence rates.

Finally, TaTME times are on average about 250 min (Table 6). Two studies suggested a significant reduction in operating times when compared to open, laparoscopic, and robotic approaches^[33,34]. In all fairness, it should be noted though that a large contributing factor lies in the resource-intense use of two teams that

Table 6 Selected transanal total mesorectal excision rectal cancer surgery studies

Ref.	Yr	Trial	Surgery	n	OR time (min)	EBL (mL)	CVR (%)	LOS (d)	Comp (%)	Mort (%)	DRM+ (%)	CRM+ (%)	LN	C-TME (%)	DFS (%)	OS (%)	LRR (%)
Denost <i>et al</i> ^[17]	2014	SC RCT	Lap	50	264		10	8	44	2	2	18	17	62			
			TaTME	50	240		4	7	32	0	8	4	17	70			
Lacy <i>et al</i> ^[31]	2015	SC O	TaTME	140			0		34			6	15	97	91	97	2.3
Chen <i>et al</i> ^[51]	2016	MC PMR	Lap	100	178	88	5	7	17			10	17				
			TaTME	50	182	68	2	7	20			4	17				
Marks <i>et al</i> ^[55]	2017	SC O	TaTME	373												90	7.4
Penna <i>et al</i> ^[87]	2017	MC O	TaTME	720	277		9	8	32	2	0	2	17	85			
Denost <i>et al</i> ^[54]	2017	SC RCT	Lap	50											71	74	4.8
			TaTME	50											73	87	2.6
Persiani <i>et al</i> ^[42]	2018	SC PSM	Lap	46	272		20	7	21			0		84			
			TaTME	46	276		0	7	23			0		87			
Perdawood <i>et al</i> ^[33]	2018	SC PSM	Lap	100	334	239	11	14			1	13	22	68			
			TaTME	100	285	82	0	8			0	7	22	58			
			Open	100	325	704		15			1	10	18	68			
Perez <i>et al</i> ^[88]	2017	SC RR	Robot	60	276		10	8	37				15	88			
			TaTME	55	291		3	7	22				15	90			
Detering <i>et al</i> ^[18]	2019	MC PSM	Lap	396			9	6	36	1		4					
			TaTME	396			2	7	42	0		4					
Law <i>et al</i> ^[34]	2019	SC PSM	Robot	40	270	150	5	6	17			1	13				
			TaTME	40	254	90	5	6	12			0	13				
Lee <i>et al</i> ^[19]	2019	MC PMR	Robot	453	189		4		35	0	1	6	16	95			
			TaTME	277	189		3		33	0	2	6	16	92			
Hol <i>et al</i> ^[56]	2019	SC O	TaTME	159					52		0	1		87	81	77	3.8
Wasmuth <i>et al</i> ^[20]	2019	MC O	TaTME	157													11.6

MC: Multicenter; RCT: Randomized controlled trial; SC: Single center; PMR: Prospective matched review; RR: Retrospective review; PSM: Propensity score matched; O: Observational study; TaTME: Transanal total mesorectal excision. OS: Overall survival; DFS: Disease-free survival; CVR: Conversion rate; Mort: Mortality; Comp: Complications; CRM: Circumferential resection margin; DRM: Distal resection margin; C-TME: Complete total mesorectal excision; LRR: Local recurrence rates.

simultaneously work transabdominally and transanally.

Intraoperative blood loss

This parameter is an estimated and self-reported variable that unsurprisingly has much more variability between studies than time. However, blood loss is generally and on average 87 mL larger in open surgery than in laparoscopic surgery. Whether this is clinically meaningful is doubtful. The largest variation was seen in the COLOR II trial with 200 mL seen in laparoscopic *vs* 400 mL in the open group^[32]. Blood loss was much less strictly reported in robotic and TaTME comparative studies. In the former, it averaged 131 mL with no major differences compared to laparoscopic surgery. For TaTME, the average in the three studies that reported blood loss was 66 mL, which was significantly less than the other modalities in two studies^[33,34].

Conversion rates

Perhaps the most relevant operative variable is the conversion rate which has been associated with substantial incremental cost^[35]. In the major RCTs, laparoscopic conversion rates average 11% with a range from (0)1-17%. Comparison between laparoscopic and robotic conversions was the primary end point in the ROLARR trial. In theory, the robotic approach was expected to reduce the number of conversions caused by obstructed vision and limited working space. However, the observed difference between conversions, which were 12% *vs* 8% for laparoscopic and robotic, respectively, did not reach statistical significance^[15]. In contrast, two retrospective studies noted lower conversion rates by robotic approach: The larger was a single center study with 200 patients per arm (2% *vs* 10%), the smaller one with 29 *vs* 37 patients (0% *vs* 20%)^[36,37]. Four larger database studies found the robotic approach to reduce the number of conversions^[38-41], which was associated with a reduced length of stay of 8 d and 15 d, respectively.

Conversions from a TaTME surgery are harder to define, especially when two teams are working and tackling the challenging sequences together. If difficulty is encountered from one direction that would warrant a conversion, the other team can finish without technically recording a conversion. As a result, the literature records a very small TaTME conversion rate averaging 2.7%. Two small matched studies comparing TaTME with laparoscopic surgeries showed zero conversions with TaTME had and 11% and 20% with the laparoscopic technique^[33,42].

SHORT TERM OUTCOMES: POSTOPERATIVE CHARACTERISTICS

Standard variables reported for the postoperative phase of care include: Return of gastrointestinal function (*e.g.*, time to food intake, flatus, and bowel movements), length of stay, postoperative complications, and mortality. It should be noted that a highly promising driver of quicker recovery, *i.e.*, intracorporeal instead of extracorporeal anastomosis, has not been yet included in most publications. The robotic approach clearly facilitates that technique and there has been a significant push towards it in the last two years. But it may be too early to see the subjective impression of a benefit substantiated by objective solid evidence.

Return of GI function

Return of bowel function has been assessed in several different ways, including tolerance of liquid or solid diet, as well as time to flatus and bowel movements. That passive approach has in recent years been revisited by the proactive ERAS protocols that were nearly invariably recognized as preferred postoperative management regardless of the surgical approach.

Different studies used different metrics, and pooling of data is difficult. Several studies showed a modest but significant reduction with the laparoscopic compared to the open approach. Specifically in the COREAN trial, laparoscopic surgery was associated with significant reductions of the time to bowel movements, flatus, and the time to solid diet by 0.8 d, 0.9 d, and 0.4 d, respectively^[7]. A smaller RCT from 2011 revealed nearly identical significant results in each category^[43]. – The impact of robotic surgery on the return of bowel function has not yet been studied as extensively, with the most robust data coming from the Kim *et al*^[16]'s RCT. There was no difference between the laparoscopic and the robotic groups with return of bowel function on day 2. – As to the TaTME trials, these data points appeared not to be in the focus of the studies and were not reported to any relevant degree. Only the Persiani study analyzed bowel function and noted a reduction in time to flatus and time to oral intake in the TaTME group compared to the laparoscopic group (1 d *vs* 3 d, respectively for both metrics)^[33].

Length of stay

The reduction in hospital stay and a quicker return of bowel function seen in the laparoscopic *vs* open colon surgery trials was expected to carry over into laparoscopic rectal surgery. There was indeed a modest but real reduction in length of stay. The COREAN and COLOR II trials both saw a one day reduction in hospital stay while the CLASICC trial recorded a two day reduction^[6,7,32]. The Z6051 trial had the shortest length of stay at 7 d and did not see a difference between the two groups^[9]. Both the laparoscopic and robotic techniques share the same advantages of minimally invasive surgery with small incisions as one of the driving forces to faster recovery. Not unexpectedly, most quality studies did not show any difference in length of stay. The average robotic length of stay is 8.5 d, the shortest was 4 d as reported in an observational study^[44]. One large database study found a shorter length of stay (4 d) in the laparoscopic group as opposed to 6 d in both the open and robotic group^[45]. However, two other similar databases reported the shortest length of stay in the robotic group (6 d), followed by the laparoscopic (7 d) and open approach (8 d)^[39,46].

The same holds true for the TaTME approach as well. The average stay is 6.8 d with only a 3-arm case-matched study from Denmark with 100 patients per approach showing a difference between the groups^[33]. The TaTME group had an 8-d stay compared to 14 and 15 d for laparoscopic and open. However, it is difficult to draw conclusions as all groups had above-average length of stay compared to US studies.

Morbidity and mortality

Open and minimally invasive colorectal surgery are generally predictable and safe. Mortality for all is fortunately equally low at 0-3%. However, the complication rates are widely variable from study to study, likely the result of varying inclusion criteria. Rectal surgery has always carried a substantial risk for complications with the most common issues related to anastomotic leak, wound infections, ileus, and stoma related problems (*e.g.*, dehydration from ileostomy). The major laparoscopic RCTs reported complication rates in the range of 19%-59%, however they did not significantly differ from open or robotic surgeries. Similar to others, the ROLARR trial found the robotic complication rate to be 33% as opposed to 31% in the laparoscopic group^[15].

Finally, TaTME appeared to be associated with a higher rate as well as unusual type of complications. An early publication noted a difference between TaTME (32%) and

laparoscopic surgery (44%) which did not reach statistical difference^[17]. Later, however, the Norwegian trial reported a significantly higher anastomotic leak rate of 8.4% when compared to their overall national averages of 4.5%^[20]. This, in part, led Norway to put a hold on the TaTME procedure. A large prospective, observational, multi-center audit study with 2579 patients found TaTME leak rates to be 10.4% when the abdominal part was done laparoscopically and 15.6% when done robotically. Both were significantly higher than the pure laparoscopic (6.7%), pure robotic (6.5%) or open (5.5%) approaches. However, the significance was lost when a mixed-effects model was applied^[47].

SHORT TERM OUTCOMES: PATHOLOGIC CHARACTERISTICS

The complete removal of the rectum and lymph nodes within an intact mesorectal envelope represents the primary goal of successful rectal cancer surgery. Pathologic assessment of the specimen is arguably a core parameter and quality benchmark. Even if residual disease after surgery is microscopic only (R1 resection), the risk of overall failure is substantial with 56% distant recurrence and disease-free survival of only 41%^[48]. There are four commonly reported elements of the pathologic results: Lymph node harvest, circumferential resection margin (CRM), distal resection margin (DRM), and quality of the TME specimen.

Lymph node harvest

A lymph node harvest of at least 12 lymph nodes is universally considered a surrogate for a complete TME, regardless of the technique. In the literature, in fact an average harvest of 15 lymph nodes is reported in all groups. No difference in nodal retrieval was noted in most studies except in the Kim RCT and a smaller trial where robotic surgery shifted the balance by three additional nodes^[16,49].

Circumferential resection margin

A negative CRM of at least 1mm is another surrogate for a complete TME. Three major trials (CLASICC, ALaCaRT, ACOSOG Z6051) showed a higher rate of positive CRMs in the laparoscopic compared to the open group. The most striking difference was 12% *vs* 6% in the CLASICC trial, even though it did not reach statistical significance^[6]. The two other trials were designed but failed to demonstrate non-inferiority based on a composite pathology index^[9,10]. Together, these trials at least early on red-flagged the use of laparoscopic surgery for rectal cancer. On long-term follow up, however, these data were less of concern as they did not translate into higher local or systemic failure rates. A recent large database audit found significantly different but overall small rates of positive CRM; 3.2% robotic, 4.1% laparoscopic, and 5.4% open^[50]. The authors concluded though that the differences were real but too small to promote one technique over the other. The major robotic *vs* laparoscopic studies have shown the robot to maintain a low rate of CRM positivity of 0-8%, which in no study was statistically inferior to laparoscopy. The ROLARR trial found CRM positivity in 5.1% (laparoscopic) and 6.3% (robotic)^[15].

In the Bordeaux trial, TaTME compared to laparoscopic specimens were associated with a much lower rate of CRM positivity of 4% *vs* 18% ($P < 0.05$)^[17]. Even though the laparoscopic comparison group had an unacceptably high recurrence rate, the authors prematurely claimed TaTME to be the new standard going forward. Also of note was that all these patients had low cancers (< 6 cm from the verge) which is the ideal setting for the TaTME. Subsequent studies could not corroborate such robust results. In fact, the large Dutch study and the recent paper by Lee both showed near identical rates of positive CRM^[18,19].

Intactness of specimen mesorectal envelope

The integrity of the TME specimens is visually graded. As with the CRM, several major laparoscopic versus open trials found the completeness of the TME to be higher in the open group but not to a significant level. TME completeness in the ALaCaRT trial was high but statistically not significantly different in both the laparoscopic and the open group (87% *vs* 92%, respectively)^[10]. The robotic and TaTME technique appeared to produce equal quality of the TME even though at a lower range. The ROLARR trial reported identical TME completeness of 75% in each group^[15]. There were no differences in TME completeness in TaTME *vs* the other groups except in a Danish study where TaTME completeness was abnormally low at 58% compared to 68% in lap and open groups^[33].

AXIAL RESECTION MARGIN

The final pathology variable is the axial and particularly the distal resection margin. This is a more nebulous variable as it is inconsistently reported as a numeric value of the absolute distance or as a binary value of positive versus negative margin. If the tumor is high enough, *i.e.*, in the mid to upper rectum, a margin of 5 cm should be targeted. For the tumors in the distal rectum, a shorter distance has generally been acceptable. Ideally, it should be at least 1-2 cm, but on occasion a negative margin of any length has been acceptable in the lowest cancers. There are no studies that show a significant difference in distal margin positivity between laparoscopic, robotic, or open techniques. However, the most recent matched trial found a higher distal margin positivity in TaTME than robotic cases of 1.8% *vs* 0.3% ($P = 0.051$), respectively^[19]. This finding is at variance with the claim that TaTME is the modality most suited to attaining a negative distal margin because the surgeon starts the dissection with this margin in mind and view usually at least 1 cm distal to the tumor. Paradoxically, the margin positivity rate was higher, but overall, the TaTME specimens had a longer DRM of 16.9 mm *vs* 15.1 mm. Two other smaller TaTME studies found no difference in DRM positivity but the TaTME DRM distance in both was larger (2.5 cm) as compared to the laparoscopic technique (1.5 cm)^[42,51].

LONG TERM OUTCOMES: SURVIVAL AND RECURRENCE

All previously discussed parameters are surrogate to the ultimate oncological long-term outcomes. The standard metrics include local recurrence rates (LRR), disease-free survival (DFS), and overall survival (OS). Most of the landmark RCTs have even published long-term data.

Four major laparoscopic trials have robust data of at least 24-36 mo follow-up comparing laparoscopic *vs* open rectal cancer surgery. Reported local recurrence rates were comparably low in the range of 2%-6%. This is especially meaningful in the Z6051 trial where the CRM positivity was by 6% higher in the laparoscopic arm. The reported 2-year local recurrence rates, DFS and OS were nearly identical at 4.6% *vs* 4.5%, 83% *vs* 85%, and 85% *vs* 86%, respectively^[11]. The other RCTs reported similar results.

Regarding robotic data, long-term data are still awaited. The ROLARR trial has not yet reported its long-term outcomes. Current information is limited to several single center, propensity score matched studies that reported on these outcomes. Robotic surgery was a positive prognostic factor for OS at 36 mo^[52], and at 60 mo was associated with increased OS of 90% *vs* 78% compared to the laparoscopic group^[53]. Whether these results can be corroborated by large RCT remains to be seen. Large database studies at least were not able to reproduce such a difference^[40,50].

As previously noted, the Bordeaux trial analyzed the respective 5-year TaTME outcomes. Even though there was a difference by 12% of CRM positivity in favor of TaTME, the local recurrence rates did not differ and were around 4% in both groups, and DFS and OS were comparable^[54]. In contrast, the Norwegian trial had not only a substantially higher leak rate, but also an alarmingly high local recurrence rate of 11.6% at 9 months as opposed to the reported average of 2.4% for all approaches in their national databases^[20]. None of the other studies replicated this high local recurrence rate^[31,55]. At five years, TaTME in 159 patients in the Netherlands resulted in a 3.8% LRR, 81% DFS, and 77% OS^[56].

In summary, laparoscopic and open technique appear to achieve comparable results, robotic surgery is likely in the same range, and the TaTME awaits further analysis. The focal areas of differences are likely a product of underpowered studies that should be overcome by better evidence to reach a final verdict.

LONG TERM OUTCOMES: BOWEL, BLADDER AND SEXUAL FUNCTION

Quality of life parameters including postoperative bowel, bladder and sexual function are a big issue and from patient perspective just as important as the oncologic outcome. This is true at any time point in a patient's journey but increasingly moves into the center of attention as the patients achieve cure from the cancer. Unfortunately, this topic has not been studied in similar detail as other outcomes for several reasons: Physicians may not assess those parameters systematically, patients may not always want to share their experiences, and the validated instruments to study these

parameters entail lengthy and cumbersome questionnaires. Finally, the functional outcomes are affected by a multitude of individual factors, both independent and dependent on the treatment as such (surgery, chemotherapy, radiation).

Bowel function after rectal cancer surgery is mainly a product of the following components: The level of the anastomosis, the absence of an anastomotic leak, the reservoir function, and the sphincter function.

The reservoir function for the very low anastomoses may in the short run be improved with a colonic J-pouch, but the benefit may wane in the long run and turn into a disadvantage with stool clustering. The post-treatment sphincter function is a function of the preexisting condition, technical preservation, and inadvertent injury (*e.g.*, stretch injury during transanal or TaTME dilation).

Sexual and urinary function are affected by multi-factorial impairment to visible and microscopic neurological networks: Hypogastric nerves, nervi erigentes, pelvic plexus, and cavernous nerves^[57]. It has been postulated that minimally invasive techniques, in particular the robotic approach, may better visualize and preserve the identifiable structures and cause less trauma.

Most of the trials to include function are small single center comparative studies. Fortunately, several of the large RCTs also included this component as part of the trial but provide limited information about the technical details of surgery. The COREAN and COLOR II laparoscopic *vs* open trials employed the EORTC quality of life questionnaires three months after surgery. Although this was early, there were fewer problems with urination and defecation in the laparoscopic group^[7]. In 2015, a follow-up to the COLOR II trial specifically reported on genitourinary function up to two years after surgery^[58]. There was no difference in urinary or sexual function between open and laparoscopic surgery at any time point between four weeks and two years follow-up. Sexual function, which also contains a psychological component, suffered a greater impact than urinary function but both improved with time.

There is decent body of evidence on this field in the robotic *vs* laparoscopic literature. The ROLARR study and two smaller single-center RCTs included sexual and urinary function as secondary end points. In the ROLARR trial, patients self-reported their bladder and sexual function using well-established scoring systems at baseline and at six months. The validated instruments included the international prostate scoring systems (IPSS), international index of erectile function (IIEF), and the female sexual function index (FSFI). The specific minimally invasive approach did not make a difference^[15]. A 2015 Chinese single center RCT with urinary and male sexual function as primary end points noted worse IPSS/IIEF scores in 66 laparoscopic compared to 71 robotic patients. On multivariate analysis, only the laparoscopic approach was predictive of worse sexual function^[59]. In contrast, another single-center RCT based on EORTC instruments showed no difference in quality of life, bowel or bladder function at 12 mo; however, sexual function was better in the robotic cohort^[16]. Finally in 2018, a propensity score study comparing 130 matched pairs reported at six and twelve months worse sexual and urinary function in the laparoscopic group as compared to the robotic group^[60]. Even though these trials suggest that the robotic approach may offer a more gentle approach to better preserve sexual and urinary function, it is hard to corroborate in the absence of a difference in the largest trial (ROLARR).

Finally, comparative studies have also looked at the impact of TaTME versus the laparoscopic technique on sexual and urinary function. Proponents suggest that the initial approach that immediately encroaches on the nerves in question might help to prevent nerve injury. Critics, on the other hand, pointed out the significant stretch injury to the pelvic floor and sphincter complex. The current data remain sparse. Based on EORTC questionnaires, two small prospective single center trials in 2019 found no difference in urinary or sexual function at six months after TaTME and laparoscopic surgery; however, fecal incontinence scores were worse in the TaTME group^[61]. Using numerous questionnaires, TaTME and laparoscopic surgery had no difference in low anterior resection syndrome score or IPSS scores; on individual questions, though, the laparoscopic group fared better on diarrhea, clustering, and urgency scores at eight months, whereas the TaTME group had improved urinary status^[62]. These trials were small and only compiled 54 and 85 patients in total. Larger studies and systematic before and after assessment will be needed before the functional concerns about TaTME are either found to be unsubstantiated or corroborated.

TRANSANAL MINIMALLY INVASIVE SURGERY

Radical oncological resection with removal of the rectum and its corresponding mesorectal envelope including lymph nodes is the appropriate and default intervention of choice for rectal cancer. However, a local excision may be appropriate for precancerous lesions and plays a clarifying role for lesions of uncertain behavior and for an organ-preserving watch and wait approach after chemoradiation^[63]. Local excision may be appropriate but remains controversial for very early presentations of invasive rectal cancer. T1 lesions comprise about 15% of the total rectal lesions^[50]. The concerns with local excision relate to the significant incidence of local recurrences, related to retained positive lymph nodes (7%-13%) and possibly the direct implantation of cancer cells into the excision site.

There are different surgical platforms to perform a transanal local excision: (1) Conventional transanal excision under direct vision for relatively low lesions; (2) Transanal endoscopic microsurgery (TEMs)^[64]; or (3) Transanal minimally invasive surgery (TAMIS). For the latter, there is the standard laparoscopic instrumentarium (L-TAMIS), and more recently, a robotic approach (R-TAMIS) has been utilized. These techniques offer improved visualization, reach and allow for a finer and better controlled dissection in the limited rectal space, which likely explains the trend to overall lower recurrence rates of 4%-6%, but still up to 24%^[65,66].

Due to the longer history, data on TEMs are more robust even though there is no RCT. In 2018, a series of 70 cases over a ten year period reported a 16% complication, a 14% positive margin rate, and a local recurrence rate of 8% at 60 mo^[67]. A recent meta-analysis of six such studies included a total of 927 local excisions^[68]. TEMs was superior to standard local excision with less specimen fragmentation, less positive margins, and a lower recurrence rate. Local recurrence rates vary and in reviews have been summarized in the range of (0-)4-6%, but still up to 24%^[65,66].

The TAMIS approach was originally described in 2010^[69], subsequently grew in popularity, and with a 6% complication rate is considered a safe and effective option. The research remains in evolution; there are no RCTs to compare TEMs to TAMIS. The rate of positive margins is around 6%, and the local recurrence 4% at 18 mo^[70]. Comparison of 53 TEMs and 68 TAMIS patients showed some differences in operative times but no difference in positive margins or fragmentation^[71]. A larger study from 2017 comparing 247 TEMs with 181 TAMIS showed identical recurrence rates of 7% and similar five-year disease-free survival of 80% *vs* 78%, respectively^[72].

More recently, the robot has been used to perform a TAMIS robotically (R-TAMIS): The docked ports are placed through a TAMIS port to accomplish a local excision from the console. R-TAMIS in 58 patients achieved the excisions with a 1.7% fragmentation, 5.2% positive margin and a 5.5% local recurrence rate^[73]. A recent paper compared L-TAMIS and R-TAMIS in 40 patients which found no difference in outcome except an \$880 increase in cost with the robot^[74].

Regardless of the platform, local excision for proven invasive cancer carries a substantial risk of local recurrence. Addition of multimodality treatment for these stage I cancers (that by means of an oncological resection have a greater than 90% cure rate) is not justified for the sole purpose to avoid a proper operation. Local excisions should be limited to highly select circumstances where the downsides are outweighed by the expected benefits.

SYNOPSIS AND FUTURE DIRECTIONS

As we enter the fourth decade of minimally invasive surgery, its popularity for rectal cancer surgery continues to grow. Of the four surgical modalities to perform an oncological resection, there is no convincing evidence to rate one superior to any other as long as the set goals are met. More impact on outcomes than the choice of approach appears to have (1) whether or not to perform an oncological resection versus a local excision by any means; and (2) the selection, tailoring, and timing of non-surgical treatment modalities.

Unquestionably, the rates of minimally invasive surgery continue to increase worldwide each year despite initial skepticism and concerns about noninferiority and cost. The exact path and role each modality will take remains to be seen. The early driving force and support most prominently come from both patients' and surgeons' perceptions that patients tend to experience a smoother short- and long-term recovery while achieving comparable oncological outcomes. Unfortunately, this biased impression remains a major obstacle to systematic randomized research. Maintaining

support in the longer run therefore mostly builds on at least equal if not superior outcomes, and the force of routine and familiarity of the surgical community that individually and collectively move past the learning curves. Except for the Norwegian moratorium on TaTME, there has been no large study that has shown significant inferiority of the minimally invasive approach to open surgery.

The main factors that will drive the future of rectal cancer surgery are selection, outcomes, access, technology, and standardization. It is highly unlikely that there will be a trial to compare all different modalities in a direct fashion. Further trials will continue to give us a patchy picture with select comparisons. For example in regard to defining the role of TaTME, there are currently two large RCTs underway comparing it to a laparoscopic TME. The first is the European COLOR III trial with the target enrollment of 1098 patients and the primary outcome the positive CRM^[75,76]. The second is the smaller French GRECCAR 11 trial with similar setup^[77].

The major factor defining the future of minimally invasive surgery for rectal cancer is access and costs. With each new modality, new instruments, tools and infrastructure are developed with respective associated costs. This was the case with the introduction of laparoscopic surgery and has equally been a major target of criticism in rolling out robotic surgery. The current robotic platform costs approximately \$2.5 million in capital investment along with many add-ons, service contracts, and disposable instruments which average \$80000-170000 annually^[78]. Almost every early cost study pertaining to robotic surgery has shown significantly increased costs (1.5-2.4 times) when compared to laparoscopic surgery^[79]. With limited healthcare resources, robotic surgery is at risk if costs continue to remain high unless there is a measurable benefit beyond the operating room. In particular, the push to perform an anastomosis intracorporeally has been facilitated by the robotic platform and appears to be associated with an accelerated recovery.

Minimally invasive surgery is not equally available to the entire population with a significant urban-rural and potentially socio-economic difference. A recent study has shown a significant proportion of patients who received robotic rectal cancer surgery were white, male, privately insured, and in a metropolitan area^[80].

Probably the most crucial factor affecting the future of minimally invasive surgery is the technology itself. Currently, there is a dominance of the DaVinci robot systems who initiated the revolution and carry an advantage of almost two decades over their competitors. Nonetheless, competition is forming and expected to advance the technology and to stimulate a reduction in price. And as new systems come online, there likely will be numerous comparative studies. Areas of desired improvements include haptic feedback, better and multilevel internal articulation, less external arm movements and collisions, as well as force reduction across the abdominal wall, decrease of instrument diameters, increased versatility through multiple quadrant surgeries, and 3D vision for all team members. New optics and robotic-endoscopic instrumentation will be crucial to advance the field of endoluminal surgical interventions (ELSI). Transanal work for local excision or TaTME will likely benefit from the newly introduced da Vinci SP (single port) robot. And finally, artificial intelligence with advance planning capabilities, fusion of imaging with the surgical field, and highlighting of key structures (ureters, lymph nodes, blood vessels) are exciting features that will contribute to increased safety of complex procedures.

CONCLUSION

The role of minimally invasive surgery for rectal cancer is constantly changing and advancing. There are currently four main modalities for minimally invasive rectal cancer surgery. Laparoscopic and robotic approaches do require a longer operative time. Conversion data is conflicted but there does appear to be a trend toward reduced times with robotic surgery. There are no differences in morbidity and mortality between any group. In addition, all short- and long-term oncologic outcomes appear to be similar. Urinary and sexual function also appear to have better recovery with robotic surgery. Finally, there are three minimally invasive approaches to local excision. They all are comparable to one another and the conventional local excision at this point. In conclusion, minimally invasive surgery for rectal cancer has evolved to an accepted and well researched practice over the last thirty years. However, there are still many questions to be answered about superiority of any modality over another. In addition, future technologies are likely to challenge the current platforms already in place.

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Accelerating the elimination of hepatitis C in Kuwait: An expert opinion

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Abstract

The hepatitis C virus (HCV) is estimated to affect 71 million people worldwide. In 2016, the World Health Organization adopted the first global health sector strategy to eliminate viral hepatitis as a public health threat by 2030. In December 2018, the European Association for the Study of the Liver, International Liver Foundation convened an expert panel to address the elimination of HCV in Kuwait. Several steps have already been taken to eliminate HCV in Kuwait, including free HCV treatment for Kuwait's citizens, high blood safety standards, and the implementation of screening and awareness programs. The expert panel made several recommendations aimed at accelerating the elimination of HCV in Kuwait: The development of a national strategy and action plan to guide all HCV elimination activities; the formation of a coordination mechanism to support collaboration between hepatitis working committees; the prioritization of micro-elimination at primary, secondary or tertiary facilities, in prisons and rehabilitation centers; and ensuring the involvement of multiple stakeholders – including relevant civil society groups – in all activities. Enhanced screening and linkage to care should be prioritized in Kuwait, with the expansion of the prescriber base to primary healthcare providers and nurse practitioners to be considered. Raising awareness and educating people about HCV infection also remain essential to achieve the goal of HCV elimination. Lastly, a national HCV registry should be developed to help monitor the implementation of viral hepatitis plans and progress towards achieving national and international targets.

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Core tip: Globally, hepatitis C remains a public health threat, causing substantial morbidity and mortality. In 2016, World Health Organization adopted a strategy to eliminate hepatitis C virus (HCV) infection as a public health threat by 2030. The expert panel convened in Kuwait explored how to eliminate HCV and developed seven recommendations to accelerate the response, which included developing a national strategy and action plan which outline national priorities for eliminating HCV; increasing awareness and educating people about HCV infection; micro-eliminating HCV in high-risk population groups; ensuring linkage to care; and developing a national HCV registry to monitor progress towards national and international goals in Kuwait.

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INTRODUCTION

The hepatitis C virus (HCV) remains a public health threat in many countries, causing substantial morbidity and mortality^[1,2]. While 15%-45% of the infected population become HCV free within six months without treatment, the rest develop chronic infection^[3,4]. According to the World Health Organization (WHO), an estimated 71 million people have chronic HCV infection globally^[5], which left untreated can lead to cirrhosis and hepatocellular carcinoma (Figure 1)^[3,4]. According to the WHO global hepatitis report, in 2015, 1.75 million new HCV infections occurred^[5], while in 2016, HCV led to an estimated 399000 deaths globally^[5].

In response to the global burden of HCV and the associated morbidity and mortality, the WHO adopted the goal of eliminating viral hepatitis as a public health threat by the year 2030^[6]. In 2016, 194 countries, including Kuwait, adopted the first global health sector strategy to combat viral hepatitis (Table 1)^[6,7]. Figure 2 shows the continuum of viral hepatitis services and retention cascade, which forms the basic blocks by which these elimination targets will be achieved^[6,7]. However, in 2019, only nine of the 194 countries to adopt the strategy were on track to reach the elimination targets. Kuwait, was one of the countries not on track to reach elimination targets before 2050^[8].

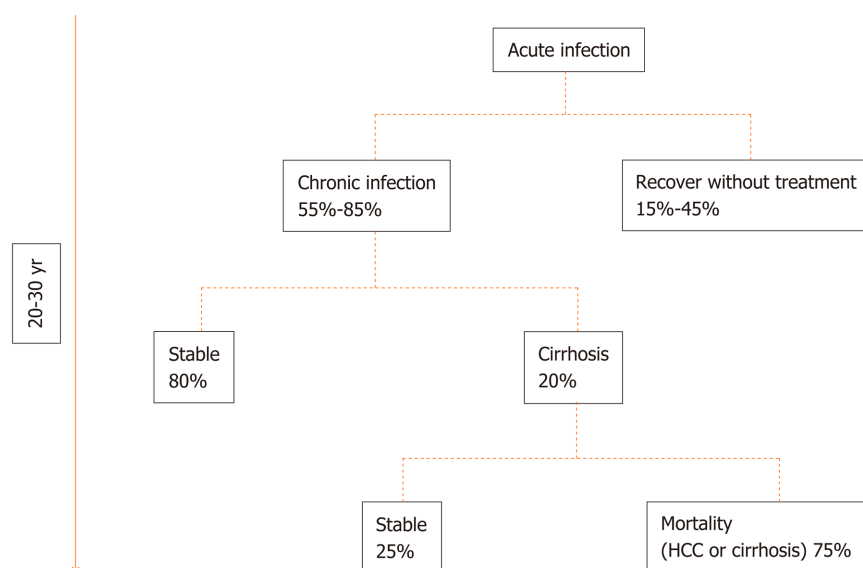
Estimated at 1%, the prevalence of HCV in the Arabian Gulf is considered high^[9]. In Kuwait, HCV remains a pressing public health issue. The country witnesses high inward migration from HCV-endemic areas^[10], with 75% of the population of 2 million being expatriates^[11]. Mohamoud *et al*^[9] 2016 estimate the prevalence of HCV among Kuwaiti nationals at 0.44% [95% confidence interval (CI): 0.29-0.62], rising to 1.45% (95% CI: 0.75-2.34) among the entire resident populations including nationals and expatriates.

In December 2018, the European Association for the Study of the Liver, International Liver Foundation (EILF) convened an expert panel in Kuwait^[7] to discuss the country's HCV elimination efforts and to determine urgent priorities. The expert panel was comprised of 12 local key opinion leaders and other stakeholders, including EILF board members, and gastroenterologists and experts from various health departments in Kuwait^[7]. This meeting had the following four objectives: (1) To assess the current state of HCV elimination efforts in Kuwait; (2) To review the action plan of HCV elimination followed in other countries; (3) To identify factors for successes, including knowledge gaps and barriers to HCV elimination in Kuwait; and (4) To develop recommendations to advance HCV elimination efforts in Kuwait^[7].

Table 1 Global health sector strategy on viral hepatitis targets

Target area	2020 target	2030 target
Impact targets		
Incidence: New cases of chronic HCV	30% reduction	80% reduction
Mortality: HCV related deaths	10% reduction	65% reduction
Service coverage targets		
Blood safety: Donations screened in a quality assured manner	95%	100%
Safe injections: Percentage of injections administered with safety-engineered devices in and out of health facilities	50%	90%
Harm reduction: Number of sterile needles and syringes provided per person who injects drugs per year	200	300
HCV diagnosis: Percent of people aware of their diagnosis	30%	90%
HCV treatment: Percent of people treated	-	80%

HCV: Hepatitis C virus.

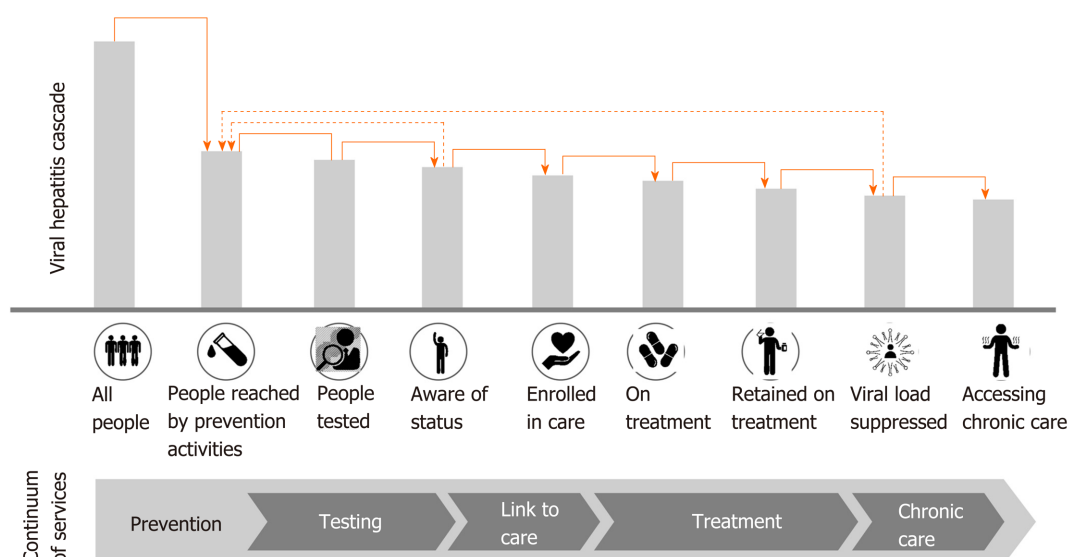
**Figure 1** Natural history of hepatitis C infection. HCC: Hepatocellular carcinoma.

CURRENT EFFORTS TO ADDRESS HCV IN KUWAIT

The panel identified several targets that have been achieved in Kuwait including mandatory HCV screening for all prisoners followed by free treatment for infected individuals; free HCV treatment for citizens of Kuwait, individuals born or married to a Kuwaiti national, and stateless individuals; high blood safety standards with 100% of blood donations screened for HCV, HBV, and HIV since 1992, 1978, and 1987, respectively; and implementation of several HCV awareness and screening efforts at national and local levels^[7].

The Kuwait Hepatology Association, which includes healthcare practitioners from six major hospitals of Kuwait, has proposed standards of practice for screening. According to these standards, screening should be initiated using an antibody test; in cases with a positive antibody test, confirmation should be made by PCR^[10].

In Kuwait, direct-acting antivirals (DAAs) have been introduced as an effective and shorter HCV treatment option with limited side-effects. There are various DAA regimes available in Kuwait including Pro [Paritaprevir (75 mg)/ritonavir (50 mg) + ombitasvir (12.5 mg)], PrOD [Paritaprevir (75 mg)/ritonavir (50 mg) + ombitasvir (12.5 mg)/dasabuvir (250 mg)], SOF/LDV [sofosbuvir (400 mg) and ledipasvir (90 mg)] and DAC/SOF [daclatasvir (60 mg)/sofosbuvir (400 mg)]. Ribavirin may be administered in combination with these regimes; the decision is based on the previous therapy, the



Source: WHO Global Health Sector Strategy on Viral Hepatitis 2016-2021. Available at <http://www.who.int/hepatitus/strategy2016-2021/ghss-hep/en/at>

Figure 2 The continuum of hepatitis services and the retention cascade.

genotype, demographic profile and any comorbidities. However, the cost and accessibility of these therapies are barriers to ensuring treatment for all HCV patients. Combination of grazoprevir/elbasvir has received approval from the European Medical Agency in 2016 but is not available in Kuwait. Moreover, simeprevir/sofosbuvir regimen is not often prescribed in Kuwait as the test for Q80K polymorphism is not available, making it challenging to prescribe for non-cirrhotic patients^[10]. In Kuwait, only gastroenterologists and hepatologists can prescribe HCV treatment. Moreover, there is no national strategy or action plan for the prevention and control of HCV. These and the aforementioned barriers hinder Kuwait's efforts to achieve HCV elimination. Based on these challenges, the panel made recommendations on how to accelerate the elimination of HCV in Kuwait^[7].

RECOMMENDATIONS TO ELIMINATE HCV BURDEN IN KUWAIT

The expert panel discussed the major challenges and bottlenecks around HCV care in Kuwait and strategized priority actions to accelerate its elimination. Informed by the country's current HCV elimination efforts and evidence of effective approaches from around the world, the panel developed seven recommendations to achieve HCV elimination in Kuwait (Figure 3)^[7].

A national strategy and action plan are critical to guide and drive action for the elimination of HCV as a public health threat in Kuwait by 2025

Ambitious health goals, such as disease elimination, require sustained efforts and targeted action, with a national strategy to guide these efforts. This should be informed by the specific context, including the opportunities and challenges to eliminating HCV, and should include specific objectives and targets^[7]. A plan of action plays a critical role in guiding actions towards achieving these goals, providing direction for all stakeholders and ensuring that all critical factors are addressed in parallel^[12,13].

Several countries have developed and implemented strategies and action plans to guide efforts for HCV elimination^[14,15]. For example, the United States National Academies, with the support of partners, developed a national strategy for addressing HCV. In the first phase of this process, stakeholders considered the feasibility of HCV elimination from a biological perspective and the specific barriers and challenges the virus presents, such as it being typically asymptomatic. In the second phase, stakeholders outlined the strategy by which elimination could be achieved; this process included establishing targets and measurable indicators to monitor the

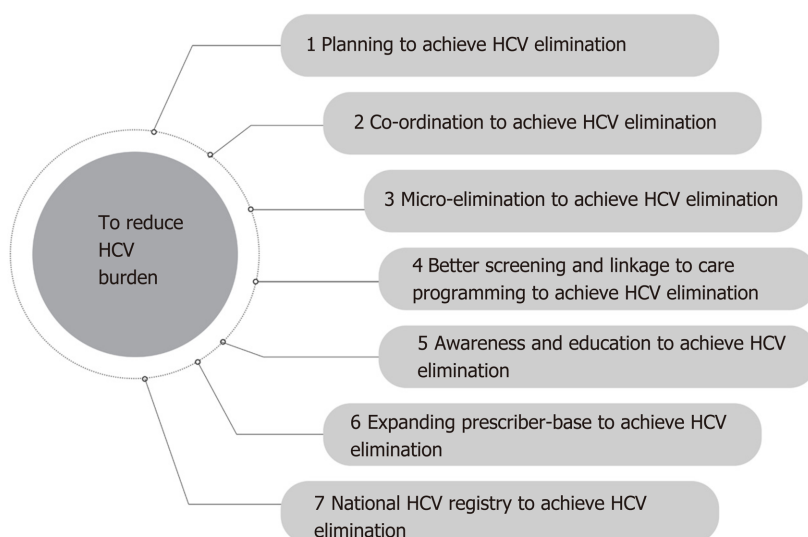


Figure 3 Steps to eliminate hepatitis C virus from Kuwait. HCV: Hepatitis C virus.

implementation of the strategy and the overall progress towards elimination^[14,15]. Action plans are commonly co-developed by a core team of stakeholders who are directly working to achieve a specific health goal. However, the successful implementation of such plans is only feasible with the support and commitment of a wide range of stakeholders across the public and private sectors, from those engaged in public health approaches (*e.g.*, policymakers, patient groups, social workers and researchers) to those involved in clinical management (*i.e.*, multidisciplinary clinical teams)^[7].

In 2016, at the first European Policy Summit on HCV, the HCV elimination manifesto was launched^[16]. A key intent of the manifesto is to highlight the importance of engaging all relevant stakeholders in the development of national elimination strategies. From national working committees, including governmental, medical, and civil society representatives, to specialty care and primary care clinicians, a wide range of stakeholders must be engaged in the development of national HCV elimination action plans. At the clinical level, the management of HCV requires a multi-disciplinary clinical team. **Figure 4**, adapted and modified from Nazareth *et al*^[17], highlights the complex nature of HCV management and emphasizes the need for engagement and coordination across health systems. The European HCV elimination manifesto also highlights the need for integrated care pathways incorporated into national elimination strategies^[16].

With respect to HCV elimination, the strategic plan informs the development of the action plan, which outlines the specific activities that will prevent new infections, effectively diagnose and treat existing infections, and ensure infection control^[11]. The action plan should outline in detail the steps to achieve HCV elimination, and the roles and responsibilities of the various stakeholders. In 2018, the New York State Department of Health prepared an action plan for HCV elimination. To achieve its aims, a task force was established with five workgroups to cover the following: (1) HCV prevention; (2) HCV care and treatment access; (3) HCV testing and linkage to care; (4) Surveillance, data and metrics; and (5) Social determinants^[18]. Qatar has also published a plan for the elimination of HCV, which includes primary prevention, early detection, clinical management, and continuous monitoring^[19]. Evidence from Greece also highlights the importance of an action plan to ensure the effective use of resources and to enhance HCV screening and treatment^[20].

Effective HCV elimination plans have also been shown to benefit the management of liver diseases more generally. Modeling of the HCV elimination plan for Italy's Liguria region shows that the plan's successful implementation would lead to significant savings in healthcare costs by averting the need to treat HCV associated liver-related disease^[21]. A study from the Netherlands also documented the introduction of the national plan for HCV elimination. The plan provided a structural framework for the implementation of elimination activities and outlined targets related to each step in the HCV healthcare cascade: (1) Awareness and prevention; (2) Testing and diagnosis; (3) Linkage to care; (4) Access to medication; (5) Monitoring

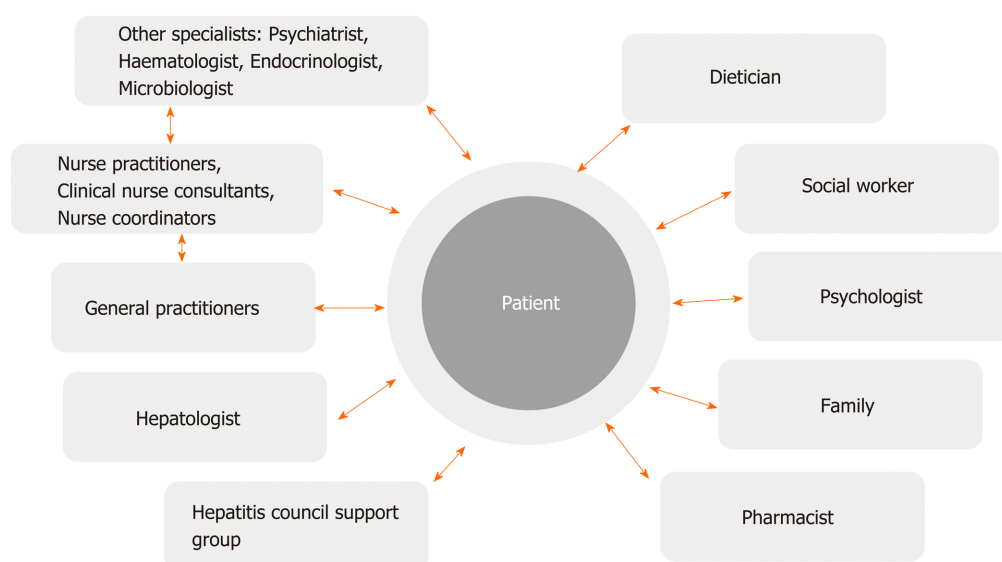


Figure 4 Multidisciplinary team for management of hepatitis C.

and evaluation; and (6) Social welfare and participation for all affected groups^[16,22].

To achieve HCV elimination, tracking of operational performance across the viral hepatitis service continuum is critical. This allows for challenges and bottlenecks to be identified and addressed in a timely manner and enables stakeholders to determine when a reprioritization or refocusing of efforts is required. At the national and municipal level, Rwanda and New York, respectively, have engaged a task force to provide input to healthcare departments regarding the progress of HCV programs^[17,23]. Chicago's task force for HCV elimination has set the aim of reducing the number of new HCV transmissions to zero^[24].

Cascade-of-care (CoC) monitoring is also essential to understand the effectiveness and impact of strategies and to determine improvement or deterioration in the HCV epidemic^[25,26]. Defined targets and high-quality data are central to effective monitoring and informing strategic decisions about the program^[26]. It is recommended that when devising national targets, including in Kuwait, these align with the goals and indicators of the WHO Global Health Sector Strategy on Viral Hepatitis 2016-2021 and the WHO regional action plan^[6,7].

In summary, a strategy will be essential for guiding hepatitis elimination efforts in Kuwait while a multi-stakeholder plan of action will be required to effectively implement this strategy. For accountability, a committee or taskforce can be established to oversee the implementation of the plan and to monitor progress, in line with the predefined targets. A principal or central person leading these efforts can help to further reinforce accountability.

A coordination mechanism is critical to provide operational oversight of all screening and linkage to care programs to ensure intensive action to eliminate HCV

HCV programs at national and local levels require effective and efficient coordination across and between multiple agencies and disciplines. The key characteristics of a well-coordinated HCV mechanism include the identification of roles and responsibilities of key organizations, individuals, and working groups; quantification of the service needs (*i.e.*, prevention, diagnosis, treatment, and care); and establishing agreed goals and targets. All of this should be accompanied by adequate investment, including in project management expertise, and be underpinned by a robust accountability framework^[27].

Many countries working towards HCV elimination need to ensure proper coordination among the working committees by reporting to a national oversight committee. Such a structure promotes efficiency by minimizing the duplication of efforts with respect to planning and implementing activities to achieve HCV elimination. The US Department of Health and Human Services has made efforts to track the progress of their HCV elimination strategy. The department assisted stakeholders engaged in raising awareness to identify additional volunteers, who spread awareness among the population, local jurisdictions, and non-governmental

organizations to support the attainment of the elimination target^[28]. One of the goals of the US national viral hepatitis action plan specifically focuses on coordinating, monitoring, and reporting on the implementation of planned activities, highlighting the importance of coordination within efforts to achieving HCV elimination^[29]. Similarly, a study from Australia in 2019 used the Delphi method to identify the critical organizational and operational elements of community-based HCV treatment models. The authors reported that integration and coordination of care and support were important from the perspective of both patients and healthcare providers^[30].

As per the expert panel, several HCV screening and linkage to care programs are presently implemented in Kuwait; however, there is currently limited coordination of activities and information sharing among programs^[7]. To ensure that every individual receives proper diagnosis and treatment, it is essential to have robust, coordinated linkage to care for all HCV diagnosed individuals. It is also important to differentiate between coordination mechanisms at the national level (*i.e.*, coordination between primary, secondary, and tertiary care) and between specialists (*e.g.*, gastroenterologists, hepatologists, *etc.*). Moreover, the development of an inventory of existing and proposed screening and linkage to care programs (from the national strategy/action plan) operating in Kuwait would be helpful^[7]. Overall, strong coordination mechanisms are key to delivering effective large-scale HCV elimination programs. Coordination mechanisms need to include all key stakeholders (*e.g.*, policy makers, public health professionals, multi-disciplinary clinical teams) and should prioritize the effective utilization of resources and the timely and efficient sharing of information between stakeholders^[7].

Enhanced HCV screening and linkage to care programming is important to achieve HCV elimination

There are various definitions of linkage to care within the HCV literature. Saab *et al*^[31] defined linkage to care as confirmation of HCV diagnosis by an HCV RNA test and establishment of a follow-up appointment for treatment with a specialist. Others defined linkage to care as confirming HCV by HCV RNA testing^[32] or referral to a specialist and attending the first appointment within six months of diagnosis^[33]. Some include both screening and attending the first appointment^[34].

Generally, HCV infection is detected following an algorithm (Figure 5, adapted from Wilkins *et al*^[35], 2015). All patients diagnosed with chronic HCV infection should then be considered for treatment. HCV treatment is chosen based upon the virus genotype, the extent of fibrosis or cirrhosis, prior therapy received, comorbid conditions, and adverse effects. However, the principal aim of all therapies is to reduce the complications and mortality associated with HCV^[35].

The development of an inventory of available data to examine the viral hepatitis care cascade (Figure 2) and to determine areas of drop-out is key for improving linkages to care and guiding actions to specifically address bottlenecks and challenges^[36]. Monitoring the prevalence of HCV is key to enable effective and efficient resource allocation and utilization. Adequate resource allocation to health care providers as well as diagnostic laboratories and hospitals is critical to enable screening and treatment. Screening and treatment capacity depend upon the resources available in the country^[37,38]. It is especially critical that available resources are utilized in the most effective and efficient way.

As part of Rwanda's initiative to eliminate HCV the country has developed a five-year plan which includes several activities aimed at enhancing screening and linkage to care^[23]. The plan includes an expansion of the workforce of general practitioners, nurses, and laboratory personnel, a reduction in the price of DAAs, and the provision of training to the key individuals and those engaged in conducting awareness campaigns. Through these activities, it is estimated that more than 4 million people will be screened and around 112000 adults treated, averting 10638 new infections, and more than 35000 premature deaths^[23].

In certain population groups, such as incarcerated individuals and people who inject drugs (PWID), timely screening and linkage to care can be challenging. For example, in many prison hospitals HCV treatment is provided to all new positive cases in order to prevent re-infection, a concept known as "treatment as prevention"^[39]. However, once released from prison, treatment is generally discontinued due to a lack of follow-up^[39]. For these types of population groups there is a need to have multiple, parallel approaches for identifying and linking individuals to programs, such as drug harm reduction services for PWID^[40].

Modeling studies can be useful to quantify the screening targets and resources required to meet national and international goals. For example, a recent publication for

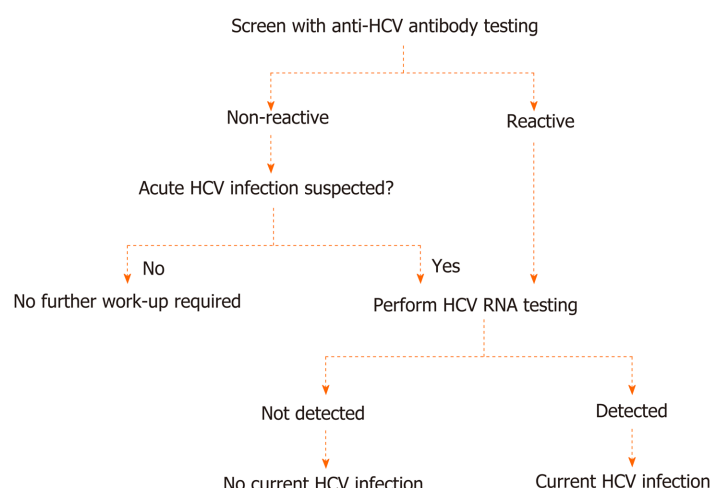


Figure 5 Algorithm for hepatitis C virus diagnosis. HCV: Hepatitis C virus.

Malaysia modeled a stepwise approach to scale up HCV screening in order to reach the WHO 2030 targets. The model estimated screening of < 1 million PWID per year between 2018-2026, before expanding to the general population, with a total of 6 million people screened between 2018-2030. The total cost of screening was estimated at \$58 million^[41]. Zuckerman *et al*^[42], suggested that there should be established recommendations for screening and diagnosis of HCV to help overcome disparities in diagnosis and linkages to care. A modeling study on the impact of enhanced screening and treatment on HCV in the US estimated a significant reduction in HCV-related deaths. Increasing treatment four-fold was projected to result in an 80% reduction in HCV prevalence by 2025, with significant reduction in liver outcomes including cirrhosis and hepatocellular carcinoma^[43].

To meet the WHO HCV elimination targets, it is essential to implement successful prevention interventions, outreach screening programs, improve infection control, design initiatives for high-risk subpopulations, such as PWID, and link these programs to other harm reduction services, and conduct extensive screening for HCV followed by treatment^[44].

Micro-elimination programs can be a key element of the national strategy and action plan to fast-track HCV elimination in high-risk populations

The micro-elimination approach focuses on eliminating HCV in high-risk subpopulations and can be applied in settings such as hospitals, prisons, and rehabilitation centers^[37]. Micro-elimination enables “quick-wins” in a long-term national plan by eliminating HCV in high-risk groups. The approach can be initiated taking geographic areas, sub-populations, or age-cohorts into consideration. As done in screening programs, modeling studies can be utilized to identify and estimate the impact of HCV infection in high-risk regions or population groups^[37].

Lazarus *et al*^[45,46] (2017 and 2018) propose that target groups suitable for micro-elimination include patients with advanced liver disease, hemophilia and/or engaged with drug treatment units, prisoners, children, migrant communities from high prevalence regions, PWID in networks, men who have sex with men, and generational cohorts of high prevalence and geographically defined areas. Hemodialysis patients should also be assessed for HCV infection^[47]. People visiting barber shops, beauty salons, tattoo parlors, and fitness centers (the latter because of injectable steroid use) should also be assessed as per the expert panels recommendations^[7]. Adults born between 1945 and 1965, known as “baby boomers”, are also considered a cohort with a high prevalence of HCV and should be screened at least once for HCV^[33]. In the US, the prevalence of HCV is expected to be five-fold higher in baby boomers compared to other adults as they are thought to have become infected during its outbreak between 1960-1980^[48]. Crowley *et al*^[49] reported screening of prisoners in an Irish prison to be a useful case finding model that enabled the diagnosis of new cases and facilitated linkage to HCV care. Currently, in Kuwait, all prisoners are screened for HCV infection and, if positive, provided free treatment^[7].

The prevalence of HCV is significantly higher in PWID than in the general population^[50]. Analysis from the US highlighted the need for PWID to be universally

screened for HCV in order to achieve elimination^[43]. In the Netherlands, micro-elimination has been at the forefront of HCV elimination efforts, with PWID a key population group screened and linked to care. This pragmatic and efficient approach has taken the country closer to the goal of HCV elimination^[22]. In Iceland, the universal HCV treatment program has been combined with an additional screening of PWID in order to achieve the national HCV elimination targets^[51].

To accelerate HCV elimination in PWID, the micro-elimination program should adhere to recognized best practice as follows: (1) Access to sterile equipment used for injecting (this would form a part of a large-scale harm reduction program); (2) Testing of volunteers after a regular time period (*e.g.*, 12 mo); and (3) Following up individuals who reported having a high-risk injection episode^[7]. Micro-elimination of HCV in PWID involves expanding and integrating harm reduction services to HCV screening and treatment^[52]. In Australia, DAAs were approved for use through the Pharmaceutical Benefits Scheme, which significantly boosted the treatment uptake in marginalized communities, including PWID^[53].

Micro-elimination programs are also associated with numerous challenges. Targeting vulnerable and often disenfranchised populations, such as PWID, homeless people, and prisoners, is resource intensive and poses particular monitoring and linkage to care-related challenges attributable to inaccessibility of those infected, lack of HCV knowledge, impaired mental health, drug-phobia, financial constraints, and a lack of motivation to be screened and treated. Moreover, these programs require multiple visits and follow-up steps, which require additional staff. In some contexts, this may also require new or different models of service delivery^[54,55]. Micro-elimination programs need to utilize existing resources and services to overcome these challenges.

Micro-elimination plans should incorporate realistic, annual goals for screening and treatment. As with national action plans, multiple stakeholders, including government officials, health service providers, and civil society groups, should be involved in the design of the micro-elimination programs. Progress should be monitored and reported on a regular basis^[37]. Where national action plans are hampered by political and logistical challenges, micro-elimination programs can help to build momentum by delivering tangible results quickly and efficiently^[56].

Overall, the micro-elimination of HCV in high-risk populations in Kuwait should be considered as an essential component of the overall elimination strategy. Involvement of multiple stakeholders, volunteers, and healthcare practitioners is essential. Micro-elimination efforts should be monitored closely to ensure they are contributing to the boarder goal of HCV elimination in the general population.

Expanding the prescriber-base to achieve HCV elimination

Evidence supports the expansion of the HCV treatment prescriber base, which in Kuwait is currently restricted to gastroenterologists and hepatologists, to include primary care providers and nurse practitioners. A non-randomized, open-label clinical study from the US District of Columbia determined that HCV treatment provided by non-specialists (nurse practitioners and primary care physicians) was as safe and effective as treatment provided by specialists^[56]. Nazareth *et al*^[17] also support the use of nurse practitioners to increase the prescriber-base to support HCV elimination efforts. Furthermore, to increase treatment capacity Koren *et al*^[57] reported utilizing clinical pharmacists for HCV treatment, which resulted in high rates of sustained virologic response (95.1%).

Evidence suggests that with adequate planning, training, and capacity building, non-specialists can provide treatment comparable to that given by specialists. Kuwait can consider expanding the prescriber base to accelerate HCV elimination efforts. Such a decision will require careful consideration which should be taken in consultation with all relevant stakeholders within the country.

Increased awareness and education on HCV will help to accelerate eradication efforts

Lack of awareness around HCV is one of the major challenges in achieving elimination. A significant number of people living with HCV are unaware of the viral infection and global knowledge of the disease in the general population is low^[58,59]. Training and educational courses for health care providers are essential to ensure they have the most up to date knowledge on standards of care and novel approaches for HCV elimination^[17,55]. Public information and awareness campaigns are key to address misconceptions about HCV, its modes of transmission, and the treatment options^[60].

Georgia and Egypt, which have both agreed specific targets for the elimination of

HCV, have implemented advocacy, awareness, and education activities as part of their elimination programs^[59]. Such efforts can focus on the general population or specific population groups such as potential targets for micro-elimination. Novel approaches to HCV awareness creation and education may be required for specific population groups, such as PWID. Integration of awareness creation efforts with other social services or through civil society groups working with the affected population may be beneficial^[61,62].

Development and implementation of a national HCV registry is key for guiding elimination efforts

HCV registries provide vital information to monitor programs and to track progress towards national and international goals^[63]. Several countries, including Taiwan and the US, have conducted clinical trials to develop national HCV registries^[64,65]. In the US, the Clinical Case Registries (CCR) consist of two registries, CCR: HIV and CCR: HCV. Both are designed to provide population-based data on Veterans Affairs patients infected with HIV or HCV, which share common risk factors^[66]. Rizk *et al*^[67] (2019) highlight how disease registries enable follow-up and allow identification of barriers to care, with innovative solutions such as co-located HIV and HCV clinics being developed as a result.

A national HCV registry can guide strategic decision making at national and local levels. Registries support the monitoring of programs and allows stakeholders to review and revise strategies and plans based on the evolving context (*e.g.*, changes in HCV epidemiology) and changing priorities (*e.g.*, transition from micro-elimination to a national program)^[7]. They play a vital role in the treatment of hard to reach population groups that present additional challenges related to tracking and follow-up^[68] and assist in assessing the rate of diagnosis for HCV. Central disease registries also provide opportunities for assessing screening efforts and identifying future opportunities for expansion^[69].

In the Middle East, there is a lack of reliable information regarding the prevalence of HCV infection. In Kuwait, determining an accurate HCV prevalence is further complicated by the constant migration of expatriates, a proportion of whom are from HCV-endemic regions^[10]. To address this challenge, the Ministry of Health can seek to upgrade the medical record systems to capture adequate information and recruit specialists for processing and analyzing data. Registries can also be used to support real word studies, as done in Germany, to determine the effectiveness and safety of different HCV treatments^[70].

An HCV registry is an essential source of information to guide strategic decision making and track the implementation of action plans. The data can also be used to review and revise strategies and plans as the context evolves or new opportunities or challenges emerge. The HCV registry should be designed to incorporate relevant indicators in line with the WHO HCV elimination strategy.

CONCLUSION

Concerted action can substantially reduce the high prevalence of HCV in Kuwait. To eliminate HCV in the country, key stakeholders have followed the WHO strategy and identified the major challenges and opportunities for advancing progress. Informed by these challenges, an expert panel developed seven recommendations to accelerate the elimination of HCV as a public health threat in Kuwait: (1) Developing and implementing a national strategy and action plan; (2) Establishing a coordination mechanism between viral hepatitis working committees; (3) Following a micro-elimination approach; 4) Enhancing screening and linkage to care; (5) Expanding the prescriber base; (6) Raising awareness and educating the population; and (7) Developing a national HCV registry.

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Retrospective Cohort Study

Vedolizumab for ulcerative colitis: Real world outcomes from a multicenter observational cohort of Australia and Oxford

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Abstract

BACKGROUND

Vedolizumab (VDZ), a humanised monoclonal antibody that selectively inhibits *alpha4-beta7* integrins is approved for use in adult moderate to severe ulcerative colitis (UC) patients.

AIM

To assess the efficacy and safety of VDZ in the real-world management of UC in a large multicenter cohort involving two countries and to identify predictors of achieving remission.

METHODS

A retrospective review of Australian and Oxford, United Kingdom data for UC patients. Clinical response at 3 mo, endoscopic remission at 6 mo and clinical remission at 3, 6 and 12 mo were assessed. Cox regression models and Kaplan Meier curves were performed to assess the time to remission, time to failure and the covariates influencing them. Safety outcomes were recorded.

RESULTS

Three hundred and three UC patients from 14 centres in Australia and United Kingdom, [60% $n = 182$, anti-TNF naïve] were included. The clinical response was 79% at 3 mo with more Australian patients achieving clinical response compared to Oxford (83% *vs* 70% $P = 0.01$). Clinical remission for all patients was 56%, 62% and 60% at 3, 6 and 12 mo respectively. Anti-TNF naïve patients were more likely to achieve remission than exposed patients at all the time points (3 mo 66% *vs* 40% $P < 0.001$, 6 mo 73% *vs* 46% $P < 0.001$, 12 mo 66% *vs* 51% $P = 0.03$). More Australian patients achieved endoscopic remission at 6 mo compared to Oxford (69% *vs* 43% $P = 0.01$). On multi-variate analysis, anti-TNF naïve patients were 1.8 (95% CI: 1.3-2.3) times more likely to achieve remission than anti-TNF exposed ($P < 0.001$). 32 patients (11%) had colectomy by 12 mo.

CONCLUSION

VDZ was safe and effective with 60% of UC patients achieving clinical remission at 12 mo and prior anti-TNF exposure influenced this outcome.

Key words: Vedolizumab; Ulcerative colitis; Outcomes

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Core tip: Vedolizumab (VDZ) is a gut selective anti-integrin used for treatment of Ulcerative colitis (UC). Evidence is needed to support its use in real life setting involving multiple centers and two countries to reduce physician, site and country biases. This is a retrospective review of prospectively collected database involving 303 UC patients from Australia and Oxford, United Kingdom treated with VDZ. Clinical response was observed in 79% of patients at 3 mo and clinical remission in 56%, 62% and 60% at 3 mo, 6 mo and 12 mo respectively. Anti-tumor necrosis factor (anti-TNF) naïve patients were 1.8 times more likely to achieve remission than anti-TNF exposed and 11% of patients required colectomy by 12 mo. We concluded that VDZ is a safe and effective biologic medication used for treatment of UC.

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INTRODUCTION

The aim of treatment in ulcerative colitis (UC) is to achieve sustained clinical, mucosal and histological healing^[1,2]. The choice of treatment depends on several factors including induction, or maintenance, of disease remission, severity of disease, extent and location of bowel involvement, disease phenotype and individual characteristics of the drug and patient. The use of conventional medications may be limited either by a lack of efficacy (5-aminosalicylates) or side effects [steroids/azathioprine (AZA)/6 mercaptopurine (6MP)/methotrexate (MTX)]^[3]. Its' use, however, is not without potential side effects including, development of opportunistic infections, reactivation of tuberculosis and an increased risk of melanoma^[4].

Vedolizumab (VDZ), a humanised monoclonal antibody, selectively inhibits the migration of *alpha4-beta7* inflammatory cells to the gastrointestinal tract, making it a biological agent without systemic immunosuppression and thus potentially reducing side-effects. In GEMINI 1, the randomised, double-blind placebo-controlled trial of VDZ in UC, the response rate for induction at week six was 47.1% with a response rate of 41.8% at week fifty two after eight-weekly VDZ treatments^[5]. Patients enrolled in clinical trials, however, do not entirely represent the patients seen in routine clinical practice as demonstrated by a retrospective study where only 31% of 206 patients with moderate-to-severe inflammatory bowel disease (IBD) were eligible to participate in such a clinical trial^[6].

Our aim was to assess the response and remission rates to VDZ in the real world, the time taken to achieve this, mucosal healing rates, adverse/serious events, the rates of colectomy and the predictors influencing remission in the first 12 mo of VDZ therapy through a multicenter consortium in a real-world setting.

MATERIALS AND METHODS

Study design

This was a multicenter retrospective review of prospectively collected data involving 14 IBD centers in Australia New Zealand inflammatory bowel disease consortium and data was also collected at a major IBD center in United Kingdom, thus reducing physician, site and country bias. All the centers involved in the study had a dedicated IBD team. In Australia, patients with UC refractory to conventional treatment, which was defined as failure of three, or more, mo of a 5-aminosalicylate and failure of three or more mo of an immunomodulator (AZA, 6MP or MTX) and 6 wk weaning dose of prednisolone that commenced at 40mg per day or more, were able to access VDZ from 2015 through the government funded pharmaceutical benefit scheme (PBS). In the United Kingdom, VDZ was given to patients at the physicians' discretion if the

conventional treatment and/or anti-tumor necrosis factor (anti-TNF) medications had failed to control the disease.

Consecutive patients with UC diagnosed as per the standard criteria^[7] who received at least induction VDZ therapy were considered for the study. All patients who finished VDZ induction therapy were included in the study for analysis. VDZ was given as standard intravenous (IV) induction dosing of 300mg at 0, 2 and 6 wk followed by maintenance therapy of 8 weekly IV infusions. Patients continued to take, or wean off, steroids, 5-aminosalicylates (oral and rectal therapy) as deemed appropriate by the treating physician. Patients taking immunosuppressant medications, including AZA, 6MP, MTX orally, or rectal tacrolimus, continued on these medications under the treating physicians' preference as guided by the disease control. There were no mandated changes to a patient's regular IBD medications. The use of steroids and/or immunomodulators and their time of cessation was recorded for analysis.

A retrospective review of the IBD databases that contained prospectively-entered data included baseline patient demographics and disease characteristics classified by the Montreal classification^[8], concomitant use of steroids and immunomodulator medications, prior exposure to anti-TNF medications, adverse events and colectomy rates.

Assessments tools and criteria

The Montreal classification was used to classify UC^[8]. The Partial Mayo clinical score was used to assess disease control and is composed of 3 items, which includes stool frequency, rectal bleeding and the physician global assessment which were each scored individually from 0 to 3 at baseline, 3, 6 and 12 mo. The higher the score, more severe the disease and maximum score was 9. The Mayo endoscopic score (MES) is classified into four levels of severity from 0-4 based on mucosal friability, vascular pattern, friability and erosions. Mayo 0-1 was inactive disease while Mayo 2 and Mayo 3 were mild-moderate and moderate-severe disease respectively. Ulcerative colitis endoscopic index of severity (UCEIS) is composed of 3 items, which includes vascular pattern, bleeding and erosions/ulcers with score ranging from 0-2 for vascular pattern and scores 0-3 for bleeding and erosions/ulcers with higher scores indicating severe disease and the maximum score was 9. The response and remission to VDZ was assessed clinically using partial Mayo clinical score in both Australia and the United Kingdom sites. MES was used for assessing endoscopic appearance in Australia, and the UCEIS was used in the United Kingdom.

Evaluation of clinical efficiency at 3, 6 and 12 mo

Induction: Clinical efficiency of VDZ induction therapy was assessed as either clinical response or clinical remission at 3 mo. A response to VDZ was defined as a decrease in the Partial Mayo score of ≥ 3 from baseline, while clinical remission was defined as Partial Mayo score of < 2 .

Maintenance

Clinical efficacy of VDZ was also assessed at 6 mo and 12 mo of VDZ therapy. The Partial Mayo score again was used to assess clinical response and clinical remission. Data was collected if VDZ was ceased due to side-effects, or loss of response (LOR) that resulted in a switch of the therapy away from VDZ, or surgery if it was required.

Endoscopic assessment of disease control was undertaken after approximately 6 mo of VDZ. An MES of 0 or 1 was defined as an endoscopic remission with a score > 1 indicating active disease. An UCEIS score of 0-1 was also defined as endoscopic remission with a score > 1 indicating active disease.

Corticosteroid and Immunomodulator therapy

Corticosteroid therapy was defined as the use of any oral steroid including prednisolone and budesonide. Immunomodulator therapy includes any oral or rectal immunomodulator which includes AZA, 6MP, MTX (oral or parenteral), tacrolimus (oral or rectal), ciclosporin and mycophenylate. Corticosteroid and immunomodulator use was assessed at baseline, 3, 6 and 12 mo.

Safety

The development of infusion reactions, adverse events and serious adverse events were all recorded. Infusion reactions were defined as any adverse event that occurred on the day, or the day after, the infusion. Adverse events were defined as any untoward medical occurrence not resulting in discontinuation of the VDZ or

hospitalization. Adverse events were graded as serious if they resulted in discontinuation of VDZ, hospitalization of the patient, or patient death.

Statistical analysis

Data for each patient from their first dose of VDZ to either last infusion within the study period or cessation of the VDZ were included for analysis. All statistical analysis was conducted using SPSS version 24 (IBM Corp, released 2016). Statistical significance was set at $P < 0.05$. Patient demographic and disease profile information was described using frequency and percent for categorically classified variables, with mean and standard deviation and median and range used to describe scale variables. VDZ treatment outcomes are described at baseline, 3, 6 and 12 mo. Sample size varied across measures and was reported accordingly.

Two Cox Regression models and Kaplan Meier curves were performed separately for each site. The first model examined time (weeks) to remission. Time to censor was calculated as the difference between the date of remission (censor event) and the date the patient started the study. The second model examined time (weeks) to failure. Failure was defined as Partial Mayo clinical score ≥ 2 or MES ≥ 2 , or the need for a change in the biologic agent, or requiring colectomy. Time to censor was calculated as the difference between this date of failure (censor event) and the date the patient started the study, truncated to 60 wk. Both models examined the covariates gender, disease duration (year), smoking (non-smoker/current smoker or ex smoker), disease location, age at which VDZ was given (year), and previous immunomodulator exposure. For the failure model, a remission covariate was also included (median split $\leq 13 / > 13$ wk of time to remission). For both models where remission or failure occurred at the start of the study, a small constant (0.01) was added to the time to event variable. Cox model proportional hazard assumption was tested using Schoenfeld residuals with no violations. A chi square analysis was used to investigate the remission and failure censor variables with anti-TNF, and site differences across categorical variables. Disease duration, age vedolizumab started, remission time and failure time were examined for normality using Shapiro Wilk and violations were noted. Therefore site differences for these continuous variables were examined using the non-parametric alternative Mann Whitney *U* Test. Further Cox Regression models and Kaplan Meier curves were performed using the combined data set with site included as a variable.

RESULTS

Patient characteristics

Three hundred and thress UC patients (Australia $n = 210$ and Oxford, United Kingdom, $n = 93$) from 15 centers (Australia $n = 14$) were included in the study. Of the 303 patients, patient data was available in 278 at 3 mo, 250 patients at 6 mo and 209 patients at 12 mo. Of the 303 patients, 15 patients were in remission at the start of VDZ and VDZ was commenced due to side effects to the anti-TNF agents, these patients were analysed separately at each time point (Figure 1).

Patient demographics and clinical characteristics

Of the total 303 patients, 60% ($n = 182$) were anti-TNF naïve and VDZ was their first biologic agent, while 40% ($n = 121$) had prior anti-TNF exposure with a secondary LOR in 20% ($n = 61$) and primary LOR in 15% ($n = 45$). VDZ was commenced in 5% ($n = 15$) of patients due to side-effects from anti-TNF therapy and these patients were in clinical remission at VDZ induction, so were analyzed separately. A total of 47% ($n = 143$) were female (Table 1).

The median age at which VDZ was started was 35 years (range 16-84 years), while the median disease duration was 6 years (range 0.2-48 years) prior to the commencement of VDZ. A family history was present in 12% ($n = 29$) and 81% ($n = 226$) were non-smokers at the time of commencing VDZ. All patients were classified by the Montreal classification^[9] and 56% were diagnosed with UC between the ages of 17-40 years ($n = 170$, A2) compared to younger than 17 years in 11% ($n = 34$, A1) and 33% older than 40 years ($n = 99$, A3). Disease extent was extensive in 56% of patients ($n = 170$, E3) with 38% with suffering left-sided colitis ($n = 114$, E2) and 6% patients with proctitis ($n = 18$, E1). Of all patients, 63% ($n = 191$) were on steroids at the commencement of VDZ, 53% ($n = 162$) were taking prednisone and 10% ($n = 29$) budesonide. 58% ($n = 175$) of the patients were on an immunomodulator, with the thiopurines (AZA/6MP) being the most commonly used in 45% ($n = 136$), followed by

Table 1 Clinical characteristics of study population

Characteristic	Total (n = 303)	Australia (n = 210)	Oxford (n = 93)
Gender			
Female, n (%)	143 (47)	95 (45)	48 (52)
Median age VDZ given (range, yr)	35 (16-84)	36 (19-78)	35 (16-84)
Median disease duration (range, yr)	6 (0.2-48)	7 (1-48)	5.4 (0.2-39.2)
Montreal classification, n (%)			
Age			
A1	34 (11)	33 (16)	1 (1)
A2	170 (56)	120 (57)	50 (54)
A3	99 (33)	57 (27)	42 (45)
Location			
E1	18 (6)	15 (7)	3 (3)
E2	114 (38)	72 (34)	42 (45)
E3	170 (56)	122 (58)	48 (52)
Missing	1	1	0
Family History, n (%)	29 (12)	22 (15)	7 (7)
First degree	19	12	7
Second degree	10	10	0
None	212	126	86
Smoking, n			
Never	226	140	86
Current	9	6	3
Ex smoker	45	41	4
Anti-TNF naïve, n (%)	182 (60)	122 (58.1)	60 (65)
Anti-TNF exposed, n (%)	121 (40)	88 (41.9)	33 (35)
Primary LOR	45 (15)	29 (13.8)	16 (17)
Secondary LOR	61 (20)	47 (22.4)	14 (15)
Side-effects	15 (5)	12 (5.7)	3 (3.2)
Steroids at VDZ initiation, n (%)	191 (63)	134 (64)	57 (61.2)
Prednisone	162 (53)	108 (51)	54 (58)
Budesonide	29 (10)	26 (12)	3 (3)
Immunomodulation at VDZ initiation, n (%)	175 (58)	135 (64)	40 (43)
AZA/6MP	136 (45)	108 (51)	28 (30)
Methotrexate	19 (6)	11 (5)	8 (9)
Tacrolimus	17 (6)	16 (8)	1 (1)
Others (Cyclo&Myco)	3 (1)	0	3 (3)
Mean Partial Mayo before VDZ initiation	5 (2-9)	6 (2-9)	5 (2-9)

VDZ: Vedolizumab; min: Minimum; max: Maximum; TNF: Tumor necrosis factor; Primary LOR: Primary loss of response; Secondary LOR: Secondary loss of response; AZA: Azathioprine; 6MP: 6-mercaptopurine; Cyclo: Ciclosporine; Myco: Mycophenolate; Init, Initiation.

MTX, tacrolimus, ciclosporine and mycophenolate. The mean partial Mayo score was 5 (range 2-9) at commencement of VDZ.

No significant differences were observed between the Australian and Oxford

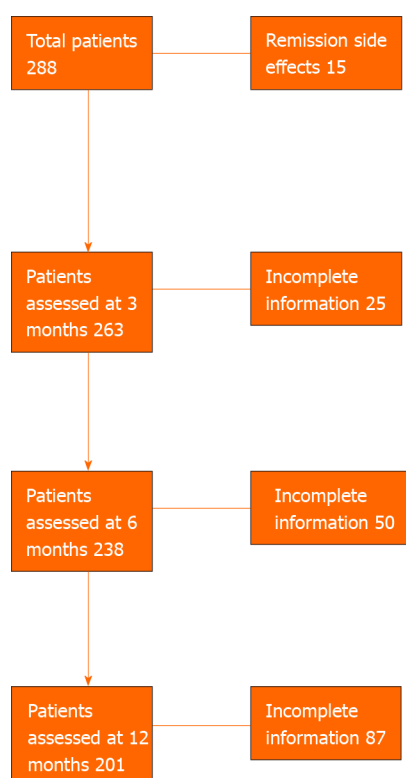


Figure 1 Flowchart.

patients for prior anti-TNF exposure ($P = 0.36$), sex ($P = 0.3$), family history ($P = 0.43$), and age at which VDZ was started ($P = 0.35$). Significant differences between the Australian and Oxford patients, however, were observed for smoking with more Oxford patients having never smoked ($P < 0.001$) but there was no difference in the current smokers at time of VDZ commencement. Immunomodulator usage at the commencement of VDZ was greater in Australian than Oxford patients ($P < 0.001$), and the disease duration in Australian patients was longer prior to VDZ commencement ($P < 0.048$).

Assessment at 3 mo, 6 mo and 12 mo

A total of 263 patients were not in remission at commencement of VDZ, and of these 79% ($n = 208$) achieved a clinical response and 56% ($n = 148$) achieved clinical remission by 3 mo. Three mo data was missing for 25 patients (9 Australia, 16 Oxford), and thus these were not included in the induction analysis but there was data for the clinical status of these patients at 6 and 12 mo.

At 3 mo, Australian patients were more likely to achieve response ($P = 0.01$), but were not more likely to achieve remission than Oxford patients ($P = 0.58$) (Table 2). Anti-TNF naïve patients were more likely to achieve both response ($P = 0.03$) and remission ($P < 0.001$) at 3 mo compared to patients who had prior anti-TNF exposure. Within the anti-TNF exposed group, there was no significant difference between the patients who had a primary or secondary LOR to an anti-TNF agent in achieving clinical response ($P = 0.9$) or remission ($P = 0.8$) to VDZ at 3 mo.

Of the total 238 patients assessed at 6 mo, 62% ($n = 147$) were in remission and of the 201 patients assessed at 12 mo, 60% ($n = 120$) were in remission (Table 3). No significant difference was found between Australia and Oxford in the number of patients in remission at 6 mo ($P = 0.3$) or at 12 mo ($P = 0.09$). Anti-TNF naïve patients were more likely to achieve remission both at 6 mo ($P < 0.001$) and 12 mo ($P = 0.03$) than those previously exposed. Within the anti-TNF exposed group, there was no significant difference between the patients who had primary or secondary LOR to anti-TNF agents in clinical remission at 6 mo ($P = 0.2$) or 12 mo ($P = 0.3$).

Of the 15 patients who were in remission at the start of VDZ, where the indication was side effects to anti-TNF agents, 66% ($n = 10/15$) at 3 mo, 58% ($n = 7/12$) at 6 mo, 50% ($n = 4/8$) at 1 year were still in clinical remission.

Table 2 Response and remission at 3 mo of vedolizumab therapy

	Total	Australia	Oxford	P value
Response, <i>n</i> (%)	208 (79)	157 (83)	51 (70)	0.01
Remission, <i>n</i> (%)	148 (56)	104 (55)	44 (59)	0.58
	Total	Anti-TNF naïve	Anti-TNF exposed	P value
Response, <i>n</i> (%)	208 (79)	138 (83)	70 (72)	0.03
Remission, <i>n</i> (%)	148 (56)	109 (66)	39 (40)	< 0.001
	Anti-TNF exposed	Primary LOR	Secondary LOR	P value
Response, <i>n</i> (%)	70 (72)	30 (73)	40 (71)	0.85
Remission, <i>n</i> (%)	39 (40)	16 (39)	23 (41)	0.83

VDZ: Vedolizumab; TNF: Tumor necrosis factor; Primary LOR: Primary loss of response to anti-TNF agent; Secondary LOR: Secondary loss of response to anti-TNF agent.

Table 3 Remission at 6 mo and 12 mo of vedolizumab therapy

	Total	Australia	Oxford	P value
Remission at 6 mo, <i>n</i> (%)	147 (62)	110/173(64)	37/65 (57)	0.34
Remission at 12 mo, <i>n</i> (%)	120/201 (60)	87/138 (63)	33/65 (52)	0.09
	Total	Anti-TNF naïve	Anti-TNF exposed	
Remission at 6 mo, <i>n</i> (%)	147/238 (62)	103/142 (73)	44/96 (46)	< 0.001
Remission at 12 mo, <i>n</i> (%)	120/201 (60)	76/115 (66)	44/86 (51)	0.03
	Anti-TNF exposed	Primary LOR	Secondary LOR	
Remission at 6 mo, <i>n</i> (%)	44/96 (46)	16/42 (38)	28/54 (52)	0.18
Remission at 12 mo, <i>n</i> (%)	44/86 (51)	17/38 (44)	27/48 (56)	0.28

VDZ: Vedolizumab; TNF: Tumor necrosis factor; Primary LOR: Primary loss of response to anti-TNF agent; Secondary LOR: Secondary loss of response to anti-TNF agent.

Endoscopy

A total of 108 patients had endoscopy at 6 mo, 78 in Australia and 30 in Oxford. Of the Oxford patients, 43% (13/30) had an UCEIS of ≤ 1 indicating endoscopic remission. Of the Australian patients, 69% (54/78) had an MES of 0-1 indicating endoscopic remission. A significantly greater percentage of patients achieved endoscopic remission in Australia compared to Oxford ($P = 0.01$). A MES score of 0 was achieved in 31% (24/78) of the Australian cohort. A MES ≥ 2 was reported in 31% ($n = 24$) of Australian patients and a UCEIS ≥ 2 was reported in 57% ($n = 17$) of Oxford patients.

Time to remission

A total of 224 (73.9%) patients were censored as being in "remission". While controlling for the site, univariate cox regression models for time to remission found no significant associations for gender ($P = 0.3$), disease duration ($P = 0.6$), smoking status ($P = 0.9$), age at which VDZ was given ($P = 0.7$), immunomodulator exposure ($P = 0.8$) and these were also not significant when considered with anti-TNF exposure. The final model included anti-TNF exposure and the site with the Log Rank (Mantel-Cox) reporting a significant difference ($P < 0.001$) between time-to-remission for anti-TNF exposure, with anti-TNF naïve patients 1.8 times more likely to achieve clinical remission (95%CI: 1.3-2.3) (Figure 2).

Time to failure

A total of 84 (27.7%) patients were censored as 'failure'. Controlling for site, univariate cox regression models for time-to-failure found no significant associations for gender ($P = 0.4$), smoking status ($P = 0.3$), age at which VDZ was given ($P = 0.9$),

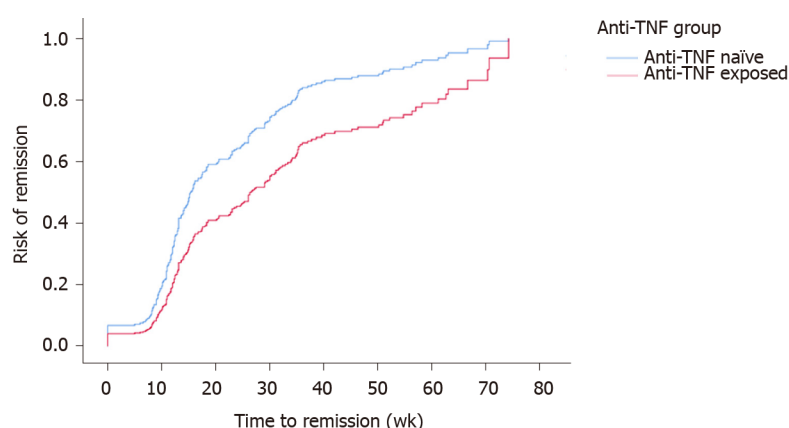


Figure 2 Cumulative remission rate of anti-tumor necrosis factor naïve patients to vedolizumab therapy (vs anti-tumor necrosis factor exposed patients, $P < 0.001$).

immunomodulator exposure ($P = 0.2$). These factors remained not significant when considered with anti-TNF exposure. Disease duration was significant (OR = 0.95, 95%CI: 0.93-1.00 $P = 0.048$), however, was no longer significant when considered with anti-TNF exposure. The final model included anti-TNF exposure and site with the Log Rank (Mantel-Cox) reporting significant difference ($P = 0.011$) between time-to-failure for anti-TNF groups with anti-TNF exposure patients 1.8 times more likely to lose response (95%CI: 1.16-2.75) (Figure 3).

Safety

The tolerability of VDZ was high with only 8% ($n = 25$) of all patients reporting an adverse event. Infections (7%, $n = 21$) were by far the most common adverse event. Two patients reported a serious infection, one was Haemophagocytic syndrome due to Cytomegalovirus and another was from Klebsiella sepsis, both from Australia and both patients were on dual immunosuppression thorough out the study period. A total of 9 patients who received VDZ reported respiratory complications of whom 4 patients reported sinusitis, 2 patients an upper respiratory tract infection, one patient each of nasopharyngitis, pharyngitis and pneumonia. Gastrointestinal infections were reported in 8 patients. Clostridium difficile was the most common gastrointestinal infection (4 patients) followed by Strongyloides (one patient), Campylobacter (one patient), and Salmonella (one patient). A buttock abscess was reported in one patient. Oral thrush was reported in an Oxford patient was attributed to VDZ use. The other complications due to VDZ use reported in our cohort during the study period include rash in one patient, delayed hypersensitivity in one patient and arthralgia and headaches in another patient (Table 4). No deaths were attributed to VDZ use.

A colectomy was undertaken in 11% ($n = 32$) patients by 12 mo. No significant difference ($P = 0.25$) in the number of patients requiring colectomy in Australia ($n = 19/210$) and Oxford ($n = 13/93$) was observed. Anti-TNF exposed patients (23/121) were more likely to require colectomy compared to anti-TNF naïve (9/182) by 12 mo ($P = 0.0005$) but when patients with primary and secondary LOR to anti-TNF agents were compared, no significant difference was noted (Table 5).

DISCUSSION

In this study of 303 UC patients treated with VDZ from 15 specialist IBD centers in two countries, VDZ was noted to be both safe and effective. This study, and the GETAID studies^[9], are the only studies where VDZ data is collected from multiple centers encompassing two countries, thus effectively reducing physician, site and country biases.

The key findings in this cohort were that the week 12 response in UC was 79% while remission rates were 56%, 62% and 60% at 3, 6 and 12 mo respectively. No differences were observed between the two countries in achieving remission at all time points. Anti-TNF-exposed patients, however, were almost twice as likely to lose response to VDZ compared to anti-TNF naïve patients but no difference in VDZ outcomes were observed between patients who had a primary and secondary LOR to anti-TNF agents.

Table 4 Complications of vedolizumab therapy

Complication	Australia (n = 20)	Oxford (n = 5)
Respiratory infections	URTI (2)	Pneumonia (1)
	Sinusitis (4)	Pharyngitis (1)
	Nasopharyngitis (1)	
Gastrointestinal Infections	Strongyloidis (1)	Gastroenteritis (1)
	Clostridium difficile (4)	Buttock abscess (1)
	Campylobacter (1)	Oral Thrush (1)
	Salmonella (1)	
Serious infections	Haemophagocytic syndrome due to CMV (1)	NA
	Klebsiella (1)	
Others	Rash (1)	NA
	Delayed hypersensitivity reaction (1)	
	Arthralgia and Headaches (1)	

VDZ: Vedolizumab; URTI: Upper respiratory tract infection; NA: Not applicable.

Table 5 Colectomy at 12 mo of vedolizumab therapy

Colectomy, n	Australia	Oxford	P value
	19/210	13/93	0.25
Colectomy, n	Anti-TNF naïve	Anti-TNF exposed	P value
	9/182	23/121	0.0005
Colectomy, n	Primary LOS	Secondary LOS	P value
	10/45	23/61	0.795

VDZ: Vedolizumab; TNF: Tumor necrosis factor; Primary LOR: Primary loss of response; Secondary LOR: Secondary loss of response.

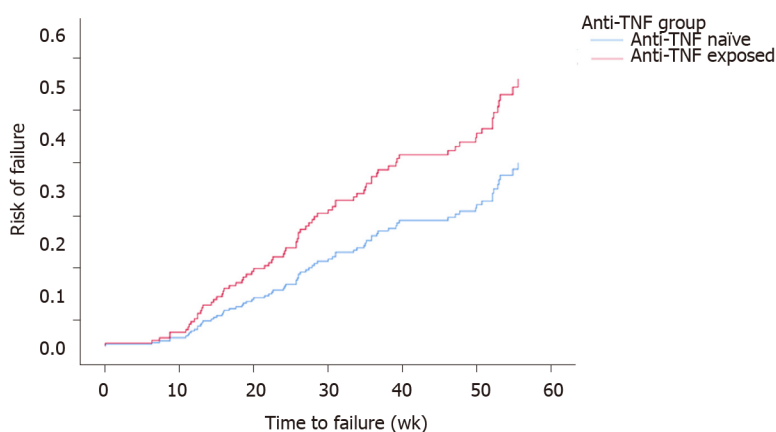


Figure 3 Cumulative loss of response of anti-tumor necrosis factor exposed patients to Vedolizumab therapy (vs anti-tumor necrosis factor naïve patients, $P = 0.011$).

Adverse events were observed in 8% of patients and 11% patients required colectomy by 12 mo.

Our results were comparable to prior work from other real world studies. A Swedish real world study observed that 64% of UC patients achieved clinical remission at the end of the follow-up period^[10], while a French study demonstrated

that 40.5% were in steroid-free clinic remission at week 54^[11]. The discrepancy in the clinical response rates could be explained by the difference in clinical characteristics of the patients entering the study and also different clinical criteria (steroid-free remission in French study) used to assess the patients. Similarly, other real world data have shown that prior exposure to anti-TNF agents reduces VDZ effectiveness, but no difference in VDZ outcomes when the patients had either primary or secondary LOR to anti-TNF agents is in line with our findings^[12]. The adverse event profile with VDZ treatment was also similar to what has been previously reported^[13].

Mucosal healing in UC is associated with improved long-term clinical outcomes and STRIDE guidelines identified it as a therapeutic goal^[1]. With MES as an endpoint, we report mucosal healing rates of 69% in Australian Cohort at 6 mo compared to 50% in ACT1, 46% in ACT2 at week 30 with Infliximab^[14], 59% in PURSUIT at week 30 with Golimumab^[15], 25% in ULTRA2 at week 52 with adalimumab^[16], 51% in GEMINI1 with VDZ at week 52^[5]. The high mucosal healing rates observed in our study could be due to high concomitant immunomodulator use in Australia (64%), however further prospective studies are needed to prove the role of immunomodulator use with VDZ.

We chose week 12 to assess the response and remission, in contrast to GEMINI 1 time of assessment for response at week 6^[5]. This was done in accordance with Australian PBS criteria, which stipulates that patients must be in remission after induction at clinic review before applying for maintenance VDZ. It appears, however, that the full effect of VDZ may take longer than 12 wk as a longer duration of treatment is associated with a higher rate remission at 6 (62%) than at 3 mo (56%). This study thus suggests that the PBS funding criteria may need to be re-attended to benefit the patients. Compared to its predecessor Natalizumab, an *alpha4* antagonist, VDZ is a more selective integrin antagonist blocking only *alpha4beta7* and thus does not effect lymphocyte trafficking to central nervous system, thereby theoretically eliminating the risk of progressive multifocal leukoencephalopathy (PML), a catastrophic side effect of Natalizumab^[17]. No case of PML occurred in our study or any other previous studies with VDZ^[18,19].

More Australian patients compared to Oxford patients achieved clinical response at 3 mo (83% *vs* 70% $P = 0.01$) and endoscopic remission at 6 mo (69% *vs* 43% $P = 0.01$). This may be due to more patients on concomitant immunomodulation in Australia compared to Oxford (64% *vs* 43% at VDZ initiation). Further analysis of our data and more prospective studies need to be done to define the role of concomitant immunomodulation with VDZ.

Our observed rate of VDZ efficacy at 12 mo in UC (60%) was comparable to the rates reported with anti-TNF agents^[20,21]. While there are no head to head randomized control trials comparing VDZ and infliximab in UC, VDZ showed a significantly better durable clinical response (OR = 3.18, 95%CI: 1.14-9.20) and clinical remission (OR = 2.93, 95%CI: 1.03-8.28) when compared to infliximab in a network meta-analysis^[22]. With no major safety concerns^[23], in the treatment algorithm ladder of UC, we argue that VDZ should be considered as the first biologic when conventional treatments fail due to its gut selectivity. This is most relevant in patients at high risk of serious infections such as the elderly, those with chronic obstructive airway disease or cardiac disease. If cost allows, it should even be considered before conventional treatment such as AZA due to the same feature and with more and more biosimilars reducing the cost of biologics we may see this in future.

VDZ is also an attractive option in patients who have failed prior anti-TNF agent. Anti-TNF therapy is effective for the treatment of moderate to severe UC, however a significant proportion of patients either fail to respond to anti-TNF therapy termed or lose response with time^[24]. Second and third anti-TNF agents can be used in such patients, however, it is a game of diminishing returns, as Golimumab efficacy data has shown that clinical response diminishes with each subsequent anti-TNF agent^[25]. Rather than giving another anti-TNF agent, VDZ provides a unique mechanism of action with gut selectivity and less side effects. VDZ does work in anti-TNF refractory IBD patients^[26] and our study supports this with 51% of TNF exposed patients achieving remission at 12 mo with VDZ therapy.

There are several limitations to our study, the most significant of which is retrospective review of the data (although the data in Australia pertaining to each VDZ application to PBS was collected prospectively). Different endoscopic assessment scores were used in Australia and United Kingdom, although a significant correlation was found between the two scores in a recent study^[27]. There was consistency, however, in the clinical and endoscopic outcomes across the institutions. One another limitation is we did not report the number of patients who were steroid free and on immunomodulators at different time points in our study although our aim is to look at that in future by obtaining further information from the centers.

As more and more biologic agents become available for the treatment of IBD, the role of VDZ needs to be defined. Real-world data is important in developing treatment algorithms, which will ultimately help physicians make important treatment decisions in complex IBD patients. This study has shown that VDZ is safe and effective in achieving clinical and endoscopic remission in UC patients.

ARTICLE HIGHLIGHTS

Research background

Vedolizumab (VDZ) is a gut selective anti-integrin used for treatment of ulcerative colitis (UC). Evidence needed to assess its efficacy and safety in a real world setting.

Research motivation

Efficacy and safety of VDZ needs to be assessed, involving multiple inflammatory bowel disease (IBD) centers from two countries to reduce physician, site and country biases.

Research objectives

In this real world study, we aim to assess the clinical response, clinical, endoscopic remission and the factors influencing them in UC patients from Australia and Oxford in United Kingdom treated with VDZ.

Research methods

Retrospective review of prospectively entered patient database, treated with VDZ. Three hundred and three UC patients from 14 Australian centers and Oxford (United Kingdom) were included. Clinical response and remission was assessed at 3, 6 and 12 mo using Mayo score across all centers. Endoscopic remission was assessed at 6 mo using Mayo endoscopic score in Australia and ulcerative colitis endoscopic score of severity score in Oxford. Cox regression models and Kaplan Meier curves were performed to assess the time to remission, time to failure and the covariates influencing them. Safety was assessed through adverse event reporting.

Research results

Clinical response for all patients was 79% at 3 mo and number of patients achieving clinical remission increased from 3 mo (56%) to 6 mo (62%) and remained almost stable at 12 mo (60%). No significant difference was observed between the two countries in achieving clinical remission at all points and a significantly greater proportion of Australian patients achieved mucosal healing compared to Oxford, which could be due to more patients using concomitant immunomodulation in Australia. Anti-tumor necrosis factor (anti-TNF) exposed patients were almost twice more likely to lose response to VDZ compared to anti-TNF naïve patients but no difference in outcomes were observed between patients who had a primary and secondary loss of response to anti-TNF agents. The role of concomitant immunomodulation in achieving above outcomes need to be elucidated in future prospective studies.

Research conclusions

VDZ can be safely and effectively used to treat UC patients in a real world setting. However patients who had prior anti-TNF therapy were more likely to fail compared to anti-TNF naïve patients.

Research perspective

VDZ use was reviewed in real world setting involving multiple IBD centers from two countries. This study helps physicians find VDZ its place in the treatment algorithm of complex IBD patient management. Future prospective studies are needed to evaluate the benefit of using concomitant immunomodulation with VDZ.

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Retrospective Cohort Study

Predictive model for acute abdominal pain after transarterial chemoembolization for liver cancer

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Institutional review board

statement: This single-center retrospective study was approved as an expedited chart review study and obtained ethical approval from the institutional review board of the First Affiliated Hospital, Zhejiang University School of Medicine.

Informed consent statement: This was a retrospective study and

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Abstract**BACKGROUND**

Transarterial chemoembolization (TACE) is the first-line treatment for patients with unresectable liver cancer; however, TACE is associated with postembolization pain.

AIM

To analyze the risk factors for acute abdominal pain after TACE and establish a predictive model for postembolization pain.

METHODS

From January 2018 to September 2018, all patients with liver cancer who underwent TACE at our hospital were included. General characteristics; clinical, imaging, and procedural data; and postembolization pain were analyzed. Postembolization pain was defined as acute moderate-to-severe abdominal pain within 24 h after TACE. Logistic regression and a classification and regression tree were used to develop a predictive model. Receiver operating characteristic curve analysis was used to examine the efficacy of the predictive model.

RESULTS

We analyzed 522 patients who underwent a total of 582 TACE procedures. Ninety-seven (16.70%) episodes of severe pain occurred. A predictive model built

exemption from the need for signed informed consent was approved by the Institutional Review Board of the First Affiliated Hospital, Zhejiang University School of Medicine.

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based on the dataset from classification and regression tree analysis identified known invasion of blood vessels as the strongest predictor of subsequent performance, followed by history of TACE, method of TACE, and history of abdominal pain after TACE. The area under the receiver operating characteristic curve was 0.736 [95% confidence interval (CI): 0.682-0.789], the sensitivity was 73.2%, the specificity was 65.6%, and the negative predictive value was 92.4%. Logistic regression produced similar results by identifying age [odds ratio (OR) = 0.971; 95%CI: 0.951-0.992; $P = 0.007$], history of TACE (OR = 0.378; 95%CI: 0.189-0.757; $P = 0.007$), history of abdominal pain after TACE (OR = 6.288; 95%CI: 2.963-13.342; $P < 0.001$), tumor size (OR = 1.978; 95%CI: 1.175-3.330; $P = 0.01$), multiple tumors (OR = 2.164; 95%CI: 1.243-3.769; $P = 0.006$), invasion of blood vessels (OR = 1.756; 95%CI: 1.045-2.950; $P = 0.034$), and TACE with drug-eluting beads (DEB-TACE) (OR = 2.05; 95%CI: 1.260-3.334; $P = 0.004$) as independent predictive factors for postembolization pain.

CONCLUSION

Blood vessel invasion, TACE history, TACE with drug-eluting beads, and history of abdominal pain after TACE are predictors of acute moderate-to-severe pain. The predictive model may help medical staff to manage pain.

Key words: Liver cancer; Predictive model; Pain; Transarterial chemoembolization; Postembolization syndrome

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Core tip: Transarterial chemoembolization (TACE) is associated with postembolization pain. We analyzed the risk factors for acute abdominal pain after TACE and established a predictive model for it. The predictive model built based on the dataset from a classification and regression tree identified known invasion of blood vessels as the strongest predictor of subsequent performance, followed by history of TACE, method of TACE, and history of abdominal pain after TACE. Our predictive model is simple to use and provides a more rational reference to improve the quality of pain management after TACE.

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INTRODUCTION

Primary liver cancer (PLC) is the seventh most common carcinoma worldwide and the third most common cause of cancer-related mortality^[1]. More than half of new cases of liver cancer occur in China. Transarterial chemo-embolization (TACE) is the most widely used treatment for unresectable PLC. TACE plays an important role in the treatment of tumors, improving quality of life and prolonging patient survival^[2]. According to statistics, more than 600000 people undergo TACE in China each year^[3].

TACE is a procedure that consists of local delivery of a high dose of chemotherapeutic agents to the tumor, which can be associated with particulate and/or oily embolization of feeding arteries, which results in exposure of the tumor to a higher concentration of chemotherapeutic agent and subsequent tumor infarction and necrosis due to vascular occlusion^[4,5]. Postembolization syndrome, which is characterized by abdominal pain, nausea, vomiting, and fever, is the most frequently reported adverse event after TACE^[6]. Approximately 60%-80% of patients complained of different levels of pain after TACE. Among those patients, more than 25% experienced moderate-to-severe pain^[7,8]. While TACE is generally understood to require hospital admission and at least a one-night in-patient stay^[9], postembolization pain is primarily associated with an extended hospital stay^[10]. Painkillers, such as opioids, are effective and safe^[11]. Clearly identifying factors associated with

postembolization pain could help predict its occurrence and improve analgesic treatment.

At present, few studies have examined the related risk factors or predictive models for postembolization pain after TACE; thus, no conclusions about the risk factors for postembolization pain have been reached. The present study aimed to evaluate the risk factors for postembolization pain and to establish a predictive model for postembolization pain in patients undergoing TACE.

MATERIALS AND METHODS

This single-center retrospective cohort study was approved as an expedited chart review study and obtained ethical approval from the institutional review board at our hospital.

Patients

Patients with PLC who underwent TACE at our hospital between January 2018 and September 2018 were analyzed retrospectively. Some patients underwent the procedure more than once during this period. A diagnosis of liver cancer was confirmed either histologically or based on consistent findings obtained from at least two imaging techniques, including ultrasonography, computed tomography, magnetic resonance imaging, and selective hepatic arterial angiography^[12]. The exclusion criteria were: (1) PLC in patients aged < 18 years; (2) Emergency embolization for rupture of liver cancer; (3) Severe complications such as bleeding after TACE; (4) Use of additional analgesics to relieve increased pain during TACE; (5) Cognitive impairment; (6) Use of psychiatric medications; and (7) Drug or alcohol abuse.

Chemoembolization procedure

All procedures were performed at a single tertiary center by board-certified interventional radiologists. All patients were administered with 10 mL of 2% lidocaine to achieve local anesthesia, and 5 mg of dezocine during surgery. An arterial catheter was inserted into the femoral artery using the Seldinger technique and subsequently placed in the hepatic artery. Tumor-feeding vessels were super-selected whenever possible. Chemotherapy drugs used were pirarubicin hydrochloride (10 mg/bottle; Shenzhen Main Luck Pharmaceuticals Inc., Shenzhen, China) at a dose of 30 mg mixed with Lipiodol (Laboratoire Guerbet, Aulnay-sous-Bois, France) for TACE, or at 60 mg for drug-eluting beads (DEB-TACE) treatment. Oxaliplatin (150 mg; Jiangsu Hengrui Medicine Co., Ltd.) was used for arterial perfusion chemotherapy. Lipiodol and/or polyvinyl alcohol particles (350-510 μ m; Ailikang Medicine Co., Ltd, Hangzhou, China) and Embosphere (Biosphere Medical, Rockland, MA, United States) were used as embolization materials. All patients received supportive treatment after TACE, including non-steroidal anti-inflammatory drugs or dezocine, liver protection, antacid agents, and antiemetics. If moderate-to-severe abdominal pain was observed, the patient received tramadol (100 mg) or other opioids by intravenous administration.

Identification of risk factors

The aim of our study was to analyze the risk factors that helped to predict moderate-to-severe postembolization pain. The numerical rating scale pain scores at rest were assessed in all patients within 24 h of embolization. The numerical rating scale pain score was used as the standard subjective evaluation using a score of 0-10, where 0 = painless; < 3 = mild pain; 4-6 = moderate pain; and 7-10 = severe pain. Additional painkillers were administered when the pain score was ≥ 4 . The pain scores were recorded 0, 2, 4, 6, 12, and 24 h after TACE. The highest score through the overall time was defined as a pain score in each patient.

Independent variables included demographics and clinical, imaging, and procedural data. Seventeen registered variables were included for each TACE procedure, including: Age and gender of the patient; tumor location (distance to liver capsule); tumor size and number; pathological properties of the tumor; invasion of blood vessels; disease type; history and number of TACE procedures; history of postembolization pain; drug delivery method (traditional TACE *vs* DEB-TACE); dosage of lipiodol; and complementary embolization (blank microsphere and/or polyvinyl alcohol particles; and postoperative prophylactic analgesics).

Statistical analysis

Quantitative data are described as the mean \pm standard deviation or as medians (min, max). Qualitative data are described by the number of cases (proportion, %). Patient characteristics were compared using the χ^2 test or Fisher's exact test for categorical data, and the Wilcoxon rank-sum test or *t*-test were used to analyze continuous data. Two statistical methods were used to develop the predictive model: A primary analysis using classification and regression tree (CART), and a conjoint predictive model using logistic regression. In this research, cross-validation was used to select the regression model in which the mean cross-validated error was within one standard error of the minimum. The area under the receiver operating characteristic (ROC) curve, specificity, sensitivity, and negative predictive value of the predictive model were calculated by ROC curve analysis to evaluate its performance. Two-sided *P* values of < 0.05 indicated statistical significance. Analyses were conducted using statistical software (IBM SPSS Statistics 22.0).

RESULTS

Patient cohort

A total of 522 patients who underwent a total of 582 TACE procedures were enrolled in the study. Patient demographics, baseline clinical and laboratory data, and procedural details are listed in Table 1 and 2. As shown in Table 1, the age range of patients was 23-87 years (average, 60.1 ± 11.4 years). The median number of TACE procedures in the 582 patients was two. As shown in Table 2, the data set comprised 81 females (13.9%) and 501 males (86.1%). Ninety-seven (16.7%) patients had acute moderate-to-severe abdominal pain after TACE. A total of 57.2% (333/582) of patients had a history of TACE and 12.5% (73/582) had a history of abdominal pain after TACE. Blood vessel invasion occurred in 176 (30.2%) patients. Approximately 57.7% (336/582) of patients used traditional TACE and 42.3% (246) used DEB-TACE.

Distribution of demographic and clinical factors associated with acute moderate and severe abdominal pain after TACE

The results of the univariate analysis are shown in Table 3. Younger patients ($P = 0.002$) and those patients who had not undergone hepatectomy ($P = 0.010$) were more likely to have acute moderate-to-severe abdominal pain after TACE compared with older patients and those who had tumor recurrence after hepatectomy. History of TACE ($P < 0.001$), history of abdominal pain after TACE ($P < 0.001$), tumor size ($P < 0.001$), tumor number ($P = 0.010$), invasion of blood vessels ($P < 0.001$), use of the DEB-TACE method ($P < 0.001$), and the number of TACE procedures ($P < 0.001$) were significantly associated with moderate-to-severe abdominal pain. The pathological properties of the tumor was not associated with moderate-to-severe abdominal pain.

A predictive model built based on the dataset from the classification and regression trees identified known blood vessel invasion as the strongest predictor of subsequent performance, followed by history of TACE, method of TACE, and history of abdominal pain after TACE (Figure 1). We used ROC curve analysis to examine the efficacy of the predictive model. We set an optimal predictive probability threshold of 0.18, and demonstrated a sensitivity of 73.2% (71/97; 95% confidence interval [CI]: 64.4%-82.0%), specificity of 65.6% (318/485; 95%CI: 61.3%-69.8%), negative predictive value of 92.4% (318/344; 95%CI: 89.6%-95.2%), and area under the curve of 0.736 (95%CI: 0.682-0.789) (Figure 2). Logistic regression produced similar results by identifying age (odds ratio [OR] = 0.971; 95%CI: 0.951-0.992; $P = 0.007$), history of TACE (OR = 0.378; 95%CI: 0.189-0.757; $P = 0.007$), history of abdominal pain after TACE (OR = 6.288; 95%CI: 2.963-13.342; $P < 0.001$), tumor size (OR = 1.978; 95%CI: 1.175-3.330; $P = 0.01$), multiple tumors (OR = 2.164; 95%CI: 1.243-3.769; $P = 0.006$), blood vessel invasion (OR = 1.756; 95%CI: 1.045-2.950; $P = 0.034$), and the DEB-TACE method (OR = 2.05; 95%CI: 1.260-3.334; $P = 0.004$) as independent predictive factors for postembolization pain.

DISCUSSION

Although painkillers were used prophylactically during and after TACE in our study, the incidence of moderate-to-severe abdominal pain in the first 24 h after TACE procedures remained as high as 16.7%. This conclusively demonstrated that use of a

Table 1 Demographic, clinical, laboratory, and procedural variables of patients (1)

Variable	<i>n</i>	mean ± SD	Median (IQR)	Min	Max
Age (yr)	582	60.1 ± 11.4	60 (53, 67)	23	87
Number of TACE	582	2.5 ± 2.2	2 (1, 3)	1	15
Dose of Lipiodol (mL)	582	4.7 ± 6.8	3 (0, 6)	0	30

TACE: Transarterial hepatic chemoembolization; IQR: Interquartile range.

single non-steroidal anti-inflammatory drug or dezocine is often not sufficient for effective pain control. Multimodal analgesia was associated with superior pain relief and decreased opioid consumption when compared with use of a single pain medication^[13]. Guo *et al*^[8] demonstrated that patients who used preemptive parecoxib and a sufentanil-based multimodal analgesia regimen had better pain relief, evidenced by a lower incidence of severe pain (11.9%)^[8]. Similar to a previous study^[14], we observed that effective pain management could reduce the length of hospital stay. The prediction model can be used to predict the risk of postembolization pain after TACE, thus providing medical staff with a reference for pain management.

The cause of postembolization pain is not fully understood; however, it is believed to be caused by local tissue hypoxia, tumor necrosis, swelling of the capsule, ectopic embolization, or consequent cytokine release and the inflammatory response^[15,16]. Identification of preoperative predictors of postembolization pain is challenging. In our pain predictive model using CART methods, blood vessel invasion was the strongest predictor of postembolization pain, followed by history of TACE, the DEB-TACE method, and history of abdominal pain after TACE. Besides these four risk factors, age, tumor size (> 5 cm), and presence of multiple tumors were identified as predictors of postembolization pain by logistic regression.

Invasion of blood vessels means portal vein tumor thrombosis

Primary liver cancer has a great propensity to invade the portal venous system, which leads to portal vein tumor thrombosis. Portal vein tumor thrombosis is found in the trunk or branches of the portal vein, and TACE is considered if the portal vein trunk is not completely blocked or portal collateral circulation is already present in the hepatic hilar region^[17]. No sources of data on blood vessel invasion as a risk factor for pain were found when conducting a literature review; thus, our study is the first in this respect, identifying blood vessel invasion as a predictor of pain. Tumor invasion of the portal vein is more common in the late stages of cancer^[17], and is often accompanied by tumors that are large in size and/or numerous, which may be attributable to tumor necrosis and a more marked inflammatory response, which is caused by embolization of a larger site^[18-20].

The conclusions that can be drawn from the two statistical approaches are generally consistent. The tree graph output from CART is intuitive and easy to explain in terms of the interaction between variables and the influence of different factors on outcome variables. The four predictors of the model can be easily extracted as predictive risk factors prior to TACE. It is beneficial to provide a comprehensive analgesic plan for patients who are at a high risk of postembolization pain. The risk factors for postembolization pain identified in this study are similar to those identified by Khalaf *et al*^[21].

Although DEB-TACE is considered less toxic and better standardized compared with traditional lipiodol-TACE, tolerance caused by DEB-TACE is controversial^[22]. Traditional TACE is performed using lipiodol loaded with chemotherapy drugs to embolize blood vessels and kill tumor cells. DEB-TACE depends on drug-loaded microspheres to precisely control the release of drugs to maximize tumor necrosis and minimize adverse effects^[23]. A randomized study performed by Lammer *et al*^[23] reported that tolerance was better with DEB-TACE compared with traditional TACE^[23]. The Precision Italia Study Group compared two types of TACE in 177 patients. The results showed that the probability of abdominal pain with DEB-TACE is lower than that of traditional TACE^[24]. In contrast to this result, our analysis showed that patients who underwent DEB-TACE experienced increased postembolization pain and required more painkillers within 24 h of the procedure. This is in accordance with the data from two other studies, which showed that severe pain occurred significantly more frequently in patients who underwent DEB-TACE than in the traditional TACE

Table 2 Demographic, clinical, laboratory, and procedural variables of patients (2)

Variable	<i>n</i>	%
Sex		
Female	81	13.9
Male	501	86.1
Disease type		
No surgery	371	63.7
Recurrence after surgery	211	36.3
TACE history	333	57.2
History of abdominal pain after TACE	73	12.5
Pathological properties		
HCC	280	48.1
ICC	13	2.2
Unknown	265	45.5
Other	24	4.1
Tumor size		
≤ 5 cm	334	57.4
> 5 cm	248	42.6
Number of tumors		
≤ 2 (non-multiple)	197	33.8
> 2 (multiple)	385	66.2
Invasion of blood vessels	176	30.2
Blank microsphere	138	23.7
PVA particles	63	10.8
Method of TACE		
Traditional TACE	336	57.7
DEB-TACE	246	42.3
Prophylactic analgesics		
Tenay	74	12.7
Kaffin	112	19.2
Dezocine	252	43.3
Pentam	29	5
None	58	10
Two painkillers	57	9.8
Pain after TACE		
No pain/mild pain	485	83.3
Moderate pain/severe pain	97	16.7

TACE: Transarterial hepatic chemoembolization; DEB-TACE: Transarterial chemoembolization with drug-eluting beads; HCC: Hepatocellular carcinoma; ICC: Intrahepatic cholangiocarcinoma; PVA: Polyvinyl alcohol.

group^[21,25]. Some studies showed that the total dose of chemotherapeutic agents administered for TACE is related to the pain score^[19,21,26]. In this study, the chemotherapy drug used was pirarubicin hydrochloride administered at a dose of 30 mg for traditional TACE and 60 mg for DEB-TACE, similar to the report of Benzakoun *et al*^[19], and that may be one of the reasons why postembolization pain was worse for

Table 3 Effects of variables on outcomes (univariate analysis)

Variable	No pain/mild pain (n = 485)	Moderate pain/severe pain (n = 97)	P value
Age (yr), mean ± SD	60.79 ± 11.39	56.87 ± 10.99	0.002
Sex (%)			0.870
Female	68 (14)	13 (13.4)	
Male	417 (86)	84 (86.6)	
Disease type (%)			0.010
No surgery	298 (61.4)	73 (75.3)	
Recurrence after surgery	187 (38.6)	24 (24.7)	
TACE history (%)	293 (60.4)	40 (41.2)	< 0.001
History of abdominal pain after TACE (%)	51 (10.5)	22 (22.7)	< 0.001
Pathological properties (%)			0.110
HCC	231 (47.6)	49 (50.5)	
ICC	8 (1.6)	5 (5.2)	
Unknown	224 (46.2)	41 (42.3)	
Other	22 (4.5)	2 (2.1)	
Tumor location (distance to liver capsule) (%)			0.085
> 1 cm	245 (81.40)	50 (90.91)	
≤ 1 cm	56 (18.60)	5 (9.09)	
Tumor size (%)			< 0.001
≤ 5 cm	294 (60.6)	40 (41.2)	
> 5 cm	191 (39.4)	57 (58.8)	
Number of tumors (%)			0.010
≤ 2 (non-multiple)	175 (36.1)	22 (22.7)	
> 2 (multiple)	310 (63.9)	75 (77.3)	
Invasion of blood vessels (%)	131 (27)	45 (46.4)	< 0.001
Blank microsphere (%)	112 (23.1)	26 (26.8)	0.430
Polyvinyl alcohol particles (%)	54 (11.1)	9 (9.3)	0.590
Method of TACE (%)			< 0.001
Traditional TACE	296 (61)	40 (41.2)	
DEB-TACE	189 (39)	57 (58.8)	
Prophylactic analgesics (%)			0.780
Parecoxib Na	58 (12)	16 (16.5)	
Flurbiprofen	97 (20)	15 (15.5)	
Dezocine	209 (43.1)	43 (44.3)	
Pentazocine	24 (4.9)	5 (5.2)	
None	48 (9.9)	10 (10.3)	
Two painkillers	49 (10.1)	8 (8.2)	
Number of TACE, median (IQR)	2 (1, 3)	1 (1, 2)	< 0.001
Dosage of lipiodol, median (IQR)	3 (0, 6)	0 (0, 8)	0.179

TACE: Transarterial hepatic chemoembolization; HCC: Hepatocellular carcinoma; ICC: Intrahepatic cholangiocarcinoma; PVA: Polyvinyl alcohol; DEB: Drug-eluting beads; IQR: Interquartile range.

DEB-TACE.

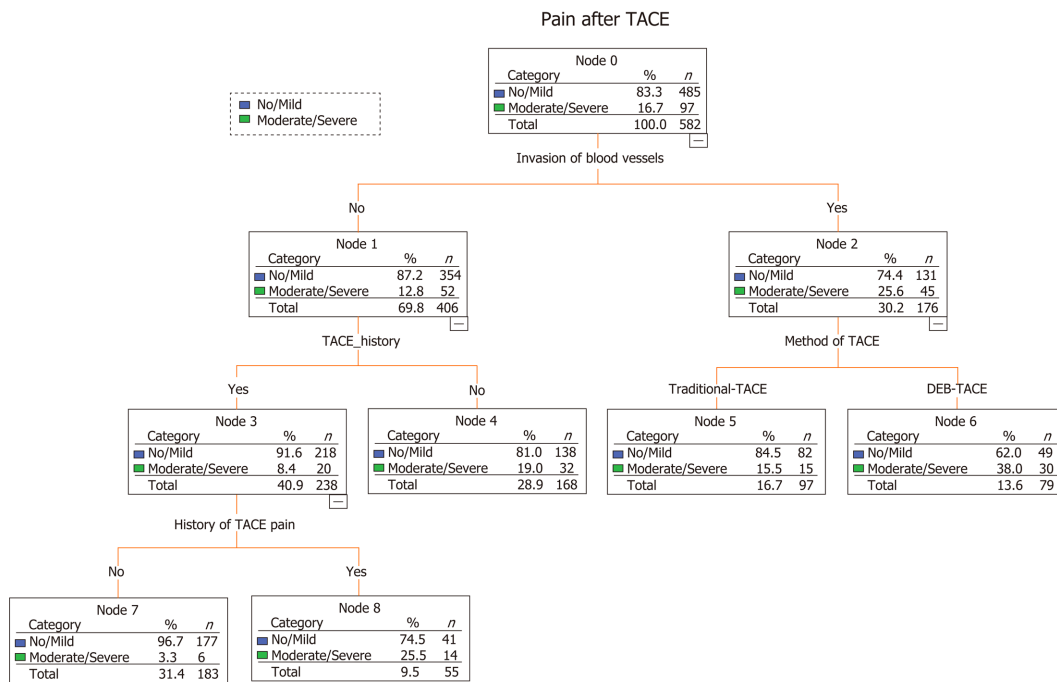


Figure 1 A predictive model built using a classification and regression tree. TACE: Transarterial chemoembolization; DEB-TACE: Transarterial chemoembolization with drug-eluting beads.

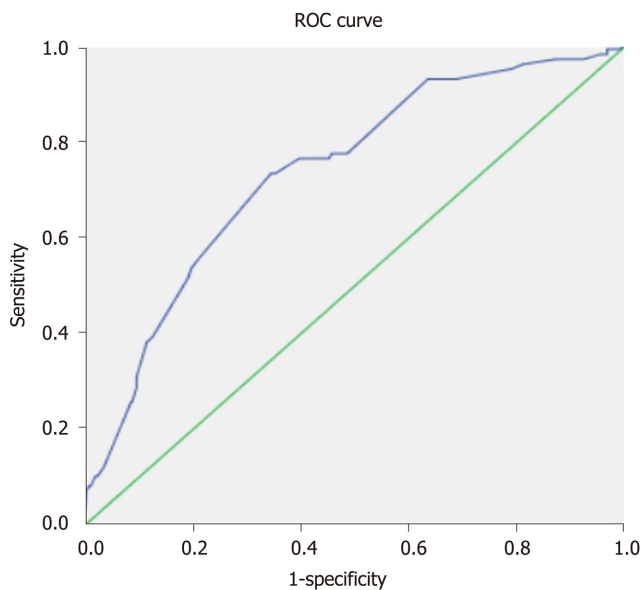


Figure 2 Receiver operating characteristic curve illustrating the performance of the predictive model. The area under the curve was 0.736 (95% confidence interval: 0.682-0.789). ROC: Receiver operating characteristic.

Our findings suggest that first-time TACE patients were more likely to experience pain than those with previous experience of TACE and this is consistent with a recent study^[27]. There are two possible reasons for this; first, the pain threshold is increased according to the time that TACE treatment is carried out and second, the tolerance to TACE is increased by repeated treatments. Patients with pain after TACE are more likely to develop pain in the future, which may be related to the individual's pain threshold and the presence of liver disease.

The predictive model that used CART was examined by ROC curve analysis. The area under the ROC curve was used to predict postembolization pain (0.736; 95%CI: 0.682-0.789). The model had a good sensitivity and specificity, and a high negative predictive value of 92.4%.

Our study has several notable limitations common to retrospective, single-center

studies. First, prior epidemiologic findings indicate that chronic liver disease, performance status, and psychological factors may contribute to postembolization pain after TACE. However, we did not control for or investigate these factors as part of our analysis. Future investigations with larger sample sizes should aim to develop more robust prediction models that include other potential contributing factors to further elucidate the risk factors for this disorder. Second, our patient population was obtained from a regional tertiary care center, which may not be representative of the general population. Finally, our model was not validated using an external population. Therefore, future studies with a larger sample size, a multicenter design, and using an external cohort are needed to confirm our findings.

Despite these limitations, our predictive model is simple to use and provides a more rational reference to improve the quality of pain management after TACE. It is suggested that more comprehensive analgesic interventions should be provided for patients who are at a high risk of pain, such as multimodal analgesic therapy.

ARTICLE HIGHLIGHTS

Research background

Transarterial chemoembolization (TACE) is the first-line treatment for patients with unresectable liver cancer. However, approximately 60%-80% of patients complain of different levels of postembolization pain after TACE.

Research motivation

Clearly identifying factors associated with postembolization pain could help predict its occurrence. Prediction model could be used to predict the risk of abdominal pain after TACE, thus providing medical staff with a reference for pain management.

Research objectives

To analyze the risk factors for acute abdominal pain after TACE and establish a predictive model for postembolization pain.

Research methods

From January 2018 to September 2018, all patients with liver cancer who underwent TACE at our hospital were included. General characteristics; clinical, imaging, and procedural data; and postembolization pain were analyzed. Postembolization pain was defined as acute moderate-to-severe abdominal pain within 24 h after TACE. Logistic regression and a classification and regression tree were used to develop a predictive model. Receiver operating characteristic curve analysis was used to examine the efficacy of the predictive model.

Research results

We analyzed 522 patients who underwent a total of 582 TACE procedures. Ninety-seven (16.70%) episodes of severe pain occurred. A predictive model built based on the dataset from classification and regression tree analysis identified known invasion of blood vessels as the strongest predictor of subsequent performance, followed by history of TACE, method of TACE, and history of abdominal pain after TACE. The area under the receiver operating characteristic curve was 0.736, the sensitivity was 73.2%, the specificity was 65.6%, and the negative predictive value was 92.4%.

Research conclusions

Blood vessel invasion, TACE history, TACE with drug-eluting beads, and history of abdominal pain after TACE are predictors of acute moderate-to-severe pain. Our predictive model is simple to use and provides a more rational reference to improve the quality of pain management.

Research perspectives

Future studies with a larger sample size, a multicenter design, and using an external cohort are needed to confirm our findings.

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Retrospective Study

Risk prediction platform for pancreatic fistula after pancreatoduodenectomy using artificial intelligence

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Abstract

BACKGROUND

Despite advancements in operative technique and improvements in postoperative managements, postoperative pancreatic fistula (POPF) is a life-threatening complication following pancreatoduodenectomy (PD). There are some reports to predict POPF preoperatively or intraoperatively, but the accuracy of those is questionable. Artificial intelligence (AI) technology is being actively used in the medical field, but few studies have reported applying it to outcomes after PD.

AIM

To develop a risk prediction platform for POPF using an AI model.

METHODS

Medical records were reviewed from 1769 patients at Samsung Medical Center who underwent PD from 2007 to 2016. A total of 38 variables were inserted into AI-driven algorithms. The algorithms tested to make the risk prediction platform were random forest (RF) and a neural network (NN) with or without recursive feature elimination (RFE). The median imputation method was used for missing

Samsung Medical Center (number: SMC 2017-01-017).

Informed consent statement:

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

Conflict-of-interest statement:

There are no financial or any potential personal conflicts of interest to declare for any of the authors.

Data sharing statement:

No additional data are available.

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values. The area under the curve (AUC) was calculated to examine the discriminative power of algorithm for POPF prediction.

RESULTS

The number of POPFs was 221 (12.5%) according to the International Study Group of Pancreatic Fistula definition 2016. After median imputation, AUCs using 38 variables were 0.68 ± 0.02 with RF and 0.71 ± 0.02 with NN. The maximal AUC using NN with RFE was 0.74. Sixteen risk factors for POPF were identified by AI algorithm: Pancreatic duct diameter, body mass index, preoperative serum albumin, lipase level, amount of intraoperative fluid infusion, age, platelet count, extrapancreatic location of tumor, combined venous resection, co-existing pancreatitis, neoadjuvant radiotherapy, American Society of Anesthesiologists' score, sex, soft texture of the pancreas, underlying heart disease, and preoperative endoscopic biliary decompression. We developed a web-based POPF prediction platform, and this application is freely available at <http://popfrisk.smchbp.org>.

CONCLUSION

This study is the first to predict POPF with multiple risk factors using AI. This platform is reliable (AUC 0.74), so it could be used to select patients who need especially intense therapy and to preoperatively establish an effective treatment strategy.

Key words: Postoperative pancreatic fistula; Pancreatoduodenectomy; Neural networks; Recursive feature elimination

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Core tip: Postoperative pancreatic fistula (POPF) is a life-threatening complication following pancreatoduodenectomy. This is a retrospective study to develop a risk prediction platform for POPF using an Artificial intelligence (AI) model. Compared with established POPF risk prediction methods, this machine learning algorithms better predict the POPF risk correctly (AUC 0.74). This AI-driven platform can identify patients who need especially intense therapy and aid in the establishment of an effective treatment strategy.

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INTRODUCTION

Despite advancements in surgical technique and operative management, postoperative pancreatic fistula (POPF) is still widely considered to be the greatest contributor to major morbidity and mortality after pancreatoduodenectomy (PD), with an incidence of 10%-30%^[1-5]. Furthermore, it frequently delays the timely delivery of adjuvant therapies, and reduces overall patient survival^[6]. The indications of PD have been widening, and the procedure is offered to an increasing number of elderly patients with multiple comorbidities^[7,8], prompting the need to accurately define which patients are fit for PD and could tolerate a potentially life-threatening POPF.

Recently, the management of POPF has undergone a paradigm shift from a standardized and uniform approach that could not reflect an individual's characteristics to a proactive mitigation strategy. The new strategy uses various predictive systems to enable early prediction and prevention and optimize individual treatment decisions^[6,9,10]. Previous predictive systems^[6,9,10] might reflect POPF incidence and had the merit of simplicity, but their predictive accuracy is somewhat questionable^[11,12]. Therefore, to more accurately predict POPF, further research is needed.

Machine-learning (ML) is an artificial intelligence (AI) technology that has been

adopted in many areas of modern society, including medical science. In ML, computational models composed of multiple processing layers learn various data representations with multiple levels of abstraction^[13]. ML is currently being used in not only surgery^[5,14,15] but also other areas, such as, pharmacogenomics, image classification, and medical decision support systems^[16-20]. Therefore, for this study we aimed to develop a new risk prediction platform for POPF after PD using ML algorithms. If so, we expected that a patient's predicted POPF risk could direct their clinical management and prevent or mitigate untoward outcomes.

MATERIALS AND METHODS

Patient selection

Under institutional review board approval (No. SMC 2017-01-017), we retrospectively collected clinicopathological variables for 1846 patients who underwent PD to treat various periampullary tumors at Samsung Medical Center (Seoul, Republic of Korea) between January 2007 and December 2016. Among them, we excluded 77 (4.2%) patients who had metastasis from sites other than the primary tumor origin, had direct invasion from the primary tumor into adjacent organs, underwent surgery for a recurrence, or lacked medical information about POPF. We analyzed the remaining 1769 patients (1079 men and 690 women).

Definition and Selection of pre- and intraoperative input variables

Data on preoperative, intraoperative, and postoperative outcomes were collected and maintained on a web-based database (MDB, Seoul, Korea). We originally analyzed 38 preoperative and intraoperative variables that could be associated with POPF. Preoperative laboratory data, such as serum C-reactive protein, amylase, lipase, and carbohydrate antigen (CA) 19-9 level just before the operation was entered to algorithms. Co-existing pancreatitis was defined as classic feature of pancreatitis on preoperative Computed Tomography (CT) scan or intraoperative findings. Underlying heart diseases included hypertension on medication, coronary or valvular heart disease, or various arrhythmic diseases. The location of tumors and pancreatic duct (p-duct) diameter were determined or measured by preoperative CT scan. Total intraoperative fluid infusion consisted of total amount of intravenous crystalloid, colloid, volume expanders, or blood transfusion. The pancreatic texture was determined as soft or hard by the surgeon during the operation.

Among the continuous variables, there was some level of missing data. Median imputation^[19], which is a common approach for dealing with missing values in ML algorithms, was used. None of the categorical variables had missing values. We used the one-hot encoding technique to encode the categorical variables when only one of the categories was assigned. When a categorical variable had three exclusive choices, then we transformed the categorical variable into three individual binary variables. When the choices within a categorical variable are not exclusive (*e.g.*, "A", "B", "both A and B", and "none"), binary encoding was used by splitting each choice into separate columns and converting to binary codes^[21,22]. As a result, 44 encoded variables were input in the ML models (Table 1).

Surgical techniques and perioperative management

In cases of cholangitis or jaundice, preoperative endoscopic or percutaneous biliary drainage was performed. After the introduction of a definition of borderline resectable pancreatic cancer, 24 (1.4%) patients received neoadjuvant treatment using various regimens. All surgical procedures were performed by experienced 5 pancreatic surgeons at Samsung Medical Center who underwent more than 50 PDs annually. To create pancreatic anastomosis, 1761 (99.5%) patients underwent pancreaticojejunostomy (PJ) and 8 (0.5%) patients underwent pancreaticogastrostomy. Pancreatoenteric anastomosis with stents was conducted in 1185 patients (70.0%) (Table 1). At the end of each surgical procedure, two or three drains were placed adjacent to the PJ anastomosis and on the right side of the superior mesenteric arterial resection margin. Serum and drain fluid amylase levels were routinely measured on postoperative days 1-3 and 5, 6, or 7, if the drains were maintained. In this study, we used 'the definition of POPF in the 2016 update of the International Study Group (ISGPS) definition and grading of POPF'^[23]. As a result, the grade A fistula has been removed from the POPF classification in this study. Drains adjacent to the PJ anastomosis were removed if no evidence of a leak was found in an abdominal CT scan on postoperative days 5-7. Patients who experienced POPF received proper

Table 1 Clinicopathologic variables included in the machine learning algorithms

Variables	Values	Variables	Values
Age (yr)	67.7 ± 10.1	Preoperative ERBD/ENBD (<i>n</i> , %)	470 (26.6)
Sex (male/female)	1079: 690	Preoperative PTBD (<i>n</i> , %)	254 (14.4)
Body mass index (kg/m ²)	22.5 ± 3.8	Preoperative ERPD (<i>n</i> , %)	19 (1.1)
Heart disease including hypertension (<i>n</i> , %)	735 (41.5)	Neoadjuvant therapy (RT/chemotherapy/CCRT/No)	2: 6: 16: 1745
Diabetes mellitus (<i>n</i> , %)	472 (26.7)	Operative time (min)	443.2 ± 90.1
Pulmonary disease (<i>n</i> , %)	153 (8.6)	†Intraoperative fluid infusion (mL)	3129 ± 3495
Liver disease (<i>n</i> , %)	99 (5.6)	Intraoperative transfusion (<i>n</i> , %)	171 (9.7)
Cerebrovascular disease (<i>n</i> , %)	73 (4.1)	Estimated blood loss (ml)	962.4 ± 665.1
Chronic kidney disease (<i>n</i> , %)	17 (1.0)	Soft pancreas (<i>n</i> , %)	750 (43.2)
ASA score (1-4)	372:1267: 128: 2	Pancreatic duct diameter (mm)	4.2 ± 2.8
White blood cell count (x10 ³ /μL)	6.6 ± 2.3	Type of surgery (PPPD/PRPD/PD)	1254: 244: 271
Hemoglobin (g/dL)	10.9 ± 1.5	Combined organ resection (<i>n</i> , %)	67 (3.8)
Platelet count (x10 ³ /μL)	280.2 ± 52.7	Combined vascular resection(<i>n</i> , %)	188 (10.6)
Albumin (d/dL)	3.5 ± 0.4	C- reactive protein (mg/dL)	1.9 ± 10.9
Total bilirubin level (mg/dL)	3.5 ± 4.3	CA 19-9 (U/mL)	1786.5 ± 7141.5
Combined portal vein resection (<i>n</i> , %)	175 (9.9)	P-duct stent (Internal/external/none)	1051: 134: 584
Amylase (U/L)	94.5 ± 5586.9	Co-existing pancreatitis (<i>n</i> , %)	370 (20.9)
Lipase (U/L)	160.5 ± 277.0	‡Location of tumor (Pancreas/others)	856: 913
Anastomotic methods (1) (Duct-to-mucosa/Dunkin)	1756: 13	Anastomotic methods (2) (P-J/P-G/Others)	1761: 8: 0

Total fluid infusion consisted of total amount of intravenous crystalloid, colloid, transfusion. 377 ampulla of Vater cancers, 446 bile duct cancers, 90 duodenal cancers. Pancreatic cystic tumors and neuroendocrine tumors belonged to pancreas. RBD: Endoscopic retrograde biliary drainage; ENBD: Endoscopic nasogastric biliary drainage; PTBD: Percutaneous transhepatic biliary drainage; ERPD: Endoscopic retrograde pancreatic drainage; RT: Radiotherapy; CCRT: Concomitant chemo- and radiotherapy; PP- or PRPD: Pylorus-preserving or pylorus-resecting pancreaticoduodenectomy; CA: Carbohydrate antigen; P-J: Pancreaticojejunostomy; P-G: Pancreaticogastrostomy.

management, including conservative, interventional, or surgical treatment, depending on each patient's clinical condition.

Machine learning algorithms as artificial intelligence

Two ML algorithms, random forest (RF) and neural network (NN), were used to predict POPF. RF method is a kind of ensemble learning algorithm that builds multiple decision trees expecting better performance by taking mode or mean of individual trees^[24]. An NN is a ML algorithm that emulates the synaptic structure of the brain^[13]. It contains singular or multiple hidden layers, between the input and output layers^[13]. Recursive feature elimination (RFE), which is a feature selection method that removes the weakest features until the maximum area under the curve (AUC) is reached^[25,26], was used to identify the subset of features used in the final NN model. We tuned hyperparameters of NN (such as number of hidden layers, number of nodes, learning rates, batch size, dropout rate, and so on) to maximize the performance by grid search algorithm on each RFE step^[27].

These AI-driven POPF prediction algorithms were developed using MATLAB Release 2017 and Python software with Tensorflow library.

Data analysis and statistical methods

The characteristics of the study population were described for each dataset, including the mean and standard deviation for each variable. For the development of the ML algorithms, the total dataset was split into a training set and a test set. The training set was used to derive the POPF prediction algorithms, and the test set was used to evaluate the derived algorithms. In order to evaluate our ML approaches, we used a

stratified 5-fold cross-validation test. This randomly divides all the data into 5 partitions (folds) keeping each one with similar positive and negative data distribution. Then, we train a model with four of the partitions and test the model with the remaining fold. By changing the folds for training and testing, this process is performed 5 times. Also, the whole cross-validation was repeated 10 times by random split of the dataset, evaluating the performance of the models at the end. These processes ensure the generalized performance of a model by preventing overfitting to the samples. Because the outputs from the ML-driven POPF prediction algorithms are probabilistic estimates of risk, the performance for the test data was evaluated using AUC. The clinical meaning of the AI-driven risk factors for POPF was identified by sliding window approach^[28]. All statistical and mechanical analyses assessing algorithm performance were done by Cho K and Cho BH from Medical AI Research Center, Samsung Medical Center using GraphPad Prism version 5.00 for Windows (GraphPad Software, SD, CA) and MATLAB Release 2017b (MathWorks, Inc., Natick, MA) software.

RESULTS

Clinicopathological characteristics and outcomes

Table 1 provides the clinicopathologic details of the 1769 patients. Among them, grade B or C POPF occurred in 221 (12.5%) patients according to the ISGPF 2016 definition, and 130 (7.3%) patients had an American Society of Anesthesiologists' (ASA) score ≥ 3 . The mean value of body mass index (BMI) was 22.5 kg/m², and the mean albumin level was 4.0 g/dl. The mean operating time was 443.2 min. The mean total fluid input and estimated blood loss during the operation were 3129.5 and 962.4 mL, respectively. A soft pancreas was observed in 750 (42.4%) patients. The mean diameter of the pancreatic duct was 4.2 mm. The most common tumor location was the pancreas, which occurred in 568 (32.1%) patients. Presumed pancreatitis was observed in 370 (20.9%) patients (**Table 1**). 30-d postoperative mortality was observed in 23 (1.3%) patients.

Development of machine learning models using random forest and neural network

Table 2 summarizes the results from each algorithm using three different configurations of the dataset. Firstly, the data with complete values for the 38 original variables were input into the two ML algorithms. The average AUCs over the 5-fold cross validation with 10 repetition were 0.67 with the RF and 0.74 with the NN, respectively. When complete data for 34 original variables (without serum C-reactive protein, amylase, lipase, and CA 19-9 level) were input into the algorithms, the 5-fold average AUCs were 0.67 with the RF and 0.72 with the NN. For the configuration of missing values treatment, we input data from 1769 patients into the ML algorithms. Those 5-fold average AUCs increased to 0.68 with the RF and 0.71 with the NN. All those AUCs are summarized in **Table 2**.

Machine learning models using neural network with recursive feature elimination

Using all 1769 data samples after missing data treatment, we could further improve the AUC from 0.71 to 0.74 using NN with RFE method (**Table 2**). Sixteen risk factors for POPF were identified using NN with RFE method: Pancreatic duct diameter, BMI, preoperative serum albumin, lipase level, amount of intraoperative fluid infusion, age, platelet count, extrapancreatic location of tumor, combined venous resection, co-existing pancreatitis, neoadjuvant radiotherapy, ASA score, sex, soft texture of the pancreas, underlying heart disease, and preoperative endoscopic biliary decompression. (**Figure 1**). The post hoc analysis revealed a nonlinear relationship by showing the response of NN model to each input variable at every RFE step. Ten discrete points cover the observed range of variation for each corresponding variable. We found several patterns of NN output response, in which the predicted POPF risk seemed to have a positive, negative, or biphasic relationship with each variable. The contribution profiles of the top 16 variables are shown in **Supplement Figure 1**. Based on these multiple and complex relationships among the risk factors for POPF after PD, we made a network connections illustration to improve understanding (**Figure 2**).

Establishment of risk prediction platform for postoperative pancreatic fistula using artificial intelligence

NN algorithm using RFE that had the best performance across the metrics of

Table 2 Prediction performance of the various dataset for postoperative pancreatic fistula

Dataset and algorithm	Number of original variables	Number of encoded variables	Number of samples	Area under curve
Random forest with complete cases	38	44	889	0.670.02
Neural network with complete cases				0.740.02
Random forest with complete variables	34	40	1769	0.670.01
Neural network with complete variables				0.720.02
Random forest with missing data treatment	38	44	1769	0.680.02
Neural network with missing data treatment				0.710.02

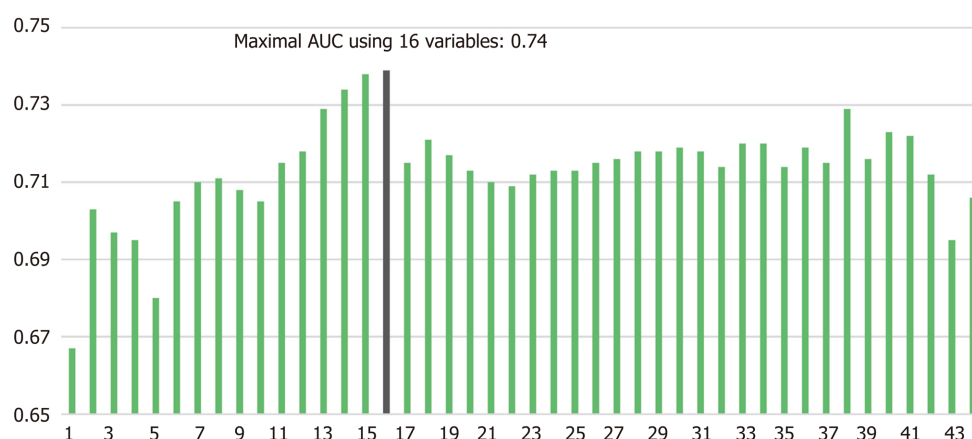


Figure 1 Performance of the neural network models optimized within each recursive feature elimination step. 1: Pancreatic duct diameter; 2: Body mass index; 3: Serum albumin; 4: Amount of intraoperative fluid infusion; 5: Age; 6: Platelet count; 7: Extrapaneatic location of tumor; 8: Combined venous resection; 9: Co-existing pancreatitis; 10: Serum lipase; 11: Neoadjuvant radiotherapy; 12: ASA score; 13: Sex; 14: Soft texture of pancreas; 15: Underlying heart disease; 16: Preoperative endoscopic biliary decompression; 17: Hemoglobin; 18: Serum total bilirubin; 19: Operative time; 20: Intraoperative transfusion; 21: Neoadjuvant chemotherapy; 22: Anastomotic methods (1); 23: Serum amylase; 24: Anastomotic methods (2-1); 25: Pancreatic duct stent (1); 26: White blood cell count; 27: Type of surgery (1); 28: Serum carbohydrate antigen 19-9; 29: Serum C- reactive protein; 30 Estimated blood loss; 31: Combined vascular resection; 32: Pancreatic duct stent (2); 33: Preoperative percutaneous biliary drainage; 34: Underlying cerebrovascular disease; 35: Combined organ resection; 36: Type of surgery (2); 37: Type of surgery (3); 38: Anastomotic methods (2-2); 39: Underlying liver disease; 40: Underlying chronic kidney disease; 41: Underlying pulmonary disease; 42: Underlying cerebrovascular disease; 43: Diabetes mellitus; 44: Preoperative endoscopic pancreatic drainage; ASA: American Society of Anesthesiologists; AUC: Area under the curve.

discrimination, calibration, and overall performance was integrated into an interactive interface. We designed our clinical decision tool to collect values entered by a clinician, feed those values into the pre-trained algorithm, retrieve the result, and output that result to the clinician in real time. This POPF prediction platform is available as an open-access, web-based application programmed to be accessible and adaptable for use on desktops, tablets, and smartphones. It is freely available at <https://popfrisk.smchbp.org/>.

DISCUSSION

POPF is a serious inherent risk of a pancreatic resection. The best option for managing POPF is undoubtedly prevention using a preoperative and intraoperative POPF risk assessment that guides response measures postoperatively^[6,9,10]. Theoretically, ML could offer an opportunity to improve the accuracy of risk assessment by exploiting the complex interactions among risk factors that affect POPF. To the best of our knowledge, this study presents the first ML algorithm for predicting POPF using multiple pre- and intraoperative variables derived from a large, single-institutional dataset. The maximum AUC of this model was considerable: 0.74 with NN with RFE method (Figure 1). Ultimately, a patient's predicted POPF risk could direct their

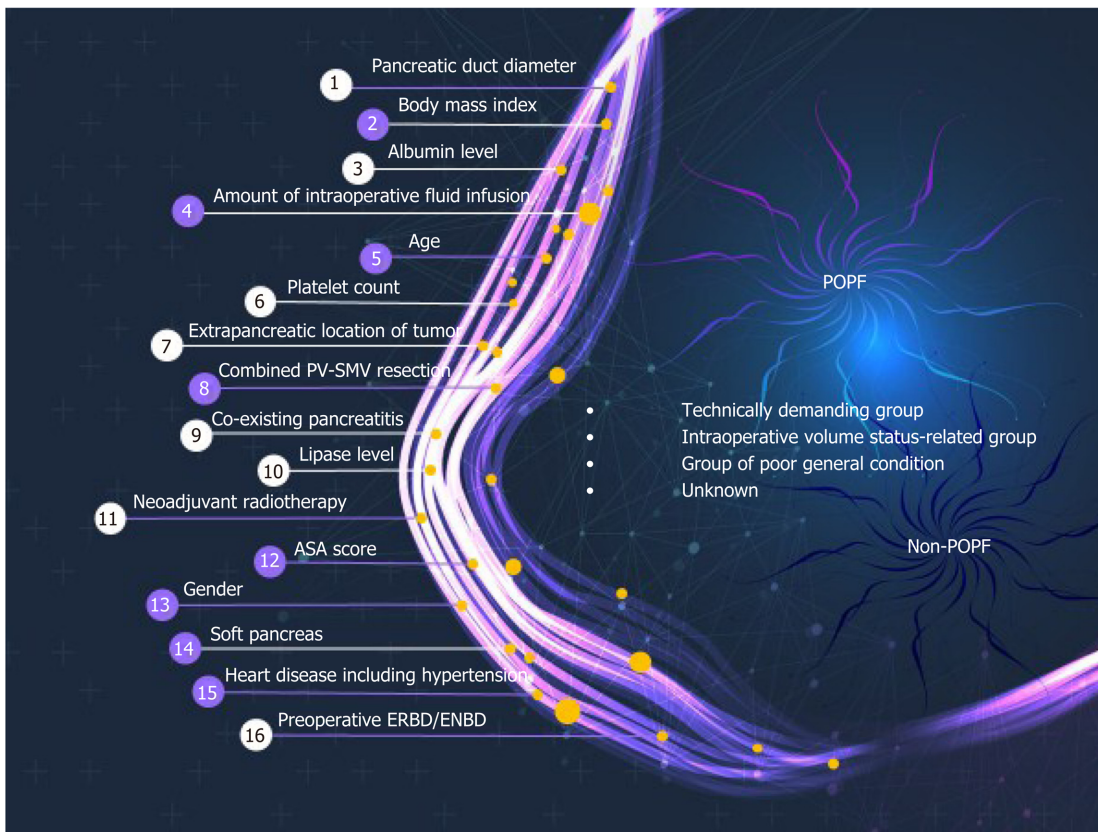


Figure 2 Illustration of artificial intelligence algorithm for 16 risk factors affecting postoperative pancreatic fistula. PV-SMV: Portal vein-superior mesenteric vein; ASA: American Society of Anesthesiologists; ERBD: Endoscopic retrograde biliary drainage; ENBD: Endoscopic nasobiliary drainage; POPF: Postoperative pancreatic fistula.

clinical management and prevent or mitigate untoward outcomes.

AI in the form of ML discovers intricate structures in large datasets by using a backpropagation algorithm to indicate how a machine should change the internal parameters it uses to compute the representation in each layer based on the representation in the previous layer^[13]. ML can identify latent variables that are unlikely to be observed but might be inferred from other variables^[18,19,24]. For example, NN with RFE algorithm found many pre- and perioperative predictors for POPF that we used in our final modeling (Figure 1). Previously developed risk assessment models^[6,9,10] implicitly assume that the risk factors are related to POPF in a linear fashion. Those models could thus oversimplify complex, nonlinear relationships among many risk factors. Even if it might be cumbersome to calculate the risk of POPF using 16 variables in actual clinical care, our AI-driven risk platform better incorporates multiple risk factors and can account for more nuanced relationships between the risk factors and POPF (Figure 2). An example of nonlinearity is shown in Supplement Figure 1, and note that each variable has a variable effect.

This ML algorithms found 16 risk factors for POPF (Figure 1). These risk factors can be categorized into 3 groups: The technically demanding group, intraoperative volume status-related group, and poor general condition group (Figure 2). The risk factors in the technically demanding group (soft pancreas^[2,5,6,9], small pancreatic duct^[6,9,29], extrapancreatic lesion^[6], absence of preoperative pancreatitis or low lipase level^[30], absence of preoperative endoscopic biliary decompression, absence of neoadjuvant radiotherapy, and high BMI^[7]) indicate potential difficulty in reconstructing the pancreatic-enteric anastomosis, which could cause POPF. Patients with pancreatic cancer, chronic pancreatitis, or neoadjuvant treatment have increased pancreatic fibrosis and a lower incidence of POPF than other PD patients^[30,31]. Also, it is well-known that preoperative endoscopic biliary drainage is frequently associated with procedure-related pancreatitis^[32]. As a result, those procedures might reduce the risk of POPF. The risk factors in the intraoperative volume status-related group (large intraoperative fluid administration, concomitant portal vein-superior mesenteric vein resection, and low platelet count) could cause ischemia and poor healing of the pancreatic-enteric anastomosis, which is compounded by tissue edema from aggressive volume replacement in a rebound fashion^[6,33]. The resultant swelling of the

anastomosis can cause duct occlusion or suture disruption. The risk factors in the poor general condition group (old age, underlying heart disease, low preoperative serum albumin level, and low ASA score) could be related to poor nutritional status, which is considered to correlate with a high risk of POPF^[34,35].

Notably, a high probability of POPF in patients characterized by only 1 or 2 classical fistula risk factors could not be determined. In this study, we found 16 risk factors by using AI algorithms (Figure 1), but controversy remains about the true risk factors for POPF. The varying results from different studies could be influenced by study design, the composition of the patient populations, or statistical methods. For example, there is still debate about whether intraoperative volume status, such as intraoperative blood loss, transfusion, or the amount of fluid administration, are risk factors for POPF^[6,9,33,36]. Some reports suggest intraoperative volume status as an independent risk factor for POPF^[6,33] because of pancreatic parenchymal and intestinal edema from aggressive volume replacement, but other studies have denied its adverse effect because estimation of blood loss during surgery is unreliable and inaccurate^[3,9]. We think this discrepancy about the prognostic value of different risk factors for POPF could be a fundamental interpretation error caused by the assumption of linearity and an attempt to simplify what isn't actually simple. Therefore, ML algorithms such as those used in the study will be an important tool for POPF risk assessments.

Other recently proposed risk prediction models^[6,9,10] have the advantage of being easily performed because they use only 3–6 variables. However, previously unknown risk factors for POPF are still being newly identified. For example, preoperative sarcopenia, an age-related decrease in muscle mass, has been identified as a risk factor for POPF^[37–39]. Existing models cannot reflect new factors for POPF as they emerge but must be re-analyzed and developed from scratch. To make matters worse, as the number of potential risk factors increases, the complexity of the conventional models can cause over-fitting, yielding implausible results. However, we addressed that possibility by using active and appropriate choices in pre-training, hyper-parameter selection, and regularization in our AI-driven algorithms^[19]. Because AI is scalable, there is no need to develop a new model; even if many new variables affecting POPF are introduced, it is possible to just continue adding them to the original model. As the amount of pancreatectomy data continues to grow, the creation and deployment of learning systems as accessible tools could significantly enhance the prognosis and management of POPF. New learning algorithms and architectures that are currently being developed, such as convolution^[40] or recurrent^[41] NNs, will accelerate this progress.

This AI-driven risk prediction platform for POPF could assist the drive toward personalized medicine by better tailoring risk management to individual patients. For example, after a risk evaluation, high-risk patients could be selected for a multiple-drain strategy and postoperative prophylactic octreotide use. In this way, we expect our platform to help select patients who need more intense therapy and establish effective (and cost-effective) treatment strategies for POPF. Various mitigation strategies have been proposed to reduce the occurrence and morbidity of POPF, including technical variations, such as, pancreaticogastrostomy reconstruction^[2,42], dunking/invaginating anastomosis^[1,43,44], absorbable mesh patches^[45,46], and the use of intraperitoneal drains^[29], anastomotic stents^[47], and prophylactic somatostatin analogues^[4,48,49]. As a part of those efforts, we have an ongoing trial of this risk score wherein we are applying a somatostatin analogue during postoperative days 0–3 in high-risk patients. Future prospective studies could stratify treatments based on the outcome of this platform and provide comprehensive treatment algorithms.

This study has several limitations. First, Co-existing pancreatitis, which is bound to be subjective, was defined as classic feature of pancreatitis on preoperative CT scan or intraoperative findings. Also, the input data used to develop risk prediction platform was pre- and intraoperative variables. In practice, it is important for both pre- and intraoperative variables to enter the algorithms in order to improve the predictability of POPF, but clinically, it may be helpful for only preoperative variables to enter the algorithms. Therefore, we will sooner or later conduct modeling for only preoperative variables using multicenter data. Second, the NN's output response to changing each input variable partially revealed the variables' nonlinear relationships to POPF risk. Therefore, the pattern of the output response should not be understood as a direct relationship between an input variable and POPF risk. Nevertheless, this process could help inform further explorations of diverse predictive risk factors and the future development of new risk prediction approaches and algorithms. Finally, the study, though a large institution, was not only conducted on patients in single center but also had the disadvantage of not performing external validation. As a result, the follow-up study will be conducted by performing an external validation on patients in multiple

institutions.

In conclusion, ML algorithms are promising tools for the prediction of POPF that can be integrated into clinically useful decision tools. Compared with established POPF risk prediction methods, our ML algorithms better predict the POPF risk correctly. After external validation, this new platform could be used to select patients who need more intense therapy and to preoperatively establish an effective treatment strategy.

ARTICLE HIGHLIGHTS

Research background

Despite advancements in operative technique and improvements in postoperative managements, postoperative pancreatic fistula (POPF) is a life-threatening complication following pancreatoduodenectomy (PD). Artificial intelligence (AI) technology is being actively used in the medical field, but few studies have reported applying it to outcomes after PD.

Research motivation

There are some reports to predict POPF preoperatively or intraoperatively, but the accuracy of those is questionable. Compared with established POPF risk prediction methods, we expect that our ML algorithms can better predict the POPF risk correctly.

Research objectives

This study aimed to develop a risk prediction platform for POPF with single center dataset using an AI model.

Research methods

A total of 38 variables from 1769 patients who underwent PD from 2007 to 2016 at Samsung Medical Center were inserted into AI-driven algorithms. The algorithms tested to make the risk prediction platform were random forest (RF) and a neural network (NN) with or without recursive feature elimination (RFE). These algorithms can better incorporate multiple risk factors and account for more nuanced relationships between the risk factors and POPF. The median imputation method was used for missing values.

Research results

The number of POPFs was 221 (12.5%) according to the International Study Group of Pancreatic Fistula definition 2016. The maximal AUC using NN with RFE was 0.74. Sixteen risk factors for POPF were identified by AI algorithm: Pancreatic duct diameter, body mass index, preoperative serum albumin, lipase level, amount of intraoperative fluid infusion, age, platelet count, extrapancreatic location of tumor, combined venous resection, co-existing pancreatitis, neoadjuvant radiotherapy, American Society of Anesthesiologists' score, sex, soft texture of the pancreas, underlying heart disease, and preoperative endoscopic biliary decompression. We developed a web-based POPF prediction platform available at <https://popfrisk.smchbp.org>.

Research conclusions

This study is the first to predict POPF with multiple risk factors using AI. This platform is reliable (AUC 0.74), so it could be used to select patients who need especially intense therapy and to preoperatively establish an effective treatment strategy.

Research perspectives

This study developed a risk prediction platform for POPF with single center dataset using an AI model. The follow-up study will be conducted by performing an external validation on patients in multiple institutions. Also, future prospective studies could stratify treatments based on the outcome of this platform and provide comprehensive treatment algorithms.

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Retrospective Study

Efficacy and safety of lenvatinib for patients with advanced hepatocellular carcinoma: A retrospective, real-world study conducted in China

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Abstract

BACKGROUND

Lenvatinib has become an indispensable part of treatment regimens for patients with advanced hepatocellular carcinoma (aHCC). Several recent real-world studies appear to have confirmed this; however, there are etiological differences. This necessitates further real-world studies of lenvatinib across diverse populations, such as in China.

AIM

To investigate the efficacy and safety of lenvatinib in a Chinese HCC patient population under real-world conditions.

METHODS

This is a retrospective and multiregional study involving patients with aHCC receiving lenvatinib monotherapy. Efficacy was assessed using the Response Evaluation Criteria in Solid Tumors version 1.1. Baseline characteristics and adverse events (AEs) were recorded throughout the entire study.

RESULTS

In total, 54 HCC patients treated with lenvatinib monotherapy were included for final analysis. The objective response rate was 22% ($n = 12$) with a progression-free survival (PFS) of 168 d; however, AEs occurred in 92.8% of patients. Multivariate analysis showed that the Barcelona Clinic Liver Cancer stage [hazard ratio (HR) 0.465; 95%CI: 0.23-0.93; $P = 0.031$], portal vein tumor thrombus (HR 0.38; 95%CI: 0.15-0.94; $P = 0.037$) and Child-Pugh classifications (HR 0.468; 95%CI:

Institutional review board

statement: The protocol of this study was compliant with the principles of the Declaration of Helsinki and was also approved by the Institutional Review Board and Ethics Committee at Peking Union Medical College Hospital.

Informed consent statement: All patients were fully informed of the objectives of this study and provided formal consent before being fully considered.

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

Data sharing statement: No additional data are available.

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0.22-0.97; $P = 0.042$) were significant factors affecting PFS. The sensitivity (56.7%) and specificity (83.3%) of decreasing serum biomarkers including alpha-fetoprotein were calculated in order to predict tumor size reduction. Gene sequencing also provided insights into potential gene mutation signatures related to the effect of lenvatinib.

CONCLUSION

Our findings confirm previous evidence from the phase III REFLECT study. The majority of patients in this Chinese sample were suffering from concomitant hepatitis B virus-related HCC. However, further analysis suggested that baseline characteristics, changes in serum biomarkers and gene sequencing may hold the key for predicting lenvatinib responses. Further large-scale prospective studies that incorporate more basic medical science measures should be conducted.

Key words: Lenvatinib; Real-world study; Hepatocellular carcinoma; Efficacy; Safety; Treatment

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Core tip: This is a real-world study of advanced hepatocellular carcinoma patients treated with lenvatinib monotherapy in China. The majority of patients in this study presented with hepatitis B virus infection. Our analysis of the safety and efficacy of this intervention confirms previous evidence from the phase III REFLECT study. A multivariate analysis of participant characteristics with changes in serum biomarkers and gene sequencing provides a more comprehensive understanding of lenvatinib responses. Although based on a small sample, this new knowledge has clinical implications and necessitates further research.

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INTRODUCTION

Primary liver cancer, which is predominantly hepatocellular carcinoma (HCC), remains one of the most common malignant tumors with approximately 841000 new cases and 782000 deaths annually^[1]. Over the past decade, sorafenib, a multikinase inhibitor, has been considered the only first-line treatment for patients with advanced HCC (aHCC). Systemic therapies for patients with aHCC are rapidly changing, with some new agents showing clinical efficacy in phase III trials^[2]. The REFLECT trial compared sorafenib to lenvatinib and, having setting noninferiority criteria as analytical endpoints, found that the overall survival (OS) for those administered lenvatinib was similar to that for those administered sorafenib^[3]. Further subgroup analysis found that lenvatinib significantly improved all secondary endpoints including the objective response rate (ORR), progression-free survival (PFS) and time-to-progression (TTP), especially in the Asian-Pacific subgroup. Based on these findings, lenvatinib has been approved worldwide and has become an alternative first-line treatment for patients with aHCC^[4].

Results from randomized controlled trials (RCTs) have tended to conflict with real-world studies, perhaps because of the nature of experimental controls and constraints. Therefore, lenvatinib monotherapy should be confirmed as efficacious in clinical practice. To date, Obi *et al*^[5] found that the early therapeutic response rate to lenvatinib reached 40% across a small sample of 16 patients. A further multicenter study conducted in Japan involving 37 participants appears to have confirmed these findings with an ORR of 32.4% and a disease control rate (DCR) of 70.3% at 12 wk^[6,7].

However, striving to maximize efficiency while avoiding side effects is proving difficult. More recently, in 2019, Sasaki *et al*^[8] suggested that lenvatinib should be administered to patients with relatively good hepatic functions because these patients

are more capable of receiving a sufficient relative dose intensity, which then significantly influences objective responses. Lenvatinib doses are generally determined by a patient's weight, and in a further related study, *Eso et al*^[9] found that the delivered dose: Intensity/body surface area ratio at 60 d can be an important factor for treatment intensity. In addition, the response to lenvatinib monotherapy has been found to be similar to that of transarterial chemoembolization (TACE), and the therapeutic action of lenvatinib in normalizing blood vessels may be more conducive to the treatment of TACE^[10,11]. Therefore, TACE and lenvatinib combined may yield more favorable results for patients with aHCC.

The aforementioned studies focused predominantly on Japanese populations; however, there are a number of not so subtle differences between populations. For example, more than 50% of the global burden of HCC occurs in China, with 76% of these patients having been infected with hepatitis B virus (HBV)^[12,13]. In the REFLECT study, researchers have also found that lenvatinib efficacy is not identical between etiological subgroups. Therefore, the massive HCC patient population with concomitant conditions in China must be examined to compare differences before developing guidelines. Lenvatinib was formally approved in China in September 2018; however, research focusing specifically on this population under real-world conditions is not readily available. It is well known that HCC patients in Japan generally also suffer concomitant HCV infection, although this is clearly not the case in the Chinese population^[13].

In this study, we investigate the efficacy and safety of lenvatinib across a Chinese HCC patient population under real-world conditions. We also attempt to develop predictions using baseline characteristics, tumor biomarkers and gene mutations, thereby incorporating basic medical research with higher levels of evidence. This novel approach was designed to develop an evidence base to guide clinicians and to gain insight into lenvatinib responses.

MATERIALS AND METHODS

Study design and participants

This is a retrospective and multiregional study involving Chinese patients diagnosed with aHCC. Participants were routinely attending multidisciplinary team consultations. All patients were fully informed about the objectives of this study and provided formal consent. Data were collected from patients during lenvatinib interventions for a period of one year from December 2018 to December 2019. The study protocol was compliant with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board and Ethics Committee at Peking Union Medical College Hospital.

A total of 113 patients were initially deemed eligible. Each of these participants had received a confirmed HCC diagnosis using pathological assessment methods or through specific HCC imaging. The initial sample included participants who had not been recommended for hepatic resection, liver transplantation or any other radical ablation. Patients with Barcelona Clinic Liver Cancer (BCLC) stage B (not applicable for TACE or progressed on locoregional therapy) or BCLC stage C, a Child-Pugh score of A-B, and an Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0-2 were included (please see [Supplementary material](#) for details). Following these criteria, we excluded 31 patients who had been treated with lenvatinib combination therapies at the beginning of treatment. Twenty-six patients were also excluded because they had received an additional antitumor therapy including systematic or locoregional therapy while receiving lenvatinib during this study.

Adverse events (AEs) were analyzed across the 56 remaining patients, of whom 54 patients provided complete information for further analysis. All 54 patients included were administered lenvatinib monotherapy until disease progression or until encountering an intolerant adverse event. The study design flow diagram is shown in [Figure 1](#).

Assessment of efficacy and adverse events

Initial lenvatinib doses were consistent with guidelines, and were administered orally at 8 mg/d when an individual patient weighed < 60 kg and 12 mg/d for those weighing ≥ 60 kg. Regimens may have been interrupted and even discontinued with the occurrence of unacceptable or serious AEs or when tumor progression was not inhibited.

Imaging examinations were conducted using enhanced computed tomography,

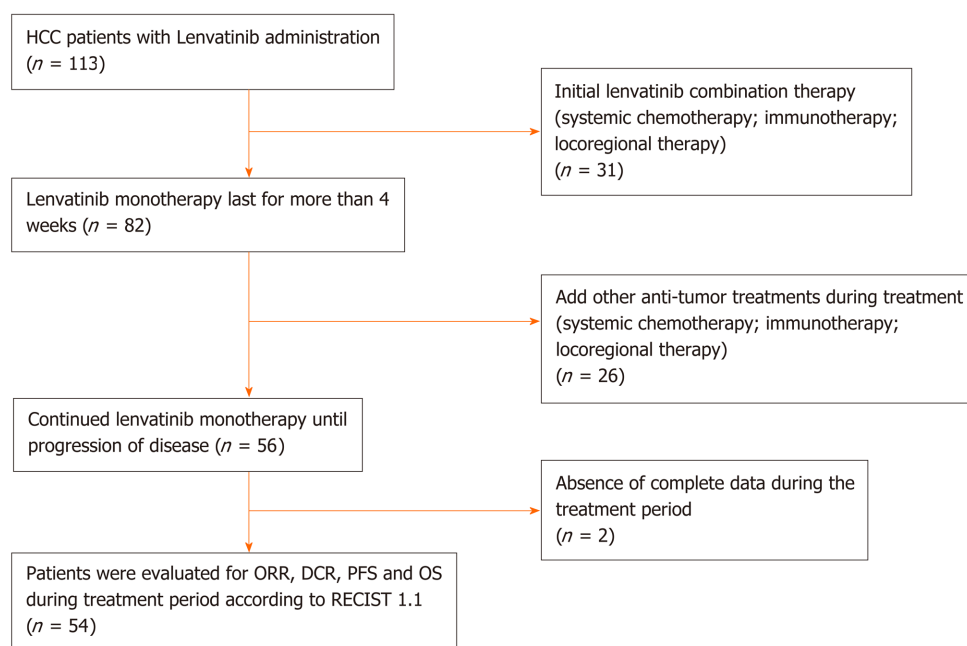


Figure 1 Flow diagram of study population. HCC: Hepatocellular carcinoma; ORR: Objective response rate; DCR: Disease control rate; PFS: Progression-free survival; RECIST 1.1: Response Evaluation Criteria in Solid Tumors version 1.1.

magnetic resonance imaging or other available imaging technologies every 4-8 wk after initiation of lenvatinib treatment. Changes in tumor size were assessed by two independent specialists using RECIST 1.1 and were categorized as a complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD).

During the observation period, AEs were collected in detail and assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.0). According to the instructions, when grade 3 or more severe AEs occurred, dose reduction took place, or a temporary interruption was commenced until symptoms subsided to pharmaceutically manageable grades 1 or 2.

Further analysis of baseline characteristics

Baseline characteristics were systematically collected and included age, gender, serum biochemistry, extrahepatic spread (EHS), tumor occupation, portal vein thrombus (PVT), history of treatment and size of the target lesion. We also recorded combined characteristics including the ECOG-PS, albumin-bilirubin stage (ALBI), Child-Pugh class and BCLC stage by reviewing histories or through calculations using the available evidence. Utilizing these enabled us to analyze potential factors affecting ORR and PFS.

The patients were divided into different subgroups and stratified according to previous treatments, liver occupation, portal vein invasion, HBV and ALBI grades. Concomitant HBV was confirmed by HBV surface antigen testing. ALBI scores were calculated using the following formula: $[\log_{10} \text{bilirubin } (\mu\text{mol/L}) \times 0.66] + [\text{albumin } (\text{g/L}) \times -0.085]$, and ALBI grade was determined as Grade I = ≤ -2.60 , Grade II = > -2.60 to -1.39 , and Grade III > -1.39 .

Generating effect predictions using tumor serum biomarkers and gene mutations

Patients with stable disease were categorized into three subgroups, which included the following: Diminished tumor size that did not reach the partial response standard (SS), stable disease without any significant tumor size change (ST) and stable disease with a tumor size increase that did not reach the progression standard (SP). SS and PR statuses were clustered into a “shrinking” group in which tumors were contracting in response to treatment. ST, SP and PD statuses were clustered into an “unshrinking” group in which participants were evidently not responding to treatment.

Recording of alpha-fetoprotein (AFP) values before and after administration of lenvatinib within 4 wk was conducted to develop response predictions in both the shrinking and unshrinking groups. Gene mutation information was collected from those who had provided samples for next generation sequencing. Genes needed to appear at least twice to be considered for further analysis. Differences in information

reflecting gene mutations were calculated for both groups and compared.

Statistical analysis

Baseline data included continuous and categorical variables, which were calculated and presented as the means with corresponding standard deviations or as simple numbers and percentages. Statistical analyses of the differences between variables were conducted using the χ^2 or Fisher's exact tests. Two tailed *P* values of less than 0.05 were considered indicative of statistical significance. Five patient characteristics that may have affected ORRs were analyzed using a multivariate logistic regression model. The Kaplan-Meier method was applied to generate PFS curves, and a log-rank test was used to compare PFS curves for different subgroups.

The variables associated with PFS were analyzed using multivariate Cox proportional hazard analysis. The results of the multivariate analysis are presented as odds ratios (ORs) or hazard ratios (HRs) with corresponding 95%CI and *P* values. The sensitivity and specificity of diagnostics were calculated to assess their predictive capabilities for tumor changes using AFP values. Mutated gene frequencies were used to construct a gene mutation map of patients with different responses to lenvatinib. All statistical analyses were performed using SPSS 22 and R software (version 3.6.1).

RESULTS

Patient characteristics

A total of 56 patients were treated with lenvatinib monotherapy until progression of disease. A further two participants were excluded due to a lack of baseline data. Complete analysis was performed using data from 54 patients. Twenty-five patients were diagnosed by the method of specific imaging. The average age was 59 (± 12) years, and 85% ($n = 46$) were male. Of this total number, 40 patients were HBV positive. The proportion of patients with cirrhosis or portal hypertension was 72% ($n = 39$) and 54% ($n = 29$), respectively. Combining serum biochemistry and baseline characteristics resulted in proportions of Child-Pugh class A and B of 81% ($n = 44$) and 19% ($n = 10$), respectively (Table 1).

In 28% ($n = 15$), liver occupation was greater than 50%. Approximately 39% ($n = 21$) had a PVT, and 33% ($n = 18$) showed EHS. In terms of treatment history, 11 patients had previously received radiotherapy, 69% ($n = 37$) received TACE, 39% ($n = 21$) received radiofrequency ablation (RFA), and 31% ($n = 17$) received another targeted therapy. The tumor size across all patients was 6.93 cm (± 4.75), and the number of patients receiving doses of 8 mg and 12 mg was 26 and 28, respectively. Approximately 33% ($n = 18$) were considered to be in stage B, and 67% ($n = 36$) in stage C, according to the BCLC criteria. In addition, 27 patients were ALBI grade I, 25 were grade II, and two patients were grade III (Table 2).

Assessment of efficacy and AEs during entire treatment period

In accordance with the RECIST 1.1 criteria, no patients achieved a CR, a PR was observed in only 12 patients, SD was observed in 36 patients, and PD was observed in six patients. The ORR was 22% ($n = 12$), and the DCR was 88% ($n = 48$). The median PFS was estimated to be 5.6 mo (95%CI: 4.3-6.8), and the TTP was 5.1 mo (95%CI: 3.8-6.3) (Figure 2).

Overall survival could not be calculated due to the death rate. Of the patients with concomitant HBV, the number with PR and SD was 10 and 26, respectively, giving an ORR of 25% and a DCR of 90%. The median PFS was 5.8 mo (95%CI: 4.1-7.5), and the TTP was 5.2 mo (95%CI: 4.2-6.2) (Table 3).

Of the 56 patients who continued to be treated with lenvatinib monotherapy, 92.86% ($n = 52$) developed AEs, and the incidence of grade 3-4 AEs was 21.15% ($n = 11$). There were no grade 5 AEs. The most common AEs encountered were hypertension in 44.64% ($n = 25$), decreased appetite in 23.21% ($n = 13$) and diarrhea in 23.21% ($n = 13$). Proteinuria was encountered by 21.43% ($n = 12$) and fatigue by 17.86% ($n = 10$), followed by hand-foot skin reaction ($n = 6$), nausea ($n = 5$), abdominal pain ($n = 4$), rash ($n = 4$), decreased weight ($n = 3$), decreased platelet count ($n = 3$), hypothyroidism ($n = 2$), dysphonia ($n = 1$) and vomiting ($n = 1$). Complete AE data with percentages are shown in Figure 3.

Among the grade 3-4 AEs, the incidence of proteinuria was the highest, reaching 9.6%, followed by diarrhea ($n = 2$), hypertension ($n = 2$), decreased appetite ($n = 1$) and rash ($n = 1$) (Table 4).

Table 1 Characteristics of patients with advanced hepatocellular carcinoma treated with lenvatinib

	All (n = 54)	HBV-related HCC (n = 40)
Age, yr	58.94 ± 12.10	57.49 ± 12.03
Gender (male:female)	46:8	35:5
Height, cm	172.04 ± 7.65	171.72 ± 7.24
Weight, kg	70.47 ± 13.72	69.21 ± 12.35
Etiology (HBV:HCV:Others)	40:5:9	40
Total bilirubin, mg/dL	29.81 ± 31.35	32.40 ± 33.17
Albumin, g/dL	38.53 ± 5.64	38.73 ± 5.01
Prothrombin time, positive, %	14 (26%)	10 (25%)
Extrahepatic spread	18 (33.3%)	12 (30%)
Lymphatic metastasis	33 (61%)	28 (70%)
Liver occupation (< 50%: > 50%)	38:15	27:13
Portal vein thrombus	21 (39%)	16 (40%)
Baseline AFP (ng/mL)(≥ 200: < 200)	32:22	22:18
Native: Recurrence	28:26	19:21
History of treatment (TACE: RFA: Targeted therapy)	37:21:17	29:14:14
History of Radiotherapy	11 (20%)	7 (18%)
Initial dose of LEN (8 mg: 12 mg)	26:28	21:19
Diagnostic method (Image: Pathology)	25:29	21:19
Size of target lesion, cm	6.93 ± 4.75	7.25 ± 4.36

AFP: α-fetoprotein; HBV: Hepatitis B virus; EHS: Extrahepatic spread; HCV: Hepatitis C virus; LEN: Lenvatinib; TACE: Transcatheter arterial chemoembolization; RFA: Radiofrequency ablation.

Table 2 Combination characteristics of patients with advanced hepatocellular carcinoma treated with lenvatinib

	All (n = 54)	HBV-related HCC (n = 40)
ECOG PS (0: 1: 2)	11:38:5	9:28:3
Child-Pugh score (5: 6: 7: 8)	29:15:7:3	21:13:4:2
ALBI grade (1: 2: 3)	27:25:2	20:19:1
BCLC stage (B: C)	18:36	12:28
TNM stage (IIIA: IIIB: IVA: IVB)	8:7:18:21	3:6:16:15

ECOG: Eastern Cooperative Oncology Group; PS: Performance Status; ALBI grade: Albumin-bilirubin grade; BCLC: Barcelona Clinic Liver Cancer; HBV: Hepatitis B virus; TNM: Tumor node metastasis; aHCC: Advanced hepatocellular carcinoma.

Multivariate and stratified analysis of ORR and PFS

There did not appear to be a significant relationship between the ORR and the factors analyzed, which were age, gender, HBV infection, first-line therapy, EHS, tumor occupation, PVT, and history of TACE. However, Cox regression analysis suggested that age (HR: 0.95, CI: 0.92-0.99, $P < 0.01$) and PVT (HR: 0.38, CI: 0.15-0.94, $P < 0.037$) were significant factors affecting PFS. The median PFS was estimated to be 6.4 mo (95%CI: 4.9-7.8) in 33 patients without PVT and 4.4 mo (95%CI: 3.5-5.3) in 21 patients with PVT (Table 5).

According to our analysis of combined factors, the ORR did not appear to have a significant relationship with ECOG-PS scores, ALBI stages, Child-Pugh classes or BCLC stages. However, changes in PFS were significantly related to patients with Child-Pugh class A or B disease (HR: 0.468; 95%CI: 0.22-0.97; $P = 0.042$) and BCLC

Table 3 Efficacy of lenvatinib in patients with advanced hepatocellular carcinoma

Investigator review according to RECIST 1.1	ALL (n = 54)	HBV-related HCC (n = 40)	P value
Progression-free survival (d, 95%CI)	168 (130-205)	175 (124-226)	0.250
Time to progression (d, 95%CI)	153 (116-189)	156 (126-186)	0.520
Objective response	22%	25%	0.753
Complete response	0	0	-
Partial response	12	10	-
Stable disease	36	26	0.866
Progressive disease	6	4	-
Disease control rate	88%	90%	0.863
Decreased AFP predicts tumor reduction			
Se	56.7%	53.8%	-
Sp	83.3%	85.7%	-

HBV: Hepatitis B virus; RECIST 1.1: Response Evaluation Criteria in Solid Tumors version 1.1; AFP: Alpha-fetoprotein; Se: Sensitivity; Sp: Specificity; CI: Confidence interval.

Table 4 Lenvatinib-related adverse events in patients with hepatocellular carcinoma, n (%)

Event	Hepatocellular carcinoma (n = 56)	
	Any grade	Grade 3-4
Any adverse event	52 (92.9)	11 (21.2)
Hypertension	25 (44.6)	2 (3.8)
Fatigue	10 (17.9)	0
Decreased appetite	13 (23.2)	1 (1.9)
Diarrhea	13 (23.2)	2 (3.8)
Proteinuria	12 (21.4)	5 (9.6)
Decreased weight	3 (5.4)	0
Hand-foot skin reaction	6 (10.7)	0
Nausea	5 (8.9)	0
Abdominal pain	4 (7.1)	0
Rash	4 (7.1)	1 (1.9)
Decreased platelet count	3 (5.4)	0
Vomiting	1 (1.8)	0
Hypothyroidism	2 (3.6)	0
Dysphonia	1 (1.8)	0

stage B or C disease (HR: 0.465; 95%CI: 0.23-0.93; $P = 0.031$). The median PFS was 7.0 mo (95%CI: 6.0-8.0 mo) in 18 patients with BCLC stage B disease, 4.4 mo (95%CI: 3.6-5.2) in 36 patients with BCLC stage C disease, 5.8 mo (95%CI: 4.3-7.3) in 44 patients with Child-Pugh class A, and 4.1 mo (95%CI: 0.8-7.4) in 10 patients with Child-Pugh class B (Figure 4 and Figure 5).

Therapeutic response predictions based on AFP and gene mutation

As previously described, the “shrinking” group consisted of 21 patients, and the “unshrinking” group consisted of 33 patients. AFP serum concentrations in 56% of patients ($n = 30$) decreased after treatment. Using this decrease in AFP concentration to predict a reduction in tumor volume, the sensitivity and specificity were calculated to

Table 5 Multivariate analysis of the objective response rate and progression-free survival in patients with advanced hepatocellular carcinoma treated with lenvatinib

Clinical factors	Category	Analysis of ORR		Analysis of PFS	
		P value	OR, 95%CI	P value	HR, 95%CI
Age (yr)	< 58.8	0.489	0.978 (0.919-1.041)	0.010	0.959 (0.929-0.990)
Gender	Male	0.571	1.828 (0.227-14.724)	0.606	1.137 (0.698-1.850)
HBV infection	HBV	0.371	2.300 (0.370-14.290)	0.151	1.844 (0.799-4.257)
First-line therapy	Sorafenib	0.212	0.324 (0.055-1.901)	0.167	1.724 (0.796-3.734)
Extrahepatic spread	Without	0.604	0.600 (0.088-4.118)	0.443	0.675 (0.247-1.844)
Tumor occupation	< 50%	0.937	1.080 (0.162-7.178)	0.169	2.043 (0.738-5.654)
Portal vein thrombus	Without	0.987	0.985 (0.167-5.817)	0.037	0.381 (0.154-0.944)
History of TACE	With	0.396	2.229 (0.350-14.186)	0.776	0.875 (0.348-2.197)
Combination factors					
ECOG-PS score	0	0.066	3.571 (0.876-14.564)	0.05	4.9 (0.998-24.193)
ALBI stage	1	0.651	-	0.462	0.574 (0.130-2.524)
Child-Pugh class	A	0.061	-	0.042	0.468 (0.225-0.973)
BCLC stage	B	0.487	1.593 (0.425-5.971)	0.031	0.465 (0.232-0.931)

ECOG: Eastern Cooperative Oncology Group; PS: Performance Status; ALBI grade: Albumin-bilirubin grade; BCLC: Barcelona Clinic Liver Cancer; TACE: Transarterial chemoembolization; HBV: Hepatitis B virus; HCV: Hepatitis C virus; EHS: Extrahepatic spread; ORR: Objective response rate; PFS: Progression-free survival; OR: Odds ratio; HR: Hazard ratio.

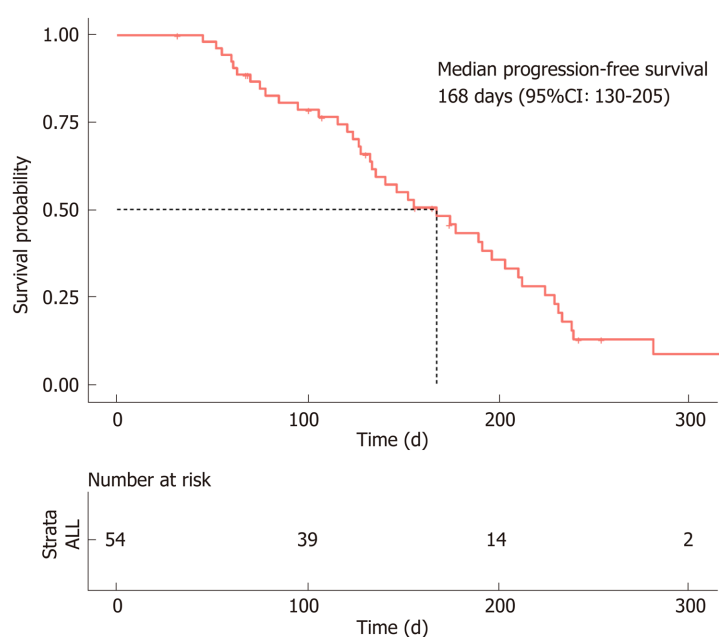


Figure 2 Progression-free survival of hepatocellular carcinoma patients treated with lenvatinib. The median progression-free survival was estimated to be 168 d (95%CI: 130–205 d).

be 56.7% and 83.3%, respectively (Table 3).

Gene sequence data were only collected from 23 patients, including 13 patients with a reduced tumor size and 11 patients without notable reduction. The high frequency mutations detected were *KMT2C*, *TP53*, and *IRS2*. Subgroup analysis demonstrated that variations in the *CHEK2*, *KRAS*, *BRCA1*, *DNMT3A*, and *JAK1* genes were relatively concentrated in patients *without* tumor reduction, while *SKHA*, *RUNX1*, *MAP3K1*, *KMT2D* and *ARAF* gene variations appeared relatively concentrated in

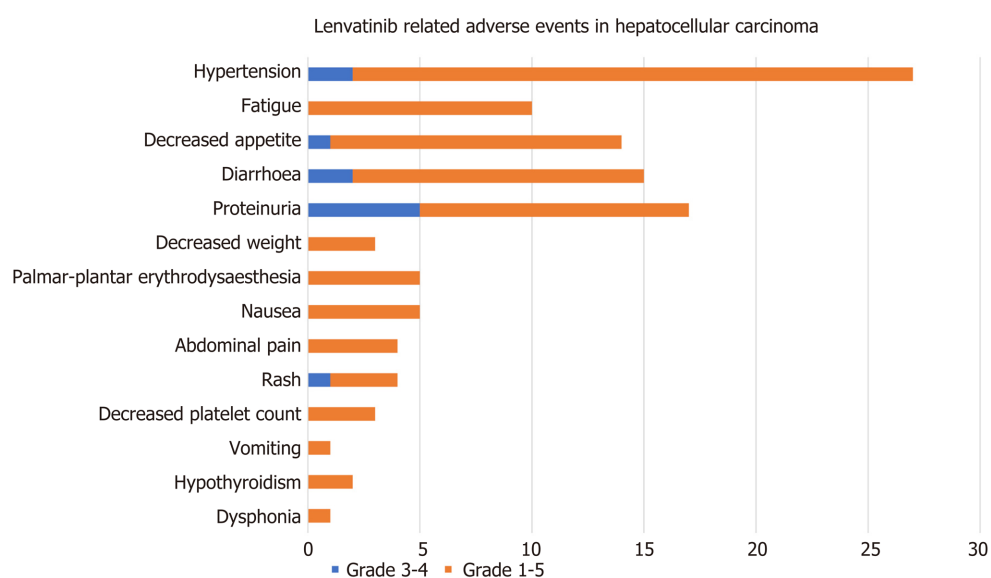


Figure 3 Lenvatinib-related adverse events in patients with hepatocellular carcinoma. The brown bar represents grade 3-4 adverse events; the blue bar represents all-grade adverse events.

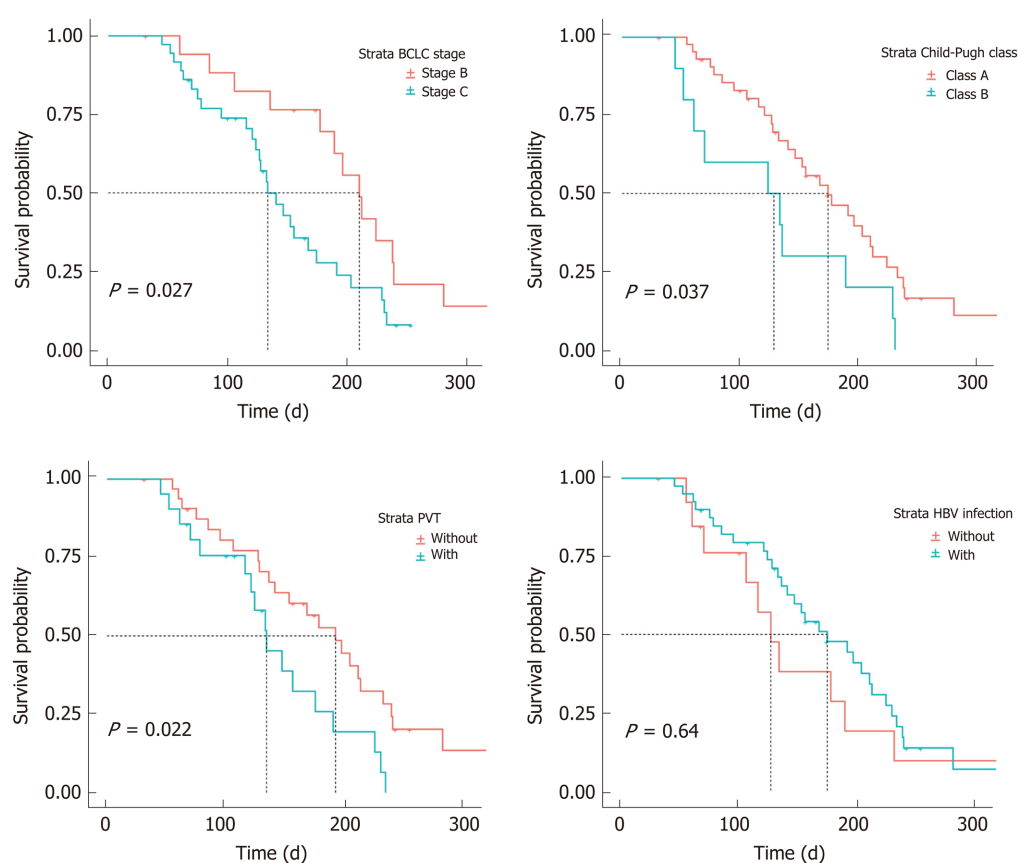


Figure 4 Progression-free survival of patients in different subgroups. BCLC: Barcelona Clinic Liver Cancer; HBV: Hepatitis B virus; PVT: Portal vein thrombus.

patients *with* tumor reduction (Figure 6).

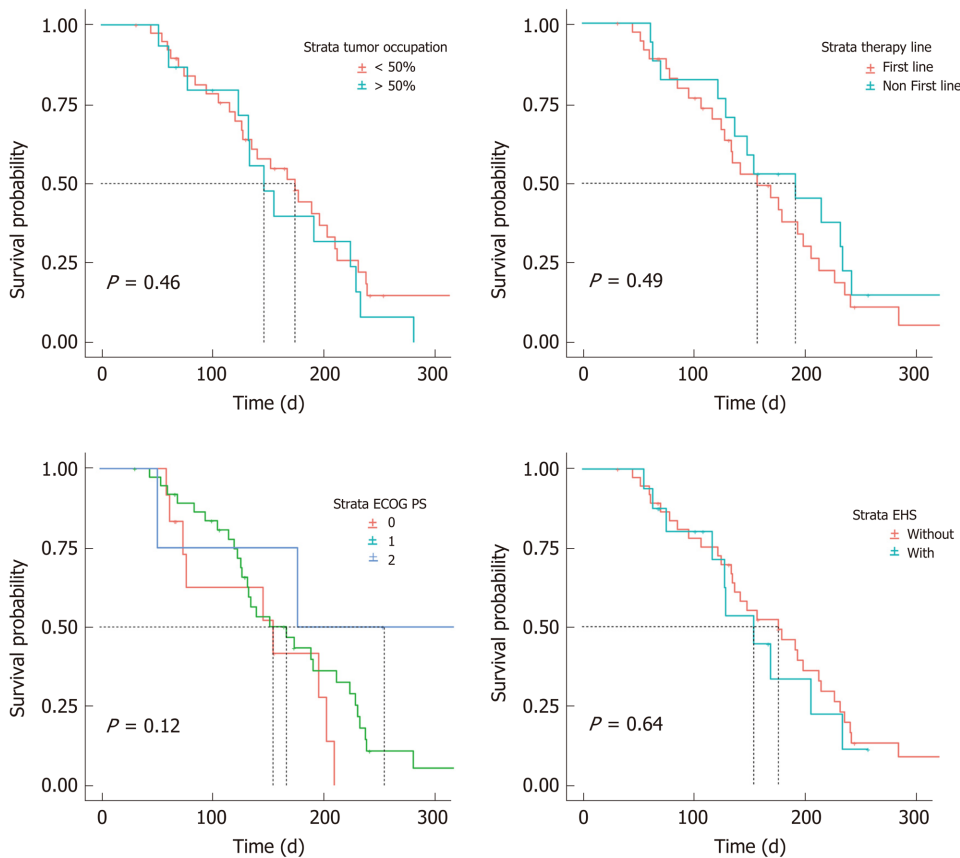


Figure 5 Progression-free survival of patients in different subgroups. PS: Performance Status; EHS: Extrahepatic spread.

DISCUSSION

The systematic treatment for hepatocellular carcinoma has dramatically changed over the past two years. To date, sorafenib and lenvatinib have been approved as first-line treatments for HCC; however, in the near future, the TA regimen (*i.e.*, atezolizumab plus bevacizumab), which has a positive effect, will also play an important role in first-line treatments^[14]. However, with this novel study design, we hoped to analyze the efficacy and safety of lenvatinib from a variety of aspects. The objective was to develop a more comprehensive understanding of its real-world effectiveness.

By contrast, sorafenib provides an ORR of less than 10%, whereas lenvatinib appears to almost double this rate according to the REFLECT trial (18.8%) and similar real-world studies that have observed ORRs ranging from 20–40% based on the mRECIST criteria^[15]. However, to date, few studies have attempted to describe the efficacy of lenvatinib monotherapy in a Chinese population. Furthermore, the apparent differences between HBV and non-HBV cases within this population raise a number of interesting questions. The aforementioned results in our study suggest that HCC carcinoma patients in China, most of whom have HBV infection (40/54), respond positively to lenvatinib (ORR, 22%; PFS, 5.6 mo). However, comparative differences between patients with and without HBV infection are not readily available due to the very limited number of non-HBV infected patients^[14]. This result may be consistent with the findings of a meta-analysis conducted by Casadei *et al*^[16] who highlighted a clear trend favoring lenvatinib over sorafenib (HR, 0.82; 95%CI: 0.60–1.15) in HBV-positive patients.

For patients with BCLC stage B disease participating in the REFLECT trial, Kudo *et al*^[11] found an ORR for lenvatinib of 61.3% with a PFS of 9.1 mo, which are higher than those achieved with any other known molecular targeted agent offered to HCC patients. Interestingly, of the patients with BCLC stage B disease, most were intolerant of chemoembolization or progressed despite previous TACE therapy. This means that most patients had good liver function and were therefore more likely to receive sustained lenvatinib treatment, which is also associated with a more favorable prognosis. However, our study appears to confirm that the mPFS for patients with BCLC stage B disease is significantly prolonged in contrast to patients with stage C.

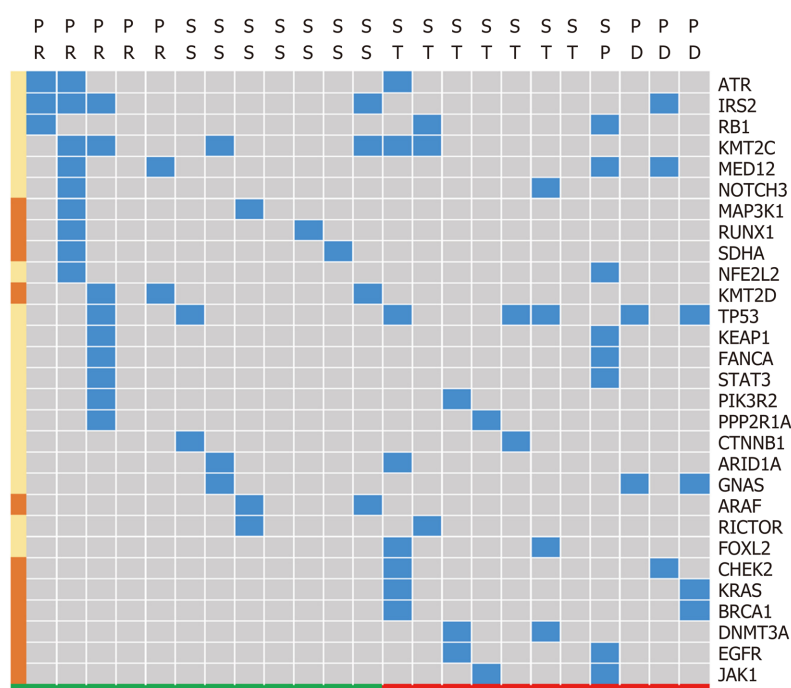


Figure 6 Signature of gene differences based on different tumor size changes. Response standard and partial response were clustered as a group encountering tumor size reduction in response to treatment, which appears green. Tumor size change, progression standard and progressive disease were clustered into a group that did not respond with tumor size reduction, which appears red. The blue block highlights the existence of specific genes, and the left brown block represents the gene that mainly appears in either the tumor reduction or without reduction groups. SS: Response standard; ST: Tumor size change; SP: Progression standard; PD: Progressive disease; PR: Partial response.

While this appears to provide valuable insight, the outcomes of the multivariate analysis in this study should be interpreted cautiously due to the small sample.

Currently, second-line therapy after sorafenib is increasingly being investigated as the majority of patients who initially receive sorafenib also require a second-line or possibly a combined intervention. Hiraoka *et al*^[6,7] found that there was no significant difference in the ORR or DCR between patients who had (or had not) previously received sorafenib. This early evidence perhaps suggests that lenvatinib provides a beneficial therapeutic response not only as a first-line treatment but also as a potential second-line intervention. Seventeen patients were treated with sorafenib in our study, and the multivariate analysis suggested that there was no significant difference in either the ORR (HR: 0.324; 95%CI: 0.055-1.901; $P = 0.212$) or PFS (HR: 1.724; 95%CI: 0.796-3.734; $P = 0.167$). These results appear to support the notion that lenvatinib can be used as an alternative second-line therapy; however, confirmatory studies are required.

Patients with $\geq 50\%$ liver occupation and portal vein invasion at the main portal branch were excluded in the REFLECT trial; however, in clinical practice, a considerable number of patients who meet these criteria are treated with lenvatinib. Therefore, to accurately analyze efficacy and identify patients who are most likely to benefit from lenvatinib, we conducted a further multivariate analysis of potentially influential factors. Stratified analysis demonstrated that liver occupation was not a significant factor affecting the ORR (HR: 1.409; 95%CI: 0.353-5.620; $P = 0.627$) or PFS (HR: 0.779; 95%CI: 0.401-1.514; $P = 0.462$). In contrast, portal vein invasion potentially affects PFS, although this finding is not completely consistent with a previous study. Hatanaka *et al*^[17] found that a PS of 0 and the presence of both macrovascular invasion and EHS were significant factors affecting overall PFS. The factors affecting both the ORR and PFS are not identical across studies, although these results all indicate that liver function and malignancy are strongly related to patient prognosis. Therefore, protecting liver function to avoid interrupting treatments due to AEs appears to be important for prolonging survival.

In this study, we utilized descriptive statistics rather than correlation analysis between AEs and clinical characteristics because there are a number of factors that could confound our interpretation. For example, Ueshima *et al*^[18] found that using a Child-Pugh score of 5 and ALBI grade I predict higher response rates and lower treatment discontinuation. However, the attributed ALBI scores were constantly

changing during the treatment period, and there was a significant decline in ALBI scores from the baseline, which was observed at 4 and 12 wk after the start of treatment^[7]. It is worth mentioning that hypertension, diarrhea, fatigue and decreased appetite were the main side effects in the present study, which are subtly different from those highlighted in the study by Hiraoka *et al*^[6]. The side effects observed here are generally more tolerable than the side effects encountered with sorafenib, which enables clinicians to prolong regimens, thereby increasing the opportunity for patients to respond positively. In addition, we found that albuminuria is particularly apparent in patients with HCC, with a rate of 10% for grade 3-4. This side effect can potentially weaken the patient's PS and cause treatment interruptions. Fortunately, this may well be manageable, if clinicians can preempt imbalanced urinary protein levels and adjust medications in a timely fashion.

AFP levels represent the activity of tumors under certain circumstances, and clinicians usually interpret AFP changes to assist in understanding treatment effects^[19,20]. The results of this study suggest that the downward trend in AFP levels from baseline after introducing lenvatinib is a direct response. Upon further analysis, we found AFP to be a potential biomarker for predicting a reduction in tumor volume. In practice, clinicians may be able to adjust lenvatinib treatments by observing changes in tumor sizes in accordance with decreasing tumor markers. However, it is important to be tentative in order to avoid false progression predictions.

Gene sequencing has been used to guide treatment planning in the field of HCC for many years, but identifying predictive genetic markers for lenvatinib treatment is frontier research and has not yet been widely considered^[21]. Even though lenvatinib is a multitarget anti-angiogenic tyrosine kinase inhibitor that can be administered without previously established gene guidelines, this certainly appears to be the next logical step for enhancing the treatment effect of lenvatinib. The inhibitory potential of fibroblast growth factor receptor (FGFR) 1-4 in lenvatinib is different to that in sorafenib and is possibly the reason for the observed improvement in the overall effect^[22]. In this study, the results of the gene mutation analysis were consistent with the published mutational landscape of HCC^[23,24].

For example, in 2017, Finn *et al*^[25] performed a study that focused on tumor gene expression clustering analysis in patients treated with lenvatinib. Patients were divided into three groups by clustering using expression levels of 36 genes involved in angiogenic and/or growth factor pathways. They found that for patients treated with lenvatinib, improvement in overall survival was seen in the group with higher vascular endothelial growth factor and FGF expression^[25]. Likewise, we found that mutations associated with the lenvatinib target, particularly FGFRs1-4, were less frequent, which may confirm previous findings^[26]; however, further research is required. An issue preventing us from carrying out correlative statistical analyses was the small number of archived tumor samples, but this study suggests some genes (and potentially intervention-related mutations) that might be used to prompt the use of lenvatinib.

While this study had a number of advantages and certainly adds to the current evidence base, it also had some limitations. Even though the research design embedded strict eligibility criteria and patients were from diverse regions of China, this was a retrospective, small-scale study with a limited number of observations, which meant there was a lack of OS data. The results of the multivariate analysis and effectiveness of predictive biomarkers, including AFP values and gene mutations, should be interpreted cautiously. In general, most participants in this study were suffering from HBV-related HCC, and lenvatinib appears effective to some degree, which confirms findings from the phase III REFLECT study. However, further analysis suggests that patients with reasonably good hepatic function may benefit more from lenvatinib treatment. Changes in AFP values and gene sequences may hold the potential to predict responses to lenvatinib during the therapeutic process although further exploratory studies are necessary.

In conclusion, the majority of this Chinese sample suffered from concomitant HBV-related HCC. Lenvatinib appears effective, which confirms previous findings from the phase III REFLECT study. However, further analysis suggests baseline characteristics, changes in serum biomarkers and gene sequencing may hold the key for predicting responses to lenvatinib. Further large-scale prospective studies that incorporate the collection and analysis of more basic medical science measures are necessary.

ARTICLE HIGHLIGHTS

Research background

Lenvatinib has become an indispensable part of regimens for patients with advanced hepatocellular carcinoma (aHCC). Recently, several real-world studies appear to have confirmed this.

Research motivation

Ethnicity appears to, at least partially, cause etiological differences, which necessitates further real-world studies of lenvatinib across diverse populations, such as in China.

Research objectives

To develop a more comprehensive understanding of the real-world effectiveness of lenvatinib by analyzing its efficacy and safety from a variety of aspects.

Research methods

This is a retrospective and multiregional study involving patients from across China who were diagnosed with aHCC and received lenvatinib monotherapy. Data were collected from patients during lenvatinib interventions from December 2018 to December 2019. After strict eligibility criteria were applied, efficacy was assessed using the RECIST 1.1 criteria. Baseline characteristics and adverse events (AEs) were recorded throughout the entire study.

Research results

In total, 54 HCC patients treated with lenvatinib monotherapy were included for final analysis. The majority of patients in this Chinese sample were suffering from concomitant HBV-related HCC. The objective response rate was 22% with a progression-free survival (PFS) of 168 d; however, AEs occurred in 92.8% of patients. The multivariate analysis showed that the Barcelona Clinic Liver Cancer stage, portal vein tumor thrombus and Child-Pugh classifications were significant factors affecting PFS. The sensitivity and specificity were calculated for decreasing serum biomarkers including alpha-fetoprotein in order to predict tumor size reduction. Gene sequencing also provided insights into potential gene mutation signatures related to the lenvatinib effect.

Research conclusions

Our findings confirm previous evidence from the phase III REFLECT study. Further analysis suggests that baseline characteristics, changes in serum biomarkers and gene sequencing may hold the key for predicting lenvatinib responses.

Research perspectives

Randomized controlled studies and real-world studies consistently report the beneficial effect of lenvatinib, but its application in HCC patients has only recently begun. Future research should focus on screening patients to ensure that we can identify those who will benefit most from lenvatinib and how the side effects can be effectively managed.

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Retrospective Study

High levels of serum interleukin-6 increase mortality of hepatitis B virus-associated acute-on-chronic liver failure

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Abstract

BACKGROUND

Patients with hepatitis B virus-associated acute-on-chronic liver failure (HBV-ACLF) present a complex and poor prognosis. Systemic inflammation plays an important role in its pathogenesis, and interleukin-6 (IL-6) as a pro-inflammatory cytokine is related with severe liver impairment and also plays a role in promoting liver regeneration. Whether serum IL-6 influences HBV-ACLF prognosis has not been studied.

AIM

To determine the impact of serum IL-6 on outcome of patients with HBV-ACLF.

METHODS

We performed a retrospective study of 412 HBV-ACLF patients. The findings were analyzed with regard to mortality and the serum IL-6 level at baseline, as well as dynamic changes of serum IL-6 within 4 wk.

RESULTS

The serum IL-6 level was associated with mortality. Within 4 wk, deceased patients had significantly higher levels of IL-6 at baseline than surviving patients [17.9 (7.3-57.6) vs 10.4 (4.7-22.3), $P = 0.011$]. Patients with high IL-6 levels (> 11.8 pg/mL) had a higher mortality within 4 wk than those with low IL-6 levels (≤ 11.8 pg/mL) (24.2% vs 13.2%, $P = 0.004$). The odds ratios calculated using univariate and multivariate logistic regression were 2.10 (95% confidence interval [CI]: 1.26-3.51, $P = 0.005$) and 2.11 (95%CI: 1.15-3.90, $P = 0.017$), respectively. The mortality between weeks 5 and 8 in patients with high IL-6 levels at 4 wk was 15.0%, which was significantly higher than the 6.6% mortality rate in patients with low IL-6

Institutional review board

statement: The study was reviewed and approved by the ethics committee of the 302 Military Hospital of China (2012 Aug 22).

Informed consent statement: The need for written informed consent was waived by the ethics committee for this study.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

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levels at 4 wk (hazard ratio = 2.39, 95% CI: 1.05-5.41, $P = 0.037$). The mortality was 5.0% in patients with high IL-6 levels at baseline and low IL-6 levels at 4 wk, 7.5% in patients with low IL-6 levels both at baseline and at 4 wk, 11.5% in patients with low IL-6 levels at baseline and high IL-6 levels at 4 wk, and 16.7% in patients with high IL-6 levels both at baseline and at 4 wk. The increasing trend of the mortality rate with the dynamic changes of IL-6 was significant (P for trend = 0.023).

CONCLUSION

A high level of serum IL-6 is an independent risk factor for mortality in patients with HBV-ACLF. Furthermore, a sustained high level or dynamic elevated level of serum IL-6 indicates a higher mortality.

Key words: Hepatitis B virus; Liver failure; Prognosis; Interleukin-6

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Core tip: To triage and prognosticate the outcome is vital for management of patients with hepatitis B virus-associated acute-on-chronic liver failure (HBV-ACLF). Interleukin-6 (IL-6) is related with the physiology and pathology of the liver. We found that a high level of serum IL-6 was an independent risk factor for mortality in patients with HBV-ACLF. HBV-ACLF patients with high levels of IL-6 showed a high mortality, especially in those with persistent high levels within 4 wk, indicating that IL-6 is an index of prognosis for HBV-ACLF.

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INTRODUCTION

Hepatitis B virus-associated acute-on-chronic liver failure (HBV-ACLF), as the major form of acute-on-chronic liver failure (ACLF) in China^[1-3], is a severe syndrome manifesting as acute exacerbation of liver dysfunction in patients with previously diagnosed or undiagnosed chronic liver disease due to the hepatitis B virus^[4]. The reported prognosis of HBV-ACLF is very poor, with a 3-mo mortality rate over 50% without liver transplantation^[5]. However, it is still a potentially reversible disease under the condition of intensive care and treatment^[6]. Therefore, distinguishing patients with a high mortality risk or reversibility from all patients is helpful for the management of HBV-ACLF, and the identification of prognostic factors is critical.

Systemic inflammatory reactions are considered to be signs of ACLF^[7,8]. Furthermore, inflammatory reactions play an important role in the pathogenesis and influence the outcome of ACLF^[8-11]. The white cell count and plasma C-reactive protein levels are higher in patients with ACLF than in those with “mere” acute decompensated cirrhosis without ACLF^[7], indicates an excessive inflammatory response at early stages of ACLF. Interleukin-6 (IL-6) as a pro-inflammatory cytokine is an important inducer of infectious defense and a measure of inflammation that is detectable earlier and is more sensitive than CRP^[12,13], and IL-6 pathway is related with the physiology and pathology of the liver. The prognostic value of IL-6 has been studied in patients with end-stage liver diseases^[14]. Our study aimed to explore whether IL-6 influences HBV-ACLF prognosis and identify the specific effects of IL-6 on HBV-ACLF outcome.

MATERIALS AND METHODS

Patients

We retrospectively analyzed HBV-ACLF patients from the National Twelve Five-Year Science and Technology Major Project of China (ChiCTR-TRC-00000766). A multicenter study was conducted from November 31, 2012 to December 31, 2014. All patients from the following 17 clinical institutions were enrolled: 302 Military Hospital, Beijing Ditan Hospital, Beijing Youan Hospital, Shanghai Public Health Clinical Center, Tongji Hospital, Tianjin Infectious Disease Hospital, Fuzhou Infectious Disease Hospital, Hubei Provincial Hospital of Traditional Chinese Medicine, Jilin Hepatobiliary Hospital, The First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine, The First Affiliated Hospital of Hunan University of Traditional Chinese Medicine, The Third Affiliated Hospital of Zhongshan University, The Sixth People's Hospital of Shenyang, Xixi Hospital of Hangzhou, Shenzhen Traditional Chinese Medicine Hospital, The Third People's Hospital of Shenzhen, and Chengdu Public Health Clinical Center.

The diagnosis of HBV-ACLF was based on both ACLF and chronic liver disease due to HBV infection. The diagnosis of ACLF complied with the diagnostic and therapeutic guidelines for liver failure established in 2014^[15]. ACLF is the main clinical manifestation of short-term acute hepatic decompensation (usually occurring within 4 wk) on the basis of an underlying chronic liver disease. The diagnostic criteria were: (1) Jaundice [serum bilirubin ≥ 5 mg/dL (≥ 85 μ mol/L)]; (2) Coagulopathy (international normalized ratio [INR] ≥ 1.5 or prothrombin activity $\leq 40\%$); and (3) Ascites and/or encephalopathy as determined by physical examination. Chronic HBV infection was diagnosed according to HBsAg positivity for more than 6 mo.

The inclusion criteria were: (1) Patients with chronic liver disease due to hepatitis B virus infection; (2) Patients with acute deteriorated liver function within 4 wk; (3) Patients with progressive jaundice (serum bilirubin ≥ 5 mg/dL); (4) Patients with a risk of bleeding (prothrombin activity $\leq 40\%$ or INR ≥ 1.5); and (5) Ascites and/or encephalopathy as determined by physical examination.

The exclusion criteria were: (1) Participation in other clinical trials within the last 3 mo; (2) Pregnancy or breastfeeding; (3) Acute or subacute hepatic failure or chronic hepatic failure; (4) Other etiologies such as autoimmunity, drugs, alcohol (a history of significant alcohol intake was identified by the Alcohol Use Disorders Identification Test^[16]), toxins, or parasites that may contribute to ACLF; (5) Hepatocellular carcinoma; (6) Other serious general or psychological diseases; (7) Human immunodeficiency virus infection; (8) Brain edema and/or infection at the time of enrollment (including septic shock and fungal infection); and (9) Type 1 hepatorenal syndrome (characterized by clinical features including severe progressive renal failure, oliguria for several days less than 2 wk, and serum creatinine > 221 μ mol/L).

A flow diagram of patient selection is shown in **Figure 1**. A total of 1059 patients with HBV-ACLF were identified between 2000 and 2014, of whom 492 who did not fulfill the criteria and 155 without consecutive records were excluded. Ultimately, 412 patients were included in the analysis.

Quantification of IL-6

IL-6 (ElecSys IL-6 kit, electrochemiluminescence immunoassay) was uniformly determined in serum using the Cobas 8000 analyzer. These samples were collected at enrollment and 4 wk, stored at -80°C , and then thawed for batched analysis at ADICON Clinical Laboratory (Shanghai, China).

Statistical analysis

Continuous variables are expressed as the mean \pm standard deviation (SD) or medians and interquartile ranges, while categorical variables are expressed as frequencies and percentages. Univariable analyses included Student's *t*-test for pairwise comparisons of parametric data distributions, the Mann-Whitney U test for pairwise comparisons of nonparametric distributions, and chi-square tests for comparisons of categorical variables. Binary logistic regression with forward elimination was used to evaluate factors related to prognosis. The choice of variables for the multivariable analysis was based on the results of univariable analysis and clinical correlation. The Cox proportional hazards regression was used for group comparisons of mortality between weeks 5 and 8. The Cochran-Armitage test for trend was used to identify whether a significant change in mortality was a dynamic change in IL-6. $P < 0.05$ was considered statistically significant for all tests. All statistical analyses were performed using IBM SPSS Statistics 20.

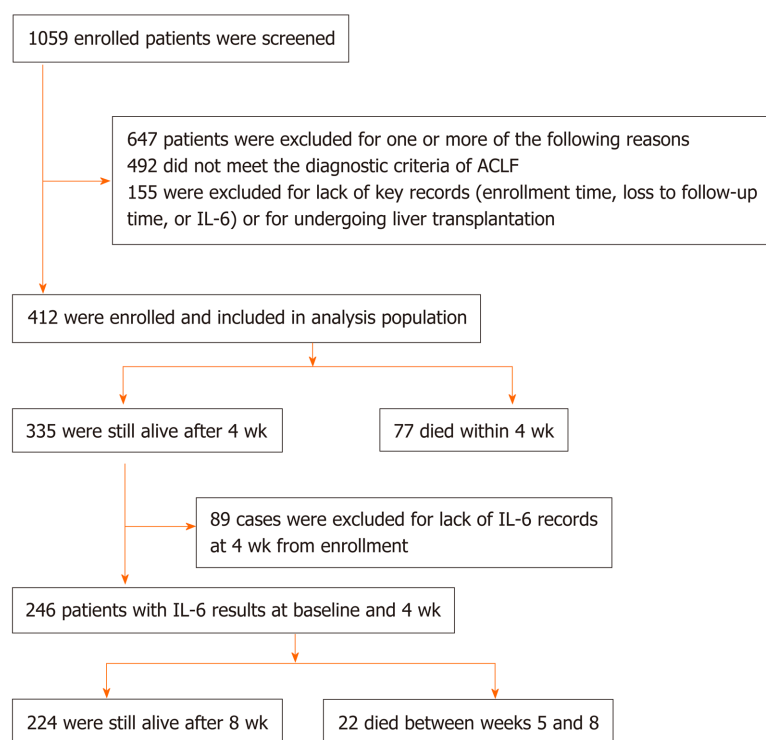


Figure 1 Study flow diagram.

RESULTS

Patient characteristics

The baseline characteristics of the cohort are summarized in [Table 1](#). We included 412 patients, of whom 84.0% were men. The average age was 44.6 ± 10.6 years. The average model for end-stage liver disease (MELD) score was 24.4 ± 4.5 . Clinical patient records revealed hyponatremia in 43.8%, spontaneous bacterial peritonitis (SBP) in 37.0%, and infection in addition to SBP which included respiratory, urinary, digestive infections, and sepsis in 20.5% of the cases.

At 4 wk from study enrollment, 18.7% (77) of the patients died, and 335 patients survived longer than 4 wk. Of the 335 surviving patients, 89 had no detectable IL-6 and were excluded. Of the 246 patients with detectable IL-6 at 4 wk, 8.9% died between weeks 5 and 8. The comparison between 89 patients without IL-6 with 246 patients with detectable IL-6 is demonstrated in [Supplementary Table 1](#).

Comparison of baseline characteristics between surviving patients and deceased patients

The baseline characteristics between surviving patients and deceased patients within 4 wk were compared in [Table 2](#). Deceased patients had higher IL-6 levels than surviving patients [17.9 (7.3-57.6) *vs* 10.4 (4.7-22.3), $P = 0.011$], as well as higher bilirubin levels (22.4 ± 8.1 *vs* 19.0 ± 7.7 , $P = 0.001$), higher creatinine levels (85.1 ± 40.8 *vs* 71.6 ± 21.7 , $P = 0.006$), a higher white blood cell count (WBC) (8.3 ± 4.1 *vs* 7.0 ± 3.0 , $P = 0.015$), a higher INR (2.7 ± 0.7 *vs* 2.7 ± 0.7 , $P < 0.001$), and a significantly higher MELD score (27.8 ± 4.5 *vs* 23.6 ± 4.2 ; $P < 0.001$). Additionally, deceased patients were older than surviving patients (47.6 ± 10.2 *vs* 43.9 ± 10.6 , $P = 0.007$).

Furthermore, deceased patients had higher proportions of patients with hepatic encephalopathy (HE) (26.7% *vs* 11.1%; $P < 0.001$), upper gastrointestinal bleeding (UGB) (7.8% *vs* 1.5%; $P = 0.002$), and renal dysfunction (11.8% *vs* 1.8%; $P < 0.001$). The presence of SBP (37.7% *vs* 34.2%; $P = 0.575$), infection excluding SBP (19.5% *vs* 25.0%; $P = 0.286$), and hyponatremia (41.9% *vs* 51.9%; $P = 0.151$) was comparable between surviving patients and deceased patients. There was no significant difference in the levels of albumin, globulin, alanine transaminase (ALT), aspartate transaminase (AST), γ -glutamyl transferase (GGT), hemoglobin, platelet count, hepatitis B virus deoxyribonucleic acid (HBV DNA), or the proportion of males.

Table 1 Comparisons of deceased and surviving patients

Parameter	All patients (n = 412)	Surviving patients (n = 335)	Deceased patients (n = 77)	P value
Age (yr)	44.6 ± 10.6	43.9 ± 10.6	47.6 ± 10.2	0.007
Male, n (%)	346 (84.0)	279 (83.3)	67 (87.0)	0.421
Albumin (g/L)	30.8 ± 12.3	30.5 ± 5.1	32.2 ± 6.7	0.587
Globulin (g/L)	29.6 ± 9.0	29.7 ± 8.9	29.2 ± 9.4	0.624
Bilirubin (mg/dL)	19.6 ± 7.9	19.0 ± 7.7	22.4 ± 8.1	0.001
ALT (U/L)	145 (63-360)	151 (63-353)	183 (74-458)	0.255
AST (U/L)	153 (86-299)	143 (85-285)	189 (110-363)	0.100
GGT (U/L)	67 (40-101)	69 (40-102)	57 (39-94)	0.226
Creatinine (μmol/L)	74.2 ± 26.8	71.6 ± 21.7	85.1 ± 40.8	0.006
INR	2.3 ± 0.6	2.7 ± 0.7	2.7 ± 0.7	< 0.001
WBC (× 10 ⁹ /L)	7.3 ± 3.3	7.0 ± 3.0	8.3 ± 4.1	0.015
Hemoglobin (g/L)	115 ± 23	117 ± 21	111 ± 29	0.095
Platelet count (× 10 ⁹ /L)	89 ± 45	90 ± 43	85 ± 53	0.367
HBV DNA (log ₁₀ IU/mL)	3.1 ± 2.3	3.0 ± 2.3	3.3 ± 2.4	0.467
MELD	24.4 ± 4.5	23.6 ± 4.2	27.8 ± 4.5	< 0.001
HE, n (%)	57 (13.9)	37 (11.1)	20 (26.7)	< 0.001
UGB, n (%)	11 (2.7)	5 (1.5)	6 (7.8)	0.002
SBP, n (%)	151 (37.0)	125 (37.7)	26 (34.2)	0.575
Infection excluding SBP, n (%) ¹	84 (20.5)	65 (19.5)	19 (25.0)	0.286
Hyponatremia, n (%)	178 (43.8)	138 (41.9)	40 (51.9)	0.151
Renal dysfunction, n (%) ²	15 (3.7)	6 (1.8)	9 (11.8)	< 0.001
IL-6 (pg/mL)	11.8 (5.4-25.9)	10.4 (4.7-22.3)	17.9 (7.3-57.6)	0.011

¹Including respiratory, urinary, and digestive infections, as well as sepsis.

²Serum creatinine level ranging from 1.5 to 1.9 mg/dL^[7]. P values shown in bold indicate statistical significance. ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: γ-glutamyl transferase; INR: International normalized ratio; WBC: White blood cell count; HBV DNA: Hepatitis B virus deoxyribonucleic acid; MELD: Model for end-stage liver disease; HE: Hepatic encephalopathy; UGB: Upper gastrointestinal bleeding; SBP: Spontaneous bacterial peritonitis; IL-6: Interleukin-6.

Characteristics of patients with different levels of IL-6

According to the median IL-6 level (11.8 pg/mL), patients were classified into two groups: Patients with high IL-6 levels and those with low IL-6 levels (Table 2). The mortality of patients with high levels of IL-6 was 24.2%, which was significantly higher than 13.2% in patients with low levels of IL-6. Additionally, patients with low levels of IL-6 presented with higher levels of albumin (32.1 ± 16.8 *vs* 29.6 ± 4.7; *P* = 0.039), GGT [74 (42, 117) *vs* 61 (38, 95); *P* = 0.018], and hemoglobin (118 ± 23 *vs* 113 ± 23; *P* = 0.036).

Factors associated with the prognosis of HBV-ACLF patients

Binary logistic regression analysis was used to determine factors independently associated with outcomes (Table 3). Characteristics that were significantly different between surviving patients and deceased patients in univariate analysis (IL-6, age, bilirubin, creatinine, INR, hemoglobin, as well as the presence of HE, UGB, and renal dysfunction) were included in the multivariate model. After forward elimination, age (odds ratio [OR] = 1.04, 95% confidence interval [CI]: 1.01-1.07, *P* = 0.005), bilirubin (OR = 1.04, 95%CI: 1.002-1.08, *P* = 0.037), creatinine (OR = 1.02, 95%CI: 1.01-1.03, *P* = 0.001), INR (OR = 3.54, 95%CI: 2.19-5.72, *P* < 0.001), presence of HE (OR = 2.47, 95%CI: 1.15-5.32, *P* = 0.021), presence of UGB (OR = 4.73, 95%CI: 1.02-21.98, *P* = 0.047), and levels of IL-6 (OR = 2.11, 95%CI: 1.15-3.90, *P* = 0.017) were independently associated with the prognosis of HBV-ACLF patients.

Table 2 Comparisons of characteristics between patients with different levels of interleukin-6

Parameter	High IL-6 level (n = 207)	Low IL-6 level (n = 205)	P value
Age (yr)	44.8 ± 10.7	44.5 ± 10.5	0.777
Male, n (%)	172 (83.1)	174 (84.9)	0.621
Albumin (g/L)	29.6 ± 4.7	32.1 ± 16.8	0.039
Globulin (g/L)	29.8 ± 9.3	29.5 ± 8.7	0.795
Bilirubin (mg/dL)	20.0 ± 8.0	19.3 ± 7.8	0.362
ALT (U/L)	119 (63, 333)	178 (64, 433)	0.070
AST (U/L)	148 (84, 277)	167 (92, 361)	0.132
GGT (U/L)	61 (38, 95)	74 (42, 117)	0.018
Creatinine (μmol/L)	75.8 ± 28.8	72.5 ± 24.7	0.222
INR	2.3 ± 0.6	2.3 ± 0.6	0.924
WBC (× 10 ⁹ /L)	7.5 ± 3.6	7.0 ± 2.9	0.149
Hemoglobin (g/L)	113 ± 23	118 ± 23	0.036
Platelet count (× 10 ⁹ /L)	89 ± 49	90 ± 42	0.815
HBVDNA (log ₁₀ IU/mL)	3.1 ± 2.4	3.1 ± 2.2	0.825
MELD	24.7 ± 4.6	24.2 ± 4.4	0.241
HE, n (%)	33 (16.1)	24 (11.8)	0.206
UGB, n (%)	7 (3.4)	4 (2.0)	0.368
SBP, n (%)	73 (35.8)	78 (38.2)	0.608
Infection excluding SBP, n (%) ¹	43 (21.0)	41 (20.1)	0.826
Hyponatremia, n (%)	94 (43.8)	90 (42.5)	0.266
Renal disfunction, n (%) ²	9 (4.4)	6 (2.9)	0.441
Mortality, n (%)	50 (24.2)	27 (13.2)	0.004

¹Including respiratory, urinary, and digestive infections, as well as sepsis.

²Serum creatinine level ranging from 1.5 to 1.9 mg/dL^[7]. P values shown in bold indicate statistical significance. ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: γ-glutamyl transferase; INR: International normalized ratio; WBC: White blood cell count; MELD: Model for End-stage Liver Disease; HE: Hepatic encephalopathy; UGB: Upper gastrointestinal bleeding; SBP: Spontaneous bacterial peritonitis; IL-6: Interleukin-6.

Impact of IL-6 on mortality of HBV-ACLF patients

Patients with high levels of IL-6 had a significantly higher 4-wk mortality than patients with low levels of IL-6 (15.0% *vs* 6.6%, *P* = 0.035). High levels of IL-6 at 4 wk (hazard ratio = 2.39, 95% CI: 1.05-5.41, *P* = 0.037) was independently associated with the high mortality between weeks 5 and 8 in patients with HBV-ACLF (Table 4).

According to the dynamic changes in IL-6 within 4 wk, patients were classified into four groups (A, B, C, and D): Patients with high IL-6 levels at baseline and low IL-6 levels at 4 wk; those with low IL-6 levels both at baseline and at 4 wk; those with low IL-6 levels at baseline and high IL-6 levels at 4 wk; and those with high IL-6 levels both at baseline and at 4 wk. The mortality rates were 5.0% in group A, 7.5% in group B, 11.5% in group C, and 16.7% in group D. There was a significant difference in the dynamic change in mortality among the four groups (*P* = 0.023) (Table 5).

DISCUSSION

Our study found that higher IL-6 at baseline was present in deceased patients with HBV-ACLF at 4 wk than in surviving patients, and IL-6 was an independent factor influencing the prognosis of HBV-ACLF. The results of the first 4 wk and the following 4 wk showed that HBV-ACLF patients with high IL-6 levels had more than twice the risk of death than those with low IL-6 levels. It indicated IL-6 could be used as an auxiliary indicator of prognosis, and dynamic changes predicted the outcomes of

Table 3 Risk factors associated with prognosis in hepatitis B virus-associated acute-on-chronic liver failure patients

Parameter	First step			Last step		
	OR	95%CI	P value	OR	95%CI	P value
Age, per yr	1.03	1.01-1.06	0.008	1.04	1.01-1.07	0.009
Bilirubin, per 1 mg/dL	1.05	1.02-1.09	0.001	1.04	1.002-1.08	0.037
Albumin, per 1 g/L	1.01	0.99-1.03	0.339	-	-	-
Creatinine, per 1 μ mol/L	1.02	1.01-1.03	< 0.001	1.02	1.01-1.03	0.001
INR, per 1 unit	3.46	2.27-5.28	< 0.001	3.54	2.19-5.72	< 0.001
ALT, per 1 U/L	1.00	1.00-1.00	0.197	-	-	-
AST, per 1 U/L	1.00	1.00-1.00	0.093	-	-	-
GGT, per 1 U/L	1.00	0.99-1.00	0.132	-	-	-
Hemoglobin, per 1 g/L	0.99	0.98-1.00	0.042	-	-	-
HE (yes vs no ¹)	2.92	1.58-5.40	0.001	2.47	1.15-5.32	0.021
UGB (yes vs no ¹)	5.54	1.65-18.67	0.006	4.73	1.02-21.98	0.047
Hyponatremia (yes vs no ¹)	1.37	0.99-1.90	0.057	-	-	-
Renal disfunction (yes vs no ^{1,2})	7.34	2.53-21.32	< 0.001	-	-	-
IL-6, pg/ml (> 11.8 vs \leq 11.8 ¹)	2.10	1.26-3.51	0.005	2.11	1.15-3.90	0.017

¹Reference value.²Serum creatinine level ranging from 1.5 to 1.9 mg/dL^[7]. P values shown in bold indicate statistical significance. OR: Odds ratio; CI: Confidence interval; INR: International normalized ratio; ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: γ -glutamyl transferase; HE: Hepatic encephalopathy; UGB: Upper gastrointestinal bleeding; IL-6: Interleukin-6.**Table 4 Mortality between weeks 5 to 8 according to interleukin 6 levels at 4 wk**

	Mortality	P value	HR (95%CI)	P value
With low levels of IL-6 (<i>n</i> = 166)	11 (6.6%)	0.035	1	
With high levels of IL-6 (<i>n</i> = 80)	12 (15.0%)		2.39 (1.05-5.41)	0.037

P values shown in bold indicate statistical significance. HR: Hazard ratio; CI: Confidence interval; IL-6: Interleukin-6.

Table 5 Mortality according to the dynamic changes in interleukin-6 within 4 wk

	Mortality	HR (95%CI)	P value	P value for trend
Group A (<i>n</i> = 60)	3 (5.0%)	1		0.023
Group B (<i>n</i> = 106)	8 (7.5%)	1.53 (0.41-5.78)	0.528	
Group C (<i>n</i> = 26)	3 (11.5%)	2.41 (0.49-12.0)	0.281	
Group D (<i>n</i> = 54)	9 (16.7%)	2.80 (0.72-10.83)	0.136	

P values shown in bold indicate statistical significance. A: Patients with high interleukin 6 (IL-6) levels at baseline and low IL-6 levels at 4 wk; B: Patients with low IL-6 levels both at baseline and at 4 wk; C: Patients with low IL-6 levels at baseline and high IL-6 levels at 4 wk; D: Patients with high IL-6 levels both at baseline and at 4 wk. HR: Hazard ratio; CI: Confidence interval.

HBV-ACLF patients.

IL-6 is produced in monocytes, macrophages, T cells, fibroblasts, and endothelial cells and initiates the production of acute-phase proteins^[17]. Elevated IL-6 is viewed as a distinctly proinflammatory cytokine, promptly activating the host defense system to perform diverse functions^[17]. Early and direct IL-6 signals were involved in aiding immune responses at the site of infection during heterosubtypic challenge^[18]. Some

studies reported that IL-6 was a more suitable parameter in cases of severe systemic inflammation for patients with severe liver impairment than WBC or CRP^[14]. However, excessive and persistent IL-6 is involved in liver injury^[19,20]. In our study, there were slight differences in albumin, GGT, and hemoglobin between the two levels of IL-6, but there was no correlation with MELD score, WBC count, presence of complications, or even infections in different locations. This could be related to the induced expression of IL-6 in the acute phase response being different from that in the chronic state^[17]. The duration of the acute phase response is normally 24–48 h; however, the infections mentioned in our study were not only involved in the acute phase. In addition, in our opinion, this indicates that the impact of IL-6 on prognosis was not only dependent on acute infections or infection-related mortality alone. The reason for this observation remains to be elucidated.

On the other hand, some experimental studies showed IL-6 as a cytokine with diverse biological functions, promoting inflammatory responses and maintaining tissue homeostasis, which was related to liver regeneration in an animal model of acute liver failure^[21]. In addition, IL-6 was considered to contribute to liver tumors. The complex function of IL-6 is involved in class or trans-signaling in the liver^[22–24]. Therefore, selective inhibition of IL-6 trans-signaling in the treatment of liver pathologies still needs further study^[17]. Our contrary findings in patients with HBV-ACLF may be explained by the pathophysiological differences between acute on-chronic and chronic liver failure, as well as by different IL-6 effects over time in our patients suffering from chronic liver diseases. Acute and only shortly increased IL-6 levels may be advantageous for liver regeneration, whereas chronic IL-6 increases may have disadvantageous effects on the liver and other organs. Our study found that dynamic changes in IL-6 were related to mortality; patients with dynamic elevated or sustained high levels of IL-6 had higher mortality, while patients with dynamic declined or sustained low levels of IL-6 had lower mortality. This result indicated that the level of IL-6 and its duration simultaneously influenced the development of disease.

This study has some limitations. It was retrospective and designed to explore the prognostic value of the inflammatory biomarker IL-6. Some parameters were not available in all patients, such as procalcitonin and CRP, and were not measured in this study as a result of no analysis between these parameters and IL-6, but this had no effect on the impact of IL-6 on prognosis. Besides, some patients lack of second IL-6 results were not included in the analysis about the impact of dynamic changes of IL-6 on mortality. However, we performed analysis between the excluded dataset ($n = 89$) and the 246 included patients, and the results showed no significance in characteristics or mortality (Supplementary Table 1). We did not distinguish infection-induced HBV-ACLF or noninfection-induced HBV-ACLF, but we analyzed the presence of infection, such as SBP, in patients with different levels of IL-6. Furthermore, despite the positive finding of IL-6 on the prognosis of HBV-ACLF, the underlying mechanism is not clear and needs further study.

In conclusion, this study showed that IL-6 is associated with the outcome of HBV-ACLF and is an independent prognostic factor. IL-6 could be a promising candidate to predict mortality in patients with HBV-ACLF. However, further studies are necessary to validate and confirm the predictive value of IL-6.

ARTICLE HIGHLIGHTS

Research background

Hepatitis B virus-associated acute-on-chronic liver failure (HBV-ACLF) has a complex and poor prognosis. Interleukin-6 (IL-6) as a pro-inflammatory cytokine is related with severe liver impairment and also plays a role in promoting liver regeneration. Whether serum IL-6 influences HBV-ACLF prognosis has not been studied.

Research motivation

HBV-ACLF is a potentially reversible disease under the condition of intensive care and treatment, therefore, an index used to triage and prognosticate the outcome will promote timely and more appropriate management of the patients.

Research objectives

This study was conducted to determine the impact of serum IL-6 on outcome of patients with HBV-ACLF.

Research methods

We analyzed 412 HBV-ACLF qualified cases from the dataset of National Twelve Five-Year Science and Technology Major Project of China "Study on HBV-ACLF Treated with Integrated Traditional Chinese Medicine and Western Medicine". The levels of serum IL-6 at baseline and 4 wk were detected and the impact of IL-6 on short-term mortality of patients with HBV-ACLF were analyzed.

Research results

Patients with high IL-6 levels (> 11.8 pg/mL) had a higher mortality within 4 wk than those with low IL-6 levels (≤ 11.8 pg/mL) (24.2% *vs* 13.2%, $P = 0.004$; odds ratio [OR] = 2.11, 95% confidence interval [CI]: 1.15-3.90, $P = 0.017$). The mortality between weeks 5 and 8 in patients with high IL-6 levels at 4 wk was 15.0%, which was significantly higher than the 6.6% mortality rate in patients with low IL-6 levels at 4 wk (hazard ratio = 2.39, 95% CI: 1.05-5.41, $P = 0.037$). The mortality was 5.0% in patients with high IL-6 levels at baseline and low IL-6 levels at 4 wk, 7.5% in patients with low IL-6 levels both at baseline and at 4 wk, 11.5% in patients with low IL-6 levels at baseline and high IL-6 levels at 4 wk, and 16.7% in patients with high IL-6 levels both at baseline and at 4 wk. The increasing trend of the mortality rate with the dynamic changes of IL-6 was significant (P for trend = 0.023).

Research conclusions

Our study demonstrated that a high level of serum IL-6 increases mortality risk in patients with HBV-ACLF.

Research perspectives

Our results suggest that IL-6 could be a promising candidate to predict mortality in patients with HBV-ACLF, as well as dynamic changes of IL-6. Further prospective studies are required to validate and confirm the predictive value of IL-6.

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Retrospective Study

Simultaneous transcatheter arterial chemoembolization and portal vein embolization for patients with large hepatocellular carcinoma before major hepatectomy

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statement: The study was approved by the ethics committee of Zhejiang Provincial People's Hospital (No.2019KY181) and followed the declaration of Helsinki.

Informed consent statement:

Written informed consent was obtained from all enrolled patients.

Conflict-of-interest statement: The

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Abstract

BACKGROUND

Sequential transarterial chemoembolization (TACE) and portal vein embolization (PVE) are associated with long time interval that can allow tumor growth and nullify treatments' benefits.

AIM

To evaluate the effect of simultaneous TACE and PVE for patients with large hepatocellular carcinoma (HCC) prior to elective major hepatectomy.

METHODS

Fifty-one patients with large HCC who underwent PVE combined with or without TACE prior to hepatectomy were included in this study, with 13 patients in the simultaneous TACE + PVE group, 17 patients in the sequential TACE + PVE group, and 21 patients in the PVE-only group. The outcomes of the procedures were compared and analyzed.

RESULTS

All patients underwent embolization. The mean interval from embolization to surgery, the kinetic growth rate of the future liver remnant (FLR), the degree of tumor size reduction, and complete tumor necrosis were significantly better in the simultaneous TACE + PVE group than in the other groups. Although the patients in the simultaneous TACE + PVE group had a higher transaminase levels after

authors declare that they have no conflict of interest.

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PVE and TACE, they recovered to comparable levels with the other two groups before surgery. The intraoperative course and the complication and mortality rates were similar among the three groups. The overall survival and disease-free survival were higher in the simultaneous TACE + PVE group than in the other two groups.

CONCLUSION

Simultaneous TACE and PVE is a safe and effective approach to increase FLR volume for patients with large HCC before major hepatectomy.

Key words: Transcatheter arterial chemoembolization; Portal vein embolization; Major hepatectomy; Hepatocellular carcinoma; Future liver remnant

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Core tip: Simultaneous transarterial chemoembolization (TACE) and portal vein embolization (PVE) is a safe and effective approach to increase the future liver remnant volume quickly in a short time, and it has achieved a longer median overall survival and disease-free survival time compared with sequential TACE and PVE or PVE-only for patients with large hepatocellular carcinoma before major hepatectomy.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly lethal invasive carcinoma arising in the liver^[1,2]. The most important risk factors for HCC are infection with hepatitis B or hepatitis C and/or preexisting liver cirrhosis^[3,4]. The incidence of HCC varies across the world and associated with the geographical distribution of hepatitis B and hepatitis C^[5-7]. The worldwide age-standardized annual mortality rates of HCC are 12.7 per 100000 in men and 4.6 per 100000 in women^[2,8].

Major hepatectomy is increasingly performed for patients with large HCC to achieve curative resection and provide the only opportunity for long-term survival^[9,10]. Nevertheless, major hepatectomy is frequently contraindicated in many patients with HCC due to insufficient future liver remnant (FLR) along with the increased possibility of postoperative liver failure, especially in patients with chronic liver disease or cirrhosis.

Preoperative portal vein embolization (PVE) aimed to induce atrophy of the embolized segments and compensatory hypertrophy of contralateral segments has been widely accepted as the standard method to reduce the risk of postoperative liver failure and convert patients with large HCC from an unresectable to a resectable status^[11,12]. The fastest liver hypertrophy was reported to appear 2 wk after PVE and could be sustained for about 8 wk^[13]. Nevertheless, the buffering increase of the hepatic arterial flow resulting from PVE might lead to rapid ipsilateral tumor growth as well as insufficient contralateral liver hypertrophy. To prevent this detrimental effect of preoperative PVE and to further facilitate FLR regeneration, transarterial chemoembolization (TACE) is recommended before PVE. Recent researches have demonstrated that sequential TACE and PVE before major hepatectomy is safe and effective for patients with large HCC and can improve their clinical outcomes and survival^[14-16]. Ogata *et al*^[17] reported that the mean increase in percentage FLR volume, the rate of complete tumor necrosis, and the 5-year disease-free survival (DFS) in the TACE+PVE group were significantly higher compared with those in the PVE alone group. Nevertheless, one obvious drawback of sequential TACE and PVE is the long wait time between TACE and PVE (3-4 wk) and before surgery (4-6 wk), which may result in tumor progression and thus nullify the obtained efficacy.

In view of the potential risk of tumor progression during the long wait time before surgery, simultaneous TACE and PVE before hepatectomy should theoretically have a stronger anticancer effect through the double-obstruction of the tumor feeding vessels and should reduce the wait time before surgery, and have been reported to be safe and effective^[18]. Nevertheless, this attempt was only made in very few patients with small HCC confined to superficial liver areas. More importantly, no study has ever compared the results of simultaneous TACE and PVE *vs* sequential TACE and PVE or *vs* PVE alone. Therefore, this is the first study to evaluate the effect of simultaneous TACE and PVE before major hepatectomy in patients with large HCC and to compare their clinical outcome with sequential TACE+PVE or PVE only.

MATERIALS AND METHODS

Patient selection

Between January 2010 and December 2018, 51 patients with large HCC and insufficient FLR were enrolled in this study. The inclusion criteria were: (1) 18-70 years of age; (2) Diagnosed with HCC based on the EASL-EORTC Recommendations^[19]; (3) Tumor size ≥ 5 cm based on computed tomography (CT) or magnetic resonance imaging (MRI); (4) Chest/abdominal-pelvic CT or positron emission tomography (PET)-CT were performed to exclude extrahepatic metastasis; (5) Major hepatectomy was deemed to be required in patients with threshold preoperative FLR $< 40\%$ and indocyanine green retention rate at 15 min (ICGR15) $< 10\%$; (6) No tumor thrombus in portal vein and branches of the retained lobe; (7) Absence of severe esophageal and gastric varices; and (8) Eastern Cooperative Oncology Group (ECOG) performance status of 0-1. The exclusion criteria were: (1) Child-Pugh C; (2) More than four lesions; (3) Tumors distributed in the two lobes; or (4) Other malignant tumors. Written informed consent was obtained from all enrolled patients. The study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital and followed the principle of declaration of Helsinki.

Transarterial chemoembolization

TACE procedures were performed under local anesthesia as selectively as possible, depending on tumor distribution. A conventional or drug-eluting beads TACE was used, with the latter being used in more recent patients. TACE procedures were performed by a team of experienced interventional radiologists. Conventional TACE included an intra-arterial injection of a mixture of chemotherapeutic agents (150 mg doxorubicin; adriamycin; Pharmacia Upjohn, Kalamazoo, MI, United States), emulsified in iodized oil (Lipiodol, Gerbet, Aulnay-sous-Bois, France). Embolization was achieved by injection of gelatin sponge (Gelitaspon, Gelita Medical B.V., Amsterdam, Netherlands) or polyvinyl alcohol particles (Bead Block, Biocompatibles, Farnham, United Kingdom). The drug-eluting beads included 100-300 μm and/or 300-500 μm sized particles (Biocompatibles, Farnham, United Kingdom), as described elsewhere^[20]. Bead loading was performed at an intended dose of 150 mg/patient.

Portal vein embolization

PVE procedures were performed 2-4 wk after TACE in the sequential TACE + PVE (seTP) group or concurrently in the simultaneous TACE + PVE (siTP) group by the same team of interventional radiologists. Procedures were performed under general anesthesia. The technical aspects of PVE have been described elsewhere^[21]. The percutaneous approach was the technique of choice. After catheterizing the main portal trunk, a portography was performed, and a mixture of N-butyl-2-cyanoacrylate and iodized oil (Lipiodol, Guerbet, Aulnay-sous-Bois, France) was used for embolization. Embolization was completed with 0.018-inch coils (MicroNester 0.018, Cook, Bloomington, United States), and polyvinyl alcohol particles (Beadblock, Biocompatibles, Farnham, United Kingdom), when necessary (Figure 1).

Major hepatectomy

Hepatectomy was considered feasible after achieving substantial hypertrophy of the FLR. Hepatectomy was not performed if the FLR was still $< 40\%$ or distant metastasis was found. Laparoscopic or open hepatectomy was performed in all patients depending on the size of the tumor and judgment of the chief surgeon. Intraoperative ultrasound was used to verify the location of the tumor and its relationship with major vascular structures as well as to detect satellite nodules. Selective hemihepatic blood

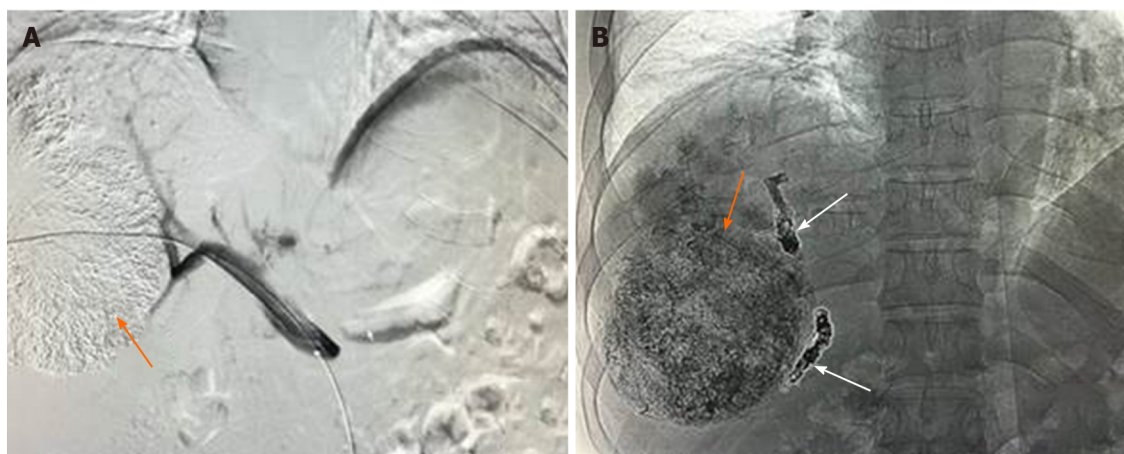


Figure 1 Transarterial chemoembolization and portal vein embolization. A: Transarterial chemoembolization in a patient with large hepatocellular carcinoma in the right liver lobe (orange arrow); B: Coils (white arrow) and polyvinyl alcohol particles were used for portal vein embolization in the same patient.

flow occlusion or intermittent Glisson pedicle clamping was routinely performed.

Data collection

Baseline patient and tumor characteristics were obtained from the prospective institutional database. All patients underwent volumetric helical computed tomographic estimation of liver volume before PVE with or without TACE and before surgery. Those with a 10% decrease before surgery compared to the baseline size were defined as with tumor size reduction. As the most accurate indicator to assess the ability of liver hypertrophy and to estimate the possibility of postoperative hepatic failure, the kinetic growth rate (KGR)^[22], which reflects the average hypertrophy of liver volume each week, was calculated for all patients. For patients who finally underwent hepatectomy, the ratio of residual tumor cells (RT) was quantitatively assessed by pathologists as the volume of the RT compared to the total surface volume of the lesion. Lesions were classified into three groups: major pathological response (MPR, RT < 10%), partial pathologic response (PPR, RT from 10% to 50%), and no pathologic response (RT > 50%)^[23]. In addition, patients who did not undergo resection because of tumor progression, insufficient FLR hypertrophy, or liver failure after PVE with or without TACE were also recorded. Other data, including liver function tests (LFTs) before and after TACE and/or PVE, perioperative results, and long-term outcomes were collected.

Follow-up information included clinical examination, liver function tests, measurement of serum alpha-fetoprotein (AFP), and abdominal MRI every month during the first half-year after liver resection and every 3 months thereafter for resected patients, but close observation every month was necessary to unresectable patients.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 (IBM, Armonk, NY, United States). Continuous data were presented as mean \pm SD or median (range), and compared with one-way analysis of variance (ANOVA) or the Kruskal-Wallis test among groups. Categorical variables were analyzed by Pearson's χ^2 or Fisher's exact test, as appropriate. Kaplan-Meier curve and the log-rank test were used to compare the survival among groups. A *P* value < 0.05 was considered statistically significant.

RESULTS

Patient and tumor characteristics

A total of 51 patients who met the eligibility criteria underwent TACE/PVE during the study period, with 13 patients in the siTP group, 17 patients in the seTP group and 21 patients in the PVE-only group. The baseline patient and tumor characteristics in the three groups are summarized in Table 1. No significant differences were found among these three groups regarding the baseline features.

Table 1 Demographic and pathological characteristics

Characteristics	siTP group (n = 13)	seTP group (n = 17)	PVE-only group (n = 21)	P value
Male, n (%)	10 (77)	13 (76)	17 (81)	0.935
Age (yr)	51.5 ± 10.0	54.0 ± 11.2	59.2 ± 8.3	0.068
Etiology, n (%)				0.928
HBV	9 (69)	12 (70)	15 (71)	
Alcoholic	1 (8)	2 (12)	2 (10)	
NASH	3 (23)	3 (18)	3 (14)	
Schistosomiasis	0	0	1 (5)	
ECOG performance status, n (%)				0.725
0	10 (77)	13 (76)	18 (86)	
1	3 (23)	4 (24)	3 (14)	
Tumor multiplicity, n (%)				0.809
Single	11 (85)	14 (82)	15 (71)	
Multiple	2 (15)	3 (18)	6 (29)	
Tumor size (cm)	8.4 ± 2.7	8.3 ± 2.3	7.7 ± 1.9	0.630
ICGR15 (%)	5.7 ± 3.0	5.0 ± 2.6	6.0 ± 2.7	0.511
Baseline FLR (%)	31.2 ± 3.7	31.8 ± 4.5	33.2 ± 3.5	0.150
AFP (ng/mL)	377 (11-3419)	487 (16-12000)	551 (9-10344)	0.640
ALT (U/mL)	30.2 ± 13.8	40.5 ± 26.0	40.4 ± 24.0	0.380
AST (U/mL)	39.5 ± 16.2	58.2 ± 40.0	49.5 ± 20.8	0.212
TBIL (μg/mL)	19.0 ± 5.1	17.4 ± 5.2	21.2 ± 6.9	0.147

HBV: Hepatitis B virus; NASH: Non-alcoholic steatohepatitis; ICGR15: Indocyanine green retention rate at 15 min; FLR: Future liver remnant; AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBIL: Total bilirubin; seTP: Sequential TACE + PVE; siTP: Simultaneous TACE + PVE.

Outcomes after preoperative TACE/ PVE

All patients underwent PVE with or without TACE. Common and minor complications included abdominal pain, fever, nausea, and transient LFTs elevation. All these complications were resolved by themselves or after symptomatic treatment. In the seTP group, one male patient suffered from acute pulmonary infarction after TACE because of an intrahepatic arteriovenous fistula. This case recovered after respiration support and PVE.

The mean ratio of FLR volume was similar in all groups before surgery ($45.9\% \pm 4.2\%$ vs $43.3\% \pm 6.6\%$ vs $43.0\% \pm 4.7\%$, $P = 0.262$). The mean interval from TACE/PVE to surgery was significantly lower in the siTP group (16.2 ± 2.7 d vs 37.9 ± 6.5 vs 35.4 ± 10.6 d, respectively, in the siTP, seTP, and PVE-only groups, $P < 0.001$), and the KGR of FLR was significantly higher in the siTP group than in the other groups ($21.1\% \pm 5.9\%$ vs $7.6\% \pm 2.9\%$ vs $6.8\% \pm 3.6\%$, $P < 0.001$) (Figure 2). The rate of tumor size reduction was 100% (13/13) in the siTP group, was 76% (13/17) in the seTP group, and was 10% (2/21) in the PVE-only group ($P < 0.001$) (Table 2).

The results of LFTs after PVE and before surgery in the three groups are shown in Figure 1. The total bilirubin (TBIL) levels were similar among the three groups before or after PVE/TACE intervention. The patients in the three groups showed comparable alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels before TACE/PVE intervention. Compared with those in the seTP group and PVE-only group, the patients in the siTP group experienced the most prominent elevation of AST (706.7 ± 376.8 U/L vs 321.3 ± 112.2 U/L vs 310.1 ± 153.0 U/L, $P < 0.001$) and ALT (641.2 ± 429.8 U/L vs 261.5 ± 95.7 U/L vs 227.4 ± 107.3 U/L, $P < 0.001$) after PVE and TACE (Figure 3). The transient elevation of AST and ALT levels in the siTP group was recovered to comparable levels with the other two groups before surgery.

Table 2 Outcomes after portal vein embolization with or without transarterial chemoembolization and before hepatectomy

Variable	siTP group (n = 13)	seTP group (n = 17)	PVE-only group (n = 21)	P value
FLR (%) (before hepatectomy)	45.9 ± 4.2	43.3 ± 6.6	43.0 ± 4.7	0.262
Interval from TACE/PVE to hepatectomy	16.2 ± 2.7	37.9 ± 6.5	35.4 ± 10.6	< 0.001
KGR (%)	21.1 ± 5.9	7.6 ± 2.9	6.8 ± 3.6	< 0.001
Tumor size reduction, n (%)	13 (100)	13 (76)	2 (10)	< 0.001
Complications, n (%)				0.875
Abdominal pain	6 (46)	9 (53)	3 (14)	
Fever	7 (54)	8 (47)	3 (14)	
Nausea	2 (15)	4 (24)	2 (10)	
Acute pulmonary infarction	0	1 (6)	0	

FLR: Future liver remnant; TACE: Transarterial chemoembolization; PVE: Portal vein embolization; KGR: Kinetic growth rate; seTP: Sequential TACE + PVE; siTP: Simultaneous TACE + PVE.

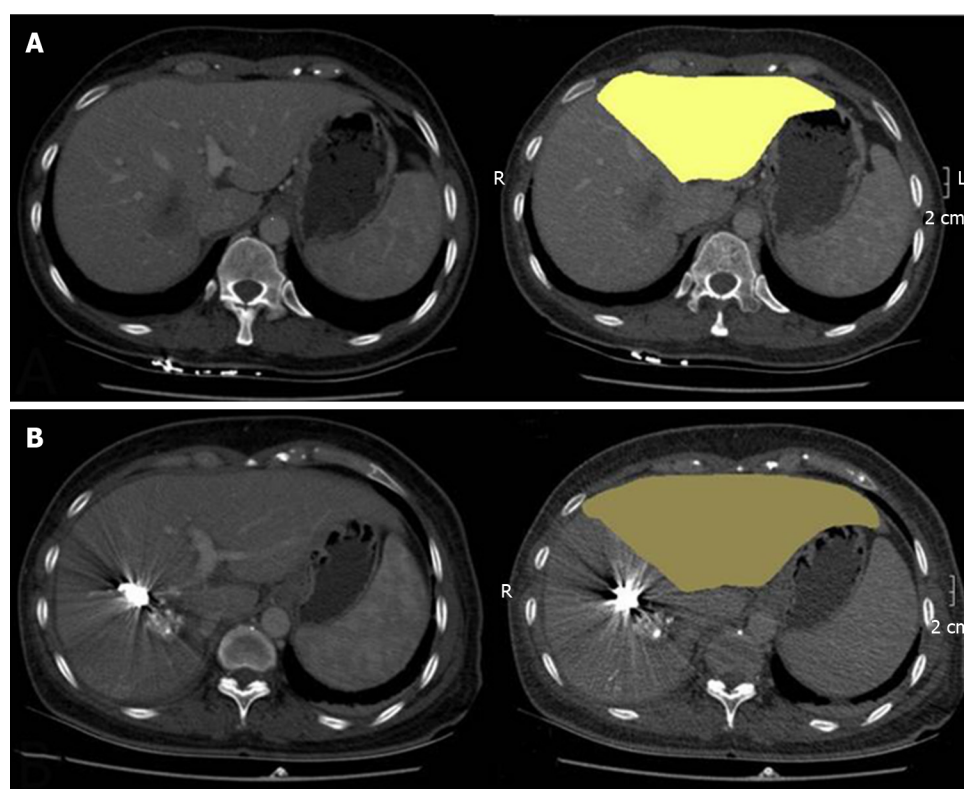


Figure 2 Future liver remnant volume of a patient was only 391 mL before simultaneous transarterial chemoembolization and portal vein embolization (A) and increased to 574 mL later by nearly 46% (B).

Operative course and overall and disease-free survival

Ten patients failed to undergo major hepatectomy due to inadequate remnant liver hypertrophy ($n = 6$) or extrahepatic metastasis ($n = 4$). The resection rate was 100% in the siTP group (13/13), which was higher than that in the seTP group (82%, 14/17) and the PVE-only group (67%, 14/21), but the difference was not significant ($P = 0.057$). Intraoperative course including operation time, blood loss, transfusion, and the incidence of complications were similar among the three groups, as shown in Table 3. One patient in the PVE-only group died of liver failure 7 days after surgery, despite medical management, but no significant difference in the mortality was observed among the groups.

The pathological examination of the resected specimens showed that MPR and PPR

Table 3 Intraoperative and postoperative outcomes of three groups

Variables	siTP group (n = 13)	seTP group (n = 17)	PVE-only group (n = 21)	P value
Patients with major hepatectomy, n (%)	13 (100)	14 (82)	14 (67)	0.057
Surgical approach, n (%)				0.868
Right hemihepatectomy	9 (69)	12 (71)	9 (43)	
Right hepatic trisegmentectomy	2 (15)	1 (6)	3 (14)	
Left hemihepatectomy	1 (8)	0	1 (5)	
Left hepatic trisegmentectomy	1 (8)	1 (6)	1 (5)	
Operation time (min)	261.4 ± 51.8	210.7 ± 53.7	246.1 ± 66.4	0.080
Intraoperative blood loss (mL)	285.0 ± 138.7	265.7 ± 153.6	343.9 ± 136.7	0.266
Transfusion, n (%)	4 (31)	4 (29)	2 (10)	0.550
Hospital stay after surgery (d)	10.9 ± 2.1	11.9 ± 3.9	12.3 ± 3.4	0.503
Complications, n (%)				0.842
Liver failure	1 (8)	0	2 (10)	
Biliary fistula	3 (23)	4 (24)	2 (10)	
Hydroperitonium	1 (8)	2 (12)	0	
Hydrothorax	2 (15)	1 (6)	1 (5)	
Abdominal infection	2 (15)	1 (6)	1 (5)	
Perioperative death, n (%)	0	0	1 (5)	0.372
Pathologic response, n (%)				< 0.001
MPR (RT < 10%)	6 (46)	2 (12)	0	
PPR (RT from 10% to 50%)	3 (23)	2 (12)	0	

MPR: Major pathological response; RT: Residual tumor cells; PPR: Partial pathological response; TACE: Transarterial chemoembolization; PVE: Portal vein embolization; seTP: Sequential TACE + PVE; siTP: Simultaneous TACE + PVE.

of tumor-induced by simultaneous TACE + PVE occurred in 9 of 13 patients, 4 of 17 in the seTP group and none in the PVE-only group ($P < 0.001$). Compared with those in the seTP or PVE-only group, the patients in the siTP group showed significantly longer median DFS ($P = 0.035$) and overall survival (OS) ($P = 0.022$) after major hepatectomy (Figure 4).

DISCUSSION

Sequential TACE and PVE are associated with long time interval that can allow tumor growth and nullify their benefits. Therefore, this study aimed to evaluate the effect of simultaneous TACE and PVE for patients with large HCC prior to elective major hepatectomy. The results suggest that simultaneous TACE and PVE is a safe and effective approach to increase FLR volume for patients with large HCC before major hepatectomy.

The risk of postoperative liver failure is increasing with more and more major hepatectomy was performed, as 70% of patients with HCC have liver cirrhosis^[4]. Therefore, the protocol of liver resection in the treatment of large liver tumors has been evaluated recently. As the most commonly used technique for increasing FLR volume, PVE is performed in many patients with HCC and has a low incidence of complications^[11]. Nevertheless, one drawback of PVE is inadequate liver hypertrophy, especially in patients with cirrhosis. More importantly, the possibility of tumor progression caused by the long interval between PVE and surgery and compensatory increase of arterial blood flow after cessation of the portal supply is unavoidable.

Sequential TACE and PVE, which was firstly reported by Makuuchi *et al*^[24], has been accepted as an effective preoperative treatment for patients with insufficient FLR and favorable hepatic reserve function. This approach has three main purposes: (1) To

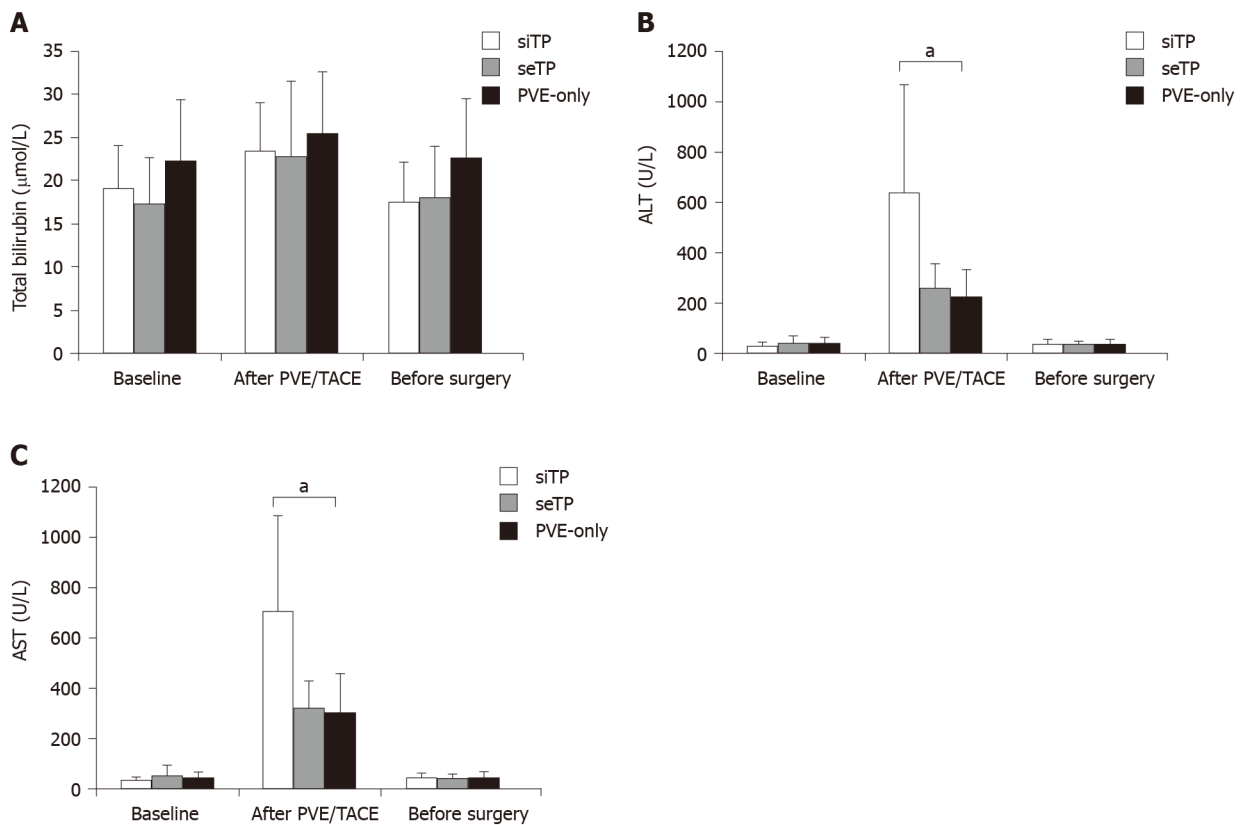


Figure 3 Liver function levels of the three groups from baseline to surgery (A-C). ^a $P < 0.05$ among the groups. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PVE: Portal vein embolization; TACE: Transarterial chemoembolization.

prevent tumor progression during the weeks between PVE and surgery; (2) To strengthen the effects of PVE by embolizing possible arterio-portal shunts (frequently found in HCC) whose blood contribution to the tumor may attenuate the effect of PVE; and (3) To improve the FLR volumetric increase through accelerating hepatocytes proliferation that may be induced by the increased inflammatory cytokine production as well as the massive HCC necrosis following the obstruction of both arterial and portal flow^[25]. Animal experiments showed that liver injury caused by TACE+PVE was mild and recoverable. TACE + PVE showed a stronger tumor-inhibiting effect than TACE or PVE alone and induced a higher level of tumor cell apoptosis^[26]. Sequential TACE and PVE before surgery is a safe and effective method to increase the rate of hypertrophy of the FLR and lead to longer OS and recurrence-free survival (RFS) in patients with large HCC. Indeed, Yoo *et al*^[14] reported the mean increase in percentage FLR volume, OS and RFS were significantly higher in the TACE +PVE group than in the PVE only group.

Nevertheless, the possibility of tumor progression caused by incomplete tumor necrosis after TACE and during the interval time between TACE and PVE remains an inevitable problem. Therefore, simultaneous TACE and PVE was proposed and performed in several liver cancer patients with small tumors located near the surface of the liver as a preoperative treatment and obtained satisfactory efficacy^[18]. One of the major concerns regarding simultaneous TACE and PVE is the risk of severe liver parenchymal necrosis resulting from the simultaneous occlusion of arterial and portal blood supply, which may influence the prognosis of patients. Therefore, the safety and efficacy of simultaneous TACE+PVE require urgent investigation in large numbers of patients, especially in those with large HCC.

In the present study, 13 patients with large HCC received simultaneous TACE + PVE before liver resection, and no liver failure occurred. TBIL level showed a minor and transient elevation in all three groups, which was consistent with previous data^[18]. Although the mean liver transaminases levels, including ALT and AST, peaked temporarily after simultaneous TACE + PVE and were significantly higher than in the seTP and PVE-only groups, they almost returned to normal before surgery. Therefore, we proposed that the transient transaminases elevation after simultaneous PVE and TACE result from tumor necrosis rather than from noncancerous liver parenchymal necrosis. This perspective is also supported by the data of higher mean RT of the

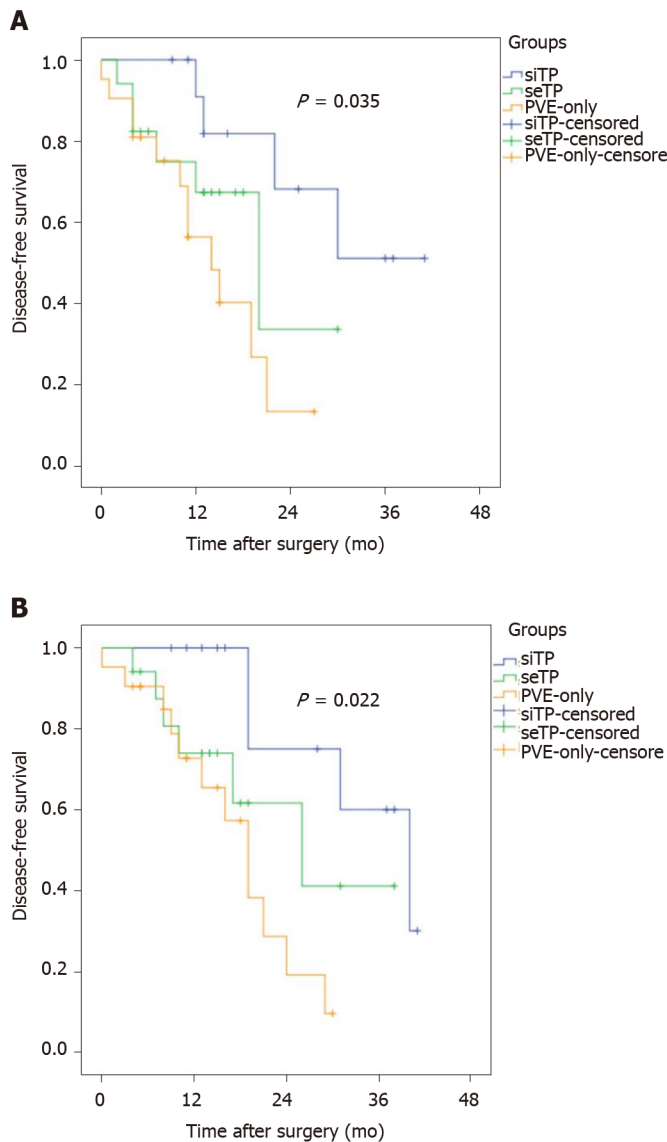


Figure 4 Disease-free survival (A) and overall survival (B) after major hepatectomy in patients with large hepatocellular carcinoma who underwent simultaneous transarterial chemoembolization + portal vein embolization, sequential transarterial chemoembolization + portal vein embolization, or portal vein embolization only. TACE: Transarterial chemoembolization; PVE: Portal vein embolization.

pathological specimens in the siTP group compared to that in the seTP and PVE-only groups, and mild liver parenchymal damage. It should be noted that all eligible patients had good liver function (ICGR15 < 10%) at baseline, which might be the reason why no liver failure occurred after TACE and/or PVE in all the three groups.

Although the mean FLR volume before surgery was similar in the three groups, the average interval time before surgery in the siTP group when the liver reached the minimum requirements of FLR volume was shorter than in the other two groups. More importantly, the KGR was significantly higher in the siTP group than in the seTP and PVE-only groups, indicating that simultaneous TACE + PVE is more efficient for increasing FLR volume than sequential TACE + PVE and PVE alone in patients with large HCC. We speculate that compared with sequential TACE + PVE or PVE alone, simultaneous PVE + TACE can lead to more thorough tumor necrosis, and thus more dramatic increase of inflammatory cytokines due to more obvious increase of blood flow of contralateral liver parenchyma. Remarkably, no significant differences were observed among the three groups regarding the operation time, blood loss, transfusion, and hospital stay after surgery, indicating that simultaneous TACE and PVE did not increase the difficulty of liver resection. In addition, no significant difference was found regarding the complications and perioperative mortality among the three groups, suggesting that simultaneous TACE and PVE did not increase postoperative complications.

We compared the survival among the three groups. The median OS and DFS were

longer in the siTP group than in seTP and PVE-only groups. This may be explained with following reasons: (1) Simultaneous TACE and PVE can completely block the hematogenous metastasis, which is a fundamental determinant of patients' survival; (2) It can induce more prominent tumor necrosis by the simultaneous occlusion of the double blood supply; and (3) It can shorten the wait time before surgery, which further reduces the chance of tumor progression.

This study has limitations. Data of portal pressure were not available to evaluate the effect of the major hepatic resections because portal pressure was not routinely measured in our center. The sample size was too small to conduct a multivariable analysis of the outcomes. Therefore, the conclusions can only be used as preliminary evidence that should be further confirmed in larger groups of patients and with longer follow-up.

In conclusion, our study demonstrates that simultaneous TACE and PVE is a safe and effective approach to increase the FLR volume quickly in a short time for patients with large HCC before major hepatectomy.

ARTICLE HIGHLIGHTS

Research background

Sequential transarterial chemoembolization (TACE) and portal vein embolization (PVE) can improve the clinical outcomes and survival of patients with large hepatocellular carcinoma (HCC). However, the sequential treatment needs long wait time that can allow tumor growth and nullify treatments' benefits.

Research motivation

No study has ever compared the results of simultaneous TACE and PVE *vs.* sequential TACE and PVE or *vs.* PVE alone.

Research objectives

To evaluate the effect of simultaneous TACE and PVE before major hepatectomy in patients with large HCC and to compare their clinical outcome with sequential TACE+PVE or PVE only.

Research methods

Fifty-one patients with large HCC who underwent PVE combined with or without TACE prior to major hepatectomy were included in this study, with 13 patients in the simultaneous TACE + PVE group, 17 patients in the sequential TACE + PVE group, and 21 patients in the PVE-only group. The outcomes of the procedures were compared and analyzed.

Research results

All patients underwent embolization. The mean interval from embolization to surgery, the kinetic growth rate of the future liver remnant (FLR), the degree of tumor size reduction, and complete tumor necrosis were significantly better in the simultaneous TACE + PVE group than in the other two groups. Although the patients in the simultaneous TACE + PVE group had a higher transaminase levels after PVE and TACE, they recovered to comparable levels with the other two groups before surgery. The intraoperative course and the complication and mortality rates were similar among the three groups. The overall survival and disease-free survival were higher in the simultaneous TACE + PVE group than in the other two groups.

Research conclusions

Simultaneous TACE and PVE is a safe and effective approach to increase FLR volume for patients with large HCC who need major hepatectomy.

Research perspectives

Although the data were extracted from a prospective database, portal pressure data were not available because portal pressure was not routinely measured in our center. Prospective studies are needed to verify the present preliminary evidence, with available data of portal pressure. Multicenter, randomized controlled trials with large sample size and long-term follow-up should be conducted to confirm our results.

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Clinical Trials Study

Efficacy of a Chinese herbal formula on hepatitis B e antigen-positive chronic hepatitis B patients

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Institutional review board

statement: The study was reviewed and approved by the Institutional Review Board of Shenzhen Traditional Chinese Medicine Hospital, The Fourth Clinical Medical College of Guangzhou University of Chinese Medicine.

Clinical trial registration statement:

This study was registered at Chinese Clinical Trial Registry. The registration identification number is ChiCTR-IPR-17011944 (11/07/2017) (<http://www.chictr.org.cn/index.aspx>).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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Abstract

BACKGROUND

No guideline recommends antiviral therapy for hepatitis B e antigen (HBeAg)-positive chronic hepatitis B patients with persistently normal alanine aminotransferase levels and a high hepatitis B virus (HBV) DNA viral load.

AIM

To evaluate the feasibility and safety of a Chinese herbal formula as a therapeutic option for chronic HBV infection.

METHODS

In total, 395 patients (30–65 years old) with confirmed HBeAg-positive chronic hepatitis B infection and persistently normal alanine aminotransferase were randomized to receive either Chinese herbal formula or placebo for 96 wk. Endpoints to evaluate therapeutic efficacy included: (1) HBV DNA levels decreased to less than 4 log₁₀ IU/mL at weeks 48 and 96; and (2) HBeAg clearance and seroconversion rates at weeks 48 and 96.

RESULTS

HBV DNA levels $\leq 4 \log_{10}$ IU/mL were 10.05% at week 48 and 18.59% at week 96 in the treatment group. The HBeAg clearance and conversion rates were 8.54% and 8.04% at week 48 and 16.08% and 14.57% at week 96, respectively. However, HBV DNA levels $\leq 4 \log_{10}$ IU/mL were 2.55% and 2.55% at weeks 48 and 96, respectively, and the HBeAg clearance rates were 3.06% and 5.61% at weeks 48 and 96, respectively, in the control group. The quantitative hepatitis B surface antigen and HBeAg levels at baseline and changes during the treatment period as well as the alanine aminotransferase elevation at weeks 12 and 24 were strong predictors of HBeAg clearance.

CONCLUSION

High rates of HBV DNA reduction, HBeAg clearance and seroconversion could be achieved with Chinese herbal formula treatments, and the treatments were relatively safe for HBeAg-positive chronic hepatitis B-infected patients with persistently normal alanine aminotransferase. The ability of the compound to modulate host immune function probably contributed to this effect.

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Core tip: Hepatitis B e antigen-positive chronic hepatitis B patients with persistently normal alanine aminotransferase levels and a high hepatitis B virus DNA viral load may progress to cirrhosis or hepatocellular carcinoma. However, no guideline recommends antiviral therapy for it because of poor efficacy. In the present study we report the feasibility and safety profiles of a Chinese herbal formula as a therapeutic option for chronic hepatitis B virus infection.

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INTRODUCTION

Most patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B virus (HBV) infection with basically normal alanine aminotransferase (ALT) levels and high viral load have no obvious clinical symptoms^[1-4]. Antiviral therapy is not recommended for these patients by any authoritative guidelines. Despite long-term normal ALT levels, a high HBV DNA viral load persists, and liver lesions progress unrecognized and advance gradually. Some patients may even progress to cirrhosis or hepatocellular carcinoma (HCC), and the risk increases with age, especially after the age of 30. Even if a chronic HBV carrier shows minimal or no necroinflammation or fibrosis in the liver (previously termed the “immune tolerant” phase), a high level of HBV DNA integration and clonal hepatocyte expansion suggests that hepatocarcinogenesis could be already underway in this early phase of the infection^[5].

Previous studies have shown that liver injury in chronic hepatitis B (CHB) patients with normal ALT levels was always mild, and the long-term clinical outcomes were not serious^[6,7]. A long-term follow-up study in Taiwan of China, which enrolled 240 HBeAg-positive patients with normal ALT levels and had a median follow-up period of 6.8 years (1-17 years) and a mean age at entry of 27 years, showed that 85% of the patients had HBeAg seroconversion and sustained remission. The cumulative incidence of cirrhosis in 17 years was 12.5%, and the cumulative incidence of HCC was 0%^[8]. However, for patients over 35 years old, another study in Taiwan (REVEAL) reported a median follow-up of 7 years, HBeAg clearance of 187 (43.4%) and an annual incidence of only 6.2%^[9]. As accumulating research data show, age is proportional to the progression of CHB. The REVEAL study also showed that HBV DNA was an independent predictor of hepatitis B progression in patients over 35 years old, and the incidence of cirrhosis increased with HBV DNA level (300 copies/mL-10⁶ copies/mL), which was 4.5%-36.2%. In this study, the corresponding cumulative incidence of hepatocellular carcinoma was 1.3% and 14.9% in patients with HBV DNA < 300 copies/mL and HBV DNA > 10⁶ copies/mL, respectively^[10,11]. HBV DNA level was independent of HBeAg status, ALT level and other risk factors^[12]. Another study concerning HBeAg seroconversion showed that in CHB patients with e antigen seroconversion before age 40, only 4.1% would progress to cirrhosis, while with e antigen seroconversion after age 40 and 50, the incidence of cirrhosis was 27.3% and 33.3%, respectively^[13]. Liver biopsy also indicated a gradient relationship between fibrosis severity and age^[14]. Therefore, in recent years, Chinese guidelines and European Association for the Study of the Liver guidelines have lowered the age for monitoring antiviral therapy in CHB patients with normal ALT from 40 years old to 30 years old^[1,4].

For HBeAg-positive CHB patients with normal ALT, antiviral therapy was not

recommended by various authoritative guidelines, mainly due to poor efficacy. The results of a small sample study of pegylated interferon for the treatment of HBV carriers reported that the seroconversion rate was below 10%^[15]. However, another lamivudine study showed that the seroconversion rate was only 2% in HBV carriers^[16]. In recent years, clinical trials of vaccines against HBV have all ended in failure^[17]. With the advent of new potent antiviral drugs, the recently published 192 wk study of patients with CHB in the immune tolerant stage of tenofovir therapy showed that 5% of patients achieved e antigen seroconversion. Although more than 50% of patients had reached HBV DNA clearance during the treatment, they all relapsed 6 mo after drug withdrawal, suggesting that the efficacy of antiviral therapy for such patients was unsatisfactory^[18].

We have more than 20 years of clinical experiences in treating CHB infection and chronic carriers with invigorating kidney and clearing away the heat and expelling superficial evils (ICE) formula. In our previous studies, we recruited 62 patients with CHB and treated with ICE. Results showed that the HBeAg clearance and virologic response of the treated group were significantly better than that of the control group^[19]. Preliminary clinical multicenter research of short courses of treatment during the national 11th five-year period project indicated that the decrease of HBV DNA was greater than 2 log by 17.5%, and the decrease of hepatitis B surface antigen (HBsAg) by 1 log was about 10% after 52 wk of ICE intervention^[20-22]. In addition, liver histological results showed significant improvements in liver fibrosis, and immunohistochemistry showed significant decreases in the expression of HBsAg and hepatitis B core antigen (HBcAg) in responding patients. These studies suggest that ICE has a better effect on interfering with chronic HBV carriers^[23].

Through this study, the effects of the ICE formula on patients with HBeAg-positive CHB with normal ALT who were over 30 were evaluated, and serological indexes, HBV DNA changes and related factors were analyzed. This study provided clinical evidence for traditional Chinese medicine (TCM) treatment for chronic HBV carriers, especially chronic HBV carriers over 30 years old with a higher risk of disease progression.

MATERIALS AND METHODS

Patient population

HBeAg-positive patients with chronic HBV infections were recruited from May 2013 to May 2014 at 20 different hospitals and medical centers for this study (Table 1). A total of 395 patients were enrolled. The inclusion and exclusion criteria used for patient selection are shown in Table 2.

The study was approved by the Ethics Committees at Shenzhen Hospital affiliated with the Fourth Clinical Medical College of Guangzhou University of TCM and was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and the Good Clinical Practice Guidelines. All enrolled patients gave written informed consent before enrollment. The clinical trial registration identifier is ChiCTR-TPR-17011944 (<http://www.chictr.org.cn/index.aspx>).

Preparation of medication

The Chinese herbal formula (ICE granules) was composed of *Phyllanthus urinaria* Linn, *Radix et caulis acanthopanacis senticosi*, *Herba Epimedii*, and so forth, which are listed in Table 3. The placebo was composed of water-soluble starch, glucosum anhydricum, edible chocolate brown pigment and lyochromes. Both were made into drug granules in Shenzhen Sanjiu Medical & Pharmaceutical Co., Ltd., China, a renowned good manufactory practice-certified state-level manufacturer of concentrated herbal extracts (its products can be purchased in China). The whole production process, from validating the raw materials to the final products, strictly complied with the standards of good manufactory practice and Chinese pharmacopoeia^[24]. Decoction and extraction of each dried medicinal herb was performed in a single batch. After extraction, the herbal preparation was separated, concentrated and spray dried into the form of a granule. The chemical compositions of the final products were analyzed, while all the herbal preparations were tested to ensure safety for human consumption, including heavy metals, microorganism contamination and insecticides. Finally, the different kinds of granules were mixed in accordance with their proportion in the Chinese herbal formula and packed in sealed plastic sachets. The composition of a sachet of granules (32.67 g) was the same as that of 190 g raw herbs, which was the daily dose of each patient. The placebo was similar to the herbal granules in shape, color, taste and

Table 1 Hospitals or medical centers that participated in this study

Name	Location (city and province)
Shanghai Shuguang hospital, Shanghai University of Traditional Chinese Medicine	Shanghai
The Second Hospital Affiliated with Zhejiang University of Traditional Chinese Medicine	Hangzhou, Zhejiang
Xiamen Hospital of Traditional Chinese Medicine	Xiamen, Fujian
Shenzhen Hospital Affiliated with Guangzhou University of Chinese Medicine	Shenzhen, Guangdong
Foshan Hospital of Traditional Chinese Medicine	Foshan, Guangdong
The Third People's Hospital of Shenzhen	Shenzhen, Guangdong
Guangdong Hospital of Traditional Chinese Medicine	Guangzhou, Guangdong
The Third Affiliated Hospital of Sun Yat-sen University	Guangzhou, Guangdong
Ruikang Hospital of Guangxi College of Traditional Chinese Medicine	Nanning, Guangxi
The First Affiliated Hospital of Guangxi College of Traditional Chinese Medicine	Nanning, Guangxi
Attached Hospital of Chengdu University of Traditional Chinese Medicine	Chengdu, Sichuan
West China Hospital, West China School of Medicine, Sichuan University	Chengdu, Sichuan
Beijing Ditan Hospital, Capital Medical University	Beijing
Xiyuan Hospital, China Academy of Traditional Chinese Medicine	Beijing
302 Military Hospital of China	Beijing
Tianjin Infectious Disease Hospital	Tianjin
Beijing Youan Hospital, Capital Medical University	Beijing
Hubei Provincial Hospital of TCM	Wuhan, Hubei
People's Hospital of Wuhan University	Wuhan, Hubei
The First Hospital of Hunan University of Chinese Medicine	Changsha, Hunan

Table 2 Inclusion and exclusion criteria of patients

Inclusion criteria	Exclusion criteria
Conform with the diagnostic criteria of HBeAg (+) chronic hepatitis B	Inactive HBsAg (+) carriers
Conform with the pathogenesis and syndromes of kidney deficiency	Serum a-fetoprotein abnormal
Age 30-65 yr	Pregnancy or breast feeding
ALT \leq 40 IU/L	Coinfection with HIV, HCV, HDV
HBsAg $>$ 10 IU/mL and $<$ 10 ⁵ IU/mL HBV DNA (10 ⁵ -10 ⁹ IU/mL)	Histologic evidence of cirrhosis; Evidence of any other chronic liver disease
Liver biopsy: Liver histology showed Knodell HAI $>$ 4, Ishak fibrosis score $>$ 3 were also included	Mental illness or any other serious systemic illness
Voluntary	Interferon- γ within 6 mo; Antivirus treatment with nucleoside
	Abuse alcohol or illegal drugs; Allergic to the drug ingredients

HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HAI: Histological activity index.

packaging.

Study design

The study was a multicenter, randomized, double-blinded and placebo-controlled clinical trial of the Chinese herbal formula versus placebo at a ratio of 1:1 for 96 wk. Each patient was instructed to dissolve a sachet of granules (32.67 g, either study drug or placebo) in 200 mL of warm water in a cup and to take 100 mL of the solution in the morning and the rest in the afternoon every day.

Table 3 The list of raw herbs composing the Chinese herbal formula

Chinese name	Latin name	Parts of plant used	Dose of dryplant (grams)	Dose after extraction (grams)
Ye xia zhu	<i>Phyllanthus urinaria</i> Linn	Whole plant	30	12.00
Ci wu jia	<i>Radix et caulis acanthopanacis senticosi</i>	Root and rhizome	10	0.50
Xian ling pi	<i>Herba Epimedii</i>	Overground part	30	1.50
Nv zhen zi	<i>Fructus ligustri lucidi</i>	Mature fruit	15	1.50
Han lian cao	<i>Herba ecliptae</i>	Overground part	15	1.50
Chai hu	<i>Radix bupleuri</i>	Root	10	1.67
Bai shao	<i>Radix paeoniae alba</i>	Root	10	1.00
Zhi shi	<i>Fructus aurantii immaturus</i>	Fruitlet	10	1.67
Tao ren	<i>Semen persicae</i>	Nuts	10	0.50
Gan cao	<i>Radix glycyrrhizae</i>	Root and rhizome	5	0.83
Hu zhang	<i>Rhizoma polygoni cuspidati</i>	Root and rhizome	15	1.00
Xi huang cao	<i>Herba rabdosiae serrae</i>	Whole plant	30	9.00
Total			190	32.67

Randomization was performed within one month after the screening had been completed using a voice interactive random assortment system^[25]. Tests were carried out at week 0, 4 and 12 and then every 12 wk thereafter through week 96. At each clinic visit, laboratory tests were performed to evaluate liver function and determine the safety of treatment and possible adverse events. Serum was assayed for HBV DNA, HBsAg, antibody to HBsAg, HBeAg and antibody to HBeAg at baseline and at weeks 24 and 48. Serum helper T1 cell and helper T2 cell cytokine levels, including interleukin (IL)-2, IL-4, IL-10 and interferon- γ (IFN- γ), were detected at baseline and at weeks 48 and 96. Patients were withdrawn from the study for any of the following reasons: Occurrence of intolerable or worsening adverse events and failure to comply with the protocol or withdrawal of consent.

Laboratory assays

All subjects undergoing blood testing were uniformly assayed in the central laboratory of Shanghai Amidikang Medical Laboratory, China. All subjects underwent complete blood counts and serum biochemistry detections, including ALT, aspartate transaminase, platelet, γ -glutamyltransferase, blood urea nitrogen and creatinine tests with the Cobas ISE 800 chemistry analyzer (Roche Diagnostics, Holliston, MA, United States)^[26]. HBsAg, hepatitis B surface antibody, HBeAg, hepatitis B envelope antibody and hepatitis B core antibody were measured with the Architect i2000 assay (Abbott Laboratories, Philippines)^[27]. The HBsAg titer in serum was quantified according to the manufacturer's instructions. An initial manual dilution of 1:100 was performed on all samples. Samples with HBsAg titers of greater than 250 IU/mL were manually diluted to 1:500 to bring the reading within the linear range. Samples with HBsAg levels of less than 0.05 IU/mL at 1:100 dilution were retested undiluted. Serum cytokine levels of IL-2, IL-4, IL-10 and IFN- γ were detected by ELISA kits (Pharmingen, San Diego, CA, United States) according to the manufacturer's instructions. Serum HBV DNA levels were quantified using the Cobas TaqMan assay (Roche Diagnostics, Branchburg, NJ, the United States) with the lowest detection limit at 20 IU/mL.

Liver biopsy

All subjects underwent percutaneous liver biopsy guided by ultrasonography^[28]. Liver biopsy was performed using 16-G Tru-Cut biopsy needles (Menghini, Bard Company of the United States). A minimum of 1.5 cm of liver tissue with at least six portal tracts was required for appropriate diagnosis. The specimens were immediately fixed, paraffin-embedded, stained with hematoxylin-eosin and sent to the Department of Pathology at the Shenzhen Traditional Chinese Medicine Hospital. The Knodell histological activity index (HAI)^[29] and Ishak's system^[30,31] were used by two experienced pathologists who were blinded to the clinical information of the subjects to grade the collected samples. The Knodell HAI was used to describe the hepatocellular necroinflammation activity with grades of 0 \pm 4, while liver fibrosis was

semiquantitatively assessed according to Ishak's system and was graded from stage 0 to stage 6.

End points

The primary efficacy end point was the proportion of patients with a virologic response at weeks 48 and 96 (including HBV DNA levels decreasing at least 2 log₁₀ units and less than 4 log₁₀ IU/mL). Secondary efficacy end points were the proportion of patients with HBeAg loss or seroconversion to anti-HBe at weeks 48 and 96. In addition, adverse events including symptoms, signs and clinical laboratory abnormalities within 96 wk were documented, and discontinuation of therapy was recorded.

Sample size determination^[32-34]

Multicenter randomized double-blind control, ICE group: the placebo group was randomized 1:1, the viral response rate (viral load decreased by 2 log after treatment) was the main effect index, and the sample content was estimated by SPSS 22.0 according to the 11th five-year "national special program for major infectious diseases" research data. The TCM treatment group 2-year virologic response rate was 25%, that of the placebo control group was 5%, and the research on the basis of the optimized treatment plan chooses a better response rate crowd (10^{-5} - 10^{-9}). Two years is expected to make the TCM group virologic response rate of 30%, and the control group was 5%. According to the rate difference between the two groups, $P_1 = 5\%$, $P_2 = 30\%$, $\alpha = 0.05$, $\beta = 0.20$, with an estimated total of 278 cases. According to our previous data of the 11th five-year "national special program for major infectious diseases," the empirical sample shedding rate was $< 10\%$, and the adjusted sample content was 306 cases. Therefore, we chose to randomly enroll 400 cases in total or 200:200 cases (experimental group:control group).

Of the 400 patients initially screened, 5 were excluded, and a total of 395 patients were included in the treatment group (199 cases) and the control group (196 cases). During 96 wk of follow-up, 13 cases and 22 cases dropped out, respectively. The dropout rate was 6.5% and 11.2%, respectively, meeting the criteria of lost to follow-up (Figure 1).

Statistical analysis

The intention-to-treat analysis included all patients who were randomly allocated to one of the two groups. A last observation carried forward analysis was conducted for any missing data on primary or secondary outcomes. Analysis of safety included data for all patients who had taken at least one dose of study medication after randomization. SPSS 22.0 package (SPSS Inc., Chicago, IL, United States) was used to perform the analysis. Continuous variables were expressed as the mean \pm standard deviation. An independent samples *t*-test was used to compare differences between the two groups. A paired samples *t*-test was performed to calculate differences between prior and after treatment in one group. Categorical variables were expressed as absolute and relative frequencies. The Chi-square test or Fisher's exact test were used to compare the differences in proportions between the two groups. Univariable and multivariable logistic regression analyses were conducted to evaluate the magnitude and significance of the association. A two-sided *P* value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

Four hundred patients were planned to be enrolled in this project, while 395 patients were actually enrolled, conforming to the inclusion criteria. Each group was balanced. The two main visit time nodes for statistical analysis were at weeks 48 and 96. All the data were statistically analyzed by the Capital Medical University School of Public Health. The project team received blinded results from the clinical evaluation center of Chinese Academy of TCM on March 13, 2015, which is group A: ICE, Group C: the placebo control. The relevant main index data are described in Table 4.

Result of treatment

Virologic response: The proportion of patients with reduced HBV DNA levels of > 2 log₁₀ IU/mL was 15.08% (30/199) at week 48 and 30.15% (60/199) at week 96 for the

Table 4 Baseline characteristics of all study patients

Variable	Treatment (ICE) group (n = 199)	Control group(n = 196)	$\chi^2/t/Z$	P value
Age (mean \pm SD), yr	38.51 \pm 7.63	38.90 \pm 7.54	-0.904	0.366 ^a
Range	30-65	30-63		
Sex, n (%)			0.013	0.910 ^b
Male	128 (64.3)	125 (63.8)		
Female	71 (35.7)	71 (36.2)		
Regions (N)			2.796	0.593 ^b
Eastern	30 (14.42)	28 (14.97)		
Western	22 (10.58)	18 (9.63)		
Southern	89 (42.79)	69 (36.90)		
Northern	40 (19.23)	48 (25.67)		
Central	27 (12.98)	24 (12.83)		
Smoking			0.491	0.484 ^b
Yes	57 (28.64)	50 (25.51)		
No	142 (71.36)	146 (74.49)		
Alcohol consumption			3.626	0.057 ^b
Yes	18 (9.05)	30 (15.31)		
No	181 (90.95)	166 (84.69)		
Genotype			0.522	0.770 ^b
B	99 (49.75)	93 (47.45)		
C	90 (45.23)	95 (48.47)		
D	10 (5.02)	8 (4.08)		
Genealogy of hepatocellular carcinoma	2 (1.00)	3 (1.53)	0.000	0.986 ^b
Clinical course (mean \pm SD) week	90.21 \pm 22.40	86.69 \pm 27.20	-1.607	0.108 ^a
Liver function (mean \pm SD)				
ALT, IU/L	29.29 \pm 8.19	30.12 \pm 6.32	1.126	0.261 ^c
AST, IU/L	24.86 \pm 7.53	25.79 \pm 6.19	1.340	0.181 ^c
TB, μ mol/L	14.34 \pm 3.25	13.98 \pm 4.15	0.961	0.337 ^c
HBV DNA baseline level, (%)			0.154	0.695 ^b
2 to < 5 log ₁₀ IU/mL	0 (0)	0 (0)		
5 to < 7 log ₁₀ IU/mL	14 (2.01)	12 (1.02)		
7 to < 9 log ₁₀ IU/mL	185 (97.99)	184 (98.98)		
HBsAg (mean \pm SD), log ₁₀ IU/mL	3.86 \pm 0.52	3.89 \pm 0.42	-0.758	0.449 ^a
HBeAg (mean \pm SD), SCO/mL	1138.18 \pm 423.99	1158.40 \pm 401.86	-0.393	0.695 ^a
HBeAb (mean \pm SD), SCO/mL	38.43 \pm 14.28	40.01 \pm 12.15	-0.904	0.366 ^a
Histological scores				
Knodell (HAI), n (%)	168 (84.40)	156 (79.60)	0.010	0.922 ^b
≥ 4	60 (35.71)	55(35.26)		
< 4	108 (64.29)	101 (64.74)		
Ishak (FIB), n (%)			0.035	0.851 ^b

≥ 2	67 (39.90)	60 (38.46)
< 2	101 (60.10)	96 (61.54)

^a: Mann-Whitney U test;

^b: Chi-square test;

^c: *t*-test. ICE: Invigorating kidney and clearing away the heat and expelling superficial evils; SD: Standard deviation; ALT: Alanine aminotransferase; AST: Aspartate transaminase; TB: Total bilirubin; HBV: Hepatitis B virus; HBsAg: Hepatitis B virus surface antigen; HBeAg: Hepatitis B virus e antigen; HBeAb: Hepatitis B virus e antibody.

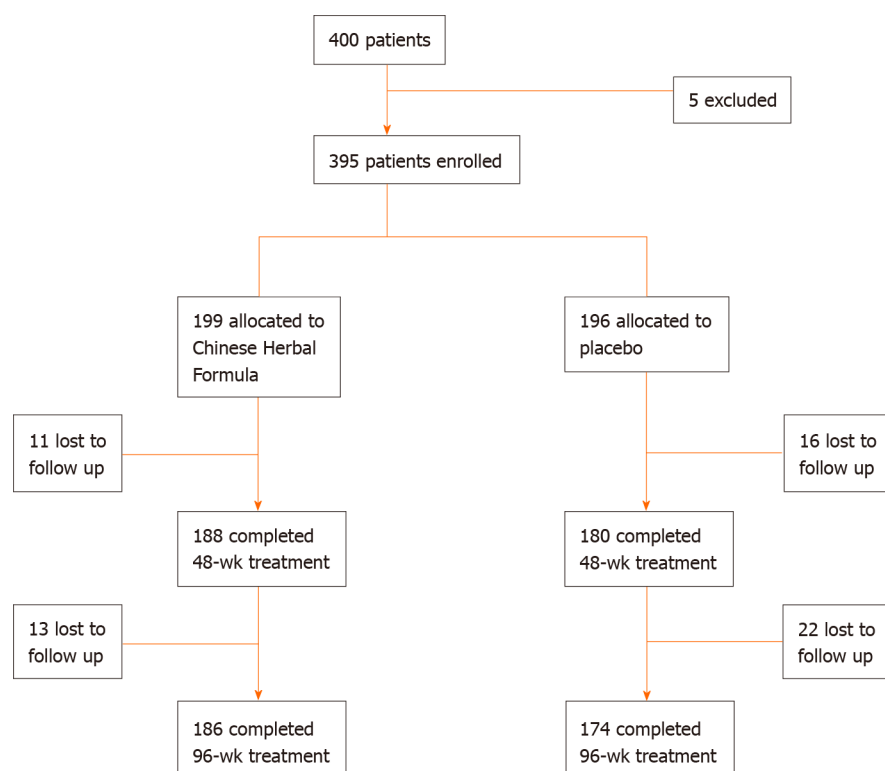


Figure 1 Study flowchart.

treatment group compared to 6.63% (13/196, $P = 0.007$) and 6.12% (12/196, $P = 0.000$), respectively, for the control group. The percentages of patients with HBV DNA levels $\leq 4 \log_{10}$ IU/mL were 10.05% (20/199) at week 48 and 18.59% (37/199) at week 96 for the treatment group compared to 2.55% (5/196, $P = 0.002$) and 3.06% (6/196, $P = 0.00$), respectively, for the control group. Among patients in the treatment group, serum HBV DNA was undetectable in 1.01% (2/199) at week 48 and 2.01% (4/199) at week 96; in the control group, it was in 0% (0/196) and 0% (0/196), respectively. There was no significant difference between the two groups ($P = 0.159$ and $P = 0.136$, respectively, Table 5).

Serological response: The proportion of patients with a decline $\geq 0.5 \log_{10}$ in HBsAg levels in the treatment group was 24.62% (49/199) at week 48 and 41.71% (83/199) at week 96 compared to the control group, which was 9.69% (19/196, $P = 0.000$) and 20.92% (41/196, $P = 0.000$), respectively. The percentages of patients with a decline $\geq 1 \log_{10}$ in HBsAg levels in the treatment group were 14.57% (29/199) at week 48 and 31.66% (63/199) at week 96 compared to the control group, which was 5.61% (11/196, $P = 0.003$) and 11.22% (22/196, $P = 0.000$), respectively. Furthermore, the proportion of patients with a decline $\geq 2 \log_{10}$ in HBsAg level in the treatment group was 3.52% (7/199) at week 48 and 8.54% (17/199) at week 96 compared to the control group, which was 1.02% (2/196, $P = 0.185$) and 0.51% (1/196, $P = 0.008$), respectively. Neither group had patients with HBsAg ≤ 0.05 at weeks 48 and 96 (Table 6).

The percentage of patients with a decline $\geq 1 \log_{10}$ in HBeAg levels in the treatment group was 22.61% (45/199) at week 48 and 25.63% (51/199) at week 96 compared to that of the control group, which was 2.55% (5/196, $P = 0.000$) and 4.59% (9/196, $P =$

Table 5 Virologic response and change in serum hepatitis B virus DNA level after treatment

Treatment response	Treatment (ICE) group (n = 199)	Control group (n = 196)	χ^2/Z	P value
48 wk				
Patients with HBV DNA level decline > 2 log ₁₀ IU/mL, n (%)	30 (15.08)	13 (6.63)	7.255	0.007
Patients with HBV DNA level ≤ 4 log ₁₀ IU/mL, n (%)	20 (10.05)	5 (2.55)	9.367	0.002
Patients with undetectable HBV DNA (≤ 20 IU/mL), n (%)	2 (1.01)	0 (0.00)	1.980	0.159
96 wk				
Patients with HBV DNA level decline > 2 log ₁₀ IU/mL, n (%)	60 (30.15)	12 (6.12)	38.249	0.000
Patients with HBV DNA level ≤ 4 log ₁₀ IU/mL, n (%)	37 (18.59)	6 (3.06)	24.555	0.000
Patients with undetectable HBV DNA (≤ 20 IU/mL), n (%)	4 (2.01)	0 (0.00)	2.227	0.136

Chi-square test. ICE: Invigorating kidney and clearing away the heat and expelling superficial evils; HBV: hepatitis B virus.

0.000), respectively. In the treatment group, 8.54% of patients (17/199) at week 48 and 16.08% (32/199) at week 96 demonstrated HBeAg clearance compared to the control group, which was 2.55% (5/196, $P = 0.009$) and 5.61% (11/196, $P = 0.001$), respectively. Seroconversion rates of HBeAg in the treatment group were 8.04% (16/199) at week 48 and 14.57% (29/199) at week 96 compared to the control group, which was 2.04% (4/196, $P = 0.007$) and 4.46% (9/196, $P = 0.001$), respectively (Table 6). In both groups, patients exhibited declines in HBsAg and HBeAg levels (Figure 2).

Serum cytokine levels: After 48 wk of ICE treatment, patients showed a significant increase in the mean levels of serum IFN- γ and IL-2 compared to the levels of these cytokines determined prior to treatment (27.80 ± 20.26 vs 19.90 ± 14.69 , $P = 0.000$; 13.76 ± 11.74 vs 9.97 ± 6.52 , $P = 0.028$, respectively). At week 96, in the ICE group, IFN- γ and IL-2 levels increased (57.54 ± 38.62 vs 20.08 ± 18.54 , $P = 0.000$; 15.92 ± 7.54 vs 8.59 ± 6.21 , $P = 0.000$, respectively). IFN- γ and IL-2 levels were not changed in the control group ($P > 0.05$). In addition, there was a marked decrease in the mean serum levels of IL-4 and IL-10 at week 48 (5.61 ± 3.83 vs 9.07 ± 6.27 , $P = 0.000$; 5.85 ± 3.14 vs 8.57 ± 4.33 , $P = 0.000$, respectively) and at week 96 (4.41 ± 3.37 vs 8.97 ± 7.75 , $P = 0.000$; 3.92 ± 2.31 vs 8.27 ± 5.49 , $P = 0.000$, respectively). In contrast, there were no differences in the levels of serum IL-4 and IL-10 before and after treatment with placebo in the control group ($P > 0.05$, Figure 3).

Moreover, among the ICE group patients, IFN- γ and IL-2 levels increased significantly ($P = 0.000$; $P = 0.000$, respectively), while IL-4 and IL-10 levels decreased significantly ($P = 0.003$; $P = 0.000$, respectively) at week 96 compared with week 48 (Figure 3).

Week 12 to week 48 ALT elevation, HBeAg and HBsAg levels and IFN- γ and IL-2 elevation associated with HBeAg clearance

At weeks 12 and 24, 15.58% (31/199) and 18.09% (36/199), respectively of the subjects in the treatment group showed an elevated ALT level (> 50 IU/L) with a maximum of 594 IU/L, and total bilirubin levels were all < 35 mmol/L. To assess the effects of the quantitative HBeAg and HBsAg levels and changes during the early period of treatment, we assessed the HBeAg and HBsAg levels at baseline, week 24 change from baseline and week 36 change from baseline using univariable logistic regression analysis. The results showed that baseline HBeAg [odds ratio (OR), 1.653, $P = 0.03$] and HBsAg (OR, 2.431, $P = 0.004$), week 24 HBeAg change from baseline (OR, 2.762, $P < 0.001$), week 36 HBeAg change from baseline (OR, 3.411, $P < 0.01$), week 24 HBsAg change from baseline (OR, 4.458, $P < 0.001$), week 36 HBsAg change from baseline (OR, 5.371, $P < 0.001$), week 12 ALT elevation (OR, 2.676, $P = 0.016$), week 24 ALT elevation (OR, 3.373, $P = 0.003$), week 48 IFN- γ elevation (OR, 2.735, $P = 0.002$) and week 48 IL-2 week 2 clearance (OR, 2.003, $P = 0.008$) were strong predictors for HBeAg clearance at week 96. Baseline sex, age and HBV DNA level were not statistically significant (Table 7).

To further evaluate baseline and changes in HBeAg and HBsAg in early treatment in predicting HBeAg clearance, multivariable logistic regressions were conducted for HBeAg and HBsAg levels at weeks 24 and 36 and HBsAg change from baseline adjusted for age, sex, HBV DNA and an increase at weeks 12 and 24 ALT. Similar to

Table 6 Virologic response and change in serum hepatitis B surface antigen and hepatitis B virus e antigen levels after treatment

Treatment response	Treatment (ICE) group (n = 199)	Control group (n = 196)	χ^2/Z	P value
48 wk				
Patients with HBsAg level decline $\geq 0.5 \log_{10}$ IU/mL, n (%)	49 (24.62)	19 (9.69)	15.443	0.000
Patients with HBsAg level decline $\geq 1 \log_{10}$ IU/mL, n (%)	29 (14.57)	11 (5.61)	8.712	0.003
Patients with HBsAg level decline $\geq 2 \log_{10}$ IU/mL, n (%)	7 (3.52)	2 (1.02)	1.758	0.185
Patients with undetectable HBsAg (≤ 0.05 IU/mL), n (%)	0 (0.00)	0 (0.00)	0.000	1
Patients with HBeAg level decline $\geq 1 \log_{10}$ S/CO, n (%)	45 (22.61)	5 (2.55)	35.947	0.000
Patients with undetectable HBeAg (≤ 1.00 S/CO), n (%)	17 (8.54)	5 (2.55)	6.740	0.009
Seroconversion rates of HBeAg ^a , n (%)	16 (8.04)	4 (2.04)	7.394	0.007
96 wk				
Patients with HBsAg level decline $\geq 0.5 \log_{10}$ IU/mL, n (%)	83 (41.71)	41 (20.92)	19.817	0.000
Patients with HBsAg level decline $\geq 1 \log_{10}$ IU/mL, n (%)	63 (31.66)	22 (11.22)	24.413	0.000
Patients with HBsAg level decline $\geq 2 \log_{10}$ IU/mL, n (%)	17 (8.54)	1 (0.51)	7.129	0.008
Patients with undetectable HBsAg (≤ 0.05 IU/mL), n (%)	0 (0)	0 (0)	0.000	1
Patients with HBeAg level decline $\geq 1 \log_{10}$ S/CO, n (%)	51 (25.63)	9 (4.59)	33.919	0.000
Patients with undetectable HBeAg (≤ 1.00 S/CO), n (%)	32 (16.08)	11 (5.61)	11.154	0.001
Seroconversion rates of HBeAg ^a , n (%)	29 (14.57)	9 (4.46)	11.962	0.001

^aPrevious HBeAg-positive patient HBeAg ≤ 1.00 S/CO and HBeAb > 1.0 S/CO. Chi-square test. ICE: Invigorating kidney and clearing away the heat and expelling superficial evils; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen.

the univariable regression analysis results, all were significantly related to HBeAg clearance. The ORs of ALT elevation at week 12 (OR, 2.049, $P = 0.006$) and week 24 (OR 3.788, $P = 0.003$) as well as week 48 IFN- γ elevation (OR, 2.171, $P = 0.007$) and week 48 IL-2 elevation (OR, 1.882, $P = 0.020$) were adjusted for age, sex and HBV DNA (Table 7).

Rates of HBeAg clearance among patients with favorable baseline, week 24 or week 36 ICE treatment response

Based on the optimal cutoff values, in our data set, the rates of HBeAg clearance were 42.9% (12/28), 52.0% (13/22), 52.4% (22/42), 45.0% (9/20), 60.0% (3/5) and 35.6% (21/59) for patients with baseline HBeAg $< 3 \log_{10}$, baseline HBsAg $< 4.36 \log_{10}$ IU/mL, week 36 HBeAg change from baseline 1 log week, 24 HBsAg change from baseline $> 1 \log_{10}$ IU/mL, week 36 HBsAg change from baseline $> 2 \log_{10}$ IU/mL and ALT elevation, respectively (Figure 4). We combined HBeAg, HBsAg decrease and ALT elevation together. Patients with a week 36 HBeAg change from baseline $> 1 \log_{10}$ S/CO/mL and ALT elevation had an HBeAg clearance rate of 64.5% (20/31), and HBsAg change from baseline $> 1 \log_{10}$ IU/mL, $> 2 \log_{10}$ IU/mL with ALT elevation had an HBeAg clearance rate of 70.6% (12/17), 80.0% (4/5), respectively. Only 11.8% (2/17) of patients with week 36 cleared HBeAg showed $> 1 \log_{10}$ S/CO/ml HBeAg change from baseline with no ALT elevation. A total of 14.3% (1/7) of patients who cleared HBeAg were among those with week 24 HBsAg change from baseline 1 log₁₀ IU/mL and no ALT elevation, and 0% (0/2) of patients had week 36 HBsAg change

Table 7 Baseline variables and change in hepatitis B virus e antigen and hepatitis B surface antigen from week 12 to week 48 associated with HBeAg clearance

Variables	Univariable analysis			Multivariable analysis		
	OR	95%CI	P	OR	95%CI	P
Sex	0.788	(0.367-1.986)	0.453			
Age	0.947	(0.886-1.198)	0.715			
HBV DNA	0.633	(0.574-1.393)	0.214			
IFN- γ	0.915	(0.837-2.131)	0.656			
IL-2	0.773	(0.512-1.318)	0.375			
Baseline HBeAg	1.653	(1.332-2.257)	0.030	1.027	(1.145-1.908)	0.047
Baseline HBsAg	2.431	(1.236-3.915)	0.004	1.339	(1.131-1.862)	0.009
Week 24 HBeAg change from baseline	2.762	(1.562-4.256)	< 0.001	2.338	(1.636-4.863)	< 0.001
Week 36 HBeAg change from baseline	3.411	(1.976-4.526)	< 0.001	3.185	(1.977-5.466)	< 0.001
Week 24 HBsAg change from baseline	4.458	(2.153-10.198)	< 0.001	3.273	(1.375-5.216)	< 0.001
Week 36 HBsAg change from baseline	5.371	(3.239-6.392)	< 0.001	5.788	(2.726-10.612)	< 0.001
Week 12 ALT elevation	2.676	(1.133-5.432)	0.016	2.049	(1.363-9.198)	0.006
Week 24 ALT elevation	3.373	(2.637-7.568)	0.003	3.788	(2.728-7.687)	0.003
Week 48 IFN- γ elevation	2.735	(1.317-6.682)	0.002	2.171	(1.163-2.961)	0.007
Week 48 IL-2 elevation	2.133	(1.171-8.616)	0.008	1.882	(1.026-2.613)	0.020

CI: Confidence interval; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; HBsAg: Hepatitis B virus surface antigen; HBeAg: Hepatitis B virus e antigen; IL-2: Interleukin-2; IFN- γ : Interferon- γ .

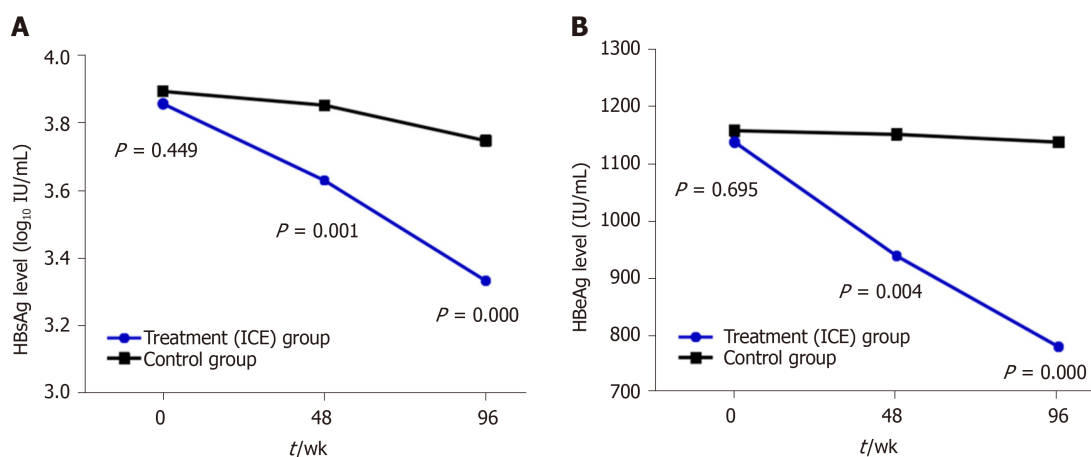


Figure 2 Concentration curves of hepatitis B virus surface antigen and hepatitis B e antigen levels during Chinese herbal formula and placebo treatment. There was no significant difference between the two groups in serum hepatitis B virus surface antigen or hepatitis B e antigen levels at baseline. A: The patients in the treatment group showed significantly decreased hepatitis B virus surface antigen level in serum compared with those in the control group at weeks 48 and 96; B: The patients in the treatment group showed significantly decreased hepatitis B e antigen level in serum compared with those in the control group at weeks 48 and 96. Mann-Whitney U test. Treatment group: Chinese herbal formula invigorating kidney and clearing away the heat and expelling superficial evils; Control group: Placebo. HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; ICE: Invigorating kidney and clearing away the heat and expelling superficial evils.

from baseline 2 log₁₀ IU/mL and no ALT elevation (Figure 4).

Liver biopsies

The present study included liver biopsies from 324 patients with chronic HBV infection, including 168 patients in the ICE group and 156 patients in the control group. A total of 138 (42.6%) patients underwent paired biopsy twice, including 72

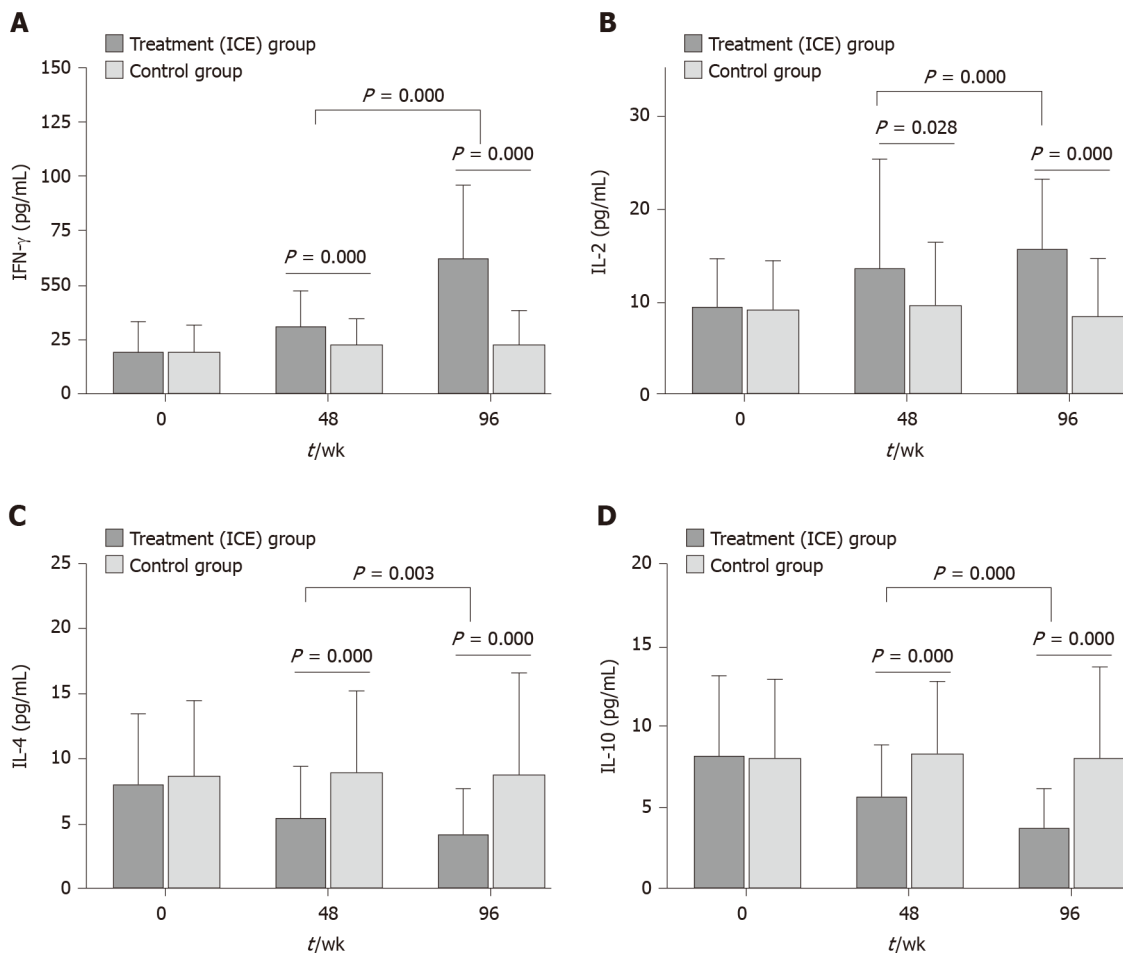


Figure 3 Serum levels of interferon- γ , interleukin-2, interleukin-4 and interleukin-10 were determined by ELISA. A: The patients in the treatment group showed significantly increased interferon- γ levels in serum compared with the placebo group; B: The patients in the treatment group showed significantly increased interleukin (IL)-2 levels in serum compared with the placebo group; C: The patients in the treatment group showed significantly decreased IL-4 levels in serum compared with the placebo group; D: The patients in the treatment group showed significantly decreased IL-10 levels in serum compared with the placebo group. Serum levels of interferon- γ , IL-2, IL-4 and IL-10 were compared between the subgroup weeks 48 and 96. Mann-Whitney U test. Treatment group: Chinese herb formula invigorating kidney and clearing away the heat and expelling superficial evils; Control group: Placebo. IL: Interleukin; IFN- γ : interferon- γ ; ICE: Invigorating kidney and clearing away the heat and expelling superficial evils.

patients in the ICE group and 66 patients in the control group. Images of two typical cases in the ICE group for these conditions are shown in Figures 5 and 6.

Changes in Knodell HAI score at week 96: As shown in Figure 7, 138 patients underwent liver biopsies twice. A total of 73 patients showed a decrease in the Knodell HAI score at 96 week, including 51 patients in the ICE group and 22 patients in the control group. There were 41 patients in the ICE group and 10 patients in the control group whose scores decreased by ≥ 2 points, and there were 10 patients in the ICE group and 12 patients in the control group whose scores decreased by < 2 points. The difference between the two groups was statistically significant ($P = 0.003$). A total of 65 patients in the ICE group and the control group showed no improvement or even deterioration in the Knodell HAI score, and 4 patients in the ICE group and 17 patients in the control group increased ≥ 2 points. The Knodell HAI scores of 6 patients in the ICE group and 13 patients in the control group rose < 2 points, and 11 patients in the ICE group and 14 patients in the control group showed an unchanged Knodell HAI score. There was no significant difference between the two groups ($P = 0.196$).

Changes in Ishak fibrosis score at week 96: As shown in Figure 7, a total of 42 patients had decreased Ishak fibrosis scores at week 96. There were 23 patients in the ICE group and 19 patients in the control group whose scores decreased by ≥ 1 point.

After 96 wk of administration, a total of 96 patients in the ICE group and the control group showed no improvement or even deterioration in Ishak fibrosis score with 13 patients in the ICE group and 23 patients in the control group increasing ≥ 1 point, respectively. The Ishak fibrosis scores of 36 patients in the ICE group and 24 patients

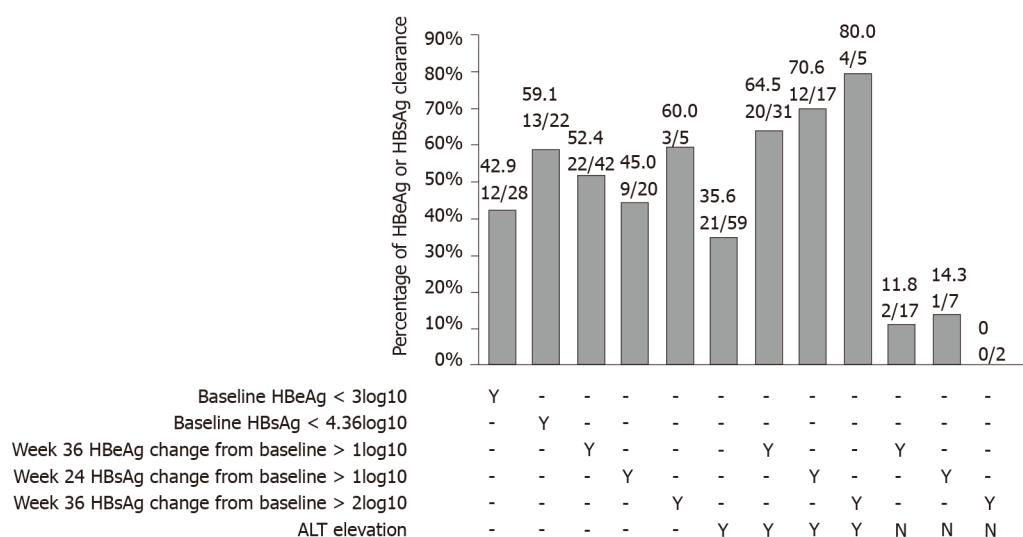


Figure 4 Percentage of hepatitis B virus surface antigen and hepatitis B e antigen clearance. Percentage of hepatitis B virus surface antigen and hepatitis B e antigen clearance among patients stratified by baseline, week 24 and week 36 hepatitis B e antigen and hepatitis B virus surface antigen levels; week 36 hepatitis B e antigen change from baseline; weeks 24 and 36 hepatitis B virus surface antigen change from baseline; and week 24 alanine aminotransferase elevation. HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase.

in the control group showed an unchanged Ishak fibrosis score. There was no significant difference between the two groups ($P = 0.070$).

Changes in liver HBsAg levels at week 96: There are five levels of liver HBsAg, HBcAg: “-,” “+,” “++,” “+++” and “++++,” which are converted to scores of 0, 1, 2, 3 and 4, respectively. After 96 wk of administration, there were a total of 85 patients in the ICE group and the control group whose liver HBsAg level decreased. There were 28 patients in the ICE group and 10 patients in the control group whose scores decreased by ≥ 2 points, and there were 34 patients in the ICE group and 13 patients in the control group whose scores decreased by ≥ 1 but < 2 points. There was no significant difference between the two groups ($P = 0.890$). After 96 wk of administration, a total of 53 patients in the ICE group and the control group showed no improvement or even deterioration in liver HBsAg levels with 3 patients in the ICE group and 8 patients in the control increasing ≥ 1 point. Seven patients in the ICE group and 35 patients in the control group showed unchanged liver HBsAg levels. There was no significant difference between the two groups ($P = 0.713$); however, there was a significant difference in liver HBsAg levels between the two groups ($P = 0.000$, Figure 7).

Changes in liver HBcAg levels at week 96: After 96 wk of administration, there were a total of 86 patients in the ICE group and the control group whose liver HBcAg level decreased. There were 21 patients in the ICE group and 9 patients in the control group whose scores decreased by ≥ 2 points, and there were 45 patients in the ICE group and 11 patients in the control group whose scores decreased by ≥ 1 but < 2 points. There was no significant difference between the two groups ($P = 0.279$).

After 96 wk of administration, a total of 52 patients in the ICE group and the control group showed no improvement or even deterioration in liver HBcAg levels with 1 patient in the ICE group and 11 patients in the control group increasing ≥ 1 point. Eleven patients in the ICE group and 35 patients in the control group showed unchanged liver HBcAg levels. There was no significant difference between the two groups ($P = 1.000$). However, there was a significant difference in liver HBcAg levels between the two groups ($P = 0.000$, Figure 7).

Adverse events and drug combination

Adverse events: During follow-up, there were five adverse events, including two cases of diarrhea (one in each group), one case of dizziness (control group) and one case of nausea (control group). Patients continued to take the medicine after symptom relief. One case was terminated due to the discovery of hepatocellular carcinoma (control group).

Drug combination: Six cases (two in the ICE group, four in the control group, bicyclol,

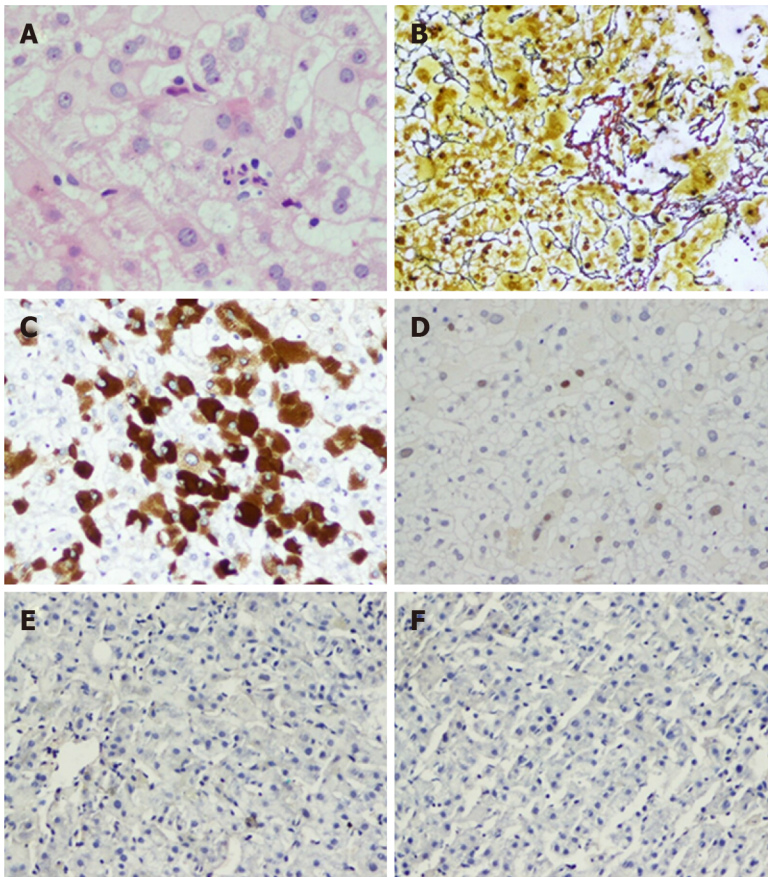


Figure 5 Typical case 1 (pathology No. liver 0372). A: Focal necrosis in hepatic lobules with inflammatory cell infiltration (G1); B: Perisinusoidal fibrosis and lobular fibrosis (S1); C: Hepatitis B virus surface antigen (+++) in one immunohistochemistry assay of liver; D: Hepatitis B virus core antigen (+) in one immunohistochemistry assay of liver; E: Hepatitis B virus surface antigen (-) in two immunohistochemistry assays of liver; F: Hepatitis B virus core antigen (-) in two immunohistochemistry assays of liver.

wuzhi tablets, glycyrrhizic acid preparations, *etc.*) were treated with drugs with hepatoprotective effects due to abnormal liver function. One case (ICE group) was treated with Contac due to cold. One case (control group) was treated with TCM due to leg injury. One case (control group) was treated with Euthyrox due to abnormal thyroid function.

Follow-up after drug withdrawal

In the treatment (ICE) group, 52 subjects were followed for 48 wk, and 43 subjects were followed for 24 wk. All subjects with cleared HBeAg maintained HBeAg clearance during the treatment, while five patients showed delayed HBeAg clearance.

However, in the control group, 23 subjects were followed for 48 wk and 36 for 24 wk; only one patient had HBeAg clearance during the period of follow-up.

In the treatment group, there was no obvious increase in HBV DNA levels after drug withdrawal, ALT levels were in the normal range, and no aggravations were observed.

DISCUSSION

Previous studies have shown that in CHB patients, liver lesions progressed with age. After the age of 30, the severity of hepatic inflammatory activity and fibrosis was significantly higher in CHB patients than in those under 30 years of age^[35-37]. To further confirm the liver histopathology of CHB patients with persistent normal ALT at enrollment, 82.0% (324/395) of patients underwent liver biopsy. A total of 35.5% (115/324) of patients with normal serum ALT had a ≥ 4 inflammation score of liver histopathology, and 39.2% (127/324) of patients had a ≥ 2 fibrosis score. Our liver biopsy data indicated that chronic hepatitis can be diagnosed in nearly 40% of HBV carriers as a serological diagnosis. Consistent with a previous study, our results

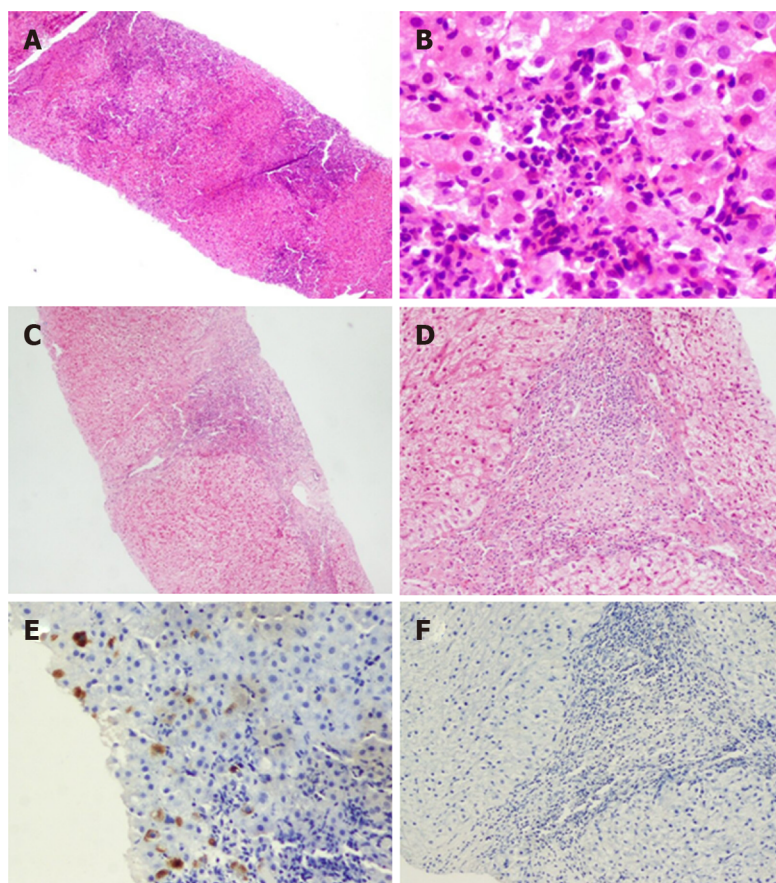


Figure 6 Typical case 2 (pathology No. liver 0178). A: Extensive necrosis involving multiple lobules and bridging necrosis at low magnification (G4); B: Necrotic cellular debris at high magnification (G4); C: Limited fusion necrosis in two immunohistochemistry assays of liver at low magnification (G3); D: Limited fusion necrosis in two immunohistochemistry assays of liver at high magnification (G3); E: Hepatitis B virus surface antigen (+) in one immunohistochemistry assay of liver; F: Hepatitis B virus surface antigen (-) in two immunohistochemistry assays of liver.

further validated that the risk of liver inflammation and fibrosis increased with age in chronic HBV carriers over 30 years old^[23], which is the reason that this study defined the age of ALT-normal HBeAg-positive chronic HBV carriers as above 30 years old. The new edition of China's 2015 guidelines and the 2017 European Association for the Study of the Liver guidelines have adjusted the age of observation in the indications of antiviral therapy, which was reduced from > 40 years old to > 30 years old. This change is consistent with the research criteria we set^[1,4].

Despite recent advances in the treatment of CHB, including multiple nucleoside/nucleotide analogs, no treatment is suitable for chronic HBV carriers with normal ALT or patients in the immune-tolerance phase. Because these patients over 30 years of age are at an increased risk of disease progression with age, most of them desperately need effective and safe treatment to reduce persistently high levels of HBV DNA and prevent progression to cirrhosis and HCC^[9-11]. Attempts and efforts have been made by many researchers in this regard, including tenofovir therapy^[14-16]. For CHB patients with normal ALT, antiviral therapy is less effective even if the histological examination indicates chronic hepatitis. A recent study showed that after treatment with interferon, 17.54% of patients with normal ALT but significant liver inflammation or fibrosis (G4, S3) demonstrated a sustained virologic response, which is significantly lower than those patients with elevated ALT (28.57%)^[38]. Our study modified the TCM compound therapy from our previous national science and technology major project during the 11th five-year plan period^[20] and extended the treatment course to 96 wk.

In this study, a total of 22 patients were lost to follow-up in the placebo group, and 13 patients were lost to follow-up in the ICE-treated group. After a 96-wk treatment, 18.59% (37/199) of CHB patients had HBV DNA levels $\leq 4 \log_{10}$ IU/mL, and the HBeAg clearance and conversion rates were 16.08% (32/199) and 14.57% (29/199), respectively. HBeAg clearance persisted in the treatment group patients at 24 or 48 wk after drug withdrawal. Extended HBeAg clearance was observed in five cases, while one case was observed in the control group. Furthermore, serum HBsAg levels and

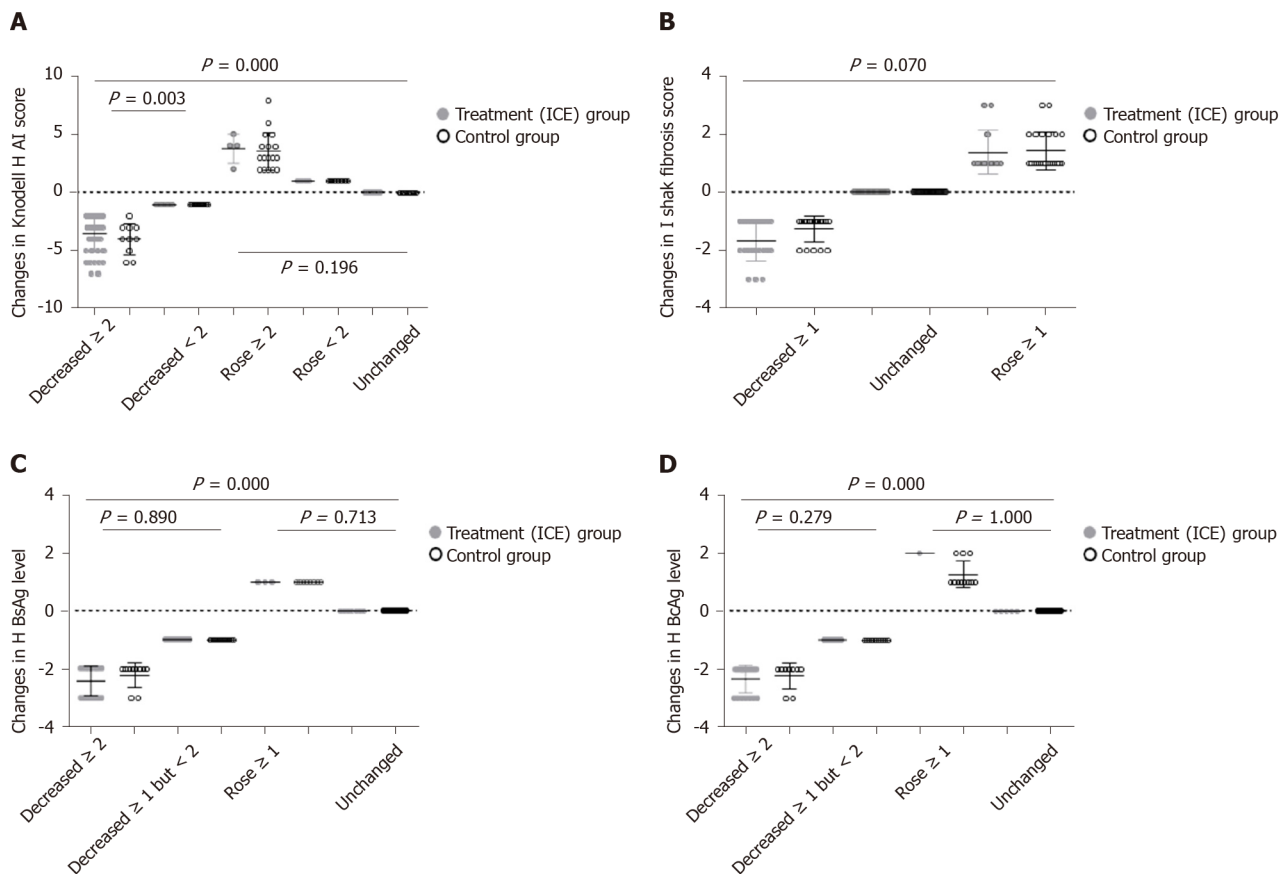


Figure 7 Changes in Knodell histological activity index score, Ishak fibrosis score, liver hepatitis B surface antigen levels and liver hepatitis B core antigen levels at 96 wk after administration in the two groups. A: Knodell histological activity index score; B: Ishak fibrosis score; C: Liver hepatitis B surface antigen levels; D: Liver hepatitis B core antigen levels. HAI: Histological activity index; HBsAg: Hepatitis B surface antigen; HBcAg: Hepatitis B core antigen.

liver HBsAg expression were decreased in the ICE treatment group. In addition, 35.6% (59/199) of patients in the ICE-treated group had increased ALT levels after the 12- or 24-wk therapy in comparison with 5.1% (10/196) of patients in the control group. The mean serum bilirubin level was < 35 mmol/L, which may be related to the immune activation and anti-HBV response during ICE therapy. Paired biopsies showed a significant difference between the treatment group and the control group after 96 wk of treatment regarding the Knodell HAI score, indicating inflammation in the liver. Although the fibrosis score decreased ≥ 1 subgroup, there was no difference between the two groups ($P = 0.08$). In the no improvement subgroup, the treatment group was significantly better than the control group ($P = 0.001$), and there was also a significant difference between the two groups in the total fibrosis score ($P = 0.000$). In this study, except for four patients with diarrhea, dizziness or nausea after initiation of the medication, no other serious adverse effects occurred, indicating that ICE treatment is relatively safe. No deterioration of liver function occurred in this study. Therefore, the reduction in HBV DNA, clearance and conversion rates of HBeAg in this study were significantly higher than those reported in previous antiviral therapies.

In ancient China, there was no unambiguous description of CHB. In recent years, according to the natural history of chronic HBV infection, clinical manifestations and the theory of TCM epidemiology^[39], we proposed that “kidney asthenia and hepatic blood prostrated by dampness-heat” is the pathogenesis of CHB. During the course of infection, dampness-heat was considered the initial pathogenic factor, deficiency of kidney qi was its underlying factor, and stagnation of the liver channel was a critical link in its pathology. In this context, the main principles of treating chronic HBV infection are invigorating the kidney, clearing away the heat evil, expelling superficial evils, activating blood and eliminating dampness. According to the above data and clinical experience, we propose the experiential effective recipe for ICE. *Radix et caulis acanthopanacis senticosi*, *Herba Epimedii*, *Fructus ligustri lucidi* and *Herba ecliptae* are the main drugs in this formula. Modern studies have proven that these herbs have liver-protective and immunity-enhancing effects^[40]. In TCM theory, *Phyllanthus urinaria* Linn

has the effects of clearing heat, removing toxicity and softening and resolving hard mass. The anti-HBV effect of this herb has been demonstrated^[41-48]. *Rhizoma polygoni cuspidati* clears heat and removes toxicity in TCM theory and has an antiviral effect in modern medicine^[49,50]. *Radix bupleuri*, *Radix paeoniae alba*, *Fructus aurantii immaturus* and *Radix glycyrrhizae* are minister drugs and have effects of eliminating pathogenic factors^[51]. *Semen persicae* is the assistant drug and promotes blood circulation to remove blood stasis and antiliver fibrosis following CHB. *Radix glycyrrhizae* is an envoy drug that removes toxicity and moderates the properties of herbs. The ICE formula exerts therapeutic effects through the concerted application of monarch, minister, assistant and envoy by tonifying, clearing, expelling and activating methods.

After 96 wk of treatment of HBeAg-positive chronic HBV carriers with normal ALT, more than 80% of patients still could not achieve HBV DNA reduction to $\leq 4 \log_{10}$ IU/mL or HBeAg clearance. Therefore, selection of the benefit population is essential for therapy outcome prediction. Quantitative determination of HBsAg and HBeAg is of great significance for antiviral therapy because it can be used as an immune control indicator to predict serum HBeAg clearance rate, HBsAg clearance rate and long-term prognosis^[52-55]. In addition, HBsAg and HBeAg level reduction at 24 and 36 wk during the treatment period were important predictors of the sustained response of ICE. Univariate logistic regression analysis showed that HBsAg levels decreased significantly at 24 and 36 wk after treatment compared with baseline. At 36 wk after treatment, the HBeAg level decreased significantly as well. Notably, the ALT level increased significantly at 12 and 24 wk. In addition, HBeAg clearance was statistically significant at baseline. As mentioned above, the changes in ALT and HBsAg were related to HBeAg clearance. CHB patients with elevated ALT and a decline $> 1 \log_{10}$ IU/mL in HBsAg level from baseline at week 24 achieved an HBeAg clearance rate of 70.6% (12/17), and for those in whom HBsAg changed from baseline $> 2 \log_{10}$ IU/mL at week 36, the rate was 80% (4/5). CHB patients with elevated ALT and a decline $> 1 \log_{10}$ IU/mL in HBeAg from baseline at week 36 achieved an HBeAg clearance rate of 64.5% (20/31). Therefore, in clinical practice, increased ALT, decreased HBsAg at week 12 and 24 and decreased HBeAg at week 24 and 36 are potential indicators for the early prediction of HBeAg clearance in TCM therapy, and this response was relatively extended compared with interferon^[56].

We further investigated the changes in immune function of patients before and after treatment. Elevated IFN- γ and IL-2 levels at week 48 were statistically significant in predicting serological clearance of HBeAg^[42,57]. At weeks 48 and 96, serum IFN- γ and IL-2 increased significantly in the ICE group compared with the control group. It has been demonstrated in several studies that IL-2 as well as IFN- γ production therapy may amplify the immune response by regulating T lymphocytes and natural killer cells, which showed clinical efficacy in Caucasian HBV DNA- and HBeAg-positive patients. Another pilot study showed that IL-2 combined with IFN- γ treatment induced HBV-specific CD4⁺ T cell proliferative responses, which could be important in controlling viremia in chronic HBV carriers. Therefore, ICE induced antiviral immune responses mainly through IL-2 and IFN- γ expression, which might lead to consequent viral elimination.

Although our study suggests that HBV DNA, HBsAg and HBeAg decreased significantly after TCM therapy, long-term prognosis, including long-term changes in HBsAg and long-term benefits in reducing the risks of cirrhosis and HCC are still unclear. However, in this study, we tried to use TCM to treat patients with unsatisfactory antiviral efficacy, controversial therapies or risks of disease progression. We provided a safe and effective therapy in this study.

In conclusion, in patients with HBeAg-positive chronic HBV infection with normal ALT, ICE therapy can achieve reduced HBV replication, increased HBeAg clearance rate and serum conversion rate and significant liver histology improvement. ICE therapy is safe and effective, and the effects may be related to host immune status modulation.

ARTICLE HIGHLIGHTS

Research background

No guideline recommends antiviral therapy for hepatitis B e antigen (HBeAg)-positive chronic hepatitis B patients with persistently normal alanine aminotransferase (ALT) levels and a high hepatitis B virus (HBV) DNA viral load. Despite long-term normal ALT levels, a high HBV DNA viral load persists, and liver lesions progress unrecognized and advance gradually.

Research motivation

The purpose of this study was to provide clinical evidence for traditional Chinese medicine treatment for chronic HBV carriers, especially chronic HBV carriers over 30 years old with a higher risk of disease progression.

Research objectives

To evaluate the feasibility and safety of a Chinese herbal formula as a therapeutic option for chronic HBV infection.

Research methods

The 395 patients (30–65 years old) with confirmed HBeAg-positive chronic hepatitis B infection and persistently normal ALT were randomized to receive either the Chinese herbal formula or placebo for 96 wk. Endpoints to evaluate therapeutic efficacy included: (1) HBV DNA levels decreased to less than 4 log₁₀ IU/mL at weeks 48 and 96; and (2) HBeAg clearance and seroconversion rates at weeks 48 and 96.

Research results

HBV DNA levels ≤ 4 log₁₀ IU/mL were 10.05% at week 48 and 18.59% at week 96 in the treatment group. The HBeAg clearance and conversion rates were 8.54% and 8.04 at week 48 and 16.08% and 14.57% at week 96, respectively. However, HBV DNA levels ≤ 4 log₁₀ IU/mL were 2.55% and 2.55% at weeks 48 and 96, respectively, and the HBeAg clearance rates were 3.06% and 5.61% at weeks 48 and 96, respectively, in the control group. The quantitative hepatitis B surface antigen and HBeAg levels at baseline and changes during the treatment period as well as the ALT elevation at weeks 12 and 24 were strong predictors of HBeAg clearance.

Research conclusions

High rates of HBV DNA reduction, HBeAg clearance and seroconversion could be achieved with Chinese herbal formula treatments, and the treatments were relatively safe for HBeAg-positive chronic hepatitis B -infected patients with persistently normal ALT. The ability of the compound to modulate host immune function probably contributed to this effect.

Research perspectives

We provided a safe and effective therapy in treating patients with unsatisfactory antiviral efficacy, controversial therapies or risks of disease progression. Traditional Chinese medicine treatment may be a therapeutic option for chronic HBV infection.

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Observational Study

Predictive value of alarm symptoms in patients with Rome IV dyspepsia: A cross-sectional study

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Informed consent statement:

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Abstract

BACKGROUND

No studies have evaluated the predictive value of alarm symptoms for organic dyspepsia and organic upper gastrointestinal (GI) diseases based on Rome IV criteria in the Chinese population.

AIM

To evaluate the predictive value of alarm symptoms for dyspeptic patients based on Rome IV criteria.

METHODS

We performed a cross-sectional study of dyspepsia patients who met the inclusion and exclusion criteria at two academic urban tertiary-care centers from March 2018 to January 2019. Basic demographic data, dyspeptic information, alarm symptoms, lifestyle, examination results, family history and outpatient cost information were collected. Dyspepsia patients with normal findings on upper GI endoscopy, epigastric ultrasound and laboratory examination and without *Helicobacter pylori*-associated dyspepsia were classified as functional dyspepsia.

RESULTS

A total of 381 patients were enrolled in the study, including 266 functional dyspepsia patients and 115 organic dyspepsia patients. There were 24 patients

of the STROBE statement have been adopted in preparing the manuscript.

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with organic upper GI disease among patients with organic dyspepsia. We found that based on the Rome IV criteria, alarm symptoms were of limited value in differentiating organic dyspepsia and organic upper GI diseases from functional dyspepsia. Age (odds ratio (OR) = 1.056, $P = 0.012$), smoking (OR = 4.714, $P = 0.006$) and anemia (OR = 88.270, $P < 0.001$) were independent predictors for organic upper GI diseases. For the comparison of epigastric pain syndrome, postprandial distress syndrome and epigastric pain syndrome combined with postprandial distress syndrome, the results showed that there were statistically significant differences in anorexia ($P = 0.021$) and previous visits ($P = 0.012$). The ClinicalTrials.gov number is NCT 03479528.

CONCLUSION

Most alarm symptoms had poor predictive value for organic dyspepsia and organic upper GI diseases based on Rome IV criteria. Gastroscopic screening should not be based solely on alarm symptoms.

Key words: Rome IV; Dyspepsia; Alarm symptoms; Prediction

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Core tip: Dyspepsia is a symptom complex referable to the upper gastrointestinal tract. Based on the Rome IV criteria, alarm symptoms were of limited value in differentiating organic dyspepsia and organic upper gastrointestinal diseases from functional dyspepsia, and gastroscopic screening should not be based solely on alarm symptoms. Age, smoking and anemia were the independent predictors for organic upper gastrointestinal diseases. The clinical characteristics of patients with epigastric pain syndrome, postprandial distress syndrome and the two combined were not significantly different.

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INTRODUCTION

Dyspepsia is a clinical symptom originating from the upper gastrointestinal (GI) tract. Dyspepsia can be divided into functional dyspepsia (FD) and organic dyspepsia. FD is a very common functional GI disorder in clinical treatment^[1,2]. It is a clinical syndrome that is characterized by chronic or recurrent gastroduodenal symptoms, without any organic or metabolic disease that may explain the symptoms^[3-5]. FD has a high incidence in the population. Dyspepsia is present in approximately 20% of the general population worldwide^[6], and a recent study showed that FD was present in 11% of the general population in Italy^[7]. FD dramatically reduces a patient's quality of life, and it also imposes a severe financial burden due to frequent clinical visits, prolonged drug use and long time off work^[8,9].

Clinical diagnosis of the underlying cause of dyspepsia based on symptoms alone is believed to be unreliable^[10,11], but a range of alarm symptoms are suggested to indicate an elevated risk of serious illness^[12]. Alarm symptoms may indicate underlying malignancy or significant pathology, such as a stricture or ulcer^[13]. However, according to the results of previous studies, the sensitivity of alarm symptoms to predict upper GI malignancies is not satisfactory^[13-15]. The predictive effect of alarm symptoms requires further research.

FD is a type of dyspepsia that has no organic, metabolic or systemic disease to explain its symptoms, but only a few studies have rigorously diagnosed FD by laboratory examination, epigastric ultrasound and upper GI endoscopy to exclude related diseases^[16,17], especially in cross-sectional studies. Further research is needed to rigorously diagnose FD through laboratory examination, epigastric ultrasound and upper GI endoscopy.

In 2016, the Rome IV criteria for dyspepsia were introduced. The Rome IV criteria

redefined the frequency and severity of each dyspeptic symptom in patients with dyspepsia, but the effectiveness of the Rome IV criteria still needs to be confirmed by relevant studies^[18]. At present, no study has assessed the predictive effect of alarm symptoms according to the Rome IV criteria. Here, we carried out a study to evaluate the predictive value of alarm symptoms in dyspeptic patients based on Rome IV.

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted at two academic urban tertiary-care centers (the Second Affiliated Hospital of Xi'an Jiaotong University and the Affiliated Hospital of Northwest University), which provide medical services to the whole of northwest China from March 2018 to January 2019. Patients who visited the gastroenterology clinics and completed upper GI endoscopy and epigastric ultrasounds during the study period were initially screened. Furthermore, patients with dyspeptic symptoms who met the Rome IV criteria were further selected. Patients who met the inclusion criteria and exclusion criteria were eventually included in our study. Oral informed consent was obtained from all included patients. The ethics committee of the Second Affiliated Hospital of Xi'an Jiaotong University approved this study. This study protocol was registered at ClinicalTrials.gov (NCT03479528). In addition, there was no funding received.

Inclusion criteria

Inclusion criteria: (1) Age was ≥ 18 years; (2) The chief complaint was dyspeptic symptoms that met the Rome IV criteria (at least one of the following symptoms was present: Bothersome postprandial fullness at least 3 d per week, bothersome early satiation at least 3 d per week, bothersome epigastric pain at least 1 d a week, bothersome epigastric burning at least 1 d a week; symptoms must have been present for at least 3 mo in the previous 6 mo); (3) Patients visited the gastroenterology clinics and completed upper GI endoscopy and epigastric ultrasounds during the study period; and (4) routine blood examination, liver function test and *Helicobacter pylori* (*H. pylori*) test were conducted within the last 6 mo (to ensure that these diagnostic tests were conducted after the onset of dyspeptic symptoms).

Exclusion criteria

Exclusion criteria: (1) History of esophageal cancer, gastric ulcer, gastric cancer or other types of organic upper GI disease, disease of the pancreas or biliary tract or metabolic disorders (thyroid dysfunction, diabetes mellitus); (2) Pregnancy, pregnancy preparation, lactation; (3) History of abdominal surgery; (4) Severe nervous system diseases, mental illness or severe liver, kidney, heart or respiratory related dysfunction; (5) Abnormal liver function, including nonalcoholic steatohepatitis, hepatitis B or hepatitis C related hepatitis; (6) Current antidepressant, steroid or nonsteroidal anti-inflammatory drug use; (7) Patients only or predominantly had reflux-related symptoms; and (8) Patients who were reluctant to participate in this study.

Data collection

All related data were obtained through a clinic visit and telephone consultation. We collected the basic demographic data (name, age, height, weight, gender, marriage), dyspeptic information (dyspeptic symptoms, duration, frequency per week), alarm symptoms [including weight loss and its extent^[19], anemia (hemoglobin < 130 g/L for men and hemoglobin < 120 g/L for women), dysphagia, melena, vomiting, anorexia], lifestyle data (including spicy foods, smoking and smoking amount, drinking and alcohol consumption, sleep quality, daily exercise duration), examination results (*H. pylori*, upper abdominal B ultrasound, upper GI endoscopy), family history and outpatient cost information. All questionnaire data were imported into the database by a trained researcher.

Definitions of FD

FD was diagnosed strictly by laboratory examination, abdominal ultrasound and upper GI endoscopy. As Rome IV criteria redefined the frequency and severity of each dyspeptic symptom in patients with dyspepsia, the dyspeptic symptoms of the included patients were all severe enough to impact usual activities, and the

questionnaire included the frequency of dyspepsia. The presence or absence of Rome IV-defined FD, epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS) were decided by the questionnaire according to the Rome IV criteria^[18,20] (see [Supplementary Table 1](#)). There was no evidence of abnormal results of upper GI endoscopy, epigastric ultrasound, laboratory examination or *H. pylori*-associated dyspepsia^[21,22]. *H. pylori*-associated dyspepsia was defined as the relief of dyspepsia symptoms after eradication of *H. pylori*^[18].

Definitions of organic dyspepsia

Dyspepsia can be divided into FD and organic dyspepsia. Organic dyspepsia occurs when clinical or laboratory tests reveal underlying organic disease that may be the cause of these symptoms^[23,24]. Organic dyspepsia was caused by abnormal results of upper GI endoscopy, epigastric ultrasound, laboratory examination and *H. pylori*-associated dyspepsia in this study. We regarded hepatic cyst (< 5 cm)^[25], hepatic hemangioma (< 5 cm)^[26], fatty liver, gallbladder wall roughness, cholesterol crystal and gallbladder polyps (< 1 cm)^[27] as normal epigastric ultrasound, and gallstone was regarded as abnormal epigastric ultrasound. Abnormal routine blood tests (anemia) were regarded as abnormal laboratory examination.

Definition of organic upper GI disease

All patients underwent complete upper GI endoscopy, and the physicians who performed upper GI endoscopy maintained a blind method for data collection. The findings were recorded using the endoscopic reporting system. Researchers reviewed these endoscopic reports and recorded the patient's endoscopic diagnosis. Upper GI endoscopy or biopsy pathology indicated that organic diseases were classified as organic upper GI disease, while upper GI endoscopy and biopsy pathology showed no evidence of organic disease were classified as nonorganic upper GI diseases. Organic upper GI diseases included gastric ulcer, gastric cancer, duodenal ulcer and esophagus cancer. Endoscopic chronic gastritis and duodenitis are considered nonorganic upper GI diseases^[18]. Gastric erosion, duodenal erosion, Barrett's esophagus and esophageal candidiasis were asymptomatic findings and were also regarded as nonorganic diseases of the upper GI diseases.

Statistical analysis

EpiData3.1 software was used to input data, and statistical analyses were performed by EmpowerStats and SPSS 20.0 software. Categorical variables were expressed as counts and percentages and analyzed using chi-square tests or Fisher's exact test. Continuous variables were expressed as the mean \pm standard deviation and analyzed using a *t*-test or Kruskal-Wallis test. Variables were first evaluated with univariate analysis, variables with $P < 0.10$ in univariate analysis were then included in the multivariate analysis (logistic regression analysis), and exact logistic regression was conducted by SAS software when appropriate. Data were presented with odds ratios (OR) and 95% confidence intervals (CI). $P < 0.05$ was considered statistically significant. We used the area under the receiver operating characteristic curve to judge the predictive value of independent risk factors.

RESULTS

Baseline of patient characteristics

Between March 2018 and January 2019, a total of 381 patients who met the inclusion and exclusion criteria were collected in this study, including 266 FD patients, 115 organic dyspepsia patients and 24 organic upper GI disease patients ([Figure 1](#)). The mean age was 49.9 ± 13.0 years, and 231 (60.6%) patients were female. The baseline characteristics of all participants are shown in [Table 1](#). Among the 381 people who met the Rome IV criteria, there were 224 with chronic gastritis, 120 with gastric erosion, 9 with gastric ulcers and 8 with Barrett's esophagus and others. The results of upper GI endoscopy are shown in [Figure 2](#), and the results of epigastric ultrasounds are shown in [Supplementary Figure 1](#). The results of routine blood tests are shown in [Supplementary Figure 2](#).

We also randomly selected the upper GI endoscopy results of 200 healthy people from the health examination center of the Affiliated Hospital of Northwest University. The upper GI endoscopy results showed that 77% of patients had chronic gastritis, duodenitis, Barrett's esophagus, esophageal candidiasis or gastric erosion, indicating a

Table 1 Baseline characteristics of all participants and univariate analyses of various predictive variables for organic dyspepsia

Characteristics	Full participants, <i>n</i> = 381	FD, <i>n</i> = 266	Organic dyspepsia, <i>n</i> = 115	<i>P</i> value
Age in yr	49.9 ± 13.0	49.6 ± 12.9	50.5 ± 13.4	0.706
BMI in kg/m ²	21.9 ± 3.3	21.9 ± 3.5	21.7 ± 2.9	0.682
Gender, M/F	150/231	107/159	43/72	0.603
Race, Han/minority	377/4	262/4	115/0	0.320
Location, Shaanxi/other	324/57	226/40	98/17	0.949
Job category				0.980
Physical	130 (34.1)	90 (33.8)	40 (34.8)	
Mental	96 (25.2)	67 (25.2)	29 (25.2)	
Middle	101 (26.5)	70 (26.3)	31 (27.0)	
Retire	54 (14.2)	39 (14.7)	15 (13.0)	
Marriage				1.000
Never married	19 (5.0)	13 (4.9)	6 (5.2)	
Married	362 (95.0)	253 (95.1)	109 (94.8)	
Daily exercise				0.048
< 1/2 h	27 (7.1)	18 (6.8)	9 (7.8)	
1/2-1 h	96 (25.2)	69 (25.9)	27 (23.5)	
1-2 h	63 (16.5)	35 (13.2)	28 (24.3)	
> 2 h	195 (51.2)	144 (54.1)	51 (44.3)	
Spicy food	206 (54.1)	145 (54.5)	61 (53.0)	0.792
Smoking				0.720
< half pack a day	335 (87.9)	235 (88.3)	100 (87.0)	
> half pack a day	46 (12.1)	31 (11.7)	15 (13.0)	
Alcohol	72 (18.9)	50 (18.8)	22 (19.1)	0.939
Sleep, good/bad	238/143	168/98	70/45	0.672
Outpatient cost				0.060
< 500	2 (0.5)	1 (0.4)	1 (0.9)	
500-1000	46 (12.1)	40 (15.0)	6 (5.2)	
1000-3000	107 (28.1)	76 (28.6)	31 (27.0)	
3000-5000	41 (10.8)	29 (10.9)	12 (10.4)	
> 5000	185 (48.5)	120 (45.1)	65 (56.5)	
Educational level				0.791
Elementary and below	175 (45.9)	122 (45.9)	53 (46.1)	
High school	84 (22.1)	61 (22.9)	23 (20.0)	
College	109 (28.6)	73 (27.4)	36 (31.3)	
Postgraduate and above	13 (3.4)	10 (3.8)	3 (2.6)	
Previous visits				0.443
0	106 (27.8)	80 (30.1)	26 (22.6)	
1	72 (18.9)	49 (18.4)	23 (20.0)	
2	32 (8.4)	20 (7.5)	12 (10.4)	
≥ 3	171 (44.9)	117 (44.0)	54 (47.0)	
Weight loss				0.238

No	277 (72.7)	199 (74.8)	78 (67.8)	
< 7 lb	50 (13.1)	30 (11.3)	20 (17.4)	
≥ 7 lb	54 (14.2)	37 (13.9)	17 (14.8)	
Anemia, yes/no	31/350	0/266	31/84	< 0.001
Anorexia, yes/no	94/287	66/200	28/87	0.923
Vomiting, yes/no	22/359	14/252	8/107	0.485
Melena, yes/no	23/358	16/250	7/108	1.000
Dysphagia, yes/no	3/378	1/265	2/113	0.218
Family history				0.204
None	331 (86.9)	235 (88.3)	96 (83.5)	
Esophagus cancer	13 (3.4)	10 (3.8)	3 (2.6)	
Gastric cancer	24 (6.3)	15 (5.6)	9 (7.8)	
Other	13 (3.4)	6 (2.3)	7 (6.1)	
Alarm symptoms				0.004
No	161 (42.3)	125 (47.0)	36 (31.3)	
Yes	220 (57.7)	141 (53.0)	79 (68.7)	
Number of alarm symptoms				0.001
0	161 (42.3)	125 (47.0)	36 (31.3)	
1	139 (36.5)	96 (36.1)	43 (37.4)	
2	59 (15.5)	37 (13.9)	22 (19.1)	
3	18 (4.7)	7 (2.6)	11 (9.6)	
4	4 (1.0)	1 (0.4)	3 (2.6)	

Values are expressed as the mean ± standard deviation or *n* (%). BMI: Body mass index; M: Male; F: Female; FD: Functional dyspepsia.

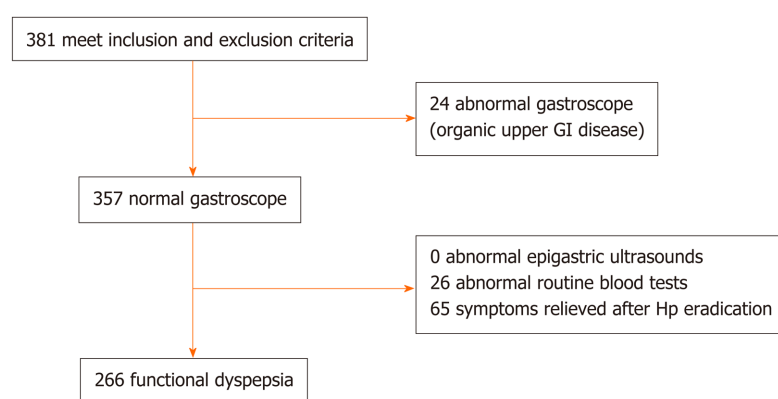


Figure 1 Flow chart of the study. GI: Gastrointestinal; Hp: *Helicobacter pylori*.

high proportion of patients in the general population (Supplementary Table 2). These data further supported our decision to treat chronic gastritis, duodenitis, Barrett's esophagus, esophageal candidiasis or gastric erosion as functional diseases.

Prediction of organic dyspepsia

For the comparison between FD and organic dyspepsia, there were 266 FD and 115 organic dyspepsia. In univariate analysis, there were statistically significant differences between FD and organic dyspepsia in daily exercise ($P = 0.048$), anemia ($P < 0.001$), alarm symptoms ($P = 0.004$) and number of alarm symptoms ($P = 0.001$). Then in the multivariate logistic regression analysis, outpatient cost was analyzed together

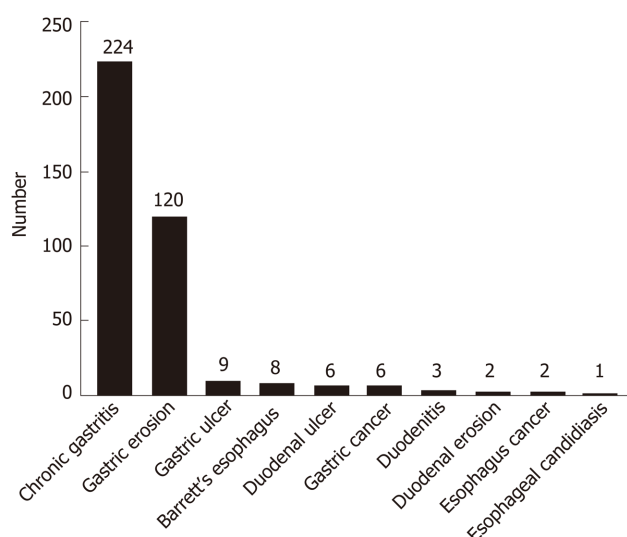


Figure 2 Endoscopy results.

with daily exercise, anemia, alarm symptoms and number of alarm symptoms. All anemia patients always had organic dyspepsia with complete separation, and exact logistic regression analysis was used. Anemia (OR = 137.700, 95%CI: 30.206- ∞ , $P < 0.001$) was still an independent predictor of organic dyspepsia (Table 1). These data suggested that most alarm symptoms had poor predictive value for organic dyspepsia based on Rome IV criteria. Moreover, there was no difference in outpatient cost between patients with FD and those with organic dyspepsia.

Prediction of organic upper GI diseases

There were 266 FD and 24 organic upper GI disease cases. Univariate analysis demonstrated that smoking ($P = 0.024$), anemia ($P < 0.001$), alarm symptoms ($P = 0.038$) and number of alarm symptoms ($P = 0.009$) were significant predictors of organic upper GI diseases. In multivariate analysis, age together with smoking, anemia, alarm symptoms and number of alarm symptoms were analyzed. Anemia belonged to organic upper GI diseases, there was complete separation, and exact logistic regression analysis was used. In multivariate regression analysis, age (OR = 1.056, $P = 0.012$), smoking (OR = 4.714, $P = 0.006$) and anemia (OR = 88.270, $P < 0.001$) were independent predictors for organic upper GI diseases (Table 2).

Additionally, the receiver operating characteristic curve was used to evaluate the predictive value of these independent risk factors. When the three criteria (age, smoking and anemia) were used together, the area under the receiver operating characteristic curve was 0.788 ($P < 0.001$, 95%CI: 0.692-0.884). These data suggested that most alarm symptoms had poor predictive value for organic dyspepsia based on Rome IV criteria, and age, smoking and anemia had certain predictive value for organic dyspepsia. Moreover, there was no difference in outpatient cost between FD patients and patients with organic upper GI diseases.

Comparison of EPS and PDS

FD was prevalent in 266 of the population who underwent complete upper GI endoscopy according to the Rome IV criteria. Among the 266 patients with dyspepsia, 174 individuals only presented with EPS, 31 individuals only met the criteria for PDS, and the remaining 61 individuals presented with both EPS and PDS. For the comparison of EPS, PDS and EPS combined with PDS, univariate analysis showed that there were statistically significant differences in anorexia ($P = 0.021$) and previous visits ($P = 0.012$), and the clinical characteristics of patients with EPS, PDS and EPS combined with PDS were not significantly different. Characteristics of patients with EPS, PDS and EPS combined with PDS are shown in Table 3.

DISCUSSION

To our knowledge, our study is the first to research the predictive value of alarm

Table 2 Univariate and multivariate analysis of various predictive variables for organic upper gastrointestinal diseases

Characteristics	FD, <i>n</i> = 266	Organic upper GI disease, <i>n</i> = 24	Univariate analysis	Multivariate analysis		
			<i>P</i> value	OR	95%CI	<i>P</i> value
Age in yr	49.6 ± 12.9	54.8 ± 14.8	0.071	1.056	1.012-1.101	0.012
BMI in kg/m ²	21.9 ± 3.5	21.5 ± 2.1	0.635			
Gender, M/F	107/159	10/14	1.000			
Race, Han/minority	262/4	24/0	1.000			
Location, Shaanxi/other	226/40	19/5	0.553			
Job category			0.629			
Physical	90 (33.8)	8 (33.3)				
Mental	67 (25.2)	4 (16.7)				
Middle	70 (26.3)	9 (37.5)				
Retire	39 (14.7)	3 (12.5)				
Marriage			0.136			
Never married	13 (4.9)	3 (12.5)				
Married	253 (95.1)	21 (87.5)				
Daily exercise			0.128			
< 1/2 h	18 (6.8)	0 (0)				
1/2-1 h	69 (25.9)	3 (12.5)				
1-2 h	35 (13.2)	6 (25.0)				
> 2 h	144 (54.1)	15 (62.5)				
Spicy food	145 (54.5)	14 (58.3)	0.719			
Smoking			0.024			
< half pack a day	235 (88.3)	17 (70.8)				
> half pack a day	31 (11.7)	7 (29.2)		4.714	1.569-14.16	0.006
Alcohol	50 (18.8)	5 (20.8)	0.788			
Sleep, good/bad	168/98	16/8	0.827			
Outpatient cost			0.363			
< 500	1 (0.4)	0				
500-1000	40 (15.0)	1 (4.2)				
1000-3000	76 (28.6)	7 (29.2)				
3000-5000	29 (10.9)	1 (4.2)				
> 5000	120 (45.1)	15 (62.5)				
Educational level			0.789			
Elementary and below	122 (45.9)	12 (50.0)				
High school	61 (22.9)	5 (20.8)				
College	73 (27.4)	7 (29.2)				
Postgraduate and above	10 (3.8)	0				
Previous visits			0.637			
0	80 (30.1)	9 (37.5)				
1	49 (18.4)	4 (16.7)				
2	20 (7.5)	3 (12.5)				
≥ 3	117 (44.0)	8 (33.3)				

Weight loss			0.380			
No	199 (74.8)	16 (66.7)				
< 7 lb	30 (11.3)	5 (20.8)				
≥ 7 lb	37 (13.9)	3 (12.5)				
Anemia, yes/no	0/266	5/19	< 0.001	88.27	15.486-∞	< 0.001
Anorexia, yes/no	66/200	9/15	0.222			
Vomiting, yes/no	14/252	3/21	0.156			
Melena, yes/no	16/250	3/21	0.200			
Dysphagia, yes/no	1/265	1/23	0.159			
Family history			0.627			
None	235 (88.3)	22 (91.7)				
Esophagus cancer	10 (3.8)	0				
Gastric cancer	15 (5.6)	2 (8.3)				
Other	6 (2.3)	0				
Alarm symptoms			0.038			
No	125 (47.0)	6 (25.0)				
Yes	141 (53.0)	18 (75.0)				
Number of alarm symptoms			0.009			
0	125 (47.0)	6 (25.0)				
1	96 (36.1)	10 (41.7)				
2	37 (13.9)	4 (16.7)				
3	7 (2.6)	3 (12.5)				
4	1 (0.4)	1 (4.2)				

Values are expressed as the mean ± standard deviation or *n* (%). BMI: Body mass index; M: Male; F: Female; FD: Functional dyspepsia; OR: Odds ratio; CI: Confidence interval.

symptoms in patients with Rome IV dyspepsia. For patients with dyspepsia, it is very important to identify early digestive tract diseases, and the ability of alarm symptoms to identify severe upper digestive tract diseases is limited, meaning that further study is necessary^[13,19,28]. In this study, patients with dyspepsia symptoms who met the Rome IV criteria were collected to evaluate the predictive value of alarm symptoms for dyspepsia.

FD was diagnosed strictly by laboratory examination, abdominal ultrasound and upper GI endoscopy. In the exclusion criteria of our study, we excluded patients with liver dysfunction, diabetes mellitus, thyroid dysfunction and other organic or metabolic diseases that were treated primarily as nondyspeptic diseases in clinical practice. In addition, severe abnormalities of white blood cells or platelets were considered as having other serious diseases and were excluded. Mild abnormalities of white blood cells or platelets were considered as normal results without causing any symptoms. Therefore, abnormal routine blood tests refer to anemia in this study.

In this cross-sectional study, we were unable to determine whether dyspeptic symptoms were relieved after treatment for anemia. As anemia is likely to explain dyspeptic symptoms and FD was diagnosed strictly by laboratory examination, abdominal ultrasound and upper GI endoscopy in this study, all anemia was considered organic disease regardless of whether it was proven to actually be associated with dyspeptic symptoms. Therefore, we not only evaluated the predictive value of alarm symptoms for organic dyspepsia, but also evaluated the predictive value of alarm symptoms for organic upper GI diseases to make the results more accurate. The results of this study showed that anemia was the only independent risk factor for organic dyspepsia and organic upper GI diseases among alarm symptoms based on the Rome IV criteria. Therefore, based on the Rome IV criteria, most alarm symptoms were of limited value in predicting organic dyspepsia and organic upper GI

Table 3 Characteristics of patients with epigastric pain syndrome and postprandial distress syndrome

Characteristics	EPS, <i>n</i> = 174	PDS, <i>n</i> = 31	EPS and PDS, <i>n</i> = 61	<i>P</i> value
Age in yr	49.2 ± 12.7	49.1 ± 12.7	50.8 ± 13.5	0.638
BMI in kg/m ²	22.3 ± 3.5	21.2 ± 2.7	21.3 ± 3.8	0.062
Gender, M/F	70/104	14/17	23/38	0.788
Race, Han/minority	170/4	31/0	61/0	0.742
Location, Shaanxi/other	147/27	28/3	51/10	0.733
Job category				0.172
Physical	59 (33.9)	11 (35.5)	20 (32.8)	
Mental	49 (28.2)	8 (25.8)	10 (16.4)	
Middle	44 (25.3)	10 (32.3)	16 (26.2)	
Retire	22 (12.6)	2 (6.5)	15 (24.6)	
Marriage				0.615
Never married	7 (4.0)	2 (6.5)	4 (6.6)	
Married	167 (96.0)	29 (93.5)	57 (93.4)	
Daily exercise				0.993
< 1/2 h	12 (6.9)	2 (6.5)	4 (6.6)	
1/2-1 h	47 (27.0)	7 (22.6)	15 (24.6)	
1-2 h	24 (13.8)	4 (12.9)	7 (11.5)	
> 2 h	91 (52.3)	18 (58.1)	35 (57.4)	
Spicy food	94 (54.0)	22 (71.0)	29 (47.5)	0.100
Smoking				0.225
No	149 (85.6)	24 (77.4)	46 (75.4)	
< half pack a day	11 (6.3)	2 (6.5)	3 (4.9)	
half pack-one pack a day	5 (2.9)	1 (3.2)	2 (3.3)	
> one pack a day	9 (5.2)	4 (12.9)	10 (16.4)	
Alcohol	29 (16.7)	9 (29.9)	12 (19.7)	0.265
Sleep, good/bad	115/59	18/13	35/26	0.393
Outpatient cost				0.672
< 500	1 (0.6)	0	0	
500-1000	30 (17.2)	5 (16.1)	5 (8.2)	
1000-3000	51 (29.3)	10 (32.3)	15 (24.6)	
3000-5000	18 (10.3)	4 (12.9)	7 (11.5)	
> 5000	74 (42.5)	12 (38.7)	34 (55.7)	
Educational level				0.166
Elementary and below	76 (43.7)	11 (35.5)	35 (57.4)	
High school	37 (21.3)	10 (32.3)	14 (23.0)	
College	53 (30.5)	10 (32.3)	10 (16.4)	
Postgraduate and above	8 (4.6)	0	2 (3.3)	
Previous visits				0.012
0	59 (33.9)	13 (41.9)	8 (13.1)	
1	36 (20.7)	3 (9.7)	10 (16.4)	
2	12 (6.9)	3 (9.7)	5 (8.2)	

≥ 3	67 (38.5)	12 (38.7)	38 (62.3)	
Weight loss				0.637
No	133 (76.4)	24 (77.4)	42 (68.9)	
< 7 lb	20 (11.5)	2 (6.5)	8 (13.1)	
≥ 7 lb	21 (12.1)	5 (16.1)	11 (18.0)	
Anorexia, yes/no	34/140	10/21	22/39	0.021
Vomiting, yes/no	11/163	0/31	3/58	0.535
Melena, yes/no	9/165	4/27	3/58	0.236
Dysphagia, yes/no	0/174	1/30	0/61	0.117
Family history				0.743
None	151 (86.8)	28 (90.3)	56 (91.8)	
Esophagus cancer	7 (4.0)	1 (3.2)	2 (3.3)	
Gastric cancer	10 (5.7)	2 (6.5)	3 (4.9)	
Other	6 (3.4)	0	0	

Values are expressed as the mean ± standard deviation or *n* (%). BMI: Body mass index; M: Male; F: Female; EPS: Epigastric pain syndrome; PDS: Postprandial distress syndrome.

diseases.

A systematic review reported that the global prevalence of FD among adults ranged between 1.8% and 57% according to the Rome criteria used to define FD. Among patients with dyspepsia, more than 70% had FD^[29]. In our study, among patients with dyspepsia, the prevalence of FD was 69.8% (266/381) according to the Rome IV criteria, and the rate of patients who were diagnosed with FD was slightly lower. The reason may be that the dyspeptic patients included had completed an upper GI endoscopy, an abdominal ultrasonography, a routine blood examination, a liver function test and an *H. pylori* test within the last 6 mo. Many FD patients with incomplete data were excluded.

Our data suggested that age was the independent predictor for organic upper GI diseases (OR = 1.056, *P* = 0.012). In a study by Gracie *et al*^[30] of the Rome III criteria, the age of organic upper GI diseases patients were older than of FD patients. In a prospective cross-sectional study of 839 patients, there was a significant difference in age between patients with FD and those with organic upper GI diseases^[31]. Our research results also showed that, based on the Rome IV criteria, smoking was an independent risk factor for organic upper GI diseases (OR = 4.714, *P* = 0.006, 95%CI: 1.569-14.16). FD epidemiological data indicated that smoking was a factor associated with the pathophysiology of FD^[32]. In an observational study, smoking was an independent predictor of organic dyspepsia, while Faintuch *et al* showed that smoking status was associated with organic dyspepsia. Several reports suggested that smoking was a risk factor for gastric or duodenal ulcer based on multivariable logistic regression analyses. Overall, the results in our study were remarkably comparable to those of other studies.

The relationship between clinical features and dyspepsia was not consistent^[33-36]. In this study, gender, BMI, race, location, marriage, spicy food, alcohol, sleep, daily exercise, educational level, outpatient cost and previous visits were not independent risk factors for organic dyspepsia and organic upper GI diseases, which may be related to the diverse clinical characteristics and the limited number of patients. No consistent results had been obtained on the relationship between FD and clinical characteristics in previous studies, which still needed to be confirmed by further clinical studies^[37-39].

Our study had some limitations. First, in our study, FD was diagnosed strictly by laboratory examination, abdominal ultrasound and upper GI endoscopy. The study inclusion criteria were very rigorous. Although our study was conducted at two centers, the relatively small sample size also limited the evidence strength of the results. The study population was mainly from northwest China. In the future, it still needs to be confirmed by larger sample studies from multicenters all over China. Second, because our study mainly compared FD with organic dyspepsia and FD with organic upper GI diseases, we only counted the number of patients with relief of dyspeptic symptoms after eradication of *H. pylori* (*H. pylori*-associated dyspepsia) as a

part of organic dyspepsia but did not further count the number of patients with no relief of dyspeptic symptoms after eradication of *H. pylori* and the rate of *H. pylori* infection in FD. To our knowledge, no study has been conducted to assess the prevalence of *H. pylori* in FD after excluding *H. pylori*-associated dyspepsia based on the Rome IV criteria making this a good direction for future research. Third, relevant data on psychological factors were not collected, which might be an important influencing factor and can be the next research direction.

In conclusion, most alarm symptoms had poor predictive value for organic dyspepsia and organic upper GI diseases based on Rome IV criteria, and gastroscopic screening should not be based solely on alarm symptoms. The clinical characteristics of patients with EPS, PDS and EPS combined with PDS were not significantly different.

ARTICLE HIGHLIGHTS

Research background

No studies have evaluated the predictive value of alarm symptoms for organic dyspepsia and organic upper gastrointestinal (GI) diseases based on Rome IV criteria in the Chinese population.

Research motivation

Previous studies have shown that the sensitivity of alarm symptoms for predicting cases with upper GI malignancies is unsatisfactory. The predictive value of alarm symptoms requires further research.

Research objectives

To evaluate the predictive value of alarm symptoms of dyspeptic patients based on Rome IV criteria.

Research methods

We performed a cross-sectional study of dyspepsia patients who met the inclusion and exclusion criteria from March 2018 to January 2019.

Research results

Based on the Rome IV criteria, alarm symptoms were of limited value in differentiating organic dyspepsia and organic upper GI diseases from functional dyspepsia.

Research conclusions

Most alarm symptoms had poor predictive value for organic dyspepsia and organic upper GI diseases based on Rome IV criteria. The clinical characteristics of patients with epigastric pain syndrome, postprandial distress syndrome and the two combined were not significantly different.

Research perspective

Gastroscopic screening of dyspepsia patients should not be based solely on alarm symptoms. In the future, the predictive value of alarm symptoms still needs to be confirmed by larger sample studies from multicenters all over China.

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Differential diagnosis of diarrhoea in patients with neuroendocrine tumours: A systematic review

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Abstract

BACKGROUND

Approximately 20% of patients with neuroendocrine tumours (NETs) develop carcinoid syndrome (CS), characterised by flushing and diarrhoea. Somatostatin analogues or telotristat can be used to control symptoms of CS through inhibition of serotonin secretion. Although CS is often the cause of diarrhoea among patients with gastroenteropancreatic NETs (GEP-NETs), other causes to consider include pancreatic enzyme insufficiency (PEI), bile acid malabsorption and small intestinal bacterial overgrowth. If other causes of diarrhoea unrelated to serotonin secretion are mistaken for CS diarrhoea, these treatments may be ineffective against the diarrhoea, risking detrimental effects to patient quality of life.

AIM

To identify and synthesise qualitative and quantitative evidence relating to the differential diagnosis of diarrhoea in patients with GEP-NETs.

METHODS

Electronic databases (MEDLINE, Embase and the Cochrane Library) were

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PRISMA 2009 Checklist statement:

This study followed PRISMA guidelines.

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searched from inception to September 12, 2018 using terms for NETs and diarrhoea. Congresses, systematic literature review bibliographies and included articles were also hand-searched. Any study designs and publication types were eligible for inclusion if relevant data on a cause(s) of diarrhoea in patients with GEP-NETs were reported. Studies were screened by two independent reviewers at abstract and full-text stages. Framework synthesis was adapted to synthesise quantitative and qualitative data. The definition of qualitative data was expanded to include all textual data in any section of relevant publications.

RESULTS

Forty-seven publications (44 studies) were included, comprising a variety of publication types, including observational studies, reviews, guidelines, case reports, interventional studies, and opinion pieces. Most reported on PEI on/after treatment with somatostatin analogs; 9.5%-84% of patients with GEP-NETs had experienced steatorrhea or confirmed PEI. Where reported, 14.3%-50.7% of patients received pancreatic enzyme replacement therapy. Other causes of diarrhoea reported in patients with GEP-NETs included bile acid malabsorption (80%), small intestinal bacterial overgrowth (23.6%-62%), colitis (20%) and infection (7.1%). Diagnostic approaches included faecal elastase, breath tests, tauroselcholic (selenium-75) acid (SeHCAT) scan and stool culture, although evidence on the effectiveness or diagnostic accuracy of these approaches was limited. Assessment of patient history or diarrhoea characteristics was also reported as initial approaches for investigation. From the identified evidence, if diarrhoea is assumed to be CS diarrhoea, consequences include uncontrolled diarrhoea, malnutrition, and perceived ineffectiveness of CS treatment. Approaches for facilitating differential diagnosis of diarrhoea include improving patient and clinician awareness of non-CS causes and involvement of a multidisciplinary clinical team, including gastroenterologists.

CONCLUSION

Diarrhoea in GEP-NETs can be multifactorial with misdiagnosis leading to delayed patient recovery and inefficient resource use. This systematic literature review highlights gaps for further research on prevalence of non-CS diarrhoea and suitability of diagnostic approaches, to determine an effective algorithm for differential diagnosis of GEP-NET diarrhoea.

Key words: Carcinoid syndrome; Diarrhea; Differential diagnosis; Neuroendocrine tumours; Serotonin; Systematic review

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Core tip: Patients with gastroenteropancreatic neuroendocrine tumours (GEP-NETs) often experience diarrhoea, which may have multiple synchronous causes. Although this has a considerable impact on patient quality of life, differential diagnosis of diarrhoea in patients with GEP-NETs is a relatively unexplored topic, and there is currently no formal clinical guidance. This systematic literature review provides valuable insight on the prevalence of causes of diarrhoea in patients with GEP-NETs, evidence on how these cause are diagnosed in this patient population specifically, the consequences if the true cause(s) of diarrhoea are not ascertained, and suggestions for improving differential diagnosis of GEP-NET diarrhoea.

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INTRODUCTION

Approximately 20% of patients with non-pancreatic neuroendocrine tumours (NETs) develop carcinoid syndrome (CS)^[1], which is characterised by dry flushing and diarrhoea^[2]. Carcinoid syndrome diarrhoea (CSD) arises mainly as a result of excess serotonin secretion, usually in the presence of liver metastases, and is the most common and debilitating symptom of CS.

Diarrhoea can be defined as passing three or more loose or liquid stools a day, or more often than is normal for the individual^[3]. As the definition and interpretation of diarrhoea can vary between patients and can have a wide range of causes, the differential diagnosis of diarrhoea can be complex^[4], particularly for specialists in other fields with less experience in gastroenterology. Uncontrolled diarrhoea can substantially impact on quality of life (QoL) and be disabling for patients^[5-7]. Although CS is often the cause of diarrhoea among patients with gastroenteropancreatic NETs (GEP-NETs), there are other potential aetiologies including, but not limited to, pancreatic enzyme insufficiency (PEI), bile acid malabsorption (BAM), small intestinal bacterial overgrowth (SIBO) and short bowel syndrome (SBS)^[8]. Patients with NETs could also have concomitant colorectal cancer, which could be causing diarrhoea^[9-11].

Long-acting somatostatin analogues (SSAs), such as lanreotide and octreotide, are the mainstay of treatment for the symptoms of CS through the inhibition of serotonin secretion, with additional efficacy for tumour growth control^[12,13]. Anti-diarrhoeals such as loperamide (Immodium®) and opioids can be used to assist in managing CSD but do not specifically target serotonin production, which can limit their effectiveness against CSD. Patients experiencing inadequate control of CSD despite the use of long-acting SSA therapy can be treated with telotristat, an inhibitor of the rate-limiting enzyme in serotonin synthesis named tryptophan hydroxylase. Telotristat has proven efficacy in reducing the frequency of bowel movements and levels of 5-hydroxyindoleacetic acid in patients with CS^[14]. If other causes of diarrhoea unrelated to serotonin secretion are mistaken for CSD, treatments that target the serotonin pathway may be ineffective, leaving diarrhoea uncontrolled.

While studies of symptomatic treatment of patients with CS often exclude patients with other potential causes of diarrhoea, such as SBS, the methods for diagnosing these gastrointestinal (GI) conditions are not reported^[14-17]. Also, the presence of one or more aetiologies of non-CS diarrhoea does not eliminate the possibility that a patient's diarrhoea is caused, completely or partially, by CS. Diarrhoea is therefore a more complex symptom than normally considered in patients with GEP-NETs, and there is currently no detailed guidance available to clinicians on the differential diagnosis of diarrhoea in this patient population.

The purpose of this systematic literature review (SLR) was to identify and synthesise qualitative and quantitative evidence relating to the differential diagnosis of diarrhoea in patients with GEP-NETs, including the proportion of patients with specific non-CS causes, associated diagnostic approaches, and consequences when the cause of diarrhoea is misdiagnosed.

MATERIALS AND METHODS

Search strategy

The SLR was conducted in accordance with a pre-specified protocol and reported in line with the Enhancing Transparency in Reporting the Synthesis of Qualitative Research guidelines^[18]. A comprehensive search strategy was planned and conducted to identify relevant articles. MEDLINE (including MEDLINE In-Process, MEDLINE Daily and MEDLINE Epub Ahead of Print), Embase, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials and Database of Abstracts of Reviews of Effect were searched from database inception to 12th September 2018. Search terms included combinations of free-text terms and database-specific subject headings related to GEP-NETs, CS and diarrhoea ([Supplementary Tables 1-4](#)).

Hand-searches of abstract books from relevant congresses from the last three years, reference lists of relevant studies and ClinicalTrials.gov were also performed. Google and websites of relevant medical associations were searched for guidelines on the diagnosis and management of NETs.

Study selection

Eligibility for inclusion was defined using the Sample, Phenomenon of Interest, Design, Evaluation, Research type (SPIDER) approach^[19]. The sample of interest

included adults with GEP-NETs who were experiencing diarrhoea and the phenomenon of interest was diagnosis of the cause of diarrhoea in this population. Any study design and article type were eligible if relevant data were presented (full eligibility criteria are presented in [Supplementary Table 5](#)). It should be noted that due to an overlap in symptoms with CS, patients with pancreatic NETs are sometimes excluded from studies of CS in the wider literature^[1]. Since many studies in patients with CS in the literature do not distinguish between gastrointestinal and pancreatic NETs as part of the GEP-NETs classification, patients with pancreatic NETs were also eligible for inclusion in this SLR to ensure that all relevant data on differential diagnosis of diarrhoea were captured.

Titles and abstracts of the search results were screened against the eligibility criteria by two independent reviewers; discrepancies were resolved by consensus, with arbitration by a third reviewer if necessary. Full-text versions of potentially relevant articles were acquired and screened using the same process.

Thematic framework

Framework synthesis was originally developed as a method for carrying out systematic reviews of qualitative evidence. It has also been reported as a way to facilitate the integration of quantitative and qualitative data from diverse sources^[20]; therefore, in this SLR, framework synthesis was adapted to include both quantitative and qualitative data. Unlike traditional framework synthesis, in which only qualitative research findings (collected and analysed using qualitative methods) are included, the definition of qualitative data was expanded to include all textual data in any section of relevant articles, to ensure that all relevant information was captured. A preliminary framework of themes that were expected to be identified was developed through a scoping search of the literature and discussion with clinical experts, to facilitate data extraction and synthesis.

Data extraction and synthesis

The preliminary framework was developed as a mind-map within Docear software^[21]. Two reviewers independently coded and indexed only relevant quantitative (proportion of patients with different causes of diarrhoea) and qualitative data against the pre-specified themes using an inductive approach, with data indexed against multiple themes if relevant. All relevant data from each study were synthesised into the framework at the same stage, which has been described as a data-based convergent approach to data synthesis^[22]. While data on the prevalence of different causes of diarrhoea were quantitative, no meta-analysis was planned. Instead, relevant passages of text containing the quantitative data were extracted to allow for understanding of context, to categorise data by whether the cause was inferred or confirmed diagnostically, and to allow for mapping of the data to other relevant themes if applicable. Any new themes or sub-themes that emerged from the literature were added to the framework iteratively, and all extracted data were considered against novel themes as well as those pre-specified. Data on population demographics, recruitment, country, and sample size were also captured. Any discrepancies were resolved by discussion, with arbitration by a third independent reviewer where required. Evidence for each theme was then interpreted, and a narrative synthesis of the available evidence was developed.

Quality assessment

Multiple study designs and article types were included, and relevant data were permitted to be extracted from any section of each article. As such, assessment of study design may not have been applicable to extracted data. Therefore, the use of a formal quality assessment checklist was not considered feasible. Instead, quality of the relevant data from each included publication was assessed by two reviewers (based on study design, location of data within each article and risk of bias), and was discussed until a consensus was reached. No studies were to be excluded based on quality appraisal.

RESULTS

Included studies

After de-duplication, 1627 unique records were suitable for title and abstract review. Following this, 424 full texts were screened. Supplementary searches of congresses,

reference lists of any relevant article, ClinicalTrials.gov and relevant guidelines yielded 1165 records. Of these, one novel record fulfilled the eligibility criteria.

In total, 47 publications (44 unique studies) were eligible for inclusion in the review (Figure 1). These comprised a broad range of study designs/article types, including observational studies ($n = 21$ publications on 18 studies), narrative reviews/guidelines ($n = 14$; of which one also contained a systematic review component), case reports/case study compendium ($n = 6$; of which one contained a literature review component), single-arm trials ($n = 2$), a randomised trial ($n = 1$), expert opinion with case series ($n = 1$), clinical roundtable monograph ($n = 1$), and commentary ($n = 1$).

Thematic results

An overview of the final framework is presented in Figure 2. In addition to the pre-determined themes the SLR identified three novel themes, described as “initial investigations into the cause of diarrhoea”, “approaches for inferring the cause of diarrhoea” and “advice and suggestions for approaching differential diagnosis of diarrhoea in patients with GEP-NETs”.

Proportion of patients with GEP-NETs and diarrhoea due to various causes: Twenty-one articles on 18 unique studies reported quantitative data related to different causes of diarrhoea in patients with GEP-NETs (Table 1)^[6,23-41]. The majority of articles reported on PEI and associated steatorrhea in patients receiving treatment with SSAs; 9.5% to 84% of patients with NETs in the included studies were reported to have experienced steatorrhea^[26,31], the primary symptom of PEI. In four articles in which the management of steatorrhea was reported, almost all patients with steatorrhea were treated for PEI (96%-100%)^[27,29,30]; however, only 62.5% of patients with steatorrhea may have been treated in one study (14.3% of the overall study cohort) (Table 1)^[40]. PEI was inferred from the reporting of steatorrhea only in patients on SSAs in two publications, for example the presence of steatorrhea “to varying degrees”^[31,40]. It is important to acknowledge that this does not necessarily mean that these patients had PEI^[31,40].

Seven articles (5 unique studies) reported a proportion of patients with GEP-NETs and non-CS diarrhoea directly confirmed by clinical tests, including PEI, SIBO, colitis and *Campylobacter coli* (*C. coli*) infection (Table 2)^[6,28,32-35,40]. BAM was diagnosed in 80% of NET patients who had been to a gastroenterologist service and were tested using the 75-selenium homocholic acid taurine (SeHCAT) scan ($n = 20$)^[33]. SIBO, diagnosed by breath tests, was reported in 23.6% to 62% of NET patients who had been tested for the condition^[32,33]. Single cases of colitis (20%) and *C. coli* infection (7.1%) were reported in two studies of patients with CS diarrhoea^[34,36].

Other causes were not reported in detail, including motility disorders, bowel obstruction and bowel resection. It should be noted that resection in itself is not a cause of diarrhoea, rather it can lead to conditions that cause diarrhoea (*e.g.*, SBS or SIBO), but the specific cause of diarrhoea that resulted from bowel resection was not reported^[26]. Ruzsniowski *et al*^[26] reported that of 79 patients (30%) who reported another cause of diarrhoea in addition to CS at study initiation, 32% had PEI (9.5% of all patients who provided a cause of diarrhoea), although how PEI had been diagnosed is unclear^[26]. Basuoy *et al*^[41] reported that one-fifth of patients with small bowel NETs or pancreatic NETs met the criteria for irritable bowel syndrome (IBS) with diarrhoea^[41], demonstrating that patients with GEP-NETs can be initially misdiagnosed with IBS or have synchronous NET and IBS diagnoses.

The SLR identified qualitative data reporting several other differential causes of diarrhoea, including laxative abuse^[42,43], dumping syndrome^[44], lymphangiectasia^[45] and pneumatosis cystoides intestinalis (PCI) induced by sunitinib treatment^[46]. Diarrhoea that can develop due to exacerbation or progression of CS was also reported, such as niacin deficiency/pellagra^[24,45,47,48], and adverse events of serotonergic medication^[49]. Of five case reports, two reported on infectious diarrhoea^[50,51], two on bowel obstruction^[52,53] and one on sunitinib-induced PCI^[46].

Initial investigations into the cause of diarrhoea: Fourteen articles recommended or described the use of approaches to facilitate identification of patients who could be experiencing diarrhoea due to a cause other than CS^[24,36-38,44,46,49,50,52-57]. Representative quotations for each sub-theme are presented in Figure 3, and full details are available in Supplementary Table 6.

The most commonly reported approach was to assess whether the progression of CS could be contributing to the diarrhoea^[24,37,38,49,50,53]. This was most often conducted by measuring the radiographic progression or urinary 5-hydroxyindoleacetic acid levels to assess hormonal production, either in isolation or in combination with the

Table 1 Proportion of patients with non-carcinoid syndrome diarrhoea inferred from symptoms/treatment or prevalence estimates

Ref.	Population	Condition	Diagnosis	Data
Diagnosis method or clinical definition of condition not reported				
Boudreaux ^[25] , 2016	Patients with NETs and abdominal pain, weight loss, bloating and diarrhoea (<i>n</i> = 100)	Bowel obstruction	NR	"More than one-third of these patients had an occult bowel obstruction that was complete or nearly complete because their primary tumour had never been resected"
Boudreaux <i>et al</i> ^[24] , 2010	NA (Guideline)	Bowel obstruction or ischaemia	Symptoms	"As many as 35% of patients with advanced carcinoid present with symptoms of obstruction, ischaemia, or both"
Iyer <i>et al</i> ^[23] , 2017	NA (Monograph)	PEI	NR	"Somatostatin analogues lead to more diarrhea from exocrine suppression in up to 30% of patients"
Ruszniewski <i>et al</i> ^[26] , 2016	Adults with NETs receiving lanreotide for at least 3 mo for relief of carcinoid syndrome (<i>n</i> = 273262 provided a cause of diarrhoea)	PEI	NR	"Note that the whole study population was selected based on a history of diarrhoea at some point prior to the study. Of those patients for whom a reason for diarrhoea was provided (<i>n</i> = 262), 30% (79) had another potential cause of diarrhoea in addition to CS. The most common were small bowel resection [44% (35/79), 13.4% (35/262)], pancreatic insufficiency [32% (25/79), 9.5% (25/262)], and ileocecal valve/colonic resection [24% (19/79), 7.3% ^a (19/262)]"
		Bowel resection	NR	
		Ileocecal valve or colonic resection	NR	
Saif <i>et al</i> ^[37] , 2020	Patients with GEP-NETs (<i>n</i> = 110)	Motility disorders	NR	"13 received PPI concomitantly while 6 started when symptoms did not improve with PER. Nutrition recommended low fat diet, 14 of 19 had improvement in diarrhoea within 4–8 wk. Two were non-compliant and 3 (2.7%) were found to have motility disorders"
Inferred from symptoms and treatment				
Chaudhry <i>et al</i> ^[40] , 2017	Patients with NETs referred to a gastroenterology NET clinic (<i>n</i> = 39)	PEI	Steatorrhea (faecal elastase used, but number of patients diagnosed with PEI using faecal elastase NR)	"78% (25/32) had been on long-acting SSA therapy and 81% (26/32) had steatorrhea"
Donnelly <i>et al</i> ^[33] , 2017	Patients with NETs referred to a NET gastroenterologist service (<i>n</i> = 57)	PEI	Faecal elastase used but number diagnosed with PEI using this test NR	"19 (33.3%) patients were commenced on either creon or colesevelam." Of the 20 patients who returned questionnaires: "95% of patients required treatment with creon or colesevelam for their steatorrhea or bile acid malabsorption respectively"
Fiebrich <i>et al</i> ^[39] , 2010	Acromegaly and carcinoid patients receiving treatment with SSAs (<i>n</i> = 35)	PEI	Steatorrhea, no tests reported	"8/35 (22.9% ^a) patients complained about steatorrhea. 12/35 patients experienced increased stool frequency (1–10 times daily). 5/35 (14.3%) carcinoid patients received supplementation of pancreatic enzymes for steatorrhea." It is inferred that 5/8 (62.5%) patients with steatorrhea received supplementation of pancreatic enzymes
Khan <i>et al</i> ^[27] , 2011	Patients with metastatic midgut NETs and carcinoids syndrome (<i>n</i> = 69 had complete data)	PEI	Steatorrhea, no tests reported	"35 (50.7% ^a) patients experienced steatorrhea which was controllable by pancreatic enzyme supplementation"
Lamarca <i>et al</i> ^[6,28]	Patients with NETs receiving treatment with SSAs (<i>n</i> = 50)	PEI	Steatorrhea and/or bloated abdomen	"Twelve patients (24%) developed SSA-related PEI (4 clinical diagnosis, 8 FE-confirmed) at a median of 2.9 mo after starting SSA: 11/12 (92%) patients received enzyme replacement." "SSA-induced PEI occurs in 1 out of 4 patients"
Lim <i>et al</i> ^[29] , 2017	Patients with NETs seen at gastroenterology and endocrinology clinics (<i>n</i> = 141)	PEI	Steatorrhea, no tests reported	"27 patients reported steatorrhea, 26 of whom were prescribed somatostatin analogues. 26 (96%) of these patients were also prescribed Creon." "27/141 NET patients (19.2% ^a) complained of steatorrhea. 26 were prescribed Creon"
Toumpanakis	Patients with metastatic NETs of midgut	PEI	Steatorrhea, no tests reported	"Twenty-eight (25.9%) patients developed clinical features of steatorrhea, which resolved after the initiation of

<i>et al</i> ^[30] , 2009	origin and symptoms of carcinoid syndrome who received octreotide LAR (<i>n</i> = 108)			pancreatic enzyme supplements ^a
Whyand <i>et al</i> ^[32] , 2018	Patients with NETs receiving an SSA (<i>n</i> = 176)	PEI	Steatorrhoea, no tests reported	"Pancreatic enzyme insufficiency is one cause of fat loss in stools. When the fat is obvious, it causes greasy and frothy loose stools called steatorrhoea. Among the survey respondents, 84% stated they had this to varying degrees"
Proportion of patients with non-CS diarrhoea diagnosed by clinical tests				
Donnelly <i>et al</i> ^[33] , 2017	Patients with NETs referred to a NET gastroenterologist service (<i>n</i> = 57)	PEI	Faecal elastase test (< 200 abnormal), although PEI not specifically stated in the abstract or poster	17% of patients tested (<i>n</i> = 18) had abnormal faecal elastase. Median: 296.0; Range: 14.0-64.0 (approximate)
		BAM	SeHCAT scan (> 20% = Normal)	80% of patients tested (<i>n</i> = 20) were diagnosed with BAM
		SIBO	Hydrogen breath test	62% of patients tested (<i>n</i> = 13) had SIBO
Gorbunova <i>et al</i> ^[34] , 2016	Patients with metastatic well-differentiated, functional NETs, on octreotide LAR for 5-6 mo (<i>n</i> = 5)	Colitis	CT scan	1 patient (20% ^a) diagnosed with colitis
Kiesewetter <i>et al</i> ^[35,36]	Patients given ondansetron as bridging therapy for refractory CS (<i>n</i> = 14)	Infectious diarrhoea (<i>Campylobacter coli</i>) ^a	Stool culture	1 patient (7.1% ^a) excluded after enrolment for infectious diarrhoea
Lamarca <i>et al</i> ^[6,28]	Patients receiving treatment with SSAs (<i>n</i> = 50)	PEI	Faecal elastase below the normal limit (200 µg/g)	"Twelve patients (24%) developed SSA-related PEI (4 clinical diagnosis, 8 FE-confirmed)"
Saif <i>et al</i> ^[38] , 2010	Patients with histological diagnosis of NETs (<i>n</i> = 43)	PEI	Stool studies for faecal fat	"Overall, our cohort showed that 11.6% of patients on chronic octreotide analog therapy developed pancreatic insufficiency"
Saif <i>et al</i> ^[37] , 2020	Patients with GEP-NETs following SSA therapy (<i>n</i> = 110)	PEI	Quantitative measurement of faecal fat and evidence of steatorrhoea	"19 (17.3%) had evidence of steatorrhea and received PER who received PER @ 500 units/kg/meal to a maximum of 10000 units/kg per day. 13 received PPI concomitantly while 6 started when symptoms did not improve with PER"
Whyand <i>et al</i> ^[32] , 2017	Patients with NETs undergoing HBT (<i>n</i> = 55)	SIBO	Hydrogen breath test, using glucose or lactulose substrates	"Twenty-four (24/55, 44%) had prior right hemicolectomy. Ten (10/24, 42%) of those were SIBO positive. Ten patients were positive for HBT prior to being given the glucose substrate, they all had abdominal surgery in the past. Twelve patients who tested negative for glucose HBT had repeat testing using lactulose and measured both H ₂ and CH ₄ production. This led to an additional 3 (25%) positive results". Overall, 23.6% ^a (13/55) of the overall study population were diagnosed with SIBO

^aValues were calculated by the reviewers from the available data. BAM: Bile acid malabsorption; CH₄: Methane; CS: Carcinoid syndrome; FE: Faecal elastase; CT: Computed tomography; GEP: Gastroenteropancreatic; H₂: Hydrogen; HBT: Hydrogen breath test; LAR: Long acting release; NA: Not applicable; NET: Neuroendocrine tumour; NR: Not reported; PEI: Pancreatic enzyme insufficiency; PER: Pancreatic enzyme replacement; PPI: Proton pump inhibitor; SeHCAT: Tauroselcholic [75 selenium]; SIBO: Small intestinal bacterial overgrowth; SSA: Somatostatin analogue.

assessment of patient history, presence of other symptoms of CS or physical examination^[24]. Four articles reported assessment of patient history as an important first step in investigating diarrhoea^[24,44,56,57]; for example, Gregersen *et al*^[56,57] ruled out prior resection of the small intestine or colon as a cause for diarrhoea in patients with CS, as the diarrhoea was present before the surgery.

Table 2 A summary of clinical tests used to confirm cause of diarrhoea

Condition or cause	Diagnostic test	Diagnostic criteria	Evaluation or opinion on accuracy of diagnostic test
Pancreatic enzyme insufficiency (PEI)	Faecal elastase ^[6,33,40,60-64]	Donnelly 2017 defined an abnormal test result as “< 200” but units were not specified ^[33] . Lamarca 2018: PEI defined as either an FE1 value below the normal limit (< 200 µg/g) or a reduction of ≥ 21% ^[6] . Other articles only mentioned the test in passing, for example stating that FE was evaluated or presenting a proportion of patients with abnormal FE	Chaudhry 2017: 22/32 patients had steatorrhoea with a normal faecal elastase, sensitivity of FE test for detecting steatorrhoea in patients with NETs was 15.4%. The authors concluded that there is a lack of association between FE and steatorrhoea in patients with NETs ^[40] . Donnelly 2017 reported that only 17% of patients with NETs and steatorrhoea had abnormal faecal elastase ^[33] . Lamarca 2018 acknowledged that there is a risk of false positives from diarrhoea, but concluded that faecal elastase testing is feasible, accessible and recommended for patients who develop symptoms of PEI, and report it was the basis for diagnosis in 67% of patients who developed PEI ^[6]
	Faecal fat: 72-h stool fat testing ^[37,38,63] ; Sudan stain of a spot stool measurement ^[38]	-	Faecal fat quantification is the cheapest and easiest way to confirm a diagnosis of PEI ^[38] . Sudan stain of a spot stool measurement is easier but a quantitative 72-h collection is more reliable (no clear evidence is provided to support this) ^[38] . Faecal fat test could be utilised for assessing response to PERT ^[38]
Bile acid malabsorption	SeHCAT scan ^[33,61]	SeHCAT < 20% retention	-
Colitis	CT scan ^[34]	-	-
Dumping syndrome	Provocative meal test ^[44]	-	-
Infectious diarrhoea	Bacterial: Stool culture for <i>Salmonella</i> , <i>Campylobacter</i> , <i>Shigella</i> and <i>Yersinia</i> , as well as <i>Clostridium difficile</i> , enteropathogenic <i>Vibrio</i> species, or <i>Escherichia coli</i> strains ^[50] ; Viral: Stool analyses for cytomegaly virus ^[50] ; Parasitological: Stool analysis for <i>Entamoeba histolytica</i> or <i>Giardia lamblia</i> ^[50]	-	-
Intestinal ischaemia	Angiography ^[44] (type of angiography was not further specified)	-	-
Laxative abuse	KOH stool preparation, intestinal secretion ^[42,43]	-	-
PCI (induced by sunitinib)	CT scan ^[46]	-	-
SBS	Urinary sodium (undetectable) ^[61]	-	-
SIBO	Breath tests: Hydrogen breath test ^[61] , with glucose ^[33] or lactulose substrate ^[32] ; Methane breath test ^[32,61]	-	Whyand <i>et al</i> ^[32] assessed the sensitivity of additional MBT and lactulose HBT testing on 12 (out of 55) patients who tested negative for SIBO with glucose HBT, but whose diarrhoea did not abate. This was under the rationale that patients with NETs are more likely to have distal SIBO (due to influences such as ileocecal valve resection), whereas glucose HBT may be more sensitive to proximal SIBO as glucose rarely reaches the colon. This testing yielded an additional 3 positive results, and led the authors to conclude that lactulose HBT and MBT increase sensitivity for detecting SIBO in patients with NETs who have previously undergone hemicolectomy

^aA reduction in faecal elastase value by ≥ 21% was applied by the authors in this study, but is not a standard definition in clinical practice. BAM: Bile acid malabsorption; CS: Carcinoid syndrome; CT: Computed tomography; FE: Faecal elastase; FF: Faecal fat; GEP-NET: Gastroenteropancreatic neuroendocrine tumour; HBT: Hydrogen breath test; KOH: Potassium hydroxide; LAR: Long acting release; MBT: Methane breath test; PEI: Pancreatic enzyme insufficiency; PERT:

Pancreatic enzyme replacement therapy; SeHCAT: Tauroselcholic [75 selenium] acid; SIBO: Small intestinal bacterial overgrowth; SSA: Somatostatin analogue.

Another reported approach involved assessing response to dose escalation of any treatment the patient was receiving for symptom or disease control; if GEP-NET diarrhoea fails to respond, this may be an indication that there may be another contributing cause. One article reported increasing the dose of octreotide long-acting release, observing that the diarrhoea did not improve, which partly contributed to the ultimate PEI diagnosis^[38]. By contrast, Carmona-Bayonas *et al*^[55] emphasised that other causes of diarrhoea must first be ruled out before escalating the dose of SSAs^[55].

Approaches for inferring the cause of diarrhoea: Characteristics of diarrhoea associated with specific causes were described by 14 articles^[24,31,38,39,42-45,47,48,50,58-60], and five articles described concomitant symptoms that may be present and may assist in a differential diagnosis (Supplementary Table 7)^[6,44,50,60,61]. Characteristics and symptoms described in the literature were as follows: Diarrhoea due to PEI was described as greasy, foul-smelling, floating stools^[24,42-45,47,59], known clinically as steatorrhea; patients with PEI may also have bloating, weight loss and signs of malabsorption^[6,44,60]; BAM diarrhoea may be choleric^[61]; patients with SIBO may experience a change from initially intermittent diarrhoea (caused by CS) to continuous diarrhoea, with associated flatulence, bloating and borborygmic sounds^[61]; characteristics of SBS diarrhoea were not reported, but other symptoms may include significant weight loss, electrolyte disturbance and hydration issues^[61].

Approaches for confirming the cause of diarrhoea: A total of 19 articles reported on 13 unique tests used to investigate diarrhoea for nine specific causes (Supplementary Table 8)^[6,32-34,36-38,40,42-44,46,49,50,60-64]. Of these, 12 studies had used a test for diagnosis of a condition in a population of patients with NETs^[6,32-34,36-38,40,46,49,50,60], whereas seven articles only described or recommended a test (Table 2)^[42-44,61-64].

In one study, a symptom-based diagnosis was considered acceptable for PEI in the absence of the faecal elastase test^[6]. Other general approaches included a full GI workup including upper and lower endoscopy^[44,46], and discontinuation of the treatment suspected to be causing increased diarrhoea *e.g.*, sunitinib or serotonergic medication^[46,49,65]. Lee *et al*^[46] described the use of colonoscopy, chest and abdominal X-ray, abdomino-pelvic computed tomography (CT) scan and discontinuation of sunitinib as approaches used to reach the diagnosis of PCI induced by sunitinib.

Consequences if the cause of diarrhoea is not properly ascertained: Fourteen articles described four main consequences if the cause of diarrhoea is not adequately ascertained (Supplementary Table 9)^[6,24,25,33,37-39,49-51,55,62,64,66]: Patients or clinicians may perceive CS treatment as ineffective, leading to discontinuation of treatments targeted at CS and/or addition of inappropriate interventions (which may inadvertently worsen steatorrhea); the underlying cause of diarrhoea remains undiagnosed and is

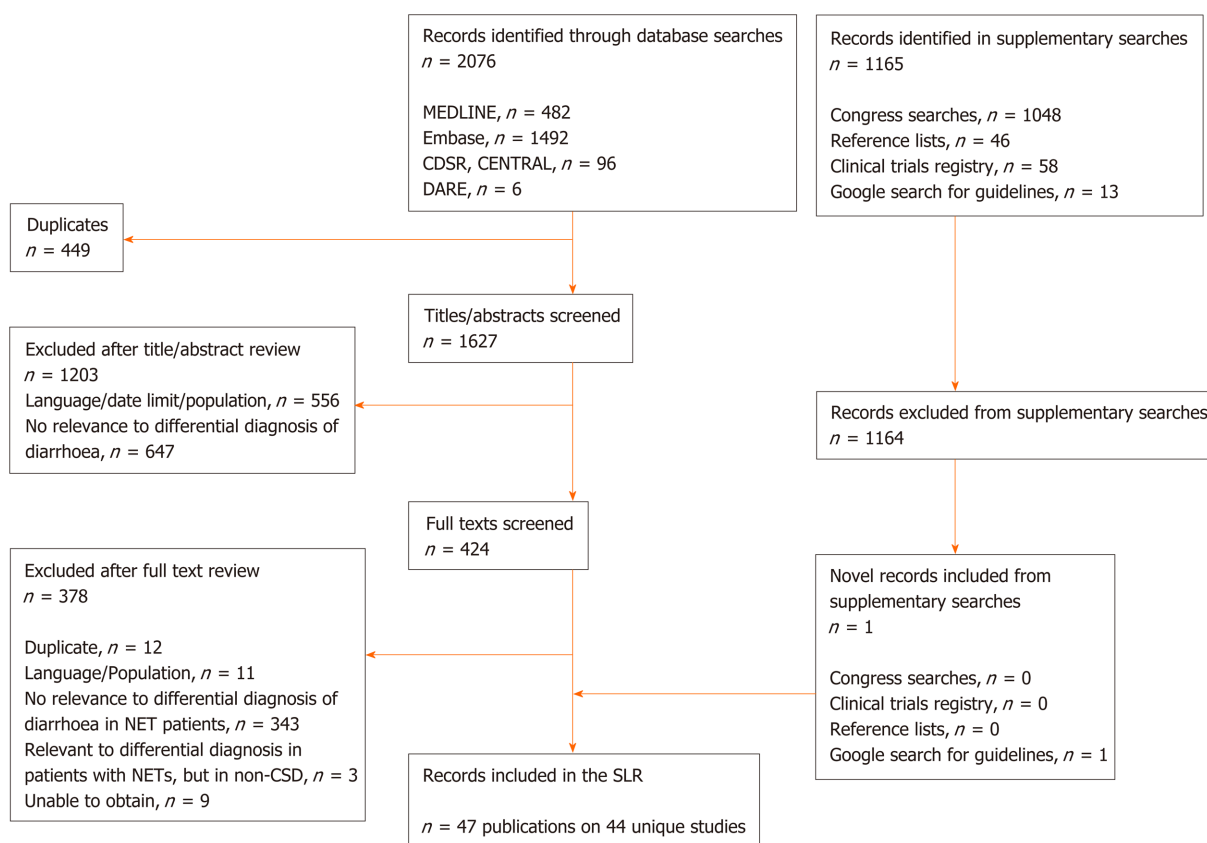


Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram. CDSR: Cochrane Database of Systematic Reviews; CENTRAL: Controlled Central Register of Controlled Trials; CSD: Carcinoid syndrome diarrhoea; DARE: Database of Abstracts of Reviews of Effects; NET: Neuroendocrine tumour; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SLR: Systematic literature review.

therefore prolonged; and patients' nutritional status may deteriorate. For example, Pavel *et al*^[67] reported that a patient discontinued telotristat due to "insufficient efficacy" as a result of persistent diarrhoea; the authors noted that there were indications that the cause of diarrhoea was PEI.

No studies directly compared outcomes in patients with and without differential diagnosis of diarrhoea. However, one study reported that 15 out of 20 (75%) patients with NETs who had systematic GI investigation and management of their diarrhoea experienced an improvement on the "impact of bowel symptoms on QoL" scale after 6 mo; among the 10 patients who completed the European Organisation for Research and Treatment of Cancer GI.NET21 questionnaire at baseline and 6 mo, there was a significant improvement in overall QoL scores^[33].

Flaherty *et al*^[51] described an outcome that could arise when a contributing cause of diarrhoea is successfully identified. In a patient undergoing chemotherapy, worsening chronic diarrhoea was diagnosed as a *Clostridium difficile* infection. In order to treat this infection, chemotherapy was halted for four months during which the patient's CS progressed, causing diarrhoea that was "difficult to control" along with weight loss and decline in performance status^[51]. Representative quotations are presented in Figure 3, and full study details are available in Supplementary Table 9.

Advice and suggestions for the differential diagnosis of diarrhoea in patients with GEP-NETs: Six articles suggested ways to approach differential diagnosis of diarrhoea in GEP-NETs, including improving patient and clinician awareness of SSA-related diarrhoea or steatorrhoea^[6,38], directly asking patients about diarrhoea^[31], regular screening for malnutrition^[31,66], and involvement of a multidisciplinary team of specialists, particularly gastroenterologists^[24,33,38]. Representative quotations are presented in Figure 3, and full study details are available in Supplementary Table 10.

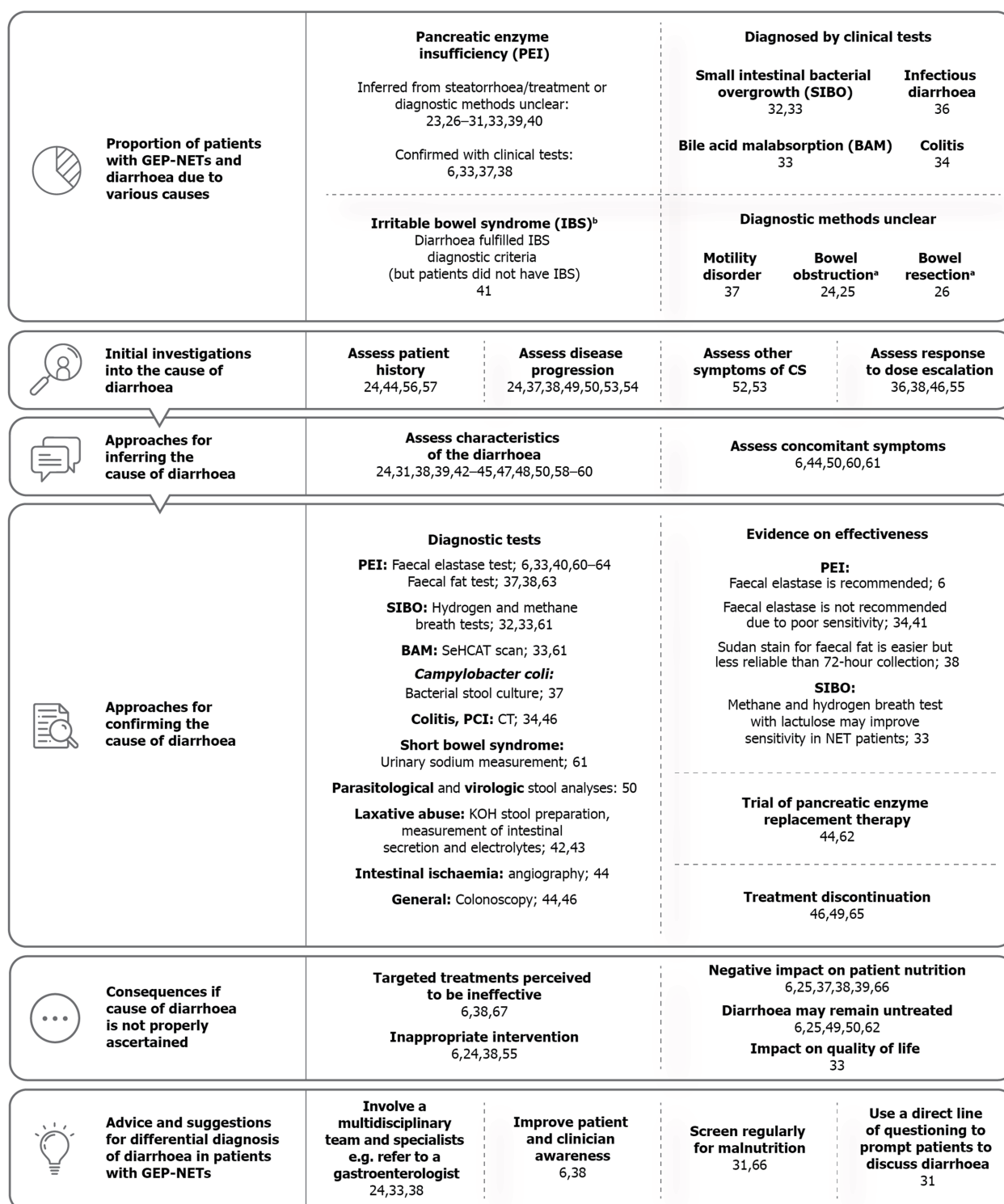


Figure 2 Overview of final framework of themes from included studies. This figure presents the thematic framework of evidence identified from the 47 included articles. It does not include all possible causes of diarrhoea or diagnostic tests that could be used to investigate diarrhoea in gastroenteropancreatic neuroendocrine tumours (GEP-NET) patients, and does not take into account the quality of the evidence for each theme. ^aBowel resection can lead to conditions that cause diarrhoea - these were not specified by the included studies. The source of bowel obstruction e.g., the NET itself, was not confirmed. ^bOne-fifth of patients with small bowel or pancreatic NETs met the criteria for irritable bowel syndrome (IBS) with diarrhoea demonstrating how patients with GEP-NETs can be initially misdiagnosed with IBS, or could have synchronous NET and IBS diagnoses^[41]. BAM: Bile acid malabsorption; CSD: Carcinoid syndrome diarrhoea; CT: Computed tomography; HBT: Hydrogen breath test; MBT: Methane breath test; GEP-NET: Gastroenteropancreatic neuroendocrine tumour; KOH: Potassium hydroxide; PEI: Pancreatic enzyme insufficiency; PERT: Pancreatic enzyme replacement therapy; SeHCAT: Tauroselcholic [75 selenium] acid.

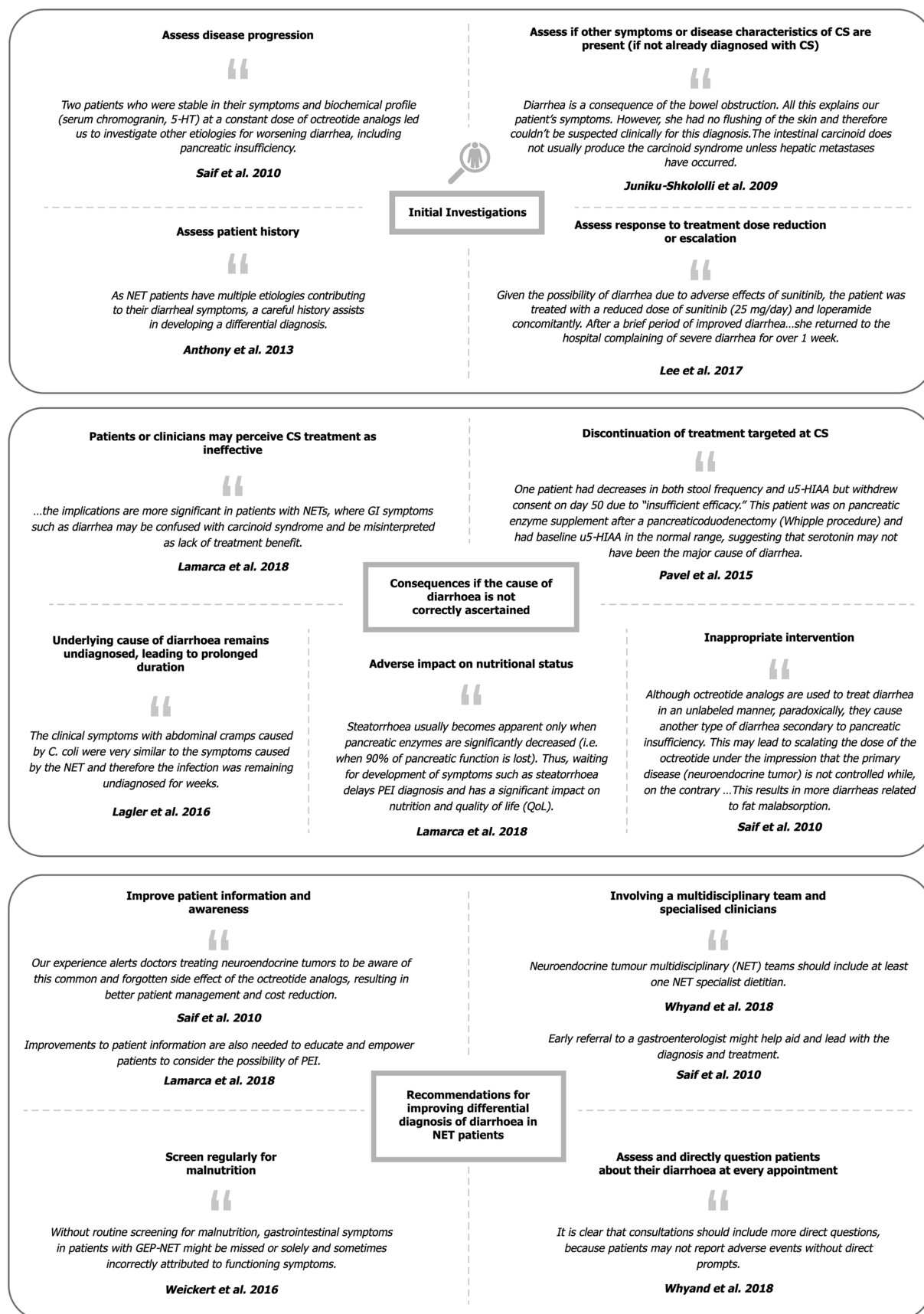


Figure 3 Representative findings for qualitative themes. CS: Carcinoid syndrome; GEP-NET: Gastroenteropancreatic neuroendocrine tumour; GI: Gastrointestinal; PEI: Pancreatic enzyme insufficiency; u5-HIAA: Urinary 5-hydroxyindoleacetic acid; 5-HT: 5-hydroxytryptamine.

DISCUSSION

This SLR is the first formal synthesis of qualitative or quantitative data related to the

differential diagnosis of diarrhoea in patients with GEP-NETs. The different causes of diarrhoea that can affect patients with GEP-NETs have been previously discussed briefly in the 2018 European Society for Medical Oncology guidelines for diarrhoea in cancer patients, and by Clement *et al*^[8] in the context of malnutrition^[4,8]. Naraev *et al*^[68] has also suggested the importance of a differential diagnosis in a narrative review on the management of CSD, highlighting PEI, BAM and SBS as a few causes to consider. However, evidence on the prevalence of these causes, the diagnostic tests that have been used to identify these causes in patients with GEP-NETs specifically, and other factors related to differential diagnosis had not yet been explored using systematic methods.

PEI-associated steatorrhea was the most common condition reported in patients with GEP-NETs; steatorrhea is a known side-effect of SSA therapy and the majority of study cohorts comprised patients who were on or had received SSA therapy. This emphasises that PEI should be considered in all patients undergoing SSA therapy as a cause of new or worsening diarrhoea (or after pancreatic resection), so that initiation of pancreatic enzyme replacement therapy can be considered in order to improve management of NET diarrhoea or steatorrhea. Not all patients with steatorrhea identified in this review were treated for PEI, suggesting that the presence of steatorrhea without consideration of severity or duration may not necessarily confirm that a patient has PEI, or impact on patients QoL sufficiently to warrant intervention. Furthermore, malabsorptive steatorrhea can also be caused by dietary factors, coeliac disease, SIBO or infections, among others. It is important to acknowledge that diarrhoea can be a short-term side effect of initiating SSAs and often resolves spontaneously^[69]; study authors emphasised the importance of patient and clinician awareness of SSA side effects, suggesting that in clinical practice, patients may not report these events without direct prompts, which may facilitate differentiation between diarrhoea and steatorrhea^[6,31].

Evaluation of test accuracy for the detection of PEI was lacking. While faecal fat testing was used or recommended for the diagnosis of PEI in three identified studies^[37,38,63], this test is no longer common in clinical practice as it is unpleasant for both patients and laboratory staff to perform^[70]. There were conflicting opinions on the efficacy of the faecal elastase test in patients with GEP-NETs, which is an issue that has also been raised by Clement *et al*^[8]. While Lamarca *et al*^[6] used and subsequently recommended the faecal elastase test for investigation of PEI in GEP-NET patients, other evidence identified in this SLR suggests that this test may not be sufficiently accurate, with poor sensitivity for the detection of steatorrhea and a high rate of false negative test results^[33,40]. Similar results have been reported previously in patients without NETs^[71], such as patients with chronic pancreatitis or patients who have undergone pancreatic resection^[72]. While suggested by only one study included in this review, empiric treatment with pancreatic enzyme therapy represents a more practical approach that is often taken in clinical practice; alleviation of symptoms may be indicative of PEI as a contributing cause to the diarrhoea^[68].

Data on other causes of diarrhoea were limited, suggesting that these causes were not considered by clinicians or study personnel, potentially due to a lack of awareness or gastroenterologist input into NET management. Randomised controlled trials that reported diarrhoea as an adverse event of treatment were not eligible for inclusion since it was unclear how many (or which) patients with GEP-NETs had diarrhoea at baseline, and methods of confirming diarrhoea as “treatment-related” were usually not reported. For example, one study reported treatment-related diarrhoea in 26% of patients with non-functional NETs receiving lanreotide; however, the same study also reported treatment-related diarrhoea in 9% of patients receiving placebo^[73], suggesting that some diarrhoea attributed to treatments may have other underlying causes. Furthermore, the Common Terminology Criteria for Adverse Events^[74] definitions for diarrhoea are not validated in patients with NETs, and do not consider relevant factors such as stool urgency or consistency.

Important approaches used in clinical practice for investigation into the cause of diarrhoea were rarely reported in patients with GEP-NETs, particularly endoscopy. Upper and lower gastrointestinal endoscopy can be used to investigate unexplained diarrhoea and to diagnose coeliac disease, colitis of different causes, colorectal cancer and is also useful in the work up for IBS. However, the use of endoscopy was only reported by two studies identified by this review. This could be due to use of colonoscopy as an index investigation for diagnosis of tumours in patients presenting with diarrhoea, rather than in patients with an existing GEP-NET diagnosis in alignment with the scope of this review.

Clinical misdiagnoses can lead to inefficient use of clinical resources and funding, or to progression of the true cause, thereby potentially risking the health and QoL of the

patient. One study captured by this review reported improvements to patient QoL after undergoing differential diagnosis of diarrhoea, and other studies have shown that a higher number of bowel movements are associated with lower QoL in patients with GEP-NETs^[7,75]. Furthermore, a real-world evidence study reported that total healthcare resource use costs increased with uncontrolled CS in patients with GEP-NETs by up to 40% per patient, when compared with controlled CS^[76]. This highlights an important opportunity for improving QoL in patients with GEP-NETs and efficient use of clinical resources; identifying the true cause(s) of diarrhoea may facilitate optimal symptom control through targeted management. Suggested approaches for improving differential diagnosis of GEP-NET diarrhoea include improving awareness of non-CS diarrhoea, or screening regularly for malnutrition. However, these approaches have not been investigated in clinical practice in patients with GEP-NETs and are therefore not evidence-based recommendations, but may represent methods to pursue in future research. It is clear that multidisciplinary team discussions including gastroenterology are necessary to ensure that clinicians with expertise on the possible causes of diarrhoea in cancer patients and subsequent management options are involved alongside oncologists, to allow for a thorough assessment and the effective use of resources in clinical practice.

Differential diagnosis of diarrhoea outside of the GEP-NET population

There are guidelines for the investigation of chronic diarrhoea outside of the NET population, such as those provided by British Medical Journal Best Practice and the British Society of Gastroenterology^[77,78]. The diagnostic approaches identified by this review do not necessarily represent the sole or optimal approaches available. Colitis was reportedly diagnosed in a patient with a GEP-NET by CT scan in one included study, but in wider clinical practice blood or stool tests and/or endoscopy may be preferred methods^[79]. Similarly, while angiography was recommended as a “definitive” diagnostic method for intestinal ischaemia by Anthony^[44], and has traditionally been considered the “gold standard”^[80], CT imaging is also a core component of investigation for intestinal ischaemia^[80,81].

Guidance on the diagnosis of specific causes of diarrhoea that were lacking in GEP-NETs can be sought from the literature, such as for SBS. Since the primary cause of SBS is surgical resection of the small intestine, patient history of this procedure, and stoma output, may be indicative of SBS, with blood tests, stool or urine analyses, imaging and biopsies conducted to confirm the diagnosis^[82]. Supporting the findings of this review, the SeHCAT scan is widely reported as a diagnostic test for BAM^[83]. It is a validated method and does not require multiple stool samples, which may improve patient compliance^[84]. Measurement of faecal bile acids has also been suggested with the caveat that it is technically challenging^[84]. Empiric therapy with bile acid sequestrants may also be given in clinical practice^[68]. For the investigation of SIBO, small bowel culture techniques such as quantitative culture of jejunal aspirate have previously been considered the gold standard for diagnosis; however, breath tests are now often favoured due to their non-invasive nature and lower costs, although they have varying sensitivities^[85-87]. Laboratory analyses such as stool cultures for the identification of infectious pathogens are reported to be inefficient and expensive^[88]; molecular panels named “culture-independent diagnostic tests” can simultaneously test for multiple pathogens and offer improved sensitivity and less time required compared with stool culture techniques^[89].

Synchronous causes of diarrhoea

It is important to acknowledge that the presence of one particular condition, for example BAM confirmed by a positive SeHCAT scan result, does not necessarily confirm that the condition is the sole or dominant cause of diarrhoea - it is possible that CS or another underlying condition is also contributing. No specific guidance for distinguishing between two simultaneous causes of diarrhoea in patients with GEP-NETs was identified, although the complexity of this scenario was demonstrated in a case of concomitant CS and infectious diarrhoea, which resulted in worsening CSD during treatment of the infectious cause^[51]. As noted in guidance for differential diagnosis of GI symptoms related to pelvic radiation disease, in which patients are also likely to be suffering with multiple causes of diarrhoea simultaneously^[90], it is likely that relying on any single diagnostic approach will be insufficient for differential diagnosis of diarrhoea in patients with GEP-NETs.

Strengths and limitations

This review used systematic methods in line with established guidelines to conduct an

exhaustive search of the literature^[9]. Although methods for combining quantitative and qualitative evidence are still under development, the novel adaptation of framework synthesis and the comprehensive inclusion approach allowed for the extraction and synthesis of heterogeneous data from diverse sources in this review. However, searches were both language- and date-limited which may have neglected relevant information and possibly limits the global applicability of the findings.

Overall, the evidence was limited and of low quality; this was largely due to the source of qualitative data (statements sourced from narrative reviews or discussion sections, and therefore not collected through robust methods) and study heterogeneity. Moreover, the use of a standardised quality assessment checklist was not feasible. Finally, it should be noted that the purpose of this review was to synthesise published literature on this topic rather than to develop any recommendations for clinical practice; therefore, no formal instrument was used to grade the identified evidence.

CONCLUSION

In conclusion, this review employed framework synthesis to synthesise heterogeneous data on differential diagnosis of diarrhoea in patients with GEP-NETs, identified by a comprehensive search of the literature. It is clear from the findings that there is a need for increased awareness and further research on the prevalence of non-CS diarrhoea aetiologies and on the suitability of diagnostic approaches, to determine the most effective algorithm for differential diagnosis of GEP-NET-related diarrhoea. Improved diagnosis of the cause(s) of diarrhoea, and the involvement of gastroenterology expertise alongside oncologists and endocrinologists, would improve the management of patients with NETs and provide opportunities for improving patients' QoL.

ARTICLE HIGHLIGHTS

Research background

Although carcinoid syndrome (CS) is often the cause of diarrhoea among patients with gastroenteropancreatic neuroendocrine tumours (GEP-NETs), other causes to consider include pancreatic enzyme insufficiency (PEI), bile acid malabsorption (BAM) or small intestinal bacterial overgrowth (SIBO). If other causes of diarrhoea unrelated to serotonin secretion are mistaken for CS diarrhoea, these treatments may be ineffective against the diarrhoea, risking detrimental effects to patient quality of life.

Research motivation

CS diarrhoea has a considerable impact on patient quality of life, but the differential diagnosis of causes of diarrhoea in patients with GEP-NETs is a relatively unexplored area of research, and there is currently no formal guidance for clinicians.

Research objectives

The objective of this research was to synthesise evidence on the differential diagnosis of diarrhoea in patients with GEP-NETs, including: (1) The prevalence of different non-CS causes of diarrhoea in patients with GEP-NETs; (2) The diagnostic approaches for diarrhoea in patients with GEP-NETs, including initial investigations and clinical testing for specific gastrointestinal conditions; (3) The potential consequences for patients if the true cause(s) of diarrhoea are not ascertained; and (4) Suggestions and advice for improving differential diagnosis of diarrhoea.

Research methods

Electronic databases were searched from inception to 12th September 2018 using terms for NETs and diarrhoea. Congresses, systematic literature review bibliographies and included articles were also hand-searched. Any study design and publication type were eligible for inclusion if relevant data on a cause(s) of diarrhoea in patients with GEP-NETs were reported. Framework synthesis was adapted to synthesise quantitative and qualitative data.

Research results

Forty-seven publications (44 studies) were included. Twenty-one articles (18 studies)

reported on the prevalence of specific causes of diarrhoea; 9.5%–84% of patients with GEP-NETs had experienced steatorrhoea or PEI. Other causes of diarrhoea included BAM (80% of patients), SIBO (23.6%–62%), colitis (20%) and infection (7.1%). Initial approaches for investigation primarily included assessing possible progression of CS and patient history. Characteristics of diarrhoea or concomitant symptoms of such causes were also described. Diagnostic approaches for diarrhoea included faecal elastase or faecal fat testing (PEI), hydrogen and/or methane breath tests (SIBO), tauroselcholic (selenium-75) acid (SeHCAT) scan (BAM) and stool culture (infectious causes). Evidence on the effectiveness or diagnostic accuracy of these tests in patients with GEP-NETs was limited. Fourteen articles described consequences if the cause of diarrhoea is not correctly diagnosed: Patients or clinicians may perceive CS treatment as ineffective, may discontinue treatment targeted at CS and/or may use inappropriate interventions; also, diarrhoea is prolonged, and patients' nutritional status may subsequently deteriorate. Improving patient and clinician awareness, directly asking patients about diarrhoea, and involving a multidisciplinary clinical team, including gastroenterologists, were reported as approaches to facilitate effective diagnosis of the underlying cause(s) of diarrhoea.

Research conclusions

PEI has been found to be relatively frequent in patients with GEP-NETs undergoing somatostatin analogues therapy, with other reported occurrences of SIBO, BAM and infectious diarrhoea. While author recommendations were available, evidence or opinion on the accuracy of diagnostic approaches in patients with GEP-NETs specifically were either contradictory or lacking completely. Furthermore, no specific guidance for distinguishing between two synchronous causes of diarrhoea was identified. Observational and/or interventional research in patients with GEP-NETs experiencing persistent diarrhoea would be beneficial, in order to investigate the most effective diagnostic and management algorithms and the subsequent impact on patient outcomes, to facilitate development of clinical guidance.

Research perspectives

There is a need for increased awareness and further research on the prevalence of non-CS diarrhoea aetiologies and on the suitability of diagnostic approaches, to determine the most effective algorithm for differential diagnosis of GEP-NET-related diarrhoea. In clinical practice, involvement of gastroenterology expertise alongside oncologists and endocrinologists would improve the management of patients with GEP-NETs and provide opportunities for improving quality of life.

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Endoscopic full-thickness resection to treat active Dieulafoy's disease: A case report

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Abstract

BACKGROUND

At present, minimally invasive endoscopic treatment is mostly used for patients with actively bleeding Dieulafoy's lesions, as it has the advantages of minimal trauma, short operation time and good hemostatic effect, although bleeding can easily recur postoperatively. Recently, extensive gastric cuneiform resection has been advocated for use in these patients because the constant-diameter artery follows a long path to the gastric mucosa.

CASE SUMMARY

A 47-year-old man was admitted to the hospital for repeated hematemesis and black stool, and he was diagnosed with Dieulafoy's disease. We chose a method that not only simulates surgical gastric cuneiform resection but also reduces trauma. We performed enlarged local endoscopic full-thickness resection of the gastric wall and abdominal constant-diameter artery and sutured the gastric wall. Postoperative follow-up showed that the constant-diameter artery had been resected from the gastric wall, which was confirmed to have no blood flow signals by endoscopic ultrasonography.

CONCLUSION

Endoscopic full-thickness resection of the gastric wall and abdominal constant-diameter artery with suturing of the gastric wall has demonstrated potential as a new treatment for Dieulafoy's disease.

Key words: Dieulafoy's disease; Endoscopic full-thickness resection; Ultrasound gastroscopy; Case report

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Core tip: Gastric Dieulafoy's disease, also known as gastric submucosal constant-diameter arterial malformation, can occur in any part of the digestive tract but is most common in the esophagus and within 6 cm of the gastroesophageal junction. The main symptoms of this disease are recurrent vomiting and tar-like stool. In severe cases, patients can develop hemorrhagic shock, and the mortality rate is high. We performed enlarged local endoscopic full-thickness resection of the gastric wall and abdominal constant-diameter artery with suturing of the gastric wall. We report a case diagnosed as Dieulafoy's disease. This is the first case of Dieulafoy's disease treated by this method.

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INTRODUCTION

Gastric Dieulafoy's disease, also known as gastric submucosal constant-diameter arterial malformation, can occur in any part of the digestive tract but is most common in the esophagus and within 6 cm of the gastroesophageal junction. The main symptoms of this disease are recurrent vomiting and tar-like stool. In severe cases, patients can develop hemorrhagic shock, and the mortality rate is high. Minimally invasive endoscopic treatment is mostly used, as it has the advantages of minimal trauma, short operation time and good hemostatic effect. Endoscopic treatment is simple and minimally traumatic, but bleeding can easily recur postoperatively. Recently, extensive gastric cuneiform resection has been advocated for use in these patients because the constant-diameter artery follows a long path to the gastric mucosa.

We performed enlarged local endoscopic full-thickness resection (EFR) of the gastric wall and abdominal constant-diameter artery with suturing of the gastric wall. Postoperative follow-up showed that the vascular lesion had been resected from the gastric wall, which was confirmed to have no blood flow signals by endoscopic ultrasonography.

CASE PRESENTATION

A 47-year-old male was admitted to the hospital due to abdominal pain for 1 wk, vomiting and black stool. Before admission, the patient had no symptoms, such as middle and upper abdominal distension, pain, fever, vomiting, and diarrhea. Three hours before admission, the patient suddenly defecated black stool twice, accompanied by vomiting dark red blood twice (approximately 500 mL), dizziness, and fatigue; thus, he made an emergency visit to our hospital. One year ago, the patient had been admitted to our hospital for vomiting dark blood. Painless gastroscopy suggested a focal mucosal ulcer on the posterior wall along the curvature of the gastric body, and blood clots could be seen. Dieulafoy's disease was considered. The wound was closed with a metal clip after high-frequency electrocoagulation (Figure 1). No rebleeding occurred, and the patient was discharged from the hospital.

The patient had no history of hypertension, coronary heart disease, diabetes, smoking or drinking, and denied a similar family history.

The examination on admission revealed the following: Body temperature, 36.7°C; pulse, 80 beats/min; respiration, 20 times/min; blood pressure, 93/64 mmHg; acute illness, an anemic appearance, a clear mind, conjunctiva, pale nail beds, normal heart and lung findings, a soft abdomen, upper abdominal tenderness, no muscle tension or rebound pain, negative mobility, and normal intestinal sounds.

The routine blood test results showed a hemoglobin level of 82 g/L, a positive result for fecal occult blood and normal findings regarding coagulation, liver and kidney function, electrolytes, and tumor markers.

Electrocardiography and chest radiography were performed. Abdominal color Doppler ultrasound suggested a left renal cyst. Painless gastroscopy suggested a focal

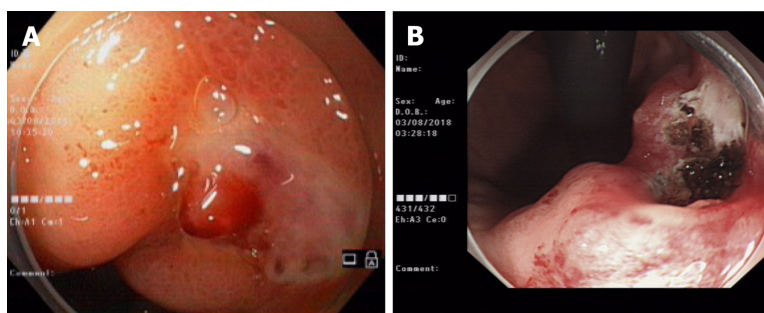


Figure 1 One year ago, bleeding in the small curvature of the stomach suggested Dieulafoy's disease (A), and high-frequency electrocoagulation was applied for hemostasis (B).

mucosal ulcer on the posterior wall along the curvature of the gastric body, and blood clots could be seen (Figure 2). Endoscopic ultrasonography revealed that the ulcer on the posterior wall of the small curvature of the stomach was approximately 0.8 cm x 1.0 cm. The structure of the first four layers of the gastric wall was not clear. There were no obvious tubular structures, but blood flow signals could be seen, and pulse Doppler showed an arterial blood flow signal; the serosa was complete (Figure 3).

FINAL DIAGNOSIS

Dieulafoy's disease.

TREATMENT

Surgery was planned and contraindications were evaluated. The patient signed an informed consent form for EFR. EFR was performed under endotracheal intubation and anesthesia. The procedures were as follows (Figure 4 and 5): (1) A DualKnife (KD-650L, Olympus, Japan) was used to mark a 1 cm margin around the lesion one week before surgery, and a disposable needle (NM-200U-0423 Olympus Japan) was used to inject fluid into the marking lateral submucous membrane to form a liquid pad; (2) The lateral side of the marking was cut layer by layer from the gastric wall to the abdominal cavity, and the small blood vessels were separated by electrocoagulation; (3) The large blood vessels on the serosal surface of the lesion were fully exposed with the use of soft tissue clamps (ROCC-D-26-195, Nanjing Minimally Invasive, China); (4) After carefully separating the soft tissue around the large vessels with the DualKnife, a disposable hemostatic clip (M00522610, Poko National Medical, United States) was placed in the medial serosa of the abdominal cavity, and the blood vessels were severed at the serosal surface. After achieving complete hemostasis, the lesion was completely resected; (5) Several soft tissue clips and nylon sutures (HX-20Q-1, MAJ-254, Olympus, Japan) were used to close the wound; and (6) A gastrointestinal decompression tube was inserted.

After the operation, the patient fasted from water, and gastrointestinal decompression, acid suppression, protection of the gastric mucosa, intravenous nutrition, anti-inflammation, and hemostasis were administered. The patient was discharged from the hospital without bleeding, abdominal pain, and infection.

OUTCOME AND FOLLOW-UP

Postoperative pathology revealed gastric Dieulafoy's disease (Figure 6). Two months after the operation, the wound was re-examined by gastroscopy, and no blood flow signals or tubular echogenicity were observed in the gastric wall (Figure 7).

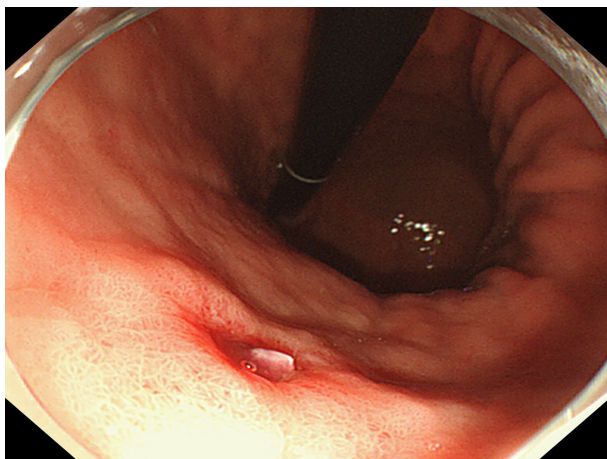


Figure 2 Dieulafoy's lesion under white light endoscopy.

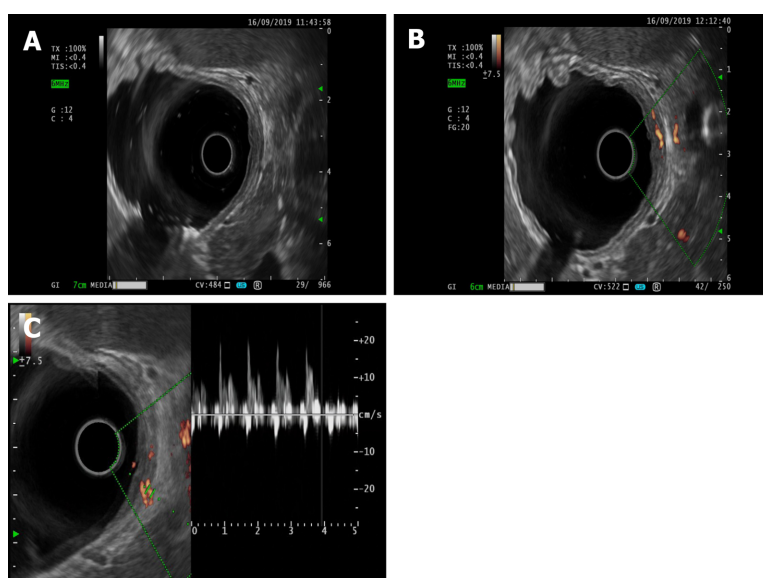


Figure 3 Endoscopic ultrasonography. A: Dieulafoy's lesion under endoscopic ultrasonography; B: Ultrasonic Doppler showing the blood flow signal of the myocombrane muscle and submucosa in the lesion area; C: Doppler spectrum representing the arterial blood flow spectrum.

DISCUSSION

Gastric Dieulafoy's disease, also known as gastric submucosal constant-diameter arterial malformation, can occur in any part of the digestive tract but is most common in the esophagus and within 6 cm of the gastroesophageal junction^[1]. The main symptoms of this disease are recurrent vomiting and tar-like stool. In severe cases, patients can develop hemorrhagic shock, and the mortality rate is high.

In our patient with Dieulafoy's disease, normal gastric mucosa was observed, no varicose veins were observed on the mucosal surface, and only the active bleeding point was found in the small curvature of the gastric cardia. Careful observation of this area revealed superficial erosion, with mucosal defects and rash-like protuberances, and while passive or active bleeding from the surface was observed, the surrounding mucosa was normal. If bleeding stops, blood clots may be attached to the mucosal surface, and the bleeding points can be found by wiping the clots away with an absorbent gelatin sponge. In some patients, arterioles protrude into the gastric cavity through superficial defects in the gastric mucosa and exhibit active bleeding. If superficial, actively bleeding lesions in the gastric mucosa are observed, Dieulafoy's disease can be considered^[2]. Emergency gastroscopy is the first choice and the most intuitive diagnostic method, and preliminary treatment can be provided according to the findings, followed by angiography, which can be used as an independent diagnostic method or a remedial method when the findings of the endoscopic

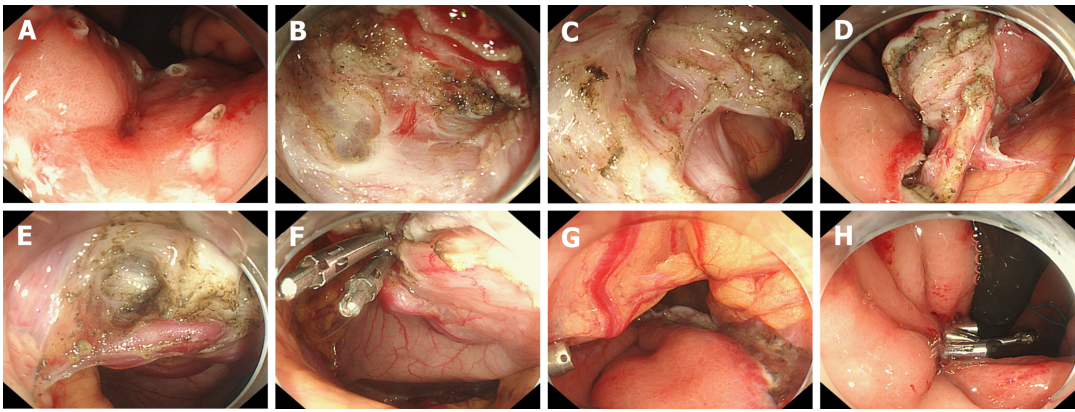


Figure 4 Operational procedures. A: Mark the lesion; B: Dissect the lesion layer by layer; C: Cut the full thickness of the lesion through the stomach wall to the abdominal cavity; D: Provide traction to the lesion; E: Expose the large vessels on the serosal surface; F: Clip thick vessels on the serosal surface; G: Examine the wound postoperatively; H: Suture the wound.

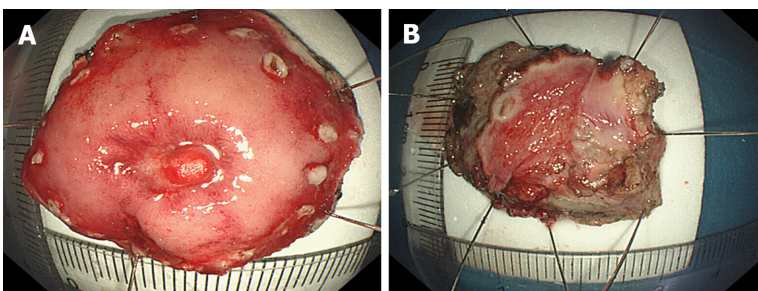


Figure 5 Excision of the specimen.

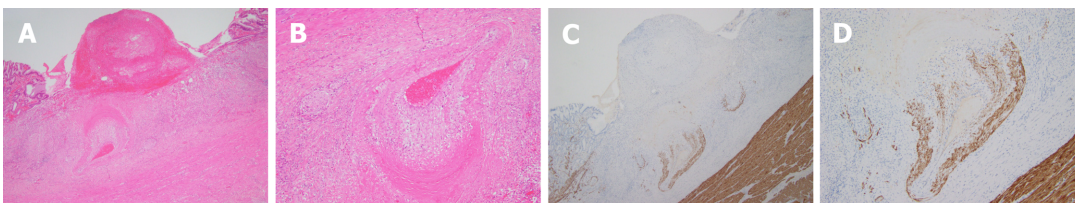


Figure 6 Postoperative pathology. A: An ulcer that reached the muscular layer of the mucous membrane. Blood clots were present in the middle. There was denatured fibrous tissue at the bottom, a dilated and tortuous artery, intimal degeneration, vitreous degeneration of the smooth muscle near the ulcer side, and uneven thickness between the left and the right. Hematoxylin and eosin (HE) staining, $\times 40$; B: Magnified image of Figure 6A. HE staining, $\times 100$; C: Immunohistochemical desmin staining showing incomplete smooth muscle of the arterial wall and varying thickness, $\times 40$; D: Magnified image of Figure 6C; magnification $\times 100$.

examination are negative^[3]. This examination should be carried out in the case of active bleeding, which can be considered when the amount of bleeding is ≥ 0.5 mL/min. During the period of intermittent bleeding, the diagnostic rate of angiography is low.

At present, for patients with actively bleeding Dieulafoy's lesions, minimally invasive endoscopic treatment is mostly used, as it has the advantages of minimal trauma, short operation time and good hemostatic effect. When endoscopic treatment is ineffective or bleeding occurs again, surgery should be selected accordingly. The surgical methods applied include subtotal resection of the proximal stomach and local cuneiform resection. Extensive gastric cuneiform resection is currently advocated in such patients. If endoscopic therapy fails or if the patient cannot tolerate surgery, angiography and embolization can be selected^[4]. However, embolization requires superselective catheterization of the left gastric artery. Angiography can be used to determine the point of bleeding without affecting collateral vessels and can be performed if the vital signs are stable and there is sufficient time for embolization. This technique is often performed, but the success rate is low.

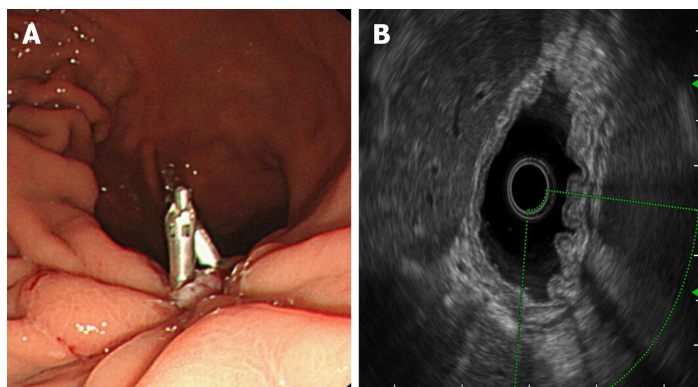


Figure 7 Endoscopic ultrasonography. A: Wound healing, residual small ulcer and metal clip; B: Focus scar area showing thickening and hyperechoic changes, with no blood flow signals on ultrasonic Doppler.

Endoscopic treatment is simple and minimally traumatic, but bleeding can easily recur postoperatively. Recently, extensive gastric cuneiform resection has been advocated for use in these patients because the constant-diameter artery follows a long path to the gastric mucosa. With resection, the cause of the disease can be ruled out, the recurrence of bleeding can be avoided, and the final diagnosis can be established by pathological examination of the resected specimen. However, because of the high degree of trauma, this surgery is not often applied. Therefore, we performed enlarged local ERF of the gastric wall and abdominal constant-diameter artery with suturing of the gastric wall. This method not only simulates surgical gastric cuneiform resection but also reduces trauma. This approach has promise as a new treatment for Dieulafoy's disease. Instead of performing endoscopic submucosal dissection for the treatment of static Dieulafoy's disease, as reported by Yang *et al*^[5] and Chen *et al*^[6], as the patient in this case had an active lesion similar to a submucosal eminence of the gastric stroma, we gradually dissected and locally expanded the range from the mucous membrane to the abdominal cavity to find, suture and detach the source of the constant-diameter artery to the abdominal cavity so that the gastric wall could be completely removed by extensive local resection of the gastric wall. This approach avoids recurrence. There are also risks associated with full-thickness resection, such as pneumoperitoneum, bleeding, abdominal infection and damage to adjacent tissue or organs. Due to the routine use of carbon dioxide gas during the operation, abdominal puncture is needed after the operation to exhaust pneumothorax or pneumoperitoneum, and attention should be paid to the treatment of exposed blood vessels. The serosal surface of the large blood vessels is particularly important. Adequate treatment of blood vessels is required to avoid serosal surface bleeding. Sufficient washing of the gastrointestinal mucosa preoperatively and the prophylactic use of antibiotics before resection of the focus to remove gastrointestinal fluid, as well as wound closure as soon as possible to reduce the operation time, can prevent abdominal infection. Endoscopic physicians with experience in EFR and natural orifice transluminal endoscopic surgery are required for this treatment. However, the branches of Dieulafoy's lesions pass through the submucosa to the mucosal layer with a constant diameter and then turn back and twist to the serosal surface, resulting in acute angles or vertical vascular loops. Therefore, how much areas of the gastric wall should be resected in the process of endoscopic surgery, with the bleeding focus as the center, to completely remove the constant-diameter artery, needs to be confirmed in more cases. We attempted to detect arterial signals on endoscopic ultrasonography and detect the shape of the artery by injecting methylene blue and other stains into the blood vessel under endoscopic ultrasonography to completely remove the lesion with the smallest surgical range and reduce the surgical trauma. The results showed that the lesion could be completely resected with the smallest surgical range and that the surgical trauma could be reduced.

CONCLUSION

EFR is a novel treatment for Dieulafoy's disease. The method simulates surgical gastric wedge resection and has value in reducing trauma, removing Dieulafoy's lesions and avoiding recurrent bleeding. However, at the same time, due to certain risks, this

method needs to be performed by an experienced endoscopic physician. The safety and efficacy of this method requires validation by further large-sample studies, and we will conduct further prospective studies after a full assessment.

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Comment on pediatric living donor liver transplantation decade progress in Shanghai: Characteristics and risks factors of mortality

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Abstract

Since the first successful liver transplantation was performed five decades ago, pediatric liver transplantation has become the gold standard treatment choice for pediatric liver disease, including metabolic diseases, liver tumors, and some acute liver failure. With improvements in immunosuppression, surgical techniques, and postoperative medical care, long-term outcomes of patients after liver transplantation have markedly improved, especially in pediatric patients.

Key words: Pediatric end stage liver disease; Living donor pediatric liver transplantation; Survival analysis; Risk factors; Living donor liver transplantation; Outcomes

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Core tip: With improvements in immunosuppression, surgical techniques, and postoperative medical care, long-term outcomes of patients after liver transplantation have markedly improved, especially in pediatric patients. We read with great interest the recent article “Pediatric living donor liver transplantation decade progress in Shanghai: Characteristics and risks factors of mortality” published by Pan and colleagues. We would like to share our opinion and criticisms about this valuable work.

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TO THE EDITOR

We read with great interest the recent article “Pediatric living donor liver transplantation decade progress in Shanghai: Characteristics and risks factors of mortality” published by Pan *et al*^[1]. In this retrospective observational study, the authors stated that they aimed to review the status of pediatric living donor liver transplantation (LDLT) and investigate the factors related to anesthetic management and survival rate in pediatric LDLT. We would like to share our opinion and criticisms about this valuable work.

The authors excluded 15 patients who were older than 12 years. The World Health Organization has stated that any individual younger than 19 years old should be considered in the pediatric age group. Therefore, the authors should have included all patients under the age of 18 years in the pediatric age group.

The authors analyzed four discrete time intervals in terms of survival analysis. The criteria for the choice of time interval are not clear from the author’s data. In our opinion, an ROC curve analysis would have determined the optimal time interval (years) in accordance with the survival of the patients^[2]. If the time intervals were chosen arbitrarily, a probability of bias during the analysis could exist. Therefore, it is no longer important to calculate clinical cutoff points today because clinical experience is the least important data in evidence-based research^[3].

Interquartile range (IQR) is the difference between the third quartile (Q3: 75%) and the first quartile (Q1: 25%) of a given variable, and therefore, IQR is supposed to be a single number. The authors have given the variable range (Q1-Q3) under IQR, which is not the IQR itself^[4,5]. This is an important error from a statistical point of view.

The authors have stated that they have performed a multivariate analysis on the parameters that had a *P* value < 0.05 in univariate analysis. In our opinion, this results in the loss of certain parameters that could have been potential risk factors during the process. Other studies have shown that certain factors that were not significant in the univariate analysis could become significant risk factors in a multivariate analysis, which is the most typical example of the interaction between variables. Therefore, we suggest that any parameter with a *P* value between 0.1 and 0.2 should be included in the logistic regression model, which will provide a more valuable result^[6-9].

The authors stated that “to identify independent predictive factors of in-hospital survival, the Chi square test with the Yates correction or the Fisher’s exact test were used”; this is statistically incorrect. Independent risk factors for any given categoric condition should always be performed using multivariate analysis methods. Any given parameter that is significant in the univariate analysis may not be an independent risk factor in the multivariate analysis.

The authors determined a cutoff value for 14 variables including age, weight, PELD score, hemoglobin level, duration of operation, and anesthesia and evaluated the relationship between these cutoff values and in-hospital mortality. The authors may have caused bias in this analysis. They should have performed an ROC curve analysis to determine optimal cutoff values with the highest sensitivity and specificity, which would result in higher reliability^[2].

We believe that there are errors in the results of the statistical analyses stated. When we analyzed the results using SPSS version 25, we found the following results, which we believe should be corrected by the authors using Yates’ correction for continuity^[9,10]: Anesthesia duration [*P* = 0.046; OR = 0.46 (0.29-0.93)], PELD score [*P* = 0.032; OR = 3.8 (1.15-12.5)], operation time [*P* = 0.006; OR = 0.38 (0.19-0.74)], ICU stay [*P* < 0.001; OR = 0.24 (0.11-0.52)], and intraoperative blood loss (*P* = 0.069).

The authors analyzed the risk factors that had an impact on 1-year and 3-year survival of the patients using univariate analysis methods. Subsequently, they selected the parameters with a *P* value < 0.05 and performed a Cox regression model. They did not perform a multivariate analysis for in-hospital mortality, which means they did not analyze the independent risk factors of mortality in the first postoperative 30-d period. In either case, they did not perform a multivariate analysis to determine the independent risk factors of mortality or factors that have an impact on survival at any time point.

The authors stated that PELD score, anesthesia duration, operation duration, intraoperative blood loss, and ICU length of stay were independent predictive factors of in-hospital patient survival. They also stated that PELD score, operation duration, and ICU length of stay were independent predictive factors of 1-year and 3-year patient survival. However, the authors did not perform any multivariate analysis to determine independent risk factors for in-hospital mortality. In addition, they did not calculate the odds ratio for the univariate analysis to determine the factors that affect in-hospital mortality. The authors stated that the risk factors related to postoperative 1-

year and 3-year mortality were PELD score, operation duration, and ICU length of stay. However, they did not include certain known factors such as biliary complications, infections, type and levels of immunosuppressive drugs, and episodes of acute rejection. We believe there are some major issues regarding this topic. The factors stated by the authors may have an effect on in-hospital mortality, but they do not have an impact on the long-term mortality of the patients.

Although the authors stated that they performed a propensity score analysis and found that ICU-stay predicted the 3-year survival, they did not discuss the rationale behind this finding. In other words, they did not theorize why a perioperative parameter would have an impact on a late outcome such as 3-year survival.

In summary, they provided the 30-d, 90-d, 1-year, and 2-year survival rates. In addition, they analyzed factors that had an impact on 3-year survival. If the factors that had an impact on the survival of the patients three years postoperatively, then the authors should have given the 3-year survival rate of the patients.

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