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The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

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REVIEW

Nonalcoholic fatty liver disease and liver transplantation - Where do we stand?

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Abstract

Nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH) is a challenging and multisystem disease that has a high socioeconomic impact. NAFLD/NASH is a main cause of macrovesicular steatosis and has multiple impacts on liver transplantation (LT), on patients on the waiting list for transplant, on post-transplant setting as well as on organ donors. Current data indicate new trends in the area of chronic liver disease. Due to the increased incidence of metabolic syndrome (MetS) and its components, NASH cirrhosis and hepatocellular carcinoma caused by NASH will soon become a major indication for LT. Furthermore, due to an increasing incidence of MetS and, consequently, NAFLD, there will be more steatotic donor livers and less high quality organs available for LT, in addition to a lack of available liver allografts. Patients who have NASH and are candidates for LT have multiple comorbidities and are unique LT candidates. Finally, we discuss long-term grafts and patient survival after LT, the recurrence of NASH

and NASH appearing *de novo* after transplantation. In addition, we suggest topics and areas that require more research for improving the health care of this increasing patient population.

Key words: Nonalcoholic steatohepatitis; Chronic liver disease; Liver transplantation; Nonalcoholic fatty liver disease; Outcome

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Core tip: Nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH) is a challenging and multisystem disease that has a high socioeconomic impact. NAFLD/NASH is a primary cause of macrovesicular steatosis and has several impacts on liver transplantation (LT), which is transmitted to transplant recipients and organ donors. Current data indicate a new trend in the area of chronic liver disease. Due to the increased incidence of metabolic syndrome (MetS) and its components, NASH cirrhosis and hepatocellular carcinoma caused by NASH will soon become a major indication for LT.

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INTRODUCTION

Parallel to the increasing prevalence of diabetes mellitus type 2 (T2DM) and obesity and a close relationship with insulin resistance (IR) and metabolic risk factors, nonalcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease (CLD) in the world over the past 30 years, with an estimated prevalence of 10%-40%[1,2]. NAFLD is characterized by increased fat depositions in the liver with clinical-histological phenotypes that range from a simple steatosis (present in > 5% of hepatocytes, as shown in histological analysis or magnetic resonance spectroscopy) to nonalcoholic steatohepatitis (NASH). NASH is a more aggressive form of the disease and includes a histological presentation of steatosis, ballooning hepatocytes and lobular inflammation that leads to advanced fibrosis and, finally, cirrhosis and hepatocellular carcinoma (HCC)[1,3]. Given the growing prevalence of NAFLD, several studies have attempted to determine the clinical course and progression of the disease, but the exact prognosis remains unclear. A recently published Swedish retrospective study was the largest biopsy-proven NAFLD study to provide insight on the long-term prognosis and outcomes of the disease, with a follow-up period of up to 40 years[4]. In that report, NAFLD patients had an increased risk for mortality and liver-related morbidity (12% of the patients developed severe liver disease, which is defined as liver failure, compensated or decompensated liver cirrhosis and HCC). Interestingly, the presence of NASH did not significantly increase the risk for liver-related morbidity or overall mortality. The fibrosis stage was highly predictive of the risk of developing sever liver disease, with a hazard ratio that ranged from 1.9 in F0 to 104.9 in F4. The primary high fibrosis stages (F3-F4) predicted overall mortality[4], which is similar to previous published research[5,6]. Compared to other etiologies of chronic liver disease, NAFLD has a slower fibrosis progression, with an estimated time for developing severe liver disease at 22-26 years for F0-1, 9.3 years for F2, 2.3 years for F3 and 0.9 years for F4 (for decompensation)[4]. The clinical burden of NAFLD extends beyond the liver, with evidence indicating that NAFLD is a multisystem disease that is closely related to cardiovascular disease (CVD), chronic kidney disease (CKD) and T2DM. It is still not clear whether NAFLD is only a risk factor or is an important component of the pathophysiological mechanisms in the development and progression of those diseases[7]. In addition, a major cause of morbidity and mortality in NAFLD patients is CVD, followed by malignancies and liver-related diseases (cirrhosis and HCC) as the third cause[7]. HCC is the sixth most common cancer in the world that is predisposed with the presence of cirrhosis, but emerging data suggest that HCC can evolve in non-cirrhotic NAFLD and is strongly associated with metabolic syndrome (MetS)[8]. The HCC that is associated with NAFLD/NASH has a distinct phenotype. It is often diagnosed at an older age and in the advanced stages of liver disease, and, compared with the HCC in viral hepatitis, is less aggressive and therefore more commonly missed on routine scans for malignancies[9]. With the continuous increase in the incidence of obesity, T2DM and MetS in United States (US) and Europe, it is predicted that NAFLD/NASH will become the most common cause of HCC in the Western world. NAFLD/NASH has already become the second leading cause of liver transplantation (LT) in the US and, importantly, the number of patients who have NAFLD/NASH and are on the waiting list for transplantation increased by 170% from 2004 to 2013. Thus, end-stage liver disease (ESLD) due to NAFLD/NASH will become the most common indicator for LT in the near future[10].

We expect groundbreaking changes in the area of LT. Therefore, this review discusses the multiple impacts of NAFLD on LT. First, due to the aging of the population and an increasing incidence of MetS and

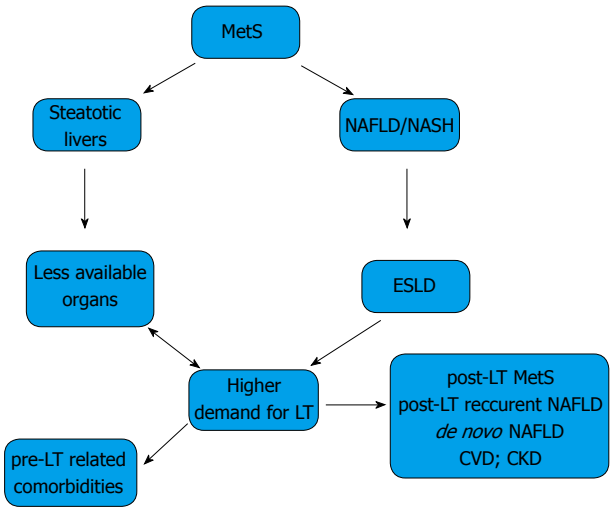

Figure 1 Higher incidence of metabolic syndrome and its complications leads to a higher incidence of nonalcoholic steatohepatitis/nonalcoholic fatty liver disease and, consequently, to more patients who have end-stage liver disease. At the same time, due to MetS and its components, we will have more steatotic livers, *i.e.*, more organs of lower quality that are available for LT. Therefore, in the future, since NAFLD will affect both the demand for LT and the supply of available organs. Patients who have NASH and are candidates for LT have several comorbidities and are unique LT candidates. Post-LT, there are several challenging issues for NAFLD: recurrent NAFLD, de novo NAFLD and the risk for CVD and CKD. MetS: Metabolic syndrome; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; ESLD: End-stage liver disease; LT: Liver transplantation; CVD: Cardiovascular diseases; CKD: Chronic kidney disease.

its liver manifestation (*i.e.*, NAFLD/NASH), ESLD as a consequence of NASH will become a primary driver of LT in the near future. Furthermore, due to the increasing incidence of obesity, and, consequently MetS, the prevalence of NAFLD in the population will also increase[1,2] As such, owing to the growing incidence of NAFLD, we can expect that there will be more steatotic donor livers and fewer high quality organs available for LT. Therefore, NAFLD affects both the demand for LT and the supply of available donors.Moreover, patients who have NASH and are candidates for LT have several comorbidities, such as obesity, T2DM and other MetS components, as well as CVD and CKD. These patients are uniquely challenging LT candidates, and transplantation specialists are continuously exposed to the challenges of transplantation from obese donors, as well as the NASH recipients with their often multiple comorbidities. Finally, we discuss long term grafts and patient survival after LT, the recurrence of NASH and NASH appearing *de novo* after transplantation[11,12] Figure 1. In addition, we suggest topics and areas for further research for improving health care for this increasing patient population.

For this Review, we identified references using PubMed and the terms "NAFLD", "NASH", and "liver transplantation." We only reviewed articles that were published in English. The references were selected based on originality and their relevance to the domain of this Review.

NAFLD RELATED END-STAGE LIVER DISEASE AND HCC AS INDICATIONS FOR LIVER TRANSPLANTATION

NAFLD patients can necessitate the need for LT in two primary ways: developing cirrhosis that manifests with decreased synthetic/excretion function(s) and signs of portal hypertension and HCC development. It is estimated that approximately one-third of the current population in industrialized countries has NAFLD as a consequence of the liver's involvement in the context of MetS. As mentioned above and according to many authors, it is clear that over the next ten or twenty years, the prevalence of NAFLD will increase due to the epidemic rise in obesity, T2DM, arterial hypertension and the prevalence of MetS, as well as people living longer[10-13]. Consequently, NAFLD-related liver disease is currently the most rapidly increasing indication for LT in the US, and it is anticipated that NAFLD-related liver disease will become the leading indication for LT in the near future[14,15]. In the context of the increasing incidence of NAFLD as an indication for LT, it is important to highlight several facts. First, due to the development of direct antiviral agents (DAA) for hepatitis C (HCV), the incidences of cirrhosis and HCC due to HCV as indications for LT will decrease over time. Three years ago, Wong *et al*[10] analyzed the United Network for Organ Sharing and Organ Procurement and Transplantation Network's (UNOS/OPTN) registry data from 2004 to 2013. There were four groups of registrants who were on the liver transplant waitlist: patients who had an HCV infection, NASH, alcoholic liver disease (ALD), or a combination of HCV infection and ALD. Over a period of nine years, the numbers of new patients on the waitlist who had NASH, ALD, and HCV increased by 170%, 45% and 14%, respectively. Moreover, the percentage of registrants who had HCV and ALD decreased by 9% (from 880 to 803)[10].

A recent study by Goldberg *et al*[16] analyzed the prevalence of HCV from 2010 to 2014 from National Health and Nutrition Examination Survey (NHANES) data. They also collected data from patients who had cirrhosis and chronic liver failure (LF) from 2006 to 2014 and were in the Health Core Integrated Research Database. In addition, they analyzed data from liver transplant recipients from UNOS from 2003 to 2015. By combining data from these three databases, the study investigated current changes in liver disease(s); HCV, alcoholic liver disease (ALD) and NAFLD/NASH through the course of liver disease; CLD - compensated cirrhosis, decompensated cirrhosis and HCC; and the waiting list for LT and LT recipients. The study authors found that there were significant changes in CLD etiology that were associated with important alterations

in the occurrence of HCV, ALD and NAFLD/NASH as indications for liver transplantation. They demonstrated that active HCV infection decreased as an indication for LT after DAA use. Subsequently, there was a decrease in the incidence of cirrhosis due to HCV in the larger population with CLD[16]. In contrast, among patients who were on the waiting list and LT recipients, NAFLD became more common. Another interesting finding from this study was that the incidence of ALD as an indication for LT increased more than NASH[16]. A retrospective study by Cholankeril *et al*[17] had similar findings after analyzing the UNOS/OPTN database from 2003 to 2014. The authors discovered that the number of LT that is secondary to NASH increased by 162% from 2003 to 2014, while the number of LT secondary to HCV increased by 33%, and the number of LT secondary to ALD increased by 55%[17].

Recently, there has been a trend of an increased incidence of HCC in developed countries, and according to the literature, this increase is most likely due to an increased incidence of MetS[8,18]. The large Bridge study included 18031 HCC patients from 2005-2012. NAFLD was one of the major risk factors for HCC development, and NAFLD was the cause of chronic liver disease for approximately 10%-12% of patients[19,20]. Similarly, a recently published US study found that HCC as a consequence of NASH is the fastest growing indication for LT. The authors of this study reported that NASH related HCC as an indication for LT had an almost fourfold increase since 2002; on the other hand, HCC that results from HCV, doubled[13,21].

In the context of LT and NAFLD, it is concerning that a recent discovery found that HCC may appear in NAFLD patients who do not have liver cirrhosis or advanced liver fibrosis[8]. Mittal *et al*[22] published data on 13% of patients who had HCC and, at the time of diagnosis, did not have cirrhosis. The primary risk factor for developing HCC was the presence of NAFLD or MetS. In addition, in a study by a group of German authors, 41.7% of the patients with NAFLD/NASH HCC previously had no diagnosis of cirrhosis[23]. Similar findings were also reported by other authors[24,25].

Another concerning issue in the context of NASH and LT is the increase in the incidence of NAFLD in children and young adults (up to age 40). Feldstein *et al*[26] analyzed long-term outcomes and survival for children who had NAFLD. In this study, children who had NAFLD had a 13.8-fold higher risk of requiring LT or dying than the general population of the same age and sex[26]. Recently, Alkhouri *et al*[27] analyzed LT in children and young adults and the frequency of NASH as an indicator for LT. They found an increased incidence of NASH as an indicator for LT in young patients. More than 100 recipients had LT before they were 34 years old, while most patients received their liver transplant closer to the age of 40 years[27].

Current guidelines do not recommend regular screenings for HCC in NAFLD patients who have no signs of liver cirrhosis or advanced fibrosis. According to recent research, NAFLD patients who have not developed cirrhosis have a risk of developing HCC; however, there are no studies that examine the cost-benefit of screening in this population of patients. However, the current data on the increasing incidence of NAFLD combined with the growing incidence of MetS and NAFLD in young people indicate that there will be a need for LT in the context of NAFLD related decompensated cirrhosis and NAFLD related HCC[13,20,21].

Due to the substantial increase in the proportion of transplants due to NAFLD, as well as new waitlist registrants with NAFLD cirrhosis complications, NAFLD/NASH cirrhosis and related HCC are the most rapidly growing indications for LT.

NAFLD PATIENTS ON THE WAITLIST FOR LIVER TRANSPLANTATION

Every CLF patient has unique characteristics and needs an individual approach in the context of LT, and the same individual approach is necessary for patients who have NASH. The risk factors for poor postoperative and long-term outcomes are age the presence of MetS components (especially T2DM and obesity), coronary artery disease (CAD) and chronic kidney disease (CKD). Patients who have NASH on the waitlist often have several or all of these risk factors. For NASH patients on the waiting list there are two problems: patient comorbidities and lower MELD than other etiologies of CLD[28].

First, NAFLD is the liver manifestation of MetS and NAFLD patients on the LT waiting list frequently have one or more components of MetS. They are often obese and have T2DM, hypertension and hyperlipidemia. In addition, NASH recipients are older than recipients who have a different CLD[28]. According to Wong *et al*[10] compared to patients who had an alcoholic, viral or alcoholic/viral etiology of CLD who were on the waitlist for LT, patients with NASH had decreased renal function, were more obese and were more likely to have T2DM. There was higher morbidity and mortality in obese patients who underwent surgical procedures. However, in the context of obesity and LT, the results were not consistent. Several studies reported worse outcomes for obese patients, while other authors found similar risks and outcomes for both obese and non-obese patient groups[28]. For example, Leonard *at al*[29] had similar results for all body mass index (BMI) categories for early and late patients and graft survival. In contrast, La Mattina *et al*[30] found that obese patients had a longer operative time, intensive care unit length of stay, and more infectious and biliary complications that required intervention. There was no significant difference in patient or graft survival for overweight Class Ⅰ and obese Class Ⅲ recipients compared to normal weight recipients. However, patients who had Class Ⅱ obesity experienced decreased patient and

allograft survival[29]. Not long ago, Conzen *et al*[31] found that morbid obesity had negative effects on long-term outcomes regardless of the short-term results. In other words, there were no differences in operative time, intensive care unit or hospital length of stay or perioperative complications. Over 3 years, recipient and graft survival rates were similar across groups. Compared to the non-obese, recipients who had a BMI > 40 kg/m^2 experienced a significantly decreased 5-year graft (49.0% *vs* 75.8%; $P < 0.02$) and recipient (51.3% *vs* 78.8%; $P < 0.01$) survival. Although between group comparisons is difficult given the different endpoints and BMIs between cohorts, in general, obese patients have increased complication rates and more resource utilization compared to non-obese recipients[19]. Given the increase in the incidence of overweight patients and MetS, we can expect an increase in the number of patients with NASH cirrhosis or HCC in NASH with high BMI who are on the transplant list in the future. In addition, the bariatric surgery (BS) methods will become more important in the context of treating obesity for the morbid obesity of NASH patients. There are promising research findings for BS in these patients. There are studies with a small number of patients who were experiencing LT and some form of BS[28]. For example, Heimbach *et al*[32] conducted a small study that combined LT with a sleeve gastrectomy, which resulted in significant weight loss for patients who were not successful with medical treatment. In addition, there were less post-LT metabolic complications[32].

Recently, 11 studies with 56 patients were analyzed in a systematic review[33]. Two studies reported that BS had been previously performed, while two studies performed it during and seven after LT. The most common procedure was the sleeve gastrectomy, while the Roux-en-Y gastric bypass, biliopancreatic diversion and gastric banding were performed in a slightly smaller number of patients. There was no mortality in the early postoperative period, with a 5.3% rate during the first postoperative year. The reoperation rate was 12.2%. Although mortality and morbidity are higher in this population, the authors agreed that BS appears to be possible[33].

In the future, there is a need for randomized studies to determine which patients on the transplant list will benefit from BS, the optimal time for BS (before, during or after LT) and the optimal type of BS. It is important to note that patients who have decompensated cirrhosis have a higher mortality rate after BS than those who have compensated cirrhosis or no chronic liver disease; thus, it is extremely important to optimize the time at which patients should undergo BS[28,34]. Future studies are also needed to demonstrate the long-term impact of BS on liver transplant recipients and graft outcomes[28].

Patients who have NASH and are on the waitlist for LT often have T2DM. Pre-transplant T2DM is a strong predictor of poor short and long-term patient and graft survival. The poor outcomes are primarily attributed to an increased incidence of postoperative infectious complications, CVD complications and kidney failure[35,36]. A recent study by Hoehn *et al*[36] indicated that recipients with pre-LT diabetes in the post-transplant period had a longer hospital length of stay, as well as higher peri-transplant mortality and 30-d readmission rates. In addition, they are less likely to be discharged home and, finally, have lower graft and patient survival than recipients who do not have diabetes[36].

For the above observations, NASH recipients often have one or more and often multiple, comorbidities that significantly affect the CVD risk in these patients so CVD risk assessment in NAFLD recipients is one of the largest problems in context of LT. According to the guidelines from European Association for the Study of the Liver (EASL), aside from obligatory electrocardiogram and transthoracic echocardiography in pre-LT evaluation, further tests need to be done to exclude asymptomatic ischemic heart disease (cardiopulmonary exercise test and if necessary in high risk patients even coronary angiography)[37]. Wray *et al*[38] showed that if coronary artery disease (CAD) is treated effectively before LT, survival after LT is not significantly different between patients with or without obstructive CAD.

Currently, many authors agree that NAFLD is a liver as well as a multisystem disease that is commonly associated with CVD, T2DM and CKD[39]. Research has shown that NAFLD is associated with an increased risk of adverse CVD events[39-42]. It is not clear whether the risk for CVD is increased in NAFLD patients due to coexisting dysmetabolic traits or whether NAFLD is actively involved in the pathogenesis of cardiovascular disease[35,39]. Previous research has shown that patients who have NASH related ESLD, compared to other ESLD recipients, have a higher CVD risk, specifically soon after LT[36]. For example, Patel *et al*[43] analyzed 420 ESLD patients that were assessed for LT: 125 had alcohol-related ESLD, and 295 had non-alcohol-related ESLD. The incidence of severe coronary artery disease (CAD) (defined by a > 70% diameter stenosis) was 13% in the non-alcohol-related ESLD group ($P < 0.005$) and 2% in the alcohol-related ESLD group. Moreover, a retrospective cohort study by Vanwagner *et al*[44] analyzed 242 LT recipients (127 alcohol-related and 115 NASH ESLD) at a post-transplant follow-up that was more than 12 mo. After controlling for recipient sex, age, smoking status, CVD, pre-transplant diabetes and the presence of MetS, the multivariate analyses shown that NASH patients were more likely to have a CVD event than alcohol-related ESLD recipients in the first year after LT. Most of the (70%) CVD events occurred in the perioperative period, and 50% of the mortality was related to the occurrence of a CVD event. However, there were no differences between the two groups in graft and patient survival[44].

According to these observations, it is important to screen all LT candidates for the presence of MeS and/or risk of CVD, especially when they have NASH related ESLD. Prospective studies are needed to answer these important questions and to provide a foundation for a

standardized approach to CVD risk assessment in the population of LT candidates[35].

An additional risk factor in the context of NAFLD is CKD, which is also a well-known CVD risk factor. Previous research has shown that patients who have NAFLD have a higher prevalence of CKD than patients who do not have NAFLD[39,45]. A recent study by Singal *et al*[46] confirmed that the most rapidly increasing indication for simultaneous liver-kidney (SLK) transplantation is NASH, which has poor renal outcomes. The authors of this study found that SLK significantly increased in the group of patients who had NASH and cryptogenic cirrhosis compared to ESLD that was related to other etiologies; the incidence increased from 6.3% from 2002 to 2003 to 19.2% from 2010 to 2011. Five-year LT recipient and graft survival rates did not differ between recipients who had NASH or cryptogenic cirrhosis and those with other etiologies of ESLD. On the other hand, in the group of patients who had NASH and cryptogenic cirrhosis, the risks for a kidney graft loss was more than 1.5-fold higher. Compared to recipients who had ESLD that was related to alcohol, primary biliary cirrhosis or primary sclerosing cholangitis, the estimated glomerular filtration rate remained lower in in the recipients who had NASH/cryptogenic ESLD[46].

When selecting LT candidates who have NASH, the largest challenge is merging these risk factors into one risk stratification tool. As such, a multi-disciplinary approach is needed to evaluate these candidates for LT.

Importantly, in the context of NASH related ESLD candidates for LT, there is an association between NASH and macrovascular venous thrombosis, especially portal vein thrombosis (PVT)[47]. In NASH patients who have cirrhosis, there is a hypercoagulable state that is characterized by increased levels of plasminogen activator inhibitor 1 and factor VIII, while anticoagulant levels of protein C are decreased in patients with cirrhosis due to NASH[47,48]. Stine *et al*[47] recently analyzed 33368 patients who have ESLD and received LT. Of these, 2096 (6.3%) patients had PVT and 12% had NASH. A comparison of NASH related ESLD recipients with all other causes of cirrhosis revealed a higher prevalence of PVT, with 10.1% in the first group versus 6% for those without NASH ($P < 0.001$). NASH cirrhosis was the strongest risk factor that was independently related to PVT in a multivariable analysis. Although the clinical significance of PVT is not entirely clear, especially whether anticoagulant therapy should be used, individual studies have shown that PVT is associated with adverse outcomes in patients who have ESLD. Specifically, several authors have shown that PVT is associated with increased pre- and post-transplant mortality, as well as with technical challenges during the transplant procedure[47,49-51]. However, the connections among NASH and PVT with hypercoagulation state is an ever-expanding field of clinical research. Additional studies on this topic are needed because there will be a significantly higher number of patients who have ESLD due to NASH on the waitlist for LT in the future, and, possibly, a higher number of thromboembolic incidents in these patients, including PVT[47].

The second important issue in the context of NASH patients who are on the waitlist for LT is competition for liver allograft allocations due to a lower MELD than other etiologies of CLD. According to current reports, patients who have ESLD due to NASH and are on the waitlist for LT have better liver functioning and, consequently, lower MELD scores than other etiologies of liver cirrhosis. In addition, these patients have a slower progression of disease[18,28]. A study by O´Leary *et al*[52] compared the data for 218 patients who had NASH or cryptogenic cirrhosis (CC) and underwent LT between 2002 and 2008, with 646 patients transplanted due to ESLD that resulted from HCV infection. Among patients who had NASH and CC, the median progression rate was 1.3 MELD points per year, and in the group of patients who had HCV, it was 3.2 MELD points per year ($P = 0.003$)[52]. Compared to patients who have HCV-related cirrhosis, patients who had NASH/CC and MELD scores $\leqslant$ 15 had fewer chances of receiving LT. They also had a higher risk of dying and a two-times higher risk of rejection or removal from the waiting list due to no suitable operative procedure given the progression of the liver disease or complications with their comorbidities. However, all patients who had MELD scores that were higher than 15 were more likely to undergo LT despite their diagnosis[52]. According to the findings from this study, the aggressive treatment of associated comorbidities is highly important; the components of MetS (hypertension, T2DM, dyslipidemia and obesity) in patients who have low MELD scores can prevent the progression of their comorbid conditions that are likely to cause death or make the patient ineligible for LT[52]. In addition, a recent study by Wong *et al*[10] demonstrated that NASH patients, compared to HCV, ALD or HCV/ALD related ESLD, are less likely to receive LT in the first 90 days on the waitlist. Another interesting finding from this study is that the one-year waiting list survival rate for ESDL patients due to NASH declined over the study period from 42.8% to 25.6%. In contrast, patients who had HCC due to NASH, compared to other etiologies of CLD with HCC, had better liver functioning and lower MELD or Child Pugh scores[18]. Taken together, these data suggest that LT candidates who have NAFLD/NASH related ESLD pose a specific challenge for the transplant community given their longer LT waiting time and associated comorbidities.

NAFLD IN DONOR LIVERS

Another challenge in the context of NASH in LT is liver allograft steatosis. Specifically, the epidemic increase in the incidence of NAFLD/NASH in the general population has a direct influence on the increased prevalence of NAFLD in deceased and living liver donors[11,28]. Based on predictions that the prevalence of MetS and its liver manifestations (*i.e.*, NAFLD) will increase in coming

years, we can expect more donors with NAFLD/NASH. We know that the availability of donor livers depends the success of the LT program. There is a global lack of organs for transplantation, as the gap between patient "demand" and organ "supply" continues to grow[53]. As such, transplant centers must use livers from "extended criteria donors" (ECD). Due to higher risk for ischemia-reperfusion injury (IRI), the severity of liver steatosis is related to a higher risk for graft failure and/or impaired graft function. Upon reperfusion, steatosis can cause microcirculatory and cellular changes in the liver graft that can lead to hepatocyte necrosis. In contrast, there is an impaired potential for regenerating steatotic livers[11,28,54-56]. For donors whose livers are more than 60% steatotic, this is almost a universal scenario; however, for those who are 30%-60% steatotic, there are controversial outcomes for donor livers[11,28,54,55]. For example, Spitzer *et al*[57] have shown that macrovesicular steatosis is an independent risk factor for graft survival. Recently, Chu *et al*[55] published a systematic review that analyzed 34 articles. The authors found that steatotic grafts that were > 60% were associated with an increased risk for poor graft functioning, while grafts that were > 30% of steatosis were related to decreased graft survival rates[55]. The lack of a standardized definition for primary non-functioning or impaired primary functioning and descriptions of the types of steatosis in research are the primary flaw in these studies. With more common utilization of ECD livers, using liver allografts that have less than 30% macrovesicular steatosis should be harmless for recipients[11,28,54,55].

There is no standardized procedure for estimating liver steatosis in potential donors; thus, evaluation procedures of liver grafts for steatosis and the use of steatotic livers for LT differ across transplant centers. Although some centers perform liver biopsies in high risk donors (abnormal liver tests, associated comorbidities, diabetes mellitus, high body mass index, older age, hepatitis B or C infections), others evaluate all potential donors[11,54,58]. Liver biopsies are the "gold standard" for detecting and assessing for steatosis. As an invasive procedure, liver biopsies can damage the organ. Moreover, it can only sample 1/50000 of the liver; thus, there is the potential for significant sampling error and limits in the numbers and sizes of biopsies. In addition, there is significant inter-observer variability for evaluating the degree of steatosis. These disadvantages place the procedure in the "silver standard" position; however, because there is not a better referential method, biopsy is still viewed as the "gold standard". Additionally, waiting for the liver biopsy results before deciding whether to accept the organ extends the cold ischemia time. Therefore, there is a need for simple, rapid and non-invasive methods for detecting steatosis in the donor[11,54,59]. Imaging methods such as ultrasonography, magnetic resonance and computed tomography are not sensitive or exact in detecting steatosis that is below 30%. Moreover, these methods cannot differentiate between micro-vesicular and macro-vesicular steatosis[11,54,58,59]. Recently, elastographic methods have been intensively investigated in the context of the noninvasive assessment of liver steatosis and fibrosis. One of the most investigated is transient elastography (TE), with a controlled attenuation parameter (CAP). In the context of donor livers, Mancia *et al*[60] examined 23 brain-dead potential donors. They analyzed TE with its CAP and reviewed liver stiffness measurements (LSM) to objectively assess liver steatosis and fibrosis. The implementation of TE with both CAP and LSM demonstrated good preoperative assessment for the histological condition and stage of the donors' liver steatosis[60]. Recently, Hong *et al*[61] investigated the usefulness of CAP as a screening tool for detecting liver steatosis in living donor livers. The author found that area under the receiver operator characteristic curve for diagnosing steatosis ($\geqslant$ S2) with CAP was 0.88, with a cutoff value of 276 dB/m. According to the findings from this study, CAP could be an adequate noninvasive method for excluding significant liver steatosis (> 33%) in liver donors[61]. There is a need for more research on using TE with CAP to evaluate steatosis and fibrosis in possible donors. A higher incidence of NAFLD/NASH in the general population will lead to a higher risk of donors who have NAFLD, which will influence on number of suitable organs from both living and deceased donors. Given the increasing incidence of NAFLD, we will face an even greater lack of LT organs or will be forced to accept liver donors that have NAFLD/NASH and are lower quality, with a high risk for poor outcomes after LT[15,54].

LIVER TRANSPLANTATION OUTCOMES FOR NAFLD PATIENTS

Although patients who are transplanted because of ESLD that is related to NASH have several comorbidities and are often older in age, post-LT survival is comparable to other etiologies of ESLD. Multiple, single-center studies of survival in ESLD related to NASH patients who had an LT, as well as several large studies were conducted over the years[28]. The studies that assess post-LT outcomes for NASH are summarized in Table 1.

One of the first studies to report outcomes for NASH patients after LT was conducted by Malik *et al*[62] and was published almost 10 years ago. This was the first study to analyze patients who had a histopathological diagnosis of NASH in the context of LT. The authors analyzed the post-LT outcomes for 98 NASH patients *vs* 686 with other etiologies, including primary biliary cirrhosis/primary sclerosing cholangitis (PBC/PSC), ALD, HCV and cryptogenic cirrhosis (CC). In 71 NASH patients, the diagnosis of NASH was based on pre-LT biopsies, and in 27 patients, the diagnosis of NASH was confirmed upon explant. The five-year survival rates

Table 1 Post liver transplantation outcomes for patients who have nonalcoholic fatty liver disease

Ref.	Study size	NASH group survival (%)	Non-NASH group survival (%)	Study period
Malik *et al*[62]	98 NASH	30-d - 93.9	30-d - 94.4-98.0	1997-2008
	686 Non-NASH group (PBC/PSC, ALD, HCV, CC)	1-yr - 79.6	1-yr - 81.6-87.2	
		5-yr - 72.4	5-yr - 65.3-80.6	
Bhagat *et al*[63]	71 NASH	1-yr - 82	1-yr - 92	1997-2007
	83 ALD	5-yr - 75	5-yr - 86	
		9-yr - 62	9-yr - 76	
Barritt *et al*[64]	21 NAFLD	30-d - 80.9	30-d - 97	2004-2007
	83 Non-NAFLD (ALD, HCV, HBV, PBC/PSC, AIH)	1-yr - 76.2	1-yr - 89.5	
		3-yr - 76.2	3-yr - 83.5	
Agopian *et al*[65]	144 NASH	90-d - 90	90-d - 90-96	1993-2011
	1150 Non-NASH (HBV, HCV, ALD, CC, PBC/PSC)	1-yr - 84	1-yr - 79-87	
		5-yr - 75	5-yr - 54-70	
Kennedy *et al*[66]	129 NASH	1-yr - 90	1-yr - 92	1999-2009
	775 Non-NASH - etiologies not defined	3-yr - 88	3-yr - 86	
		5-yr - 85	5-yr - 80	
Park *et al*[67]	71 NASH	1-yr - 78	1-yr - 87	1998-2008
	472 Non-NASH	2-yr - 78	2-yrs - 85	
Vanwagner *et al*[44]	115 NASH	1-yr - 81.3	1-yr - 88.1	1993-2010
	127 ALD	3-yr - 73.3	3-yr - 85.3	
		5-yr - 60.3	5-yr - 68.8	
Afazali *et al*[68]	1810 NASH	1-yr - 87.6	Variable	1997-2010
	3843 CC	3-yr - 82.2		
	48.085 Non-NASH	5-yr - 76.7		
Charlton *et al*[14]	1959 NASH	1-yr - 84	1-yr - 87	2001-2009
	33822 Non-NASH	3-yr - 78	3-yr - 78	

NAFLD: Nonalcoholic fatty liver disease; ALD: Alcoholic liver disease; HCV: Hepatitis C virus; HBV: Hepatitis B virus; CC: Cryptogenic cirrhosis; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis.

after the LT were similar between the patients who were transplanted for NASH and the patients who were transplanted for other etiologies of ESLD. On the other hand, there was a tendency for higher mortality soon after the LT (30-d mortality was 6.1%), and one year after the LT (21.4%). NASH patients who were older (⩾ 60 years), obese (BMI > 30 kg/m^2), and had pre-LT hypertension and pre-LT T2DM had a higher risk for poor post-LT outcomes. Another important finding was that infection was the most common cause of death in the NASH patients compared to the controls[62]. In 2009, Bhagat *et al*[63] published a retrospective study that reported the post-LT outcomes for the NASH and ALD groups of patients who underwent LT. The authors found that overall survival and death rates due to CVD events was higher in the NASH group, but this difference was not significant. Interestingly, acute rejection crises and recurrent steatohepatitis occurred significantly more often in the NASH group but did not lead to higher rates of re-transplantation[63]. Two years later, Barritt *et al*[64] published another retrospective, but small, study. The primary finding of this study was that NASH, as an indication for LT, was the independent factor that influenced early post-LT mortality[28,64]. In 2012, Agopian *et al*[65] published a large, single-center study and found that the frequency of ESLD due to NASH as an indication for LT increased from 3% in 2002 to 19% in 2011. They reported that patients who were transplanted for NASH had a longer operative time, more operative blood loss and a longer post-LT length of stay. On the other hand, recipient and graft survival rates at one, three and five years were comparable to patients who were transplanted for other causes of ESLD. The predictors of poor outcomes for the recipient and its graft were pre-LT obesity and pre-LT hemodialysis[28,65]. Early postoperative mortality due to infections and CVD events in the recipients who were transplanted for ESLD due to NASH was reported in Kennedy *et al*[66]. This study also highlighted that an older age (> 60 years), pre-LT obesity, hypertension and T2DM were associated with lower five-year survival rates after LT. However, the overall survival rates at one, three and five years were comparable to other etiologies of ESLD[28,66]. VanWagner *et al*[44] discovered that NASH recipients had an increased risk for adverse CVD events in the first year after the LT compared to recipients who had ALD. The presence of MetS before LT was the most important risk factor[42]. One of the largest national US studies that addressed the outcomes of LT for ESLD due to NASH was published by Afazali *et al*[67]. The author used the UNOS database and analyzed 1810 LT recipients who had ESLD due to NASH, 3843 recipients who had ESLD due to CC, and 48085 recipients who had ESLD due to other etiologies of ESLD. The author reported an increased proportion of LTs for NASH patients; from 1.2% in 1997-2003 to 7.4% in 2010. NASH and CC recipients had good survival rates that were comparable to other etiologies of CLD. Consistent with other studies, there was a higher rate of early mortality in the NASH patients. In addition, in line with earlier, small studies, an older age, pre-LT T2DM, obesity and pre-LT hypertension were risk factors

for higher mortality rates in the first year after LT[68]. Another large national US study that used the SRTR database and was performed by Charlaton *et al*[14] had similar findings.

Finally, a meta-analysis that was published four years ago by Wang *et al*[69] showed that similar number of patients with and without NASH survived for 1, 3, and 5 years after LT; however, those who had NASH were more likely to die due to adverse CVD events or sepsis[69].

In most studies, patients who were transplanted for ESLD related to NASH had very good survival rates. One-year survival rates were between 85% and 90%, while five-year survival ranged from 70% to 80% in most studies. In addition, patients who underwent LT due to NASH-related ESLD had almost the same outcomes as other etiologies of CLD. It is interesting that NASH recipients, despite multiple comorbidities, have survival comparable to that of other etiologies of CLD. One possible explanations is that the rate of NASH and cirrhosis recurrence is lower than the recurrence of HBV or HCV[35,68]. Another consideration is that these patients undergo a very extensive pre-transplantation screening for risk evaluation and cardiovascular status, thus; those who have significant cardiovascular morbidity are excluded from the transplant list. However, according to the results, overall survival after LT is good in the NASH recipient group, and a higher incidence of post LT CVD events are noted in NASH recipients. However, infections (sepsis) were observed more frequently in this group of recipients. When selecting NASH patients for LT, there is a need for more attention and careful consideration combined with the radical management of sepsis and CVD complications after LT[11,68,69].

NONALCOHOLIC FATTY LIVER DISEASE AFTER LIVER TRANSPLANTATION

Progress in surgical techniques for transplant surgery, as well as the development of immunosuppressive therapy, led to decreased early post-LT mortality and, consequently, to improved survival rates after LT, with a 90% survival rate at the first year and a survival rate of more than 70% five years after LT. The development of metabolic comorbidities, combined with this higher post-LT survival, contributes to morbidity and mortality rates. Subsequently, the focus of research is changing to long-term complications, such as CVD[70-72]. CVD can be initiated with every insulin resistance (IR) associated component of MetS. Furthermore, the clinical features and prevalence of MetS, such as T2DM, hypertension, rapid weight gain and dyslipidemia, often deteriorate in the post-LT period based on transplant specific factors, for example, adverse events in immunosuppression. They are also related to the recipients' morbidity and mortality[70,72]. For metabolic balance, for hyperglycemia, weight gain, hypertension and hyperlipidemia, immunosuppressant drugs, such as corticosteroids, calcineurin inhibitors (CNIs) (cyclosporine (CSA), tacrolimus (TAC)) and mammalian target of rapamycin inhibitors (mTORs) (such as sirolimus (SIR) and everolimus), have a crucial role. Corticosteroids stimulate gluconeogenesis. CNI stimulates the post-LT occurrence of new-onset diabetes (NOD) that is more likely related to TAC use compared to CSA. CNI also initiates the development of post-LT hypertension, and it appears that CSA is highly related to the development of hypertension after LT. For dyslipidemia, CSA has a higher risk of causing dyslipidemia than TAC. Finally, for dyslipidemia, mTORs are the most unfavorable immunosuppressive drugs. These groups of immunosuppressive drugs may, to an extent, affect the development of CVD through metabolic complications[70-72]. Most transplanted patients become obese after LT, with the highest increase in weight occurring after the first six months, as well as one and three years after LT[70,72,73]. Of the liver recipients, 10%-64% develop T2DM, 45%-69% experience hyperlipidemia, and approximately 50%-100% develop hypertension after LT[70-72]. Thus, a significant number of liver recipients met the criteria for MetS, which indicates that these patients have a higher risk for CVD[70-72]. Based on the literature, MetS is present in approximately 50%-60% of transplant patients[71]. Therefore, MetS is an important post transplantation problem. Because NAFLD is a liver manifestation of MetS, it is not surprising that both recurrent and *de novo* NAFLD can be found after LT[70-72]. According to the abovementioned observations, MetS components (*i.e*., NAFLD risk factors) may persist or worsen after LT due to the high incidence of MetS after LT. NAFLD can affect the post-LT course in two ways. First, post-transplant NAFLD can develop as a recurrence of a pre-LT condition, and can progress to cirrhosis and lead to ESLD when re-transplantation is necessary. Second, due to the high incidence of MetS components after LT, NAFLD can also occur *de novo* and complicate the course of the recipients who are transplanted for other etiologies of CLD[28,70-72,74]. More than 25 years ago, Burke *et al*[75] were the first to describe recurrent NAFLD, and authors from San Francisco, CA, United States, reported the first case series of *de novo* NAFLD in 2003[76].

According to the literature, recurrent NAFLD is a relatively common diagnosis after LT. Across reports, the rates of recurring steatosis and NASH range from 30%-100%[28]. For example, Bhagat *et al*[70] found that 33% of patients who were transplanted due to NASH cirrhosis had steatohepatitis in biopsy specimens during the first six months after the LT. On the other hand, none of these patients developed cirrhosis or required re-transplantation during the 10-year follow-up period[70]. A group of Dallas authors[77] conducted a retrospective study and analyzed post-LT outcomes for 257 patients undergoing LT for CC or NASH cirrhosis.

After comparing patients who had NASH/CC with patients who underwent LT due to other etiologies of CLD, they found that more NASH/CC patients developed graft steatosis at one, two, five and 10 years post-LT (8.2%, 13.6%, 24.9% and 32.9%) than those who were transplanted for other etiologies (3.1%, 5.9%, 9.6% and 10%). Of the 257 NASH/CC patients, 13 developed NASH, and 5% and 10% developed bridging fibrosis or cirrhosis after 5 and 10 years. This outcome was more common in patients who had NASH than in those who developed steatosis per se or had no fat (3%). The survival rate during the 10-year follow-up was similar for patients who underwent LT for CC or NASH or LT for other indications. However, the cause of death differed between those two groups, as the NASH group had more adverse CVD events[77]. Moreover, Dureja *et al*[78] evaluated 88 liver transplant recipients that underwent LT due to NAFLD-related cirrhosis from 1993 to 2007. There was recurrent NAFLD in 34 liver transplants, isolated steatosis in 9 and steatohepatitis in 25 recipients, while there was advanced fibrosis in 3 recipients. The survival rate after LT was not affected by NAFLD recurrence, but a higher number of CVD and infectious complications were reported in this group[78]. Recently, Sourianarayanane *et al*[79] published a retrospective study and analyzed data from NASH and ALD transplant recipients between 2001 and 2006. The authors found that NASH recipients had a higher incidence of steatosis and inflammation after LT; however, the progression of fibrosis was slower in NASH than in ALD recipients[79]. Recently, Bhati *et al*[80] analyzed 103 patients who were transplanted for NASH in whom TE and liver biopsies were used to assess steatosis and fibrosis. Of 103 total patients, 56 had TE, while 34 had a liver biopsy. Implementing TE with CAP demonstrated that 87.5% of the patients who had steatosis also had recurrent NAFLD. Most patients had LSM with no fibrosis (42.9%) or F1-F2 fibrosis (30.4%). Overall, 26.8% of the patients had advanced fibrosis, while 5.4% developed cirrhosis. Of the patients who underwent a liver biopsy, 88.2% had recurrent NAFLD, while almost half (41.2%) had NASH. Bridging fibrosis was noted in 20.6% of patients; however, none of the patients had cirrhosis. In most patients, cancer (25%) or infectious complications (25%) were the cause of death in combination with CVD (21.9%). Graft cirrhosis only caused 9% of the deaths. According to this recent study, recurrent NAFLD commonly occurs after LT (88% of all patients), while nearly a quarter of the patients developed advanced fibrosis[80]. An interesting observation was published on the genetic predisposition for NAFLD recurrence. The presence of the rs738409-G allele of the Patatin-like phospholipase in LT recipients is an independent risk factor for post-LT steatosis, as well as obesity and T2DM[72,81].

Most research that investigates the prevalence of recurrent NASH in post-LT patients have shown that the incidence of recurrent NASH is between 20% and 40%, while the incidence largely depends on NASH detection methods, including liver enzymes, imaging techniques or liver biopsies. Most of the studies that investigated the incidence of recurrent NASH were retrospective, without a standard post-LT interval biopsy protocol. In addition, the histological criteria that was used for defining the diagnosis of recurrent NAFLD varied among published studies[74,81,82]. Therefore, there is a need for prospective studies that show the actual incidence and progression for recurrent NAFLD after LT. Also, it is not clear is NAFLD a primitive process, to which follows MetS, or is it just the opposite. Further research on this topic are needed.

A recently published study investigated the incidence of NASH in children and young adults as indications for LT in addition to post-LT patients and graft outcomes. Alkhouri *et al*[27] found that approximately 4% (13) of patients who were transplanted for NASH cirrhosis needed re-transplantation due to NASH recurrence.

Based on the literature, approximately one-third of patients who were transplanted for non-NASH indications developed IR and MetS (risk factors for NAFLD) in the three years post-LT. As such, researchers have attended to understanding the development of *de novo* NAFLD in recipients who underwent LT for indications other than NASH[11]. Ten years ago, Seo *et al*[83] retrospectively analyzed data from 68 recipients who experienced LT due to ESLD that was related to non-NASH indications. They reported that 18% of the recipients developed *de novo* NAFLD, while 9% developed *de novo* NASH. The data analysis showed that the utilization of angiotensin-converting enzyme inhibitors (ACE-I) was related to a decreased risk for developing NAFLD after LT. In contrast, an increased BMI of more than 10% after LT was a risk factor for NAFLD after LT[83]. The observation related to the protective effect of ACE-I in the context of *de novo* NAFLD after LT is interesting given preliminary findings that renin-angiotensin (RAAS) inhibitors have a beneficial effect on the regression of NAFLD in non-transplanted patients[84]. Recently, we have shown that using the RAAS inhibitor is associated with a lower rate of NAFLD as defined by TE with CAP in the population of renal transplant recipients[85]. However, additional research is needed on the benefits of using RAAS inhibitors to prevent the occurrence or progression of NAFLD in post-LT patients[85]. A few years ago, Dumortier *et al*[86] published a retrospective study that analyzed the prevalence of NAFLD in post-LT liver biopsies from 421 recipients who were transplanted for non-NASH indications. Histological evidence of steatosis occurred in 131 (31.1%) patients; and 53% had grade 1, 31% grade 2 and 16% grade 3 steatosis. Interestingly, 51.1% of those with steatosis had normal liver enzymes. There was perisinusoidal fibrosis in 38 patients (29.0%), while 5 patients (3.8%) were diagnosed with NASH. In contrast, there was cirrhosis or extensive fibrosis in 2.25% of recipients at the end of the follow-up. The authors noted that post-LT obesity, tacrolimus-based regimen, hyperlipidemia, hypertension, diabetes mellitus, and alcoholic cirrhosis

were the primary indications for the LT and, combined with pre-transplant liver graft steatosis, were risk factors for steatosis after transplantation[86]. This is the first study that showed an association between the presence of steatosis in the donor liver and the development of new NAFLD after the LT[28,86]. Recently, Kim *et al*[87] showed that preexisting donor graft steatosis is associated with a threefold increased risk for developing post-LT NAFLD (OR = 3.147, P = 0.022). Although the impact of donor steatosis on graft and patient outcomes remains an insufficiently explored area, the growing incidence of NAFLD in general population indicates an urgent need for further investigations on this topic[13].

Another interesting topic in the context of NAFLD after LT is the difference between recurrent and *de novo* NAFLD after LT. Vallin *et al*[88] published the first longitudinal study four years ago with a small number of patients. The authors analyzed the characteristics of 91 patients who experienced LT between 2000 and 2010. They compared biological, clinical, and histological markers for patients who had recurrent NAFLD and patients who had *de novo* NAFLD. During the study, 91 patients were given a diagnosis of post-LT NAFLD: 11 cases were classified as recurrent NAFLD, and 80 cases were classified as *de novo* NAFLD. There were no differences in sex, age and the prevalence of obesity, hypercholesterolemia or hypertension. However, in patients with recurrent NAFLD, there was a higher prevalence of diabetes mellitus (100% *vs* 37.5%). Severe fibrosis (stage 3 or 4) and steatohepatitis at 5 years had a higher incidence in patients who had recurrent NAFLD than in patients with *de novo* NAFLD [71.4% *vs* 12.5% (P < 0.01) and 71.4% *vs* 17.2% (P < 0.01), respectively]. Additionally, after 1 year, NAFLD was diagnosed in 67% of patients who had *de novo* NAFLD, while it was present in all patients who had recurrent NAFLD. For the liver biopsy, steatosis disappeared in 18 patients (22.5%) who had *de novo* NAFLD and in no patients who had recurrent NAFLD[88]. Although this was a small study, it is important to note that recurrent and *de novo* NAFLD after LT are different entities and recurrent NAFLD appears to be a more severe and irreversible condition with an earlier onset[88].

Although many drugs have been examined for treating NAFLD/NASH in the general population, there is still no efficient therapy for NAFLD. Thus, there are no studies that examine treatment options for preventing or treating the development or recurrence of NAFLD/NASH after LT. Because NAFLD is a liver manifestation of MetS, we need to prevent and treat all MetS components in post-LT patients. Given the metabolic effects of immunosuppressive drugs that are used in liver transplant recipients, this can often be challenging. For now, we can attempt to prevent and manage hypertension, dyslipidemia, diabetes and obesity, as well as individualize immunosuppressive therapy in post-LT patients to prevent NAFLD recurrence/development and CVD complications in all recipients[28,70,72].

NAFLD AND CHRONIC KIDNEY DISEASE AFTER LIVER TRANSPLANTATION

CKD is another important area and potential challenge in the context of NAFLD and LT. The survival of the graft and patient as well as the success of LT directly depends on kidney functions. Unfortunately, it is almost impossible to prevent the development of CKD after LT. For the occurrence of CKD after LT, there are three primary risk factors: pre-LT kidney disease, using immunosuppressive drugs and recipient comorbidities. Several authors reported that a risk factor for the development and progression of CVD and CKD is NAFLD[70,72,89-91]. Musso *et al*[89] performed a meta-analysis that included 33 studies 4 years ago. The study showed that NAFLD was related to an increased incidence and prevalence of CKD. There is a close relation between NAFLD and risk factors for CVD and CKD, which makes it difficult to determine whether NAFLD is only a risk marker for CVD and/or CKD or a causal factor[71,90,91]. Park *et al*[67] reported similar results for NASH patients who were on the waitlist. Patients who had ESLD due to NASH on the waiting list had significantly higher levels of serum creatinine than patients who had other etiologies of ESLD, despite similar MELD scores[67]. Moreover, NASH is also important in the context of CKD for the post-LT setting. The first study that highlighted this association was by Houlihan *et al*[91]. They demonstrated that patients who underwent LT for ESLD related to NASH developed worse renal functioning than patients who had ESLD due to other etiologies. Compared to non-NASH patients, three months after LT, NASH patients had a significantly lower estimated glomerular filtration rate (eGFR). During the next two years 31.2% of the NASH patients (15/48) developed stage IIIb CKD, which only occurred in 8.3% of the non-NASH patients (4/48)[91]. Three years later, Fussner *et al*[92] reported that female gender and NASH were independent predictors of $\geqslant$ stage 3 CKD development at 5 years post-LT.

Given the increase in the incidence of ESLD due to NASH, and based on the MELD allocation system, which favors LT for patients with higher creatinine (kidney injury), the incidence of CKD after LT is also likely to increase. In order to prevent pre- and post-LT CKD, more effective methods of treatment are needed, such as, delayed usage of CNIs or immunosuppressive protocols without CNIs which may be effective way for saving kidney function after LT. Therefore, immunosuppressive protocols should be considered in the context of LT and NASH, and more pro-perspective studies are needed on this topic[28,91,93].

CONCLUSION

NAFLD/NASH is a challenging and multisystem disease

that has a high socioeconomic impact. NAFLD/NASH, as a primary cause of macrovesicular steatosis, has several impacts on LT; on patients on the waiting list for transplant, on post-transplant setting as well as on organ donors. Current data indicate a new trend in the area of CLD. Because of the increased incidence of T2DM and obesity, *i.e.*, the growing incidence of MetS, there is a parallel rise in the HCC incidence[13,19,25,54,94]. Consequently, NASH cirrhosis and HCC due to NASH will soon become the major indications for LT. Importantly, recent investigations and observations indicate that HCC can occur in patients who have NAFLD without liver cirrhosis. Because screening for HCC is not a part of standard approach for a patient with NAFLD without cirrhosis, HCC is often diagnosed in advanced stages. One of the primary goals of health care practitioners should be to increase awareness of NAFLD/NASH and to develop and conduct useful screening programs for this increasing patient population[13,19,25,54].

An increased incidence of MetS and, consequently, NAFLD/NASH effects the demand for LT and the supply of available donors. Thus, we can expect that there will be a higher number of steatotic livers for LT in the future. The lack of organs is a global problem and could result in one of two possible scenarios. We will either choose low quality organs that have a greater risk for post-transplantation complications and, consequently, a higher risk for worse outcome of LT. The second option is that we will decrease steatotic livers but the time on the waiting list will become longer and, consequently, there will be an increase in wait-list mortality. To develop appropriate method for optimizing the allocation of steatotic grafts prior to LT, research needs to examine procedures to protect it from IRI or primary graft non-functioning and to expand the pool of available donors. Moreover, future research should identify new non-invasive diagnostic methods for the exact detection and quantification of steatosis in donor organs. In addition, more data on other potential risk factors that are associated with the development of steatotic livers is necessary[28,54].

There are two problems with keeping NASH patients on the waiting list: their comorbidities and lower MELD scores compared to other etiologies of CLD. These patients often have different metabolic risk factors and coexisting CVD and/or CKD, which makes managing these patients complicated and demanding. As such, there is a need for more detailed and personalized screening and evaluations of NAFLD/NASH patients, particularly for assessing CVD. According to available research, there are no universal guidelines or clear recommendations for the optimal screening method for CVD in patients who have NASH related ESLD and are candidates for LT. We need new prospective studies that will answer this important question and provide a basis for a standardized approach to assessing CVD risk in this population of LT candidates[13,28,35] In addition, randomized studies are needed to determine which NASH patients on the transplant list will benefit from treatment with BS, the optimal time for BS (before LT, during LT, after LT) and the type of BS to apply[28,34]. Future research is also needed to demonstrate the long-term impact of BS on LT recipients[28].

Patients who have ESLD due to NASH and underwent LT have similar post-transplant outcomes as other etiologies of CLD[35,68]. However, according to research, the total survival rates after LT are good, but NASH recipients have a higher incidence of CVD events after LT. Interestingly, infections (sepsis) were also more frequently observed in this group of recipients. The NASH LT recipients should be viewed as population at high risk for CVD, thus, there is a need for more studies on how to follow and treat these patients[11,68,69].

The prevalence of MetS clinical features, such as T2DM, hypertension, rapid weight gain and dyslipidemia, are often higher in the period after LT, are frequently caused by transplant specific factors, including immunosuppression, and can be valuable predictors of recipients' morbidity and mortality. Immunosuppressant drugs, such as corticosteroids, CNIs and mTORs, have a specific role in metabolic balance and favor hyperglycemia, weight gain, hypertension and hyperlipidemia. These groups of immunosuppressive drugs may, to an extent, contribute to the formation of CVD by affecting metabolic complications[70,72]. Most studies that examine the prevalence of recurrent NASH in the post-LT setting have shown that the incidence of recurrent NASH is between 20% and 40%, but the incidence largely depends on NASH detection methods, such as liver enzymes, imaging techniques or liver biopsies. Most of the studies that investigated the incidence of recurrent NASH have been retrospective, without the standard Post-LT interval biopsy protocol. In addition, the histological criteria that are used for the diagnosis of recurrent NAFLD varied in the published studies[74,81]. Therefore, prospective studies with well-defined biopsy protocols are needed to show the actual incidence and progression of recurrent NAFLD after LT. According to the literature, in one-third of patients who were transplanted for non-NASH indications, IR and MetS developed within three years post-LT. As such, more research has focused on understanding the development of *de novo* NAFLD in recipients who underwent LT for indications other than NASH[11]. Another interesting topic in the context of NAFLD after LT is the difference between recurrent and *de novo* NAFLD after LT. Although the results from previous studies were conducted with a small number of patients, it is important to note that recurrent NAFLD and *de novo* NAFLD after LT are different entities and that recurrent NAFLD appears to be much more severe and irreversible and has an earlier onset[88].

Preliminary data indicated that preexisting donor graft steatosis is associated with a threefold increase in the risk for developing post-LT NAFLD. However, the influence of donor steatosis on the graft and patient outcomes has been minimally explored, and given the growing incidence of NAFLD in the general population,

there is an urgent need for further investigations on this topic[13,87].

NASH is important in the context of CKD and in the post-LT setting. Preliminary data outline that NASH is an independent predictor of ⩾ stage 3 CKD development after LT[91,92]. Given the increase in the incidence of ESLD due to NASH, there is also likely to be an increase in the incidence of CKD after LT. The transplant society will have to identify a more useful approach to these patients to prevent pre- and post-LT CKD. The delayed use of CNIs or immunosuppressive protocols without CNIs may be an effective way for saving kidney function after LT. Therefore, immunosuppressive protocols should be considered in the context of LT and NASH, and more pro-perspective studies are needed on this topic[28,91,93].

REFERENCES

1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

2 **Brunt EM**, Wong VW, Nobili V, Day CP, Sookoian S, Maher JJ, Bugianesi E, Sirlin CB, Neuschwander-Tetri BA, Rinella ME. Nonalcoholic fatty liver disease. *Nat Rev Dis Primers* 2015; **1**: 15080 [PMID: 27188459 DOI: 10.1038/nrdp.2015.80]

3 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

4 **Hagström H**, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, Kechagias S. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. *J Hepatol* 2017; **67**: 1265-1273 [PMID: 28803953 DOI: 10.1016/j.jhep.2017.07.027]

5 **Ekstedt M**, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015; **61**: 1547-1554 [PMID: 25125077 DOI: 10.1002/hep.27368]

6 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-397.e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]

7 **Byrne CD**, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015; **62**: S47-S64 [PMID: 25920090 DOI: 10.1016/j.jhep.2014.12.012]

8 **Baffy G**, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012; **56**: 1384-1391 [PMID: 22326465 DOI: 10.1016/j.hep.2011.10.027]

9 **Guzman G**, Brunt EM, Petrovic LM, Chejfec G, Layden TJ, Cotler SJ. Does nonalcoholic fatty liver disease predispose patients to hepatocellular carcinoma in the absence of cirrhosis? *Arch Pathol Lab Med* 2008; **132**: 1761-1766 [PMID: 18976012 DOI: 10.1043/1543-2165-132.11.1761]

10 **Wong RJ**, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; **148**: 547-555 [PMID: 25461851 DOI: 10.1053/j.gastro.2014.11.039]

11 **Zezos P**, Renner EL. Liver transplantation and non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 15532-15538 [PMID: 25400437 DOI: 10.3748/wjg.v20.i42.15532]

12 **Udompap P**, Kim D, Kim WR. Current and Future Burden of Chronic Nonmalignant Liver Disease. *Clin Gastroenterol Hepatol* 2015; **13**: 2031-2041 [PMID: 26291665 DOI: 10.1016/j.cgh.2015.08.015]

13 **Canbay A**, Sowa JP, Syn WK, Treckmann J. NASH Cirrhosis - the New Burden in Liver Transplantation: How Should It Be Managed? *Visc Med* 2016; **32**: 234-238 [PMID: 27722159 DOI: 10.1159/000446379]

14 **Charlton MR**, Burns JM, Pedersen RA, Watt KD, Heimbach JK, Dierkhising RA. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. *Gastroenterology* 2011; **141**: 1249-1253 [PMID: 21726509 DOI: 10.1053/j.gastro.2011.06.061]

15 **Heimbach J**. Debate: A bridge too far--liver transplantation for nonalcoholic steatohepatitis will overwhelm the organ supply. *Liver Transpl* 2014; **20** Suppl 2: S32-S37 [PMID: 25155244 DOI: 10.1002/lt.23980]

16 **Goldberg D**, Ditah IC, Saeian K, Lalehzari M, Aronsohn A, Gorospe EC, Charlton M. Changes in the Prevalence of Hepatitis C Virus Infection, Nonalcoholic Steatohepatitis, and Alcoholic Liver Disease Among Patients With Cirrhosis or Liver Failure on the Waitlist for Liver Transplantation. *Gastroenterology* 2017; **152**: 1090-1099.e1 [PMID: 28088461 DOI: 10.1053/j.gastro.2017.01.003]

17 **Cholankeril G**, Wong RJ, Hu M, Perumpail RB, Yoo ER, Puri P, Younossi ZM, Harrison SA, Ahmed A. Liver Transplantation for Nonalcoholic Steatohepatitis in the US: Temporal Trends and Outcomes. *Dig Dis Sci* 2017; **62**: 2915-2922 [PMID: 28744836 DOI: 10.1007/s10620-017-4684-x]

18 **Weinmann A**, Alt Y, Koch S, Nelles C, Düber C, Lang H, Otto G, Zimmermann T, Marquardt JU, Galle PR, Wörns MA, Schattenberg JM. Treatment and survival of non-alcoholic steatohepatitis associated hepatocellular carcinoma. *BMC Cancer* 2015; **15**: 210 [PMID: 25884354 DOI: 10.1186/s12885-015-1197-x]

19 **Degasperi E**, Colombo M. Distinctive features of hepatocellular carcinoma in non-alcoholic fatty liver disease. *Lancet Gastroenterol Hepatol* 2016; **1**: 156-164 [PMID: 28404072 DOI: 10.1016/S2468-1253(16)30018-8]

20 **Park JW**, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS, Sherman M. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015; **35**: 2155-2166 [PMID: 25752327 DOI: 10.1111/liv.12818]

21 **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711 DOI: 10.1002/hep.26986]

22 **Mittal S**, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA, Davila JA. Hepatocellular Carcinoma in the Absence of Cirrhosis in United States Veterans is Associated With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2016; **14**: 124-131.e1 [PMID: 26196445 DOI: 10.1016/j.cgh.2015.07.019]

23 **Yasui K**, Hashimoto E, Komorizono Y, Koike K, Arii S, Imai Y, Shima T, Kanbara Y, Saibara T, Mori T, Kawata S, Uto H, Takami S, Sumida Y, Takamura T, Kawanaka M, Okanoue T; Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan. Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011; **9**: 428-433; quiz e50 [PMID: 21320639 DOI: 10.1016/j.cgh.2011.01.023]

24 **Kawada N**, Imanaka K, Kawaguchi T, Tamai C, Ishihara R, Matsunaga T, Gotoh K, Yamada T, Tomita Y. Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis. *J Gastroenterol* 2009; **44**: 1190-1194 [PMID: 19672551 DOI: 10.1007/s00535-009-0112-0]

25 **Perumpail RB**, Wong RJ, Ahmed A, Harrison SA. Hepatocellular Carcinoma in the Setting of Non-cirrhotic Nonalcoholic Fatty Liver Disease and the Metabolic Syndrome: US Experience. *Dig Dis Sci* 2015; **60**: 3142-3148 [PMID: 26250831 DOI: 10.1007/s10620-015-3821-7]

26 **Feldstein AE**, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut* 2009; **58**: 1538-1544 [PMID: 19625277 DOI: 10.1136/gut.2008.171280]

27 **Alkhouri N**, Hanouneh IA, Zein NN, Lopez R, Kelly D, Eghtesad B, Fung JJ. Liver transplantation for nonalcoholic steatohepatitis in young patients. *Transpl Int* 2016; **29**: 418-424 [PMID: 26402655 DOI: 10.1111/tri.12694]

28 **Patel YA**, Berg CL, Moylan CA. Nonalcoholic Fatty Liver Disease: Key Considerations Before and After Liver Transplantation. *Dig Dis Sci* 2016; **61**: 1406-1416 [PMID: 26815171 DOI: 10.1007/s10620-016-4035-3]

29 **Leonard J**, Heimbach JK, Malinchoc M, Watt K, Charlton M. The impact of obesity on long-term outcomes in liver transplant recipients-results of the NIDDK liver transplant database. *Am J Transplant* 2008; **8**: 667-672 [PMID: 18294163 DOI: 10.1111/j.1600-6143.2007.02100.x]

30 **LaMattina JC**, Foley DP, Fernandez LA, Pirsch JD, Musat AI, D' Alessandro AM, Mezrich JD. Complications associated with liver transplantation in the obese recipient. *Clin Transplant* 2012; **26**: 910-918 [PMID: 22694047 DOI: 10.1111/j.1399-0012.2012.01669.x]

31 **Conzen KD**, Vachharajani N, Collins KM, Anderson CD, Lin Y, Wellen JR, Shenoy S, Lowell JA, Doyle MB, Chapman WC. Morbid obesity in liver transplant recipients adversely affects longterm graft and patient survival in a single-institution analysis. *HPB* (Oxford) 2015; **17**: 251-257 [PMID: 25322849 DOI: 10.1111/hpb.12340]

32 **Heimbach JK**, Watt KD, Poterucha JJ, Ziller NF, Cecco SD, Charlton MR, Hay JE, Wiesner RH, Sanchez W, Rosen CB, Swain JM. Combined liver transplantation and gastric sleeve resection for patients with medically complicated obesity and end-stage liver disease. *Am J Transplant* 2013; **13**: 363-368 [PMID: 23137119 DOI: 10.1111/j.1600-6143.2012.04318.x]

33 **Lazzati A**, Iannelli A, Schneck AS, Nelson AC, Katsahian S, Gugenheim J, Azoulay D. Bariatric surgery and liver transplantation: a systematic review a new frontier for bariatric surgery. *Obes Surg* 2015; **25**: 134-142 [PMID: 25337867 DOI: 10.1007/s11695-014-1430-8]

34 **Mosko JD**, Nguyen GC. Increased perioperative mortality following bariatric surgery among patients with cirrhosis. *Clin Gastroenterol Hepatol* 2011; **9**: 897-901 [PMID: 21782772 DOI: 10.1016/j.cgh.2011.07.007]

35 **Malhi H**, Allen AM, Watt KD. Nonalcoholic fatty liver: optimizing pretransplant selection and posttransplant care to maximize survival. *Curr Opin Organ Transplant* 2016; **21**: 99-106 [PMID: 26825357 DOI: 10.1097/MOT.0000000000000283]

36 **Hoehn RS**, Singhal A, Wima K, Sutton JM, Paterno F, Steve Woodle E, Hohmann S, Abbott DE, Shah SA. Effect of pretransplant diabetes on short-term outcomes after liver transplantation: a national cohort study. *Liver Int* 2015; **35**: 1902-1909 [PMID: 25533420 DOI: 10.1111/liv.12770]

37 **European Association for the Study of the Liver.** EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol* 2016; **64**: 433-485 [PMID: 26597456 DOI: 10.1016/j.jhep.2015.10.006]

38 **Wray C**, Scovotti JC, Tobis J, Niemann CU, Planinsic R, Walia A, Findlay J, Wagener G, Cywinski JB, Markovic D, Hughes C, Humar A, Olmos A, Sierra R, Busuttil R, Steadman RH. Liver transplantation outcome in patients with angiographically proven coronary artery disease: a multi-institutional study. *Am J Transplant* 2013; **13**: 184-191 [PMID: 23126562 DOI: 10.1111/j.1600-6143.2012.04293.x]

39 **Mikolasevic I**, Milic S, Turk Wensveen T, Grgic I, Jakopcic I, Stimac D, Wensveen F, Orlic L. Nonalcoholic fatty liver disease - A multisystem disease? *World J Gastroenterol* 2016; **22**: 9488-9505 [PMID: 27920470 DOI: 10.3748/wjg.v22.i43.9488]

40 **Mikolasevic I**, Orlic L, Milic S, Zaputovic L, Lukenda V, Racki S. Non-alcoholic fatty liver disease proven by transient elastography in hemodialysis patients: is it a new risk factor for adverse cardiovascular events? *Blood Purif* 2014; **37**: 259-265 [PMID: 24993140 DOI: 10.1159/000360270]

41 **Kim D**, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology* 2013; **57**: 1357-1365 [PMID: 23175136 DOI: 10.1002/hep.26156]

42 **Targher G**, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. *Atherosclerosis* 2007; **191**: 235-240 [PMID: 16970951 DOI: 10.1016/j.atherosclerosis.2006.0]

43 **Patel S**, Kiefer TL, Ahmed A, Ali ZA, Tremmel JA, Lee DP, Yeung AC, Fearon WF. Comparison of the frequency of coronary artery disease in alcohol-related versus non-alcohol-related endstage liver disease. *Am J Cardiol* 2011; **108**: 1552-1555 [PMID: 21890080 DOI: 10.1016/j.amjcard.2011.07.013]

44 **Vanwagner LB**, Bhave M, Te HS, Feinglass J, Alvarez L, Rinella ME. Patients transplanted for nonalcoholic steatohepatitis are at increased risk for postoperative cardiovascular events. *Hepatology* 2012; **56**: 1741-1750 [PMID: 22611040 DOI: 10.1002/hep.25855]

45 **Jia G**, Di F, Wang Q, Shao J, Gao L, Wang L, Li Q, Li N. Non-Alcoholic Fatty Liver Disease Is a Risk Factor for the Development of Diabetic Nephropathy in Patients with Type 2 Diabetes Mellitus. *PLoS One* 2015; **10**: e0142808 [PMID: 26566287 DOI: 10.1371/journal.pone.0142808]

46 **Singal AK**, Hasanin M, Kaif M, Wiesner R, Kuo YF. Nonalcoholic Steatohepatitis is the Most Rapidly Growing Indication for Simultaneous Liver Kidney Transplantation in the United States. *Transplantation* 2016; **100**: 607-612 [PMID: 26479282 DOI: 10.1097/TP.0000000000000945]

47 **Stine JG**, Shah NL, Argo CK, Pelletier SJ, Caldwell SH, Northup PG. Increased risk of portal vein thrombosis in patients with cirrhosis due to nonalcoholic steatohepatitis. *Liver Transpl* 2015; **21**: 1016-1021 [PMID: 25845711 DOI: 10.1002/lt.24134]

48 **Tripodi A**, Fracanzani AL, Primignani M, Chantarangkul V, Clerici M, Mannucci PM, Peyvandi F, Bertelli C, Valenti L, Fargion S. Procoagulant imbalance in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **61**: 148-154 [PMID: 24657400 DOI: 10.1016/j.jhep.2014.03.013]

49 **Englesbe MJ**, Kubus J, Muhammad W, Sonnenday CJ, Welling T, Punch JD, Lynch RJ, Marrero JA, Pelletier SJ. Portal vein thrombosis and survival in patients with cirrhosis. *Liver Transpl* 2010; **16**: 83-90 [PMID: 20035521 DOI: 10.1002/lt.21941]

50 **Stine JG**, Argo CK, Pelletier SJ, Maluf DG, Caldwell SH, Northup PG. Advanced non-alcoholic steatohepatitis cirrhosis: A high-risk population for pre-liver transplant portal vein thrombosis. *World J Hepatol* 2017; **9**: 139-146 [PMID: 28217250 DOI: 10.4254/wjh.v9.i3.139]

51 **Ponziani FR**, Zocco MA, Senzolo M, Pompili M, Gasbarrini A, Avolio AW. Portal vein thrombosis and liver transplantation: implications for waiting list period, surgical approach, early and late follow-up. *Transplant Rev* (Orlando) 2014; **28**: 92-101 [PMID: 24582320 DOI: 10.1016/j.trre.2014.01.003]

52 **O'Leary JG**, Landaverde C, Jennings L, Goldstein RM, Davis GL. Patients with NASH and cryptogenic cirrhosis are less likely than those with hepatitis C to receive liver transplants. *Clin Gastroenterol Hepatol* 2011; **9**: 700-704.e1 [PMID: 21570483 DOI: 10.1016/j.cgh.2011.04.007]

53 **Saidi RF**, Hejazii Kenari SK. Challenges of organ shortage for transplantation: solutions and opportunities. *Int J Organ Transplant Med* 2014; **5**: 87-96 [PMID: 25184029]

54 **Mikolasevic I**, Milic S, Filipec-Kanizaj T. Fatty liver allografts are associated with primary graft non-function and high mortality after transplantation. *Liver Int* 2017; **37**: 1113-1115 [PMID: 28710818 DOI: 10.1111/liv.13453]

55 **Chu MJ**, Dare AJ, Phillips AR, Bartlett AS. Donor Hepatic Steatosis and Outcome After Liver Transplantation: a Systematic Review. *J Gastrointest Surg* 2015; **19**: 1713-1724 [PMID:

25917535 DOI: 10.1007/s11605-015-2832-1]
56 **Gehrau RC**, Mas VR, Dumur CI, Suh JL, Sharma AK, Cathro HP, Maluf DG. Donor Hepatic Steatosis Induce Exacerbated Ischemia-Reperfusion Injury Through Activation of Innate Immune Response Molecular Pathways. *Transplantation* 2015; **99**: 2523-2533 [PMID: 26285018 DOI: 10.1097/TP.0000000000000857]
57 **Spitzer AL**, Lao OB, Dick AA, Bakthavatsalam R, Halldorson JB, Yeh MM, Upton MP, Reyes JD, Perkins JD. The biopsied donor liver: incorporating macrosteatosis into high-risk donor assessment. *Liver Transpl* 2010; **16**: 874-884 [PMID: 20583086 DOI: 10.1002/lt.22085]
58 **Jun MJ**, Shim JH, Kim SY, Seo N, Kim KM, Lim YS, Lee HC, Yu E, Lee SG. Clinical implications of preoperative and intraoperative liver biopsies for evaluating donor steatosis in living related liver transplantation. *Liver Transpl* 2014; **20**: 437-445 [PMID: 24478019 DOI: 10.1002/lt.23832]
59 **Kulik U**, Lehner F, Klempnauer J, Borlak J. Primary non-function is frequently associated with fatty liver allografts and high mortality after re-transplantation. *Liver Int* 2017; **37**: 1219-1228 [PMID: 28267886 DOI: 10.1111/liv.13404]
60 **Mancia C**, Loustaud-Ratti V, Carrier P, Naudet F, Bellissant E, Labrousse F, Pichon N. Controlled Attenuation Parameter and Liver Stiffness Measurements for Steatosis Assessment in the Liver Transplant of Brain Dead Donors. *Transplantation* 2015; **99**: 1619-1624 [PMID: 25719261 DOI: 10.1097/TP.0000000000000652]
61 **Hong YM**, Yoon KT, Cho M, Chu CW, Rhu JH, Yang KH, Lee JW. Clinical usefulness of controlled attenuation parameter to screen hepatic steatosis for potential donor of living donor liver transplant. *Eur J Gastroenterol Hepatol* 2017; **29**: 805-810 [PMID: 28379854 DOI: 10.1097/MEG.0000000000000876]
62 **Malik SM**, deVera ME, Fontes P, Shaikh O, Ahmad J. Outcome after liver transplantation for NASH cirrhosis. *Am J Transplant* 2009; **9**: 782-793 [PMID: 19344467 DOI: 10.1111/j.1600-6143.2009.02590.x]
63 **Bhagat V**, Mindikoglu AL, Nudo CG, Schiff ER, Tzakis A, Regev A. Outcomes of liver transplantation in patients with cirrhosis due to nonalcoholic steatohepatitis versus patients with cirrhosis due to alcoholic liver disease. *Liver Transpl* 2009; **15**: 1814-1820 [PMID: 19938128 DOI: 10.1002/lt.21927]
64 **Barritt AS 4th**, Dellon ES, Kozlowski T, Gerber DA, Hayashi PH. The influence of nonalcoholic fatty liver disease and its associated comorbidities on liver transplant outcomes. *J Clin Gastroenterol* 2011; **45**: 372-378 [PMID: 20733515 DOI: 10.1097/MCG.0b013e3181eeaff0]
65 **Agopian VG**, Kaldas FM, Hong JC, Whittaker M, Holt C, Rana A, Zarrinpar A, Petrowsky H, Farmer D, Yersiz H, Xia V, Hiatt JR, Busuttil RW. Liver transplantation for nonalcoholic steatohepatitis: the new epidemic. *Ann Surg* 2012; **256**: 624-633 [PMID: 22964732 DOI: 10.1097/SLA.0b013e31826b4b7e]
66 **Kennedy C**, Redden D, Gray S, Eckhoff D, Massoud O, McGuire B, Alkurdi B, Bloomer J, DuBay DA. Equivalent survival following liver transplantation in patients with non-alcoholic steatohepatitis compared with patients with other liver diseases. *HPB* (Oxford) 2012; **14**: 625-634 [PMID: 22882200 DOI: 10.1111/j.1477-2574.2012.00497.x]
67 **Park CW**, Tsai NT, Wong LL. Implications of worse renal dysfunction and medical comorbidities in patients with NASH undergoing liver transplant evaluation: impact on MELD and more. *Clin Transplant* 2011; **25**: E606-E611 [PMID: 21958082 DOI: 10.1111/j.1399-0012.2011.01497.x]
68 **Afzali A**, Berry K, Ioannou GN. Excellent posttransplant survival for patients with nonalcoholic steatohepatitis in the United States. *Liver Transpl* 2012; **18**: 29-37 [PMID: 21932374 DOI: 10.1002/lt.22435]
69 **Wang X**, Li J, Riaz DR, Shi G, Liu C, Dai Y. Outcomes of liver transplantation for nonalcoholic steatohepatitis: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2014; **12**: 394-402.e1 [PMID: 24076414 DOI: 10.1016/j.cgh.2013.09.023]
70 **Mikolasevic I**, Orlic L, Hrstic I, Milic S. Metabolic syndrome and non-alcoholic fatty liver disease after liver or kidney transplantation. *Hepatol Res* 2016; **46**: 841-852 [PMID: 26713425 DOI: 10.1111/hepr.12642]
71 **Watt KD**, Charlton MR. Metabolic syndrome and liver transplantation: a review and guide to management. *J Hepatol* 2010; **53**: 199-206 [PMID: 20451282 DOI: 10.1016/j.jhep.2010.01.040]
72 **Gitto S**, Villa E. Non-Alcoholic Fatty Liver Disease and Metabolic Syndrome after Liver Transplant. *Int J Mol Sci* 2016; **17**: 490 [PMID: 27049380 DOI: 10.3390/ijms17040490]
73 **Richards J**, Gunson B, Johnson J, Neuberger J. Weight gain and obesity after liver transplantation. *Transpl Int* 2005; **18**: 461-466 [PMID: 15773968 DOI: 10.1111/j.1432-2277.2004.00067.x]
74 **Patil DT**, Yerian LM. Evolution of nonalcoholic fatty liver disease recurrence after liver transplantation. *Liver Transpl* 2012; **18**: 1147-1153 [PMID: 22740341 DOI: 10.1002/lt.23499]
75 **Burke GW 3rd**, Cirocco R, Hensley G, Ranjan D, Reddy R, Jeffers L, Schiff E, Miller J. Liver transplantation for cirrhosis following jejuno-ileal bypass--regional cytokine differences associated with pathological changes in the transplant liver. *Transplantation* 1992; **54**: 374-377 [PMID: 1496549]
76 **Poordad F**, Gish R, Wakil A, Garcia-Kennedy R, Martin P, Yao FY. De novo non-alcoholic fatty liver disease following orthotopic liver transplantation. *Am J Transplant* 2003; **3**: 1413-1417 [PMID: 14525603]
77 **Yalamanchili K**, Saadeh S, Klintmalm GB, Jennings LW, Davis GL. Nonalcoholic fatty liver disease after liver transplantation for cryptogenic cirrhosis or nonalcoholic fatty liver disease. *Liver Transpl* 2010; **16**: 431-439 [PMID: 20373454 DOI: 10.1002/lt.22004]
78 **Dureja P**, Mellinger J, Agni R, Chang F, Avey G, Lucey M, Said A. NAFLD recurrence in liver transplant recipients. *Transplantation* 2011; **91**: 684-689 [PMID: 21248661 DOI: 10.1097/TP.0b013e31820b6b84]
79 **Sourianarayanane A**, Arikapudi S, McCullough AJ, Humar A. Nonalcoholic steatohepatitis recurrence and rate of fibrosis progression following liver transplantation. *Eur J Gastroenterol Hepatol* 2017; **29**: 481-487 [PMID: 28253211 DOI: 10.1097/MEG.0000000000000820]
80 **Bhati C**, Idowu MO, Sanyal AJ, Rivera M, Driscoll C, Stravitz RT, Kohli DR, Matherly S, Puri P, Gilles H, Cotterell A, Levy M, Sterling RK, Luketic VA, Lee H, Sharma A, Siddiqui MS. Long-term Outcomes in Patients Undergoing Liver Transplantation for Nonalcoholic Steatohepatitis-Related Cirrhosis. *Transplantation* 2017; **101**: 1867-1874 [PMID: 28296807 DOI: 10.1097/TP.0000000000001709]
81 **Finkenstedt A**, Auer C, Glodny B, Posch U, Steitzer H, Lanzer G, Pratschke J, Biebl M, Steurer M, Graziadei I, Vogel W, Zoller H. Patatin-like phospholipase domain-containing protein 3 rs738409-G in recipients of liver transplants is a risk factor for graft steatosis. *Clin Gastroenterol Hepatol* 2013; **11**: 1667-1672 [PMID: 23872669 DOI: 10.1016/j.cgh.2013.06.025]
82 **Burra P**, Germani G. Orthotopic liver transplantation in non-alcoholic fatty liver disease patients. *Rev Recent Clin Trials* 2014; **9**: 210-216 [PMID: 25514913]
83 **Seo S**, Maganti K, Khehra M, Ramsamooj R, Tsodikov A, Bowlus C, McVicar J, Zern M, Torok N. De novo nonalcoholic fatty liver disease after liver transplantation. *Liver Transpl* 2007; **13**: 844-847 [PMID: 17029282]
84 **Hirata T**, Tomita K, Kawai T, Yokoyama H, Shimada A, Kikuchi M, Hirose H, Ebinuma H, Irie J, Ojiro K, Oikawa Y, Saito H, Itoh H, Hibi T. Effect of Telmisartan or Losartan for Treatment of Nonalcoholic Fatty Liver Disease: Fatty Liver Protection Trial by Telmisartan or Losartan Study (FANTASY). *Int J Endocrinol* 2013; **2013**: 587140 [PMID: 23997767 DOI: 10.1155/2013/587140]
85 **Orlic L**, Mikolasevic I, Lukenda V, Anic K, Jelic I, Racki S. Nonalcoholic fatty liver disease and the renin-angiotensin system blockers in the patients with chronic kidney disease. *Wien Klin Wochenschr* 2015; **127**: 355-362 [PMID: 25412597 DOI: 10.1007/s00508-014-0661-y]
86 **Dumortier J**, Giostra E, Belbouab S, Morard I, Guillaud O, Spahr L, Boillot O, Rubbia-Brandt L, Scoazec JY, Hadengue A. Non-

alcoholic fatty liver disease in liver transplant recipients: another story of "seed and soil". *Am J Gastroenterol* 2010; **105**: 613-620 [PMID: 20040915 DOI: 10.1038/ajg.2009.717]

87 **Kim H**, Lee K, Lee KW, Yi NJ, Lee HW, Hong G, Choi Y, You T, Suh SW, Jang JJ, Suh KS. Histologically proven non-alcoholic fatty liver disease and clinically related factors in recipients after liver transplantation. *Clin Transplant* 2014; **28**: 521-529 [PMID: 24579874 DOI: 10.1111/ctr.12343]

88 **Vallin M**, Guillaud O, Boillot O, Hervieu V, Scoazec JY, Dumortier J. Recurrent or de novo nonalcoholic fatty liver disease after liver transplantation: natural history based on liver biopsy analysis. *Liver Transpl* 2014; **20**: 1064-1071 [PMID: 24961607 DOI: 10.1002/lt.23936]

89 **Musso G**, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, Hultcrantz R, Hagström H, Yoon SK, Charatcharoenwitthaya P, George J, Barrera F, Hafliðadóttir S, Björnsson ES, Armstrong MJ, Hopkins LJ, Gao X, Francque S, Verrijken A, Yilmaz Y, Lindor KD, Charlton M, Haring R, Lerch MM, Rettig R, Völzke H, Ryu S, Li G, Wong LL, Machado M, Cortez-Pinto H, Yasui K, Cassader M. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. *PLoS Med* 2014; **11**: e1001680 [PMID: 25050550 DOI: 10.1371/journal.pmed.1001680]

90 **Bonora E**, Targher G. Increased risk of cardiovascular disease and chronic kidney disease in NAFLD. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 372-381 [PMID: 22565095 DOI: 10.1038/nrgastro.2012.79]

91 **Houlihan DD**, Armstrong MJ, Davidov Y, Hodson J, Nightingale P, Rowe IA, Paris S, Gunson BK, Bramhall SB, Mutimer DJ, Neuberger JM, Newsome PN. Renal function in patients undergoing transplantation for nonalcoholic steatohepatitis cirrhosis: time to reconsider immunosuppression regimens? *Liver Transpl* 2011; **17**: 1292-1298 [PMID: 21761549 DOI: 10.1002/lt.22382]

92 **Fussner LA**, Charlton MR, Heimbach JK, Fan C, Dierkhising R, Coss E, Watt KD. The impact of gender and NASH on chronic kidney disease before and after liver transplantation. *Liver Int* 2014; **34**: 1259-1266 [PMID: 24262002 DOI: 10.1111/liv.12381]

93 **Neuberger JM**, Mamelok RD, Neuhaus P, Pirenne J, Samuel D, Isoniemi H, Rostaing L, Rimola A, Marshall S, Mayer AD; ReSpECT Study Group. Delayed introduction of reduced-dose tacrolimus, and renal function in liver transplantation: the 'ReSpECT' study. *Am J Transplant* 2009; **9**: 327-336 [PMID: 19120077 DOI: 10.1111/j.1600-6143.2008.02493.x]

94 **Scalera A**, Tarantino G. Could metabolic syndrome lead to hepatocarcinoma via non-alcoholic fatty liver disease? *World J Gastroenterol* 2014; **20**: 9217-9228 [PMID: 25071314 DOI: 10.3748/wjg.v20.i28.9217]

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REVIEW

Hepatitis B virus pre-S/S variants in liver diseases

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Abstract

Chronic hepatitis B is a global health problem. The clinical outcomes of chronic hepatitis B infection include asymptomatic carrier state, chronic hepatitis (CH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC). Because of the spontaneous error rate inherent to viral reverse transcriptase, the hepatitis B virus (HBV) genome evolves during the course of infection under the antiviral pressure of host immunity. The clinical significance of pre-S/S variants has become increasingly recognized in patients with chronic HBV infection. Pre-S/S variants are often identified in hepatitis B carriers with CH, LC, and HCC, which suggests that these naturally occurring pre-S/S variants may contribute to the development of progressive liver damage and hepatocarcinogenesis. This paper reviews the function of the pre-S/S region along with recent findings related to the role of pre-S/S variants in liver diseases. According to the mutation type, five pre-S/S variants have been identified: pre-S deletion, pre-S point mutation, pre-S1 splice variant, C-terminus S point mutation, and pre-S/S nonsense mutation. Their associations with HBV genotype and the possible pathogenesis of pre-S/S variants are discussed. Different pre-S/S variants cause liver diseases through different mechanisms. Most cause the intracellular retention of HBV envelope proteins and induction of endoplasmic reticulum stress, which results in liver diseases. Pre-S/S variants should be routinely determined in HBV carriers to help identify individuals who may be at a high risk of less favorable liver disease progression. Additional investigations are required to explore the molecular mechanisms of the pre-S/S variants involved in the pathogenesis of each stage of liver disease.

Key words: Hepatitis B virus; Pre-S/S mutant; Pre-S deletion; Splice variant; spPS1; Chronic hepatitis; Liver cirrhosis; Hepatocellular carcinoma

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Core tip: Naturally occurring hepatitis B virus (HBV) pre-S/S variants have been identified and associated with progressive liver diseases. In this review, the author discusses five pre-S/S variants: pre-S deletion,

pre-S point mutation, pre-S1 splice variant, C-terminus S point mutation, and pre-S/S nonsense mutation. Their associations with HBV genotype and the possible pathogenesis of pre-S/S variants are also discussed. Different pre-S/S variants cause liver diseases through different mechanisms. Most cause the intracellular retention of HBV envelope proteins and induction of endoplasmic reticulum stress, resulting in liver diseases. The exact pathogenesis of pre-S/S variants requires further investigation.

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INTRODUCTION

Hepatitis B virus (HBV) infection, which causes acute and chronic liver diseases, is a global health concern. The majority of acute HBV infections are self-limited, whereas chronic HBV infection usually results in a lifelong course. Chronic HBV infection can result in numerous clinical conditions, including asymptomatic HBV carrier (ASC), chronic hepatitis (CH), liver cirrhosis (LC), and hepatocellular carcinoma (HCC)[1,2]. More than 350 million people worldwide are estimated to have chronic HBV infection, and more than 25% of the chronically infected patients in Asia die because of HBV-related chronic diseases. The outcomes of HBV infection vary, which is likely because of differences in the host and viral factors.

To date, 10 HBV genotypes, designated as genotypes A to J, have been identified based on a divergence of > 8% over the entire genomic sequence. These 10 HBV genotypes are distributed in specific geographical locations[3,4]. Genotypes A (HBV/A) and D (HBV/D) are prevalent in Africa, Europe, and the Americas; genotypes B (HBV/B) and C (HBV/C) in Asia; genotype E (HBV/E) in sub-Saharan Africa; genotypes F and H in Southern and Central America; genotype G in France, Germany, and the United States; genotype I in Vietnam and Laos; and genotype J in Japan's Ryukyu islands. All genotypes can lead to progressive liver disease, but the clinical implications of each genotype differ. For example, patients infected by the HBV/C or HBV/D strain have a higher frequency of basal core promoter mutations, a lower response rate to interferon therapy, and a more rapid progression to liver fibrosis and HCC than those infected by the HBV/B or HBV/A strain[3,4]. In addition, carriers infected by HBV/C have a higher rate of pre-S deletions than those infected by HBV/B[5,6]. Collectively, these data indicate pathogenic and therapeutic differences among the HBV genotypes[3,4].

HBV is a small (42 nm) enveloped DNA virus, whose genome consists of partially double-stranded circular DNA that is 3182-3248 bp in length (varying with the genotype). Four genes - pre-S/S, precore (PC)/core (C), Pol, and X - encode seven polypeptides, including the structural proteins of the virion envelope and core, a small transcriptional transactivator, and a large polymerase protein with reverse transcriptase (RT) and RNase H (RH) activity (Figure 1). The pre-S/S gene has three in-frame initiation codons and encodes the small (S) envelope proteins as well as the middle (M) and large (L) envelope proteins, which contain pre-S2 and pre-S (pre-S1 and pre-S2) sequences, respectively (Figure 2A). The PC/C gene has two in-frame initiation codons and encodes the core antigen plus HBe protein, which is processed to produce soluble hepatitis B e antigen[1]. HBV replicates through the reverse transcription of an RNA intermediate, but because the RT lacks a proofreading function, errors in HBV DNA replication occur at a much higher rate than for other DNA viruses. The estimated rate of nucleotide substitution is approximately 1.4-3.2 × 10^{-5} per site per year[7]. These naturally occurring mutants evolve during the course of infection under the antiviral pressure of the host immune system or exogenous factors, including immunization or specific therapy[8]. Such HBV mutants display alteration of epitopes vital to host immune recognition, enhanced virulence with increased replication of HBV, and resistance to antiviral therapies

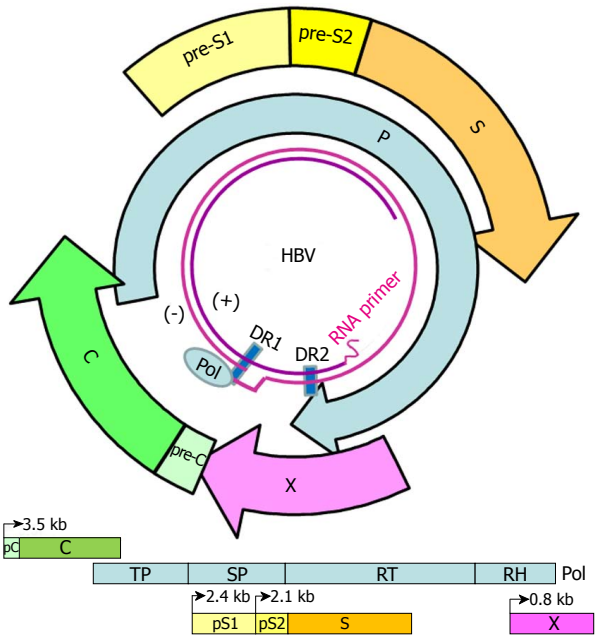

Figure 1 Genome structure and organization of hepatitis B virus. The relaxed-circular DNA genome of HBV with a complete minus strand and incomplete plus strand is shown (inner circle), along with the four main open reading frames (ORFs): pre-S/S; precore/core (pC/C); Pol, including four domains: TP, SP, reverse transcriptase (RT), and RNase H (RH); and X. The minus (-) and plus (+) DNA strands are marked. The HBV Pol and capped mRNA oligomer at the 5' end of the (-) and (+) strands as well as the DR-1 and DR-2 are illustrated. The space between the DR-1 and DR-2 is the "cohesive overlap region." The (+) strand is typically incomplete.

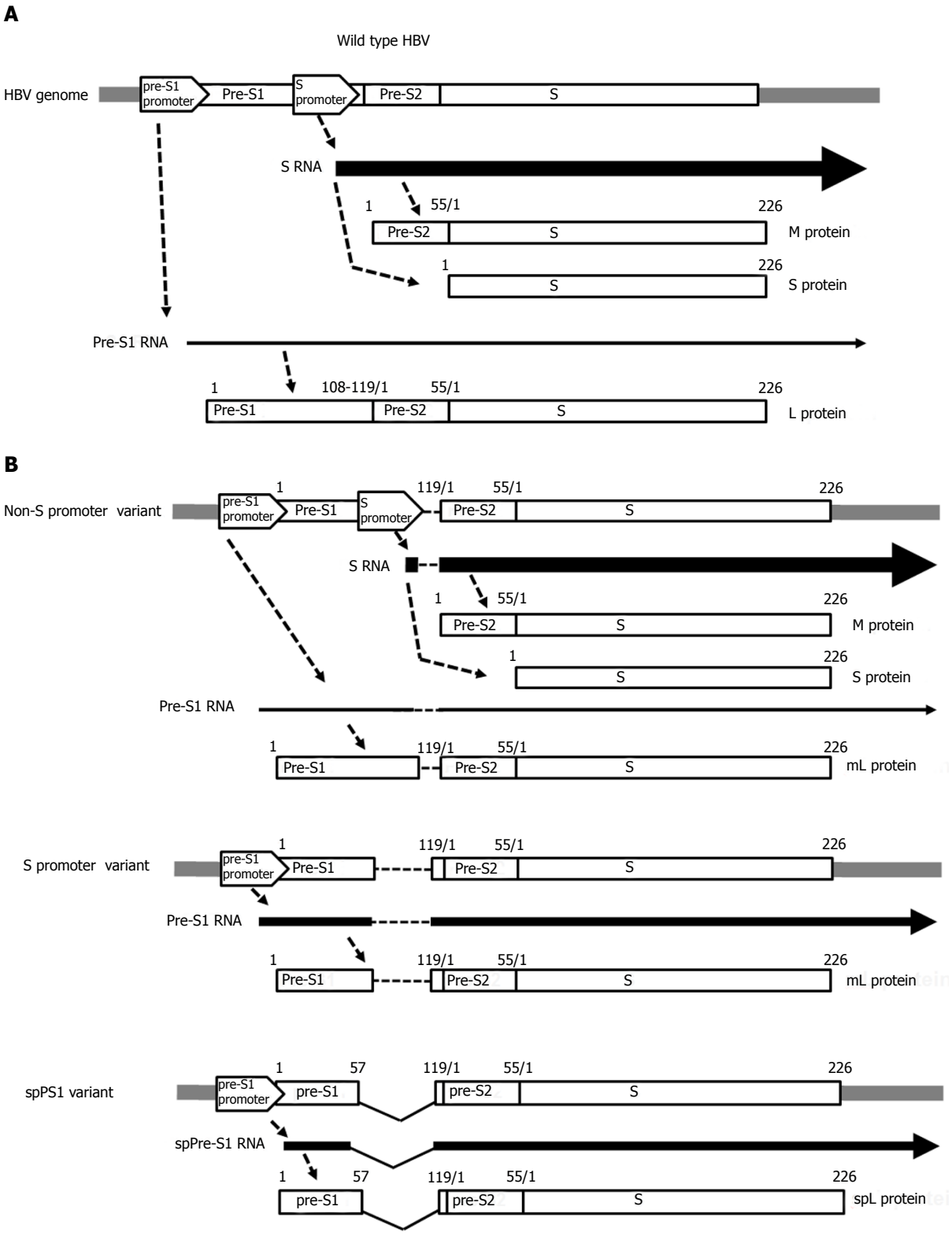

Figure 2 Gene expression of the pre-S/S gene in (A) wild-type hepatitis B virus and (B) pre-S/S variants: non-S promoter, S promoter, and spPS1.

while facilitating cell attachment or penetration[9,10]. These viral mutants, including basal core promoter, PC mutation, pre-S deletion, pre-S mutation, S mutants, and splice variants[5,6,11-27], have been associated with an increased risk of liver diseases.

The clinical significance of these naturally occurring mutants has become increasingly recognized in patients with both acute and chronic HBV infections[8-10,21,26,27]. In this article, the function of the pre-S/S region and recent findings related to the role of pre-S/S variants on

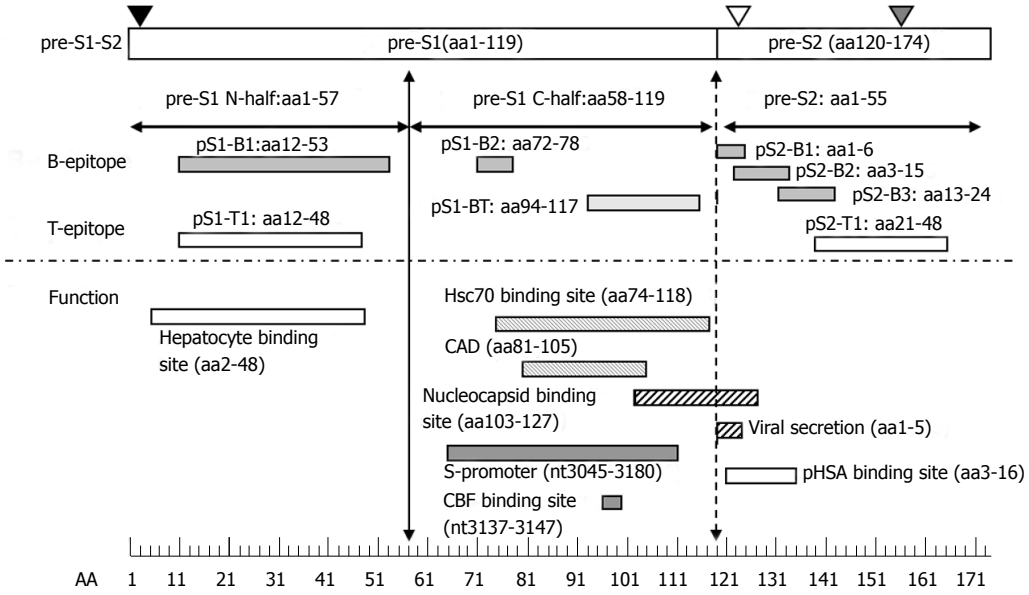

Figure 3 Immune epitopes and functional domains within the hepatitis B virus pre-S region. The pre-S region consists of the pre-S1 and pre-S2 regions. The pre-S1 region contains 119 amino acids in HBV genotypes B or C and is further divided into two parts: the N half (amino acids 1-57) and C half (amino acids 58-119). The pre-S2 region contains 55 amino acids. The pre-S domain contains many B- or T-epitopes and exerts multiple functions, as illustrated. The N-half of pre-S1 contains a hepatocyte binding site essential for infection. The C-half of pre-S1 contains a heat-shock protein 70 (Hsc70) binding site and cytosolic anchorage determinant (CAD) vital for dual topology of L proteins as well as a nucleocapsid binding site (NBS) for virion morphogenesis. The C-half of pre-S1 also contains an S-promoter and CCAAT binding factor (CBF) binding site necessary for expression of the S gene. The pre-S2 region has a polymerized human serum albumin (pHSA) binging site and viral secretion (VS) site. Black triangle, myristylation at second amino acid; white triangle, N-link glycosylation at N-4 of the M protein; gray triangle, O-link glycosylation at T-37 of the M protein. B-epitopes: pS1-B1, pS1-B2, pS2-B1, pS2-B2, and pS2-B3. T-epitopes: pS1-T1 and pS2-T1. B- and T-epitope: pS1-BT.

liver diseases is discussed and reviewed.

THE BIOLOGICAL FUNCTION OF THE PRE-S/S REGION

The pre-S/S gene has three open reading frames (ORFs) that encode three forms of hepatitis B surface antigen (HBsAg): the L, M, and S structural proteins of the viral envelope. However, these proteins are translated from different mRNAs: the L protein is translated from a long 2.4 kb pre-S1 RNA transcript, whereas the M and S proteins are translated from a slightly shorter 2.1 kb S RNA transcript (Figure 2A). The S protein consists of 226 amino acids (aa). The M protein is an extension of the S protein, with an additional 55 aa (*i.e.*, pre-S2 region). The L protein is an extension of the M protein, with an additional 108-119 aa depending on the genotype (*i.e.*, pre-S1 region). The aa sequence present at the C termini of the L and M proteins is identical to the S protein and is referred to as the S region. The pre-S (pre-S1 and pre-S2) region of the L protein is crucial for viral replication. It contains several functional sites: the hepatocyte binding site, which is essential for the attachment of HBV to liver cells; the S promoter and the CCAAT binding factor binding site, which is essential for S RNA transcription; the heat-shock protein 70 (Hsc70) binding site and the cytosolic anchorage determinant (CAD), which are essential for the dual topology (T) of L proteins; the nucleocapsid binding site (NBS), which is essential for virion morphogenesis; the site for viral secretion (VS); and the site for polymerized human albumin (pHSA) (Figure 3)[28-33].The pre-S region also plays an essential role in the interaction with the immune responses because it contains both B- and T-cell epitopes (Figure 3)[34-39]. By contrast, the biological role of M protein in the viral life cycle has been controversial. *In vitro* studies have suggested that M protein is not essential for viral replication, virion morphogenesis, or infectivity. Huang *et al*[40] defined a novel regulatory role for M protein, which may undergo a proteolytic process to generate an MHBs^{au} (aa 1-57 of M protein) species to upregulate the transcription of S promoter. In addition, the pre-S2 region of M protein binds to pHSA (aa 3-16), but the significance of this binding is unknown[34]. The S proteins are required for virion morphogenesis and secretion, and they also contain both B- and T-cell epitopes[26,41].

HBV envelope proteins are synthesized at the endoplasmic reticulum (ER). HBV envelope proteins have an unusual feature; they have multiple transmembrane domains that span the ER with loops of amino acids internal and external to the cytosol (Figure 4A)[41]. The S protein spans the ER membrane through four transmembrane domains (TM 1-4) that are linked by internal and external loops[41]. The loop of amino acids linking TM2 and TM3 is external to the ER and comprises aa 99-169. This loop is known as the "a" determinant (aa 122-148), and it is of vital virological and clinical significance as it is a major antigenic determinant of HBV. The transmembrane topology of

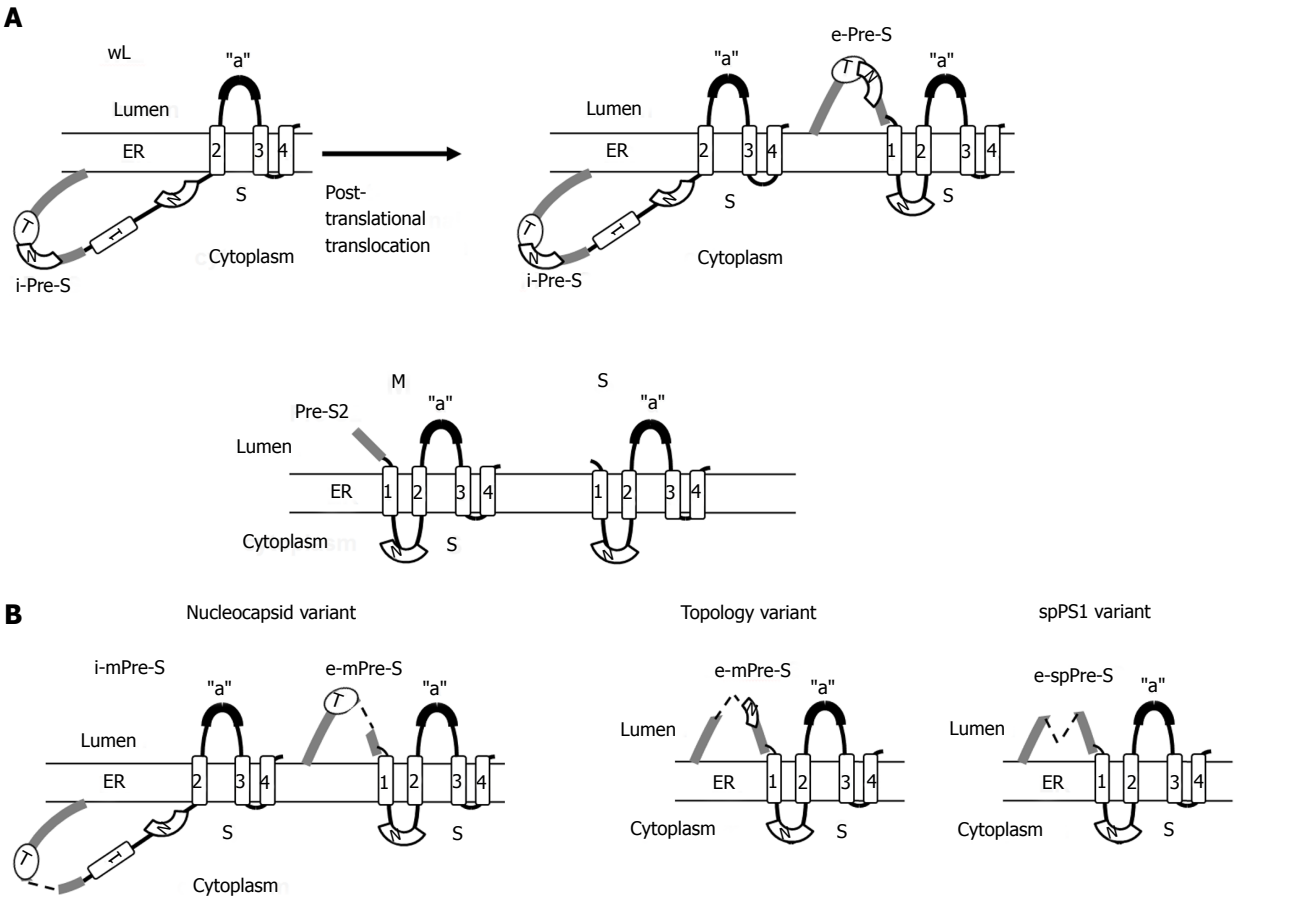

Figure 4 Topology of wild-type small (S), medium (M), and large (L) envelope proteins. The predicted four membrane-spanning segments (indicated by rectangular boxes) of S project their N and C termini into the endoplasmic reticulum lumen (A). The M proteins exhibit a topology similar to the S protein with their N-terminal pre-S2 domain protruding into the endoplasmic reticulum (ER) lumen, whereas the L proteins display a dual topology. Upon cotranslational membrane integration, the pre-S domains of L proteins are initially located on the cytosolic side of the ER membrane (i-Pre-S); they are controlled by the dual topology site (indicated by an oval). During maturation (marked by the arrow), nearly half of mature L-protein molecules posttranslationally translocate their pre-S region to the luminal space (e-Pre-S). The nucleocapsid (N) binding sites in the pre-S and S region are indicated by the white curved box. (B) The L-protein topology of pre-S/S variants. The nucleocapsid variant demonstrates a dual topology, and the topology variants and spPS1 variants display a uniform topology. The broken line indicates deletion, and "a" indicates "a" determinant.

the M protein is identical to the S protein. By contrast, the L protein has two transmembrane topologies. On biogenesis, the CAD of the pre-S1 region interacts with the cognate heat-shock protein Hsc70, thereby preventing cotranslational pre-S translocation to remain the pre-S domain of L cytosolic[42,43]. During maturation, approximately half of the L molecules posttranslationally translocate their pre-S region into the ER, thereby generating a dual topology (Figure 4A)[41-43]. The L protein serves its topological opposing functions in the virus life cycle by orientating the pre-S domain at both the cytosolic (i-Pre-S, inside the virus) and luminal (e-Pre-S, outside the virus) locations, i-Pre-S for capsid envelopment and e-Pre-S for receptor binding[41].

ASSOCIATION BETWEEN HBV PRE-S/S VARIANTS AND LIVER DISEASES

Owing to the spontaneous error rate of viral reverse transcription, naturally occurring HBV mutants arise during the course of a patient's infection under the pressure of host immunity or specific therapy[8]. Recently, many investigations have reported that pre-S/S variants are associated with the development of liver diseases[5,6,11-14,26,27]. Here, according to the mutation type, five pre-S/S variants-pre-S deletion, pre-S point mutation, pre-S1 splice variant, C-terminus S point mutation, and pre-S/S nonsense mutation-are reviewed. The pre-S region is the most variable sequence of the viral genome and changes with the genotype. The HBV genotype may influence the emergence of different pre-S variants; thus, it is also reviewed.

Pre-S deletion and genotype

Many studies have demonstrated that pre-S deletions are associated with progressive liver diseases[5,6,11-14,26,27]. Pre-S deletion is frequently found at the C-terminal half (aa 58-119) of pre-S1 and the N-terminus (aa 1-23) of pre-S2. Most are in-frame deletions[6,11-14,26,27]. Mapping of the pre-S region has revealed that all deletion regions encompassed T- and B-cell epitopes, and most of them lost one or more functional sites, including

the S promoter, T site, NBS, start codon of M, VS site, and pHSA site[6,44]. Most reports have focused on the relationship between pre-S deletion and HCC and have indicated that pre-S2 deletion is associated with HCC development in adults[5,6,11-14,21,26,27,44]. Two reports have revealed a high prevalence of HBV pre-S deletion mutation, with the mutation being recognized in 27 of 30 (90%) and 9 of 19 (47.4%) examined childhood cases of HCC[45,46]. Pre-S2 deletion also occurred frequently (20/27, 74%; 8/9, 88.8%)[45,46]. Other studies have reported a high rate of pre-S1 deletion in HBV/C-infected HCC cases[47,48]. These differences might result from the prevalence of different genotypes (or subgenotypes) in different countries. Biswas *et al*[49] investigated the association of types of pre-S mutations with HBV genotypes from 25 cases and revealed that pre-S1 deletion (5/9, 55.56%) was common in HBV/D, pre-S2 start codon mutation (5/9, 55.56%) was frequent in HBV/A, and pre-S2 deletions (3/7, 42.85%) were frequent in HBV/C. Recently, we enrolled 43 HBV/B and 43 HBV/C-infected carriers with pre-S deletion to examine the prevalence of different pre-S deletions and their associations with HBV genotypes[50]. The results showed the frequencies of some types of pre-S deletion differed between the HBV/B and HBV/C groups, whereas the frequencies of other types of pre-S deletion were similar in both genotypes[50]. Sequence alignment analysis indicated that both genotypes possessed a high frequency of deletion in the C-terminus half of the pre-S1 region and N-terminus of the pre-S2 region (86.0% and 79.1% in the HBV/B group; 69.8% and 72.1% in the HBV/C group, respectively). Epitope mapping revealed that deletion in several epitope sites was frequent in both genotypes, particularly pS1-BT and pS2-B2. Conversely, the frequency of pS2-B1 deletion was significantly higher in the HBV/B group (72.1% *vs* 37.2%, $P = 0.002$), and the frequency of pS2-T deletion was significantly higher in the HBV/C group (48.8% *vs* 25.6%, $P = 0.044$). Functional mapping revealed that the frequency of deletion in three functional sites (NBS, the start codon of M, and VS site) located in the border between the pre-S1 and pre-S2 region (aa 103-127) was significantly higher in the HBV/B group ($P < 0.05$). One variety of N-terminus pre-S1 deletion mutation demonstrating deletion of the start codon of the L protein was frequently observed in the HBV/C group (20.9% *vs* 9.3%, $P = 0.228$). The correlation of different pre-S deletion with the HBV genotype was further examined according to different clinical outcomes. Significant differences were observed between the HBV/B- and HBV/C-infected patients with LC-HCC. Deletion in the N-terminus of the pre-S2 region - including two epitope sites (pS2-B1 and pS2-B2) and three functional sites (the start codon of M, VS, and pHSA) - was significantly more frequent in the HBV/B-infected LC-HCC patients ($P < 0.05$). In Asia, HBV/B and HBV/C commonly coexist. However, their distribution differs by country[3,4]. Pre-S2 deletion has been associated with the development of HCC in Taiwan[27,45,51]. This finding may be due to HBV/B being more prevalent than HBV/C in Taiwan. HBV/C is predominant in Korea, where the N-terminus pre-S1 deletion mutant with deletion of the start codon of the L protein has been correlated with the development of HCC[47,48]. These results indicate that the tendency of different pre-S deletion varies across HBV genotypes. Therefore, the difference in genotype (or subgenotype) prevalence in different countries may influence the pattern of pre-S deletion associated with HCC.

The association of specific types of pre-S deletion with CH and LC development remains unknown. Our studies have revealed that deletion in the C-terminal half of the pre-S1 region is frequent among CH and LC patients[25,50], which is in contrast to HCC patients, who demonstrated a significantly higher frequency of deletion in the pre-S2 region[11-14,26,27,44-46]. Functional mapping showed that deletion in the S promoter was significantly frequent in CH and LC patients compared with that in ASCs[25,50]. The correlation among different pre-S deletion mutants with HBV genotypes in CH and LC patients was investigated, and deletion in the S promoter and the C-terminal half of pre-S1 was frequently observed in both genotypes[50]. In the CH patients, deletion in the pS1-BT and four functional sites (S promoter, Hsc70, CAD, and NBS), which are located in the C-terminal half of the pre-S1 region, was frequent in both genotypes. Conversely, deletion in the pHSA was more frequent in the HBV/B group than in the HBV/C group (88.9% *vs* 36.4%, $P = 0.028$). In the LC patients, no significant differences were observed between the HBV/B and HBV/C groups, except that deletion in the start codon of L was more frequent in the HBV/C group (42.9% *vs* 12.5%, $P = 0.193$)[50].

To understand the characteristics of these pre-S deletion mutants, five naturally occurring pre-S deletion mutants - namely one pre-S1 C-terminus half deletion mutant (dps1), two pre-S1/2 deletion mutants with deletion spanning pre-S1 and pre-S2 (dpS12a and dpS12b), and two pre-S2 deletion mutants (dpS2a and dpS2b) - were analyzed *in vitro*[52]. Functional analyses indicated that they could be divided into two groups: S promoter (dpS1 and dpS12a) and non-S promoter (dpS12b, dpS2a, and dpS2b) deletion mutants. Northern blot analysis revealed that S RNA could be transcribed in non-S-promoter deletion mutants and that the ratio of pre-S1 RNA to S RNA was similar to that in wild-type (WT) HBV transfected cells (Figure 2). Conversely, S promoter deletion mutants could not transcribe S RNA efficiently and had a higher level of pre-S1 RNA, causing an inverse ratio of pre-S1 RNA to S RNA (Figure 2). Western blot and ELISA analyses indicated that intracellular accumulation of envelope proteins was present in all pre-S deletion mutant transfected cells, especially in the S promoter deletion mutant transfected cells. Immunofluorescence analysis revealed that the mutant L proteins, unlike the WT L

proteins, exhibited granular staining in the S promoter deletion variants and a perinuclear staining pattern in the non-S-promoter deletion variants[52]; other studies have reported similar findings[12,26,27,53-56]. Two types of ground glass hepatocytes (GGHs) have been defined and associated with liver diseases in chronic HBV infection[27]. These GGHs contain pre-S deletion mutants that are accumulated in the ER and induce ER stress. Type I GGHs that harbor pre-S1 deletion variants display a globular or inclusion-like immunostaining pattern of HBsAg and are typical of the high viral-replicative phase of chronic HBV infection. Type Ⅱ GGHs that harbor pre-S2 deletion variants with or without point mutations at the start codon of M proteins demonstrate marginal staining patterns of HBsAg, are distributed in large clusters because of their higher proliferative activity, and are characteristic of the advanced stages of chronic liver diseases[27]. Pre-S deletion mutants have been observed to induce the ER stress response, leading to the enhanced expression of vascular endothelial growth factor-A and the activation of Akt/mammalian target of rapamycin signaling in GGHs[57]. In addition, pre-S2 deletion mutants may elicit the aberrant cyclin A expression and centrosome overduplication through ER stress induction and result in cell cycle progression, cell proliferation, and anchorage-independent growth[58-60]. In addition to the induction of ER stress signals, pre-S2 deletion L proteins may directly interact with the Jun activation domain-binding protein 1, thus triggering cyclin-dependent kinase inhibitor p27 degradation, retinoblastoma hyperphosphorylation, and cell cycle progression[61]. These studies all suggest that pre-S deletion mutants may cause the intracellular retention of HBV envelope proteins, resulting in liver diseases.

Pre-S point mutant and genotype

The pathogenic role of pre-S point mutation has been the subject of fewer studies. Chen *et al*[62] reported that, compared with control patients, patients with HCC had higher frequencies of pre-S deletions and amino acid substitutions at codon 4 (W4P/R), 7 (K7T/N), and 81 (A81T) in the pre-S1 regions; and at the start codon (M1V/I/A) in the pre-S2 regions. By contrast, patients had a lower frequency of amino acid substitution at codon2 (Q2K/R) in the pre-S2 regions compared with control patients. The correlation between different pre-S point mutation with HBV genotype was further examined; compared with patients with HBV/B infection, patients with HBV/C infection were found to have higher frequencies of amino acid substitutions at codon 4 (17 of 79 *vs* 0 of 159; $P < 0.001$), codon 7 (14 of 79 *vs* 3 of 159; $P < 0.001$), and codon 81 (16 of 79 *vs* 2 of 159; $P < 0.001$) in pre-S1 genes[62]. Zhang *et al*[44] also reported that compared with the HCC-free group, higher frequencies of pre-S deletions and point mutations at 11 codons - 4, 27, 51, 54, 60, 62, 100, 125, 137, 166, and 167 - were observed in the HCC group ($P < 0.05$) with either HBV/B or HBV/C. Multiple logistic regression analysis revealed that pre-S deletions and point mutations at codon 51 and 167 were independent factors associated with HCC. Longitudinal observation indicated that pre-S deletions and the majority of the 11 HCC-associated pre-S point mutations existed at least 10 years before HCC development, and they were more prevalent preceding HCC development in patients from the HCC groups than the HCC-free group[44]. Five amino acid sites (codon 27, 35, 54, 137, and 167) that were under positive selection pressure were identified in the HBV/C sequences, whereas no positive selection codon was detected for HBV/B[44]. Zhang *et al*[63] later used deep sequencing to examine the dynamics of HBV quasi-species and their relationship to HCC development. In total, 32 chronic hepatitis B (CHB) patients with HCC (HCC group) and 32 matched controls were recruited[63]. HCC patients were found to have a higher intrapatient prevalence of pre-S deletions and point mutations at codons 4, 27, and 167 compared with the control patients (all $P < 0.05$). Longitudinal observation in the sera of 14 HCC patients determined that quasi-species complexity ($P = 0.027$ and 0.024 at the nucleotide level and the amino acid level, respectively) and diversity ($P = 0.035$ and 0.031 at the nucleotide level and the amino acid level, respectively) increased as the disease progressed to HCC[63]. Another study in patients with either HBV/B or HBV/C indicated that point mutation C2964A, A2962G, and C3116T in the pre-S1 region; C7A and T53C in the pre-S2 region; and pre-S2 start codon mutation are associated with an increased risk of HCC, and a novel mutation C105T in the pre-S2 region is inversely associated with the risk of HCC[64]. Functional studies investigating pre-S point mutants have been conducted. Mun *et al*[65] demonstrated that amino acid substitution F141L in the pre-S2 region increases the risk of HCC in HBV/C-infected subjects. An *in vitro* study demonstrated that F141L-LHBs can induce cell cycle progression by down-regulating the p53 and p21 pathways and up-regulating cyclin-dependent kinase 4 and cyclin A. In a colony-forming assay, the colony-forming frequencies in cell lines expressing F141L-LHBs were approximately twice as high as those of the WTs[65]. This suggests that F141L-LHBs may have a vital role in the pathogenesis of HCC by inducing cell proliferation and transformation[65]. Zhang *et al*[63] proposed that these pre-S point mutants may cause imbalanced envelope protein production and intracellular retention of HBsAg, leading to ER stress and tumorigenesis. These studies were conducted in patients infected with HBV/B or HBV/C. Additional studies are required to evaluate whether these mutations exist in other HBV genotypes and whether the conclusions of previous studies are valid.

Pre-S1 splice variant and genotype

RNA splice donor and acceptor sites can be detected throughout the HBV genome. Thus, RNA splicing can occur and involve deletions of nucleotides at specific

Table 1 Putative 5′ splice donor and 3′ splice acceptor sites in hepatitis B virus used to generate the splice variant spPS1

Genotype	Position (nt)	type	Potential splice donor site	Position (nt)	Type	Potential splice acceptor site	Ref.
A	3024/3025	Donor	CAG/gtagga	3207/3208	Acceptor	tcatcctcag/GC	
B	3018/3019	Donor	AAG/gtggga	3201/3202	Acceptor	tcatcctcag/GC	[25,73]
C	3018/3019	Donor	CAG/gtagga	3201/3202	Acceptor	tcatcctcag/GC	[25,72,74]
D	2985/2986	Donor	AAG/gtagga	3168/3169	Acceptor	tcatcctcag/GC	[53,71,76]
E	3015/3016	Donor	AAG/gtagga	3198/3199	Acceptor	tcatcctcag/GC	
F	3018/3019	Donor	AAG/gtagga	3201/3202	Acceptor	acatcctcag/GC	
G	3051/3052	Donor	AAG/gtagga	3234/3235	Acceptor	tcatcctcag/GC	
H	3018/3019	Donor	AAG/gtagga	3201/3202	Acceptor	acatccacag/GC	

sites. To date, 14 types of spliced HBV genomes have been identified and isolated from the sera and liver tissues of HBV-infected patients[23,24,66,67]. Different introns are removed in different splicing variants, and the splicing variants vary by genotype. The splice sites of the HBV genome are not random: the five common splice donor sites are at nucleotide positions 2067, 2447, 2471, 2985, and 2087, and the five common splice acceptor sites are at nucleotide positions 489, 2350, 2236, 2902, and 282 (these nucleotide positions are based on HBV/D). These variants can be reverse transcribed and packaged with the help of WT virus to provide the necessary proteins[68,69]. Several studies have reported that spliced HBV variants enhance WT virus replication in patients with CH; these variants have been associated with advanced liver disease[23,24]. The most frequently detected splice variant, SP1, can encode a novel protein - the hepatitis B spliced protein - which has been associated with viral replication and liver fibrosis[24] and may induce cell apoptosis[70].

To investigate the mechanism of the generation of pre-S deletion-that is, whether these pre-S deletion mutants are generated through RNA splicing or sporadic events-the splice donor and acceptor sites of the pre-S region have been searched, and only one type of pre-S1 deletion mutant was determined to have splice donor (nt 3018) and acceptor (nt 3202)(the nucleotide positions are based on HBV/B and HBV/C) site-specific sequences at the deletion boundaries. This suggests that these pre-S1 deletion mutants (spPS1) were derived from spliced pgRNA (Figure 2B)[25]. The splice donor site was at the existing position 3018 (nucleotide position 2985 based on HBV/D), whereas the splice acceptor site at position 3202 (nucleotide position 3169 based on HBV/D) was new (Table 1). Splice mapping revealed that the splice donor and splice acceptor residues critical for spPS1 were conserved across HBV genotypes A-H (Table 1). This phenomenon explains why this splice variant is frequently found during persistent viral infection[25,53,71-76].

The molecular characteristics of the novel splice variant spPS1 are mostly unknown. The splicing event of spPS1 results in a 183-nucleotide deletion in the C-terminal half of the pre-S1 region, complete deletion of two functional sites (the S promoter and site for dual topology), partial deletion of the NBS, and generation of a spliced L protein (spL, deletion of 61 amino acids, aa 58-118) (Figure 2B). S promoter deletion should lead to a reduction in S RNAs (consequently resulting in a low level or absence of M and S proteins) and an increase in pre-S1 RNAs (consequently resulting in relative overexpression of the spL surface protein). The removal of sites for dual topology and nucleocapsid binding in the spPS1 variant leads to uniform (e-Pre-S) conformation of spL proteins (Figure 4B) and decreased secretion of HBsAg and viral particles. Our *in vitro* study revealed that spPS1 (previously named dpS1) has a defect in S RNA transcription and secretion of envelope proteins[52]. Other studies have also demonstrated that spPS1 possesses a defect in secretion of envelope proteins, viral packaging, and subsequent virion secretion[53,71,72]. Western blot analysis showed that intracellular spL proteins exhibited a heterogeneous pattern, and additional spL proteins with a higher molecular weight were detected[52]. Immunofluorescence staining revealed that spL proteins were accumulated within the ER and displayed a granular staining pattern[52].

The clinical significance of the spPS1variant remains largely unknown. This variant has been found in an occult HBV-infected child[73] and numerous chronically HBV-infected patients worldwide, and it has frequently been found in the sera of individuals with CH and cirrhosis[53,71,72,74-76]. Clinical follow-up studies conducted over a period of 10-14 years indicate that after this variant occurs, acute exacerbation of CHB occurs, which is followed by the development of liver fibrosis[71,72]. A study demonstrated that the prevalence of spPS1 was higher in CH patients (7 of 55, 12.7% *vs* 1 of 55, 1.8%; $P = 0.06$) and LC patients (8 of 55, 14.5% *vs* 1 of 55, 1.8%; $P = 0.032$) than in ASCs[25]. Logistic regression analysis revealed that spPS1 variants were highly related to CH ($P = 0.058$) and significantly related to LC ($P = 0.040$). Thus, these clinical studies strongly suggest that the spPS1 variant could cause acute exacerbation of CHB, liver inflammation, and fibrosis.

C-terminus S mutant and genotype

The C-terminus S domain (aa 179-226) is hydrophobic and assumed to be inserted in the ER membrane (Figure 4A). This domain is involved in mediating

the transit of envelope glycoproteins across the endoplasmic reticulum[77]. Mutations in this domain can result in a stable, glycosylated, but nonsecreted chain, thus affecting the biogenesis and secretion of subviral particles[77]. Two C-terminus S mutations were found and significantly correlated with HCC: P203Q (4/23, 17.4% in HCC *vs* 1/105, 1.0 in non-HCC, P = 0.004); S210R (8/23, 34.8% in HCC *vs* 4/105, 3.8% in non-HCC, $P < 0.001$); P203Q + S210R (4/23, 17.4% in HCC *vs* 0/110, 0 in non-HCC, $P = 0.001$)[78]. *In vitro* experiments revealed that P203Q, S210R, and P203Q+S210R significantly reduced the ratio of secreted and intracellular HBsAg compared with WT at each time point analyzed ($P < 0.05$); P203Q and P203Q+S210R increased the percentage of cells in S-phase compared with WT (P203Q: 26% ± 13%; P203Q+S210R: 29% ± 14%; WT: 18% ± 9%, $P < 0.01$); S210R increased the percentage of cells in the G2/M-phase (33% ± 6% for S210R *vs* 26% ± 8% for WT, $P < 0.001$)[78]. These results show that these two C-terminus S mutations, P203Q and S210R, hamper HBsAg secretion and are associated with increased cellular proliferation, supporting their involvement in HCC development. This study was conducted in patients infected with HBV/D or HBV/A. Additional studies are required to evaluate whether these mutations exist in other HBV genotypes and whether the conclusions of previous studies are valid.

Pre-S/S nonsense mutation

Pre-S nonsense mutations were also found in patients with progressive liver diseases[6]; the pathogenic impacts of these naturally occurring mutants remain unknown. Such pre-S nonsense mutations result in the occurrence of pre-S stop codon mutants and the synthesis of C terminally truncated M (MHBs^t) and L (LHBs^t) proteins. Studies have reported that MHBs^t and LHBs^t function as a transcriptional activator and result in an increased hepatocyte proliferation rate[79-81]. Results from experiments conducted on transgenic mice and hepatoma cell cultures have revealed that MHBs^t proteins retained in the ER can trigger a PKC dependent activation of the c-Raf-1/Erk2 signaling cascade, which leads to the induction of AP-1 and nuclear factor-kappa B (NF-κB) transcription factors as well as to enhanced proliferative activity of hepatocytes[82,83]. By contrast, Yeh *et al*[18] demonstrated that five patients who carried stop codons (nonsense mutation) in the pre-S region had a more favorable disease-free prognosis following multivariate analysis.

S nonsense mutations can arise as result of mutations in the P ORF that are generally caused by exposure to antivirals, a phenomenon commonly called antiviral drug-associated S gene mutations[84-87]. These mutations can cause the occurrence of S stop codon mutants and the synthesis of C terminally truncated L, M, and S proteins. For example, the HBV mutation that encodes rtA181T is selected in the viral polymerase during antiviral drug therapy and can also encode a stop codon in the overlapping S gene at amino acid 172 (sW172*), resulting in truncation of the last 55 amino acids of the C-terminal hydrophobic region of the S domain. *In vitro* study revealed that the sW172* variant had a secretory defect and exerted a dominant negative effect on WT HBV virion secretion[85]. In addition, sW172* transgenic mice developed HCC in an *in vivo* study[86]. Other S nonsense mutants such as sC69*, sL95*, sW182*, and sL216* were identified in HCC tumors[87,88]. Functional studies of sL95*, sW182*, and sL216* demonstrated that they had higher cell proliferation activities and transformation abilities than WT S, especially sW182*[87]. The sW182* mutant in HBV/C was also shown to be associated with liver cirrhosis[89].

Possible pathogenesis of pre-S/S variants

On the basis of the previous studies investigating pre-S/S variants, a model to explain the occurrences of the pre-S/S variants and the possible role of these mutants in progressive liver diseases is proposed (Figure 5). After persistent HBV infection, under the pressure of immune responses and antiviral drugs, immune epitope deletion and mutation occur along with drug-resistant mutants. Different pre-S deletion and pre-S/S mutants use different routes to cause liver diseases. Most cause the intracellular retention of HBV envelope proteins and induction of ER stress, resulting in liver diseases. Based on the region mutated, at least six pre-S/S variants occurred.

Type Ⅰ - pre-S deletion in the N-terminal pre-S1 region causes deletion of the start codon of L proteins. *In vitro* study demonstrated that the L-start codon deletion mutant resulted in the absence of L proteins and increased levels of intracellular viral mRNA and extracellular HBsAg[56]. The accumulated intracellular viral mRNA might activate the intracellular toll-like receptors, leading to the subsequent activation of NF-κB pathways, chronic inflammation, and carcinogenesis[56].

Type Ⅱ - pre-S deletion in the C-terminal half of the pre-S1 region can be separated into two groups characterized by S promoter: (Ⅱ-a) S promoter deletion variants and (Ⅱ-b) non-S promoter deletion variants (Figure 2B). (Ⅱ-a) The S promoter deletion variants that cannot transcribe S RNA efficiently result in no synthesis or reduction of the M and S proteins. Because the L protein cannot be secreted from cells efficiently when expressed by itself, it must complex with the S and M proteins to form subviral particles or mature virions, bud from intracellular post-ER pre-Golgi membranes, and be released from the cell through secretion[41]. A low level or absence of M and S proteins results in the accumulation of mutant L proteins in the ER. *In vitro* studies have revealed severe intracellular retention of mutant L proteins in S promoter deletion

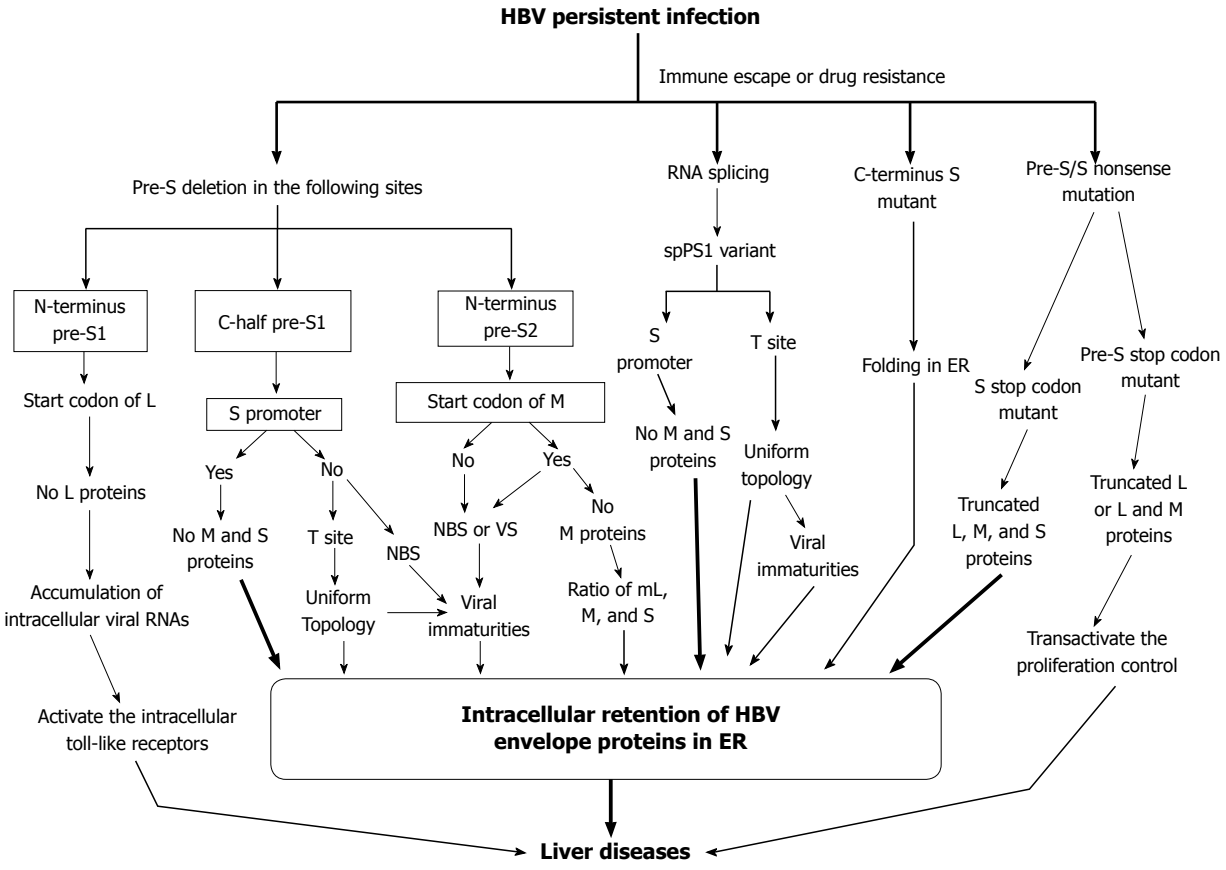

Figure 5 Proposed model for the generation of pre-S/S variants and their possible roles in liver damage and carcinogenesis. HBV: Hepatitis B virus; ER: Endoplasmic reticulum; NBS: Nucleocapsid binding site; VS: Viral secretion.

variant transfected cells[52-55]. (Ⅱ-b) The non-S promoter deletion variants can transcribe S RNA efficiently and synthesize the M and S proteins (Figure 2B), but the mutant L proteins may delete the T site to maintain a e-Pre-S form (Figure 4B, Topology variant) or delete the NBS site (Figure 4B, Nucleocapsid variant), leading to inefficient assembly of the nucleocapsid, viral immaturities, and mild intracellular retention[52].

Type Ⅲ - N-terminus pre-S2 deletion mutants can also be separated into two groups by the start codon of the M protein: (Ⅲ-a) non-M start codon deletion variants; (Ⅲ-b) M start codon deletion variants. (Ⅲ-a) non-M start codon variants with internal deletion of M proteins but the mutant L proteins may lose the NBS or VS site, resulting in viral immaturities, and slight intracellular retention[52,90]. (Ⅲ-b) The M start codon deletion variants with no M proteins change the ratio of mutant L, M, and S proteins, and lead to intracellular retention of mutant L proteins[52]. Because the M start codon is located in the NBS and VS sites, these variants may produce mutant L proteins such as the nucleocapsid variant that cannot assemble the nucleocapsid efficiently, leading to viral immaturities and slight intracellular retention of HBsAg[52].

Type Ⅳ - spPS1 variants are generated through RNA splicing of HBV pregenomic RNA. The splicing event results in a 183-nucleotide deletion in the C-terminal half of the pre-S1 region, complete deletion of two functional sites (the S promoter and T sites), partial deletion of the NBS site, and generation of spL (Figures 2B and 4B). S promoter deletion leads to absence of M and S proteins and severe intracellular retention of spL proteins[52]. T-site deletion results in uniform conformation of spL proteins (Figure 4B) and loss of i-Pre-S form for capsid envelopment, which causes viral immaturities and intracellular retention of spL proteins.

Type Ⅴ-C-terminus S mutants influence protein folding in the ER membrane, thus impairing HBsAg release, resulting in its accumulation in specific intracellular compartments (presumably represented by the ER and Golgi apparatus) and in turn contributing to cell proliferation[78]. An *in vitro* study revealed that C-terminus S mutants can also activate the proliferation control[78].

Type Ⅵ - pre-S/S nonsense mutations can be separated into two groups: (Ⅵ-a) Pre-S nonsense mutation and (Ⅵ-b) S nonsense mutation. (Ⅵ-a) Pre-S nonsense mutations can create C' truncated L and M proteins, leading to transactivation of proliferation control and causing liver diseases[79-81]. (Ⅵ-b) S nonsense mutation can create C' truncated L, M, and S proteins. *In vitro* study revealed that a stop codon in the C-terminal hydrophobic region of the S region results in truncated envelope proteins that are less glycosylated

and are defective in secretion of viral particles, causing intracellular retention of envelope proteins and liver diseases[85]. *In vitro* and *in vivo* studies have also demonstrated that these S stop codon mutants have higher cell proliferation activity[86,87].

CONCLUSION

Naturally occurring pre-S/S variants are frequently found in chronically HBV-infected patients and have been identified as influencing liver disease progression. From a review of relevant studies, pre-S/S variants should be routinely determined in HBV carriers to help identify those who may be at a higher risk of a less favorable liver disease progression. In the future, further studies are required exploring the molecular mechanisms of the pre-S/S variants involved in the pathogenesis of each stage of liver disease.

REFERENCES

1 **Seeger C**, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64**: 51-68 [PMID: 10704474]
2 **Kao JH**, Chen DS. Global control of hepatitis B virus infection. *Lancet Infect Dis* 2002; **2**: 395-403 [PMID: 12127351]
3 **Liu CJ**, Kao JH. Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J. *Semin Liver Dis* 2013; **33**: 97-102 [PMID: 23749665 DOI: 10.1055/s-0033-1345716]
4 **Lin CL**, Kao JH. The clinical implications of hepatitis B virus genotype: Recent advances. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 123-130 [PMID: 21199523 DOI: 10.1111/j.1440-1746.2010.06541]
5 **Sugauchi F**, Ohno T, Orito E, Sakugawa H, Ichida T, Komatsu M, Kuramitsu T, Ueda R, Miyakawa Y, Mizokami M. Influence of hepatitis B virus genotypes on the development of preS deletions and advanced liver disease. *J Med Virol* 2003; **70**: 537-544 [PMID: 12794715]
6 **Chen BF**, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006; **130**: 1153-1168 [PMID: 16618410]
7 **Okamoto H**, Imai M, Kametani M, Nakamura T, Mayumi M. Genomic heterogeneity of hepatitis B virus in a 54-year-old woman who contracted the infection through materno-fetal transmission. *Jpn J Exp Med* 1987; **57**: 231-236 [PMID: 3430800]
8 **Günther S**, Fischer L, Pult I, Sterneck M, Will H. Naturally occurring variants of hepatitis B virus. *Adv Virus Res* 1999; **52**: 25-137 [PMID: 10384235]
9 **Locarnini S**, McMillan J, Bartholomeusz A. The hepatitis B virus and common mutants. *Semin Liver Dis* 2003; **23**: 5-20 [PMID: 12616447 DOI: 10.1055/s-2003-37587]
10 **Hunt CM**, McGill JM, Allen MI, Condreay LD. Clinical relevance of hepatitis B viral mutations. *Hepatology* 2000; **31**: 1037-1044 [PMID: 10796877 DOI: 10.1053/he.2000.6709]
11 **Pollicino T**, Zanetti AR, Cacciola I, Petit MA, Smedile A, Campo S, Sagliocca L, Pasquali M, Tanzi E, Longo G, Raimondo G. Pre-S2 defective hepatitis B virus infection in patients with fulminant hepatitis. *Hepatology* 1997; **26**: 495-499 [PMID: 9252165 DOI: 10.1002/hep.510260235]
12 **Fan YF**, Lu CC, Chen WC, Yao WJ, Wang HC, Chang TT, Lei HY, Shiau AL, Su IJ. Prevalence and significance of hepatitis B virus (HBV) pre-S mutants in serum and liver at different replicative stages of chronic HBV infection. *Hepatology* 2001; **33**: 277-286 [PMID: 11124846 DOI: 10.1053/jhep.2001.21163]
13 **Chen CH**, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, Lu SN, Changchien CS. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. *Gastroenterology* 2007; **133**: 1466-1474 [PMID: 17915220 DOI: 10.1053/j.gastro.2007.09.002]
14 **Yeung P**, Wong DK, Lai CL, Fung J, Seto WK, Yuen MF. Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in chronic hepatitis B. *J Infect Dis* 2011; **203**: 646-654 [PMID: 21227916 DOI: 10.1093/infdis/jiq096]
15 **Kao JH**, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003; **124**: 327-334 [PMID: 12557138 DOI: 10.1053/gast.2003.50053]
16 **Liu CJ**, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, Chen DS. Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. *J Infect Dis* 2006; **193**: 1258-1265 [PMID: 16586363 DOI: 10.1086/502978]
17 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH; REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73 [PMID: 16391218 DOI: 10.1001/jama.295.1.65]
18 **Yeh CT**, So M, Ng J, Yang HW, Chang ML, Lai MW, Chen TC, Lin CY, Yeh TS, Lee WC. Hepatitis B virus-DNA level and basal core promoter A1762T/G1764A mutation in liver tissue independently predict postoperative survival in hepatocellular carcinoma. *Hepatology* 2010; **52**: 1922-1933 [PMID: 20814897 DOI: 10.1002/hep.23898]
19 **Lin CL**, Kao JH. Risk stratification for hepatitis B virus related hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013; **28**: 10-17 [PMID: 23094699 DOI: 10.1111/jgh.12010]
20 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]
21 **Zhang ZH**, Wu CC, Chen XW, Li X, Li J, Lu MJ. Genetic variation of hepatitis B virus and its significance for pathogenesis. *World J Gastroenterol* 2016; **22**: 126-144 [PMID: 26755865 DOI: 10.3748/wjg.v22.i1.126]
22 **Lee D**, Lyu H, Chung YH, Kim JA, Mathews P, Jaffee E, Zheng L, Yu E, Lee YJ, Ryu SH. Genomic change in hepatitis B virus associated with development of hepatocellular carcinoma. *World J Gastroenterol* 2016; **22**: 5393-5399 [PMID: 27340355 DOI: 10.3748/wjg.v22.i23.5393]
23 **Soussan P**, Pol J, Garreau F, Schneider V, Le Pendeven C, Nalpas B, Lacombe K, Bonnard P, Pol S, Kremsdorf D. Expression of defective hepatitis B virus particles derived from singly spliced RNA is related to liver disease. *J Infect Dis* 2008; **198**: 218-225 [PMID: 18532883 DOI: 10.1086/589623]
24 **Soussan P**, Tuveri R, Nalpas B, Garreau F, Zavala F, Masson A, Pol S, Brechot C, Kremsdorf D. The expression of hepatitis B spliced protein (HBSP) encoded by a spliced hepatitis B virus RNA is associated with viral replication and liver fibrosis. *J Hepatol* 2003; **38**: 343-348 [PMID: 12586301]
25 **Lin YM**, Chen BF. A putative hepatitis B virus splice variant associated with chronic hepatitis and liver cirrhosis. *Virology* 2017; **510**: 224-233 [PMID: 28750326 DOI: 10.1016/j.virol.2017.07.025]
26 **Pollicino T**, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. *J Hepatol* 2014; **61**: 408-417 [PMID: 24801416 DOI: 10.1016/j.jhep.2014.04.041]
27 **Su IJ**, Wang HC, Wu HC, Huang WY. Ground glass hepatocytes contain pre-S mutants and represent preneoplastic lesions in chronic hepatitis B virus infection. *J Gastroenterol Hepatol* 2008; **23**: 1169-1174 [PMID: 18505413 DOI: 10.1111/j.1440-1746.2008.05348.x]
28 **Neurath AR**, Kent SB, Strick N, Parker K. Identification and

chemical synthesis of a host cell receptor binding site on hepatitis B virus. *Cell* 1986; **46**: 429-436 [PMID: 3015414]
29 **Pontisso P**, Ruvoletto MG, Gerlich WH, Heermann KH, Bardini R, Alberti A. Identification of an attachment site for human liver plasma membranes on hepatitis B virus particles. *Virology* 1989; **173**: 522-530 [PMID: 2480688]
30 **Le Seyec J**, Chouteau P, Cannie I, Guguen-Guillouzo C, Gripon P. Role of the pre-S2 domain of the large envelope protein in hepatitis B virus assembly and infectivity. *J Virol* 1998; **72**: 5573-5578 [PMID: 9621015]
31 **Poisson F**, Severac A, Hourioux C, Goudeau A, Roingeard P. Both pre-S1 and S domains of hepatitis B virus envelope proteins interact with the core particle. *Virology* 1997; **228**: 115-120 [PMID: 9024817]
32 **Bruss V**. A short linear sequence in the pre-S domain of the large hepatitis B virus envelope protein required for virion formation. *J Virol* 1997; **71**: 9350-9357 [PMID: 9371594]
33 **Bock CT**, Kubicka S, Manns MP, Trautwein C. Two control elements in the hepatitis B virus S-promoter are important for full promoter activity mediated by CCAAT-binding factor. *Hepatology* 1999; **29**: 1236-1247 [PMID: 10094970]
34 **Sobotta D**, Sominskaya I, Jansons J, Meisel H, Schmitt S, Heermann KH, Kaluza G, Pumpens P, Gerlich WH. Mapping of immunodominant B-cell epitopes and the human serum albumin-binding site in natural hepatitis B virus surface antigen of defined genosubtype. *J Gen Virol* 2000; **81**: 369-378 [PMID: 10644835]
35 **Milich DR**, Jones JE, McLachlan A, Bitter G, Moriarty A, Hughes JL. Importance of subtype in the immune response to the pre-S(2) region of the hepatitis B surface antigen. II. Synthetic Pre-S(2) immunogen. *J Immunol* 1990; **144**: 3544-3551 [PMID: 1691763]
36 **Park JH**, Cho EW, Lee YJ, Shin SY, Kim KL. Determination of the protective effects of neutralizing anti-hepatitis B virus (HBV) immunoglobulins by epitope mapping with recombinant HBV surface-antigen proteins. *Microbiol Immunol* 2000; **44**: 703-710 [PMID: 11021401]
37 **Kuroki K**, Floreani M, Mimms LT, Ganem D. Epitope mapping of the PreS1 domain of the hepatitis B virus large surface protein. *Virology* 1990; **176**: 620-624 [PMID: 1693249]
38 **Maeng CY**, Ryu CJ, Gripon P, Guguen-Guillouzo C, Hong HJ. Fine mapping of virus-neutralizing epitopes on hepatitis B virus PreS1. *Virology* 2000; **270**: 9-16 [PMID: 10772975]
39 **Ferrari C**, Cavalli A, Penna A, Valli A, Bertoletti A, Pedretti G, Pilli M, Vitali P, Neri TM, Giuberti T. Fine specificity of the human T-cell response to the hepatitis B virus preS1 antigen. *Gastroenterology* 1992; **103**: 255-263 [PMID: 1377142]
40 **Huang TJ**, Lu CC, Tsai JC, Yao WJ, Lu X, Lai MD, Liu HS, Shiau AL. Novel autoregulatory function of hepatitis B virus M protein on surface gene expression. *J Biol Chem* 2005; **280**: 27742-27754 [PMID: 15899887 DOI: 10.1074/jbc.M502209200.]
41 **Bruss V**. Hepatitis B virus morphogenesis. *World J Gastroenterol* 2007; **13**: 65-73 [PMID: 17206755 DOI: 10.3748/wjg.v13.i1.65]
42 **Lambert C**, Prange R. Chaperone action in the posttranslational topological reorientation of the hepatitis B virus large envelope protein: Implications for translocational regulation. *Proc Natl Acad Sci U S A* 2003; **100**: 5199-5204 [PMID: 12697898 DOI: 10.1073/pnas.0930813100]
43 **Löffler-Mary H**, Werr M, Prange R. Sequence-specific repression of cotranslational translocation of the hepatitis B virus envelope proteins coincides with binding of heat shock protein Hsc70. *Virology* 1997; **235**: 144-152 [PMID: 9300046 DOI: 10.1006/viro.1997.8689]
44 **Zhang AY**, Lai CL, Huang FY, Seto WK, Fung J, Wong DK, Yuen MF. Evolutionary changes of hepatitis B virus pre-S mutations prior to development of hepatocellular carcinoma. *PLoS One* 2015; **10**: e0139478 [PMID: 26421619 DOI: 10.1371/journal.pone.0139478.]
45 **Huang HP**, Hsu HY, Chen CL, Ni YH, Wang HY, Tsuei DJ, Chiang CL, Tsai YC, Chen HL, Chang MH. Pre-S2 deletions of hepatitis B virus and hepatocellular carcinoma in children. *Pediatr Res* 2010; **67**: 90-94 [PMID: 19816238 DOI: 10.1203/PDR.0b013e3181c1b0b7]
46 **Abe K**, Thung SN, Wu HC, Tran TT, Le Hoang P, Truong KD, Inui A, Jang JJ, Su IJ. Pre-S2 deletion mutants of hepatitis B virus could have an important role in hepatocarcinogenesis in Asian children. *Cancer Sci* 2009; **100**: 2249-2254 [PMID: 19719772 DOI: 10.1111/j.1349-7006.2009.01309.x]Plos]
47 **Mun HS**, Lee SA, Jee Y, Kim H, Park JH, Song BC, Yoon JH, Kim YJ, Lee HS, Hyun JW, Hwang ES, Kook YH, Kim BJ. The prevalence of hepatitis B virus preS deletions occurring naturally in Korean patients infected chronically with genotype C. *J Med Virol* 2008; **80**: 1189-1194 [PMID: 18461612 DOI: 10.1002/jmv.21208]
48 **Ahn SH**, Yuen L, Han KH, Littlejohn M, Chang HY, Damerow H, Ayres A, Heo J, Locarnini S, Revill PA. Molecular and clinical characteristics of hepatitis B virus in Korea. *J Med Virol* 2010; **82**: 1126-1134 [PMID: 20513074 DOI: 10.1002/jmv.21844]
49 **Biswas A**, Panigrahi R, Banerjee A, Pal M, De BK, Chakrabarti S, Chakravarty R. Differential pattern of pre-S mutations/deletions and its association with hepatitis B virus genotypes in Eastern India. *Infect Genet Evol* 2012; **12**: 384-391 [PMID: 22266243 DOI: 10.1016/j.meegid.2012.01.007]
50 **Chen BF**. Different pre-S deletion patterns and their association with hepatitis B virus genotypes. *World J Gastroenterol* 2016; **22**: 8041-8049 [PMID: 27672298 DOI: 10.3748/wjg.v22.i35.8041.]
51 **Kao JH**, Liu CJ, Jow GM, Chen PJ, Chen DS, Chen BF. Fine mapping of hepatitis B virus pre-S deletion and its association with hepatocellular carcinoma. *Liver Int* 2012; **32**: 1373-1381 [PMID: 22676233]
52 **Lin CM**, Wang GM, Jow GM, Chen BF. Functional analysis of hepatitis B virus pre-s deletion variants associated with hepatocellular carcinoma. *J Biomed Sci* 2012; **19**: 17 [PMID: 22313590 DOI: 10.1186/1423-0127-19-17]
53 **Melegari M**, Scaglioni PP, Wands JR. The small envelope protein is required for secretion of a naturally occurring hepatitis B virus mutant with pre-S1 deleted. *J Virol* 1997; **71**: 5449-5454 [PMID: 9188617]
54 **Bock CT**, Tillmann HL, Maschek HJ, Manns MP, Trautwein C. A preS mutation isolated from a patient with chronic hepatitis B infection leads to virus retention and misassembly. *Gastroenterology* 1997; **113**: 1976-1982 [PMID: 9394738]
55 **Xu Z**, Yen TS. Intracellular retention of surface protein by a hepatitis B virus mutant that releases virion particles. *J Virol* 1996; **70**: 133-140 [PMID: 8523517]
56 **Kim BK**, Choi SH, Ahn SH, Chung AR, Park YK, Han KH, Kim S, Kim HS, Park JH, Kim KS, Lee HS, Cho YS, Kim KH, Ahn SH. Pre-S mutations of hepatitis B virus affect genome replication and expression of surface antigens. *J Gastroenterol Hepatol* 2014; **29**: 843-850 [PMID: 24783251]
57 **Yang JC**, Teng CF, Wu HC, Tsai HW, Chuang HC, Tsai TF, Hsu YH, Huang W, Wu LW, Su IJ. Enhanced expression of vascular endothelial growth factor-A in ground glass hepatocytes and its implication in hepatitis B virus hepatocarcinogenesis. *Hepatology* 2009; **49**: 1962-1971 [PMID: 19475690 DOI: 10.1002/hep.22889]
58 **Wang HC**, Chang WT, Chang WW, Wu HC, Huang W, Lei HY, Lai MD, Fausto N, Su IJ. Hepatitis B virus pre-S2 mutant upregulates cyclin A expression and induces nodular proliferation of hepatocytes. *Hepatology* 2005; **41**: 761-770 [PMID: 15726643 DOI: 10.1002/hep.20615]
59 **Wang LH**, Huang W, Lai MD, Su IJ. Aberrant cyclin A expression and centrosome overduplication induced by hepatitis B virus pre-S2 mutants and its implication in hepatocarcinogenesis. *Carcinogenesis* 2012; **33**: 466-472 [PMID: 22159224 DOI: 10.1093/carcin/bgr296]
60 **Hung JH**, Su IJ, Lei HY, Wang HC, Lin WC, Chang WT, Huang W, Chang WC, Chang YS, Chen CC, Lai MD. Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF-kappaB and pp38 mitogen-activated protein kinase. *J Biol Chem* 2004; **279**: 46384-46392 [PMID: 15319438 DOI: 10.1074/jbc.M403568200]

61 **Hsieh YH**, Su IJ, Wang HC, Tsai JH, Huang YJ, Chang WW, Lai MD, Lei HY, Huang W. Hepatitis B virus pre-S2 mutant surface antigen induces degradation of cyclin-dependent kinase inhibitor p27Kip1 through c-Jun activation domain-binding protein 1. *Mol Cancer Res* 2007; **5**: 1063-1072 [PMID: 17951406 DOI: 10.1158/1541-7786.MCR-07-0098]

62 **Chen CH**, Changchien CS, Lee CM, Hung CH, Hu TH, Wang JH, Wang JC, Lu SN. Combined mutations in pre-s/surface and core promoter/precore regions of hepatitis B virus increase the risk of hepatocellular carcinoma: a case-control study. *J Infect Dis* 2008; **198**: 1634-1642 [PMID: 18939932 DOI: 10.1086/592990]

63 **Zhang AY**, Lai CL, Huang FY, Seto WK, Fung J, Wong DK, Yuen MF. Deep sequencing analysis of quasispecies in the HBV pre-S region and its association with hepatocellular carcinoma. *J Gastroenterol* 2017; **52**: 1064-1074 [PMID: 28353014 DOI: 10.1007/s00535-017-1334-1.]

64 **Zhang Q**, Cao G. Genotypes, mutations, and viral load of hepatitis B virus and the risk of hepatocellular carcinoma: HBV properties and hepatocarcinogenesis. *Hepat Mon* 2011; **11**: 86-91 [PMID: 22087123]

65 **Mun HS**, Lee SA, Kim H, Hwang ES, Kook YH, Kim BJ. Novel F141L pre-S2 mutation in hepatitis B virus increases the risk of hepatocellular carcinoma in patients with chronic genotype C infections. *J Virol* 2011; **85**: 123-132 [PMID: 20962085 DOI: 10.1128/JVI.01524-10.]

66 **Bayliss J**, Lim L, Thompson AJ, Desmond P, Angus P, Locarnini S, Revill PA. Hepatitis B virus splicing is enhanced prior to development of hepatocellular carcinoma. *J Hepatol* 2013; **59**: 1022-1028 [PMID: 23811301 DOI: 10.1016/j.jhep.2013.06.018]

67 **Günther S**, Sommer G, Iwanska A, Will H. Heterogeneity and common features of defective hepatitis B virus genomes derived from spliced pregenomic RNA. *Virology* 1997; **238**: 363-371 [PMID: 9400609 DOI: 10.1006/viro.1997.8863]

68 **Ma ZM**, Lin X, Wang YX, Tian XC, Xie YH, Wen YM. A double-spliced defective hepatitis B virus genome derived from hepatocellular carcinoma tissue enhanced replication of full-length virus. *J Med Virol* 2009; **81**: 230-237 [PMID: 19107969 DOI: 10.1002/jmv.21393]

69 **Abraham TM**, Lewellyn EB, Haines KM, Loeb DD. Characterization of the contribution of spliced RNAs of hepatitis B virus to DNA synthesis in transfected cultures of Huh7 and HepG2 cells. *Virology* 2008; **379**: 30-37 [PMID: 18657840 DOI: 10.1016/j.virol.2008.06.021]

70 **Soussan P**, Garreau F, Zylberberg H, Ferray C, Brechot C, Kremsdorf D. In vivo expression of a new hepatitis B virus protein encoded by a spliced RNA. *J Clin Invest* 2000; **105**: 55-60 [PMID: 10619861 DOI: 10.1172/JCI8098]

71 **Gerner P**, Schäfer HM, Prange R, Pravitt D, Wirth S. Functional analysis of a rare HBV deletion mutant in chronically infected children. *Pediatr Res* 2003; **53**: 891-897 [PMID: 12646724 DOI: 10.1203/01.PDR.0000064906.63939.72]

72 **Kajiya Y**, Hamasaki K, Nakata K, Nakagawa Y, Miyazoe S, Takeda Y, Ohkubo K, Ichikawa T, Nakao K, Kato Y, Eguchi K. Full-length sequence and functional analysis of hepatitis B virus genome in a virus carrier: a case report suggesting the impact of pre-S and core promoter mutations on the progression of the disease. *J Viral Hepat* 2002; **9**: 149-156 [PMID: 11876799 DOI: 10.1046/j.1365-2893.2002.00335.x]

73 **Mu SC**, Lin YM, Jow GM, Chen BF. Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. *J Hepatol* 2009; **50**: 264-272 [PMID: 19070923 DOI: 10.1016/j.jhep.2008.09.017]

74 **Nakajima E**, Minami M, Ochiya T, Kagawa K, Okanoue T. PreS1 deleted variants of hepatitis B virus in patients with chronic hepatitis. *J Hepatol* 1994; **20**: 329-335 [PMID: 7516949]

75 **Takayanagi M**, Kakumu S, Ishikawa T, Higashi Y, Yoshioka K, Wakita T. Comparison of envelope and precore/core variants of hepatitis B virus (HBV) during chronic HBV infection. *Virology* 1993; **196**: 138-145 [PMID: 8356791 DOI: 10.1006/viro.1993.1462]

76 **Gerner PR**, Friedt M, Oettinger R, Lausch E, Wirth S. The hepatitis B virus seroconversion to anti-HBe is frequently associated with HBV genotype changes and selection of preS2-defective particles in chronically infected children. *Virology* 1998; **245**: 163-172 [PMID: 9614877]

77 **Bruss V**, Ganem D. Mutational analysis of hepatitis B surface antigen particle assembly and secretion. *J Virol* 1991; **65**: 3813-3820 [PMID: 2041095]

78 **Salpini R**, Surdo M, Warner N, Cortese MF, Colledge D, Soppe S, Bellocchi MC, Armenia D, Carioti L, Continenza F, Di Carlo D, Saccomandi P, Mirabelli C, Pollicita M, Longo R, Romano S, Cappiello G, Spanò A, Trimoulet P, Fleury H, Vecchiet J, Iapadre N, Barlattani A, Bertoli A, Mari T, Pasquazzi C, Missale G, Sarrecchia C, Orecchini E, Michienzi A, Andreoni M, Francioso S, Angelico M, Verheyen J, Ceccherini-Silberstein F, Locarnini S, Perno CF, Svicher V. Novel HBsAg mutations correlate with hepatocellular carcinoma, hamper HBsAg secretion and promote cell proliferation in vitro. *Oncotarget* 2017; **8**: 15704-15715 [PMID: 28152517 DOI: 10.18632/oncotarget.14944]

79 **Kim HS**, Ryu CJ, Hong HJ. Hepatitis B virus preS1 functions as a transcriptional activation domain. *J Gen Virol* 1997; **78**: 1083-1086 [PMID: 9152426 DOI: 10.1099/0022-1317-78-5-1083]

80 **Hildt E**, Urban S, Hofschneider PH. Characterization of essential domains for the functionality of the MHBst transcriptional activator and identification of a minimal MHBst activator. *Oncogene* 1995; **11**: 2055-2066 [PMID: 7478525]

81 **Hildt E**, Saher G, Bruss V, Hofschneider PH. The hepatitis B virus large surface protein (LHBs) is a transcriptional activator. *Virology* 1996; **225**: 235-239 [PMID: 8918553 DOI: 10.1006/viro.1996.0594]

82 **Hildt E**, Munz B, Saher G, Reifenberg K, Hofschneider PH. The PreS2 activator MHBs(t) of hepatitis B virus activates c-raf-1/Erk2 signaling in transgenic mice. *EMBO J* 2002; **21**: 525-535 [PMID: 11847101 DOI: 10.1093/emboj/21.4.525]

83 **Lupberger J**, Hildt E. Hepatitis B virus-induced oncogenesis. *World J Gastroenterol* 2007; **13**: 74-81 [PMID: 17206756 DOI: 10.3748/wjg.v13.i1.74]

84 **Kamili S**, Sozzi V, Thompson G, Campbell K, Walker CM, Locarnini S, Krawczynski K. Efficacy of hepatitis B vaccine against antiviral drug-resistant hepatitis B virus mutants in the chimpanzee model. *Hepatology* 2009; **49**: 1483-1491 [PMID: 19274751 DOI: 10.1002/hep.22796]

85 **Warner N**, Locarnini S. The antiviral drug selected hepatitis B virus rtA181T/sW172* mutant has a dominant negative secretion defect and alters the typical profile of viral rebound. *Hepatology* 2008; **48**: 88-98 [PMID: 18537180 DOI: 10.1002/hep.22295]

86 **Lai MW**, Liang KH, Lin WR, Huang YH, Huang SF, Chen TC, Yeh CT. Hepatocarcinogenesis in transgenic mice carrying hepatitis B virus pre-S/S gene with the sW172* mutation. *Oncogenesis* 2016; **5**: e273 [PMID: 27918551 DOI: 10.1038/oncsis.2016.77.]

87 **Huang SF**, Chen YT, Lee WC, Chang IC, Chiu YT, Chang Y, Tu HC, Yuh CH, Matsuura I, Shih LY, Lai MW, Wu HD, Chen MF, Yeh CT. Identification of transforming hepatitis B virus S gene nonsense mutations derived from freely replicative viruses in hepatocellular carcinoma. *PLoS One* 2014; **9**: e89753 [PMID: 24587012 DOI: 10.1371/journal.pone.0089753]

88 **Shirvani-Dastgerdi E**, Winer BY, Celià-Terrassa T, Kang Y, Tabernero D, Yagmur E, Rodríguez-Frías F, Gregori J, Luedde T, Trautwein C, Ploss A, Tacke F. Selection of the highly replicative and partially multidrug resistant rtS78T HBV polymerase mutation during TDF-ETV combination therapy. *J Hepatol* 2017; **67**: 246-254 [PMID: 28392234 DOI: 10.1016/j.jhep.2017.03.027]

89 **Lee SA**, Kim K, Kim H, Kim BJ. Nucleotide change of codon 182 in the surface gene of hepatitis B virus genotype C leading to truncated surface protein is associated with progression of liver

diseases. *J Hepatol* 2012; **56**: 63-69 [PMID: 21827734 DOI: 10.1016/j.jhep.2011.06.028]
90 **Tai PC**, Suk FM, Gerlich WH, Neurath AR, Shih C. Hypermodification and immune escape of an internally deleted middle-envelope (M) protein of frequent and predominant hepatitis B virus variants. *Virology* 2002; **292**: 44-58 [PMID: 11878907]

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MINIREVIEWS

Extra-intestinal manifestations of non-celiac gluten sensitivity: An expanding paradigm

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Abstract

Non celiac gluten sensitivity (NCGS) is a syndrome characterized by a cohort of symptoms related to the ingestion of gluten-containing food in subjects who are not affected by celiac disease (CD) or wheat allergy. The possibility of systemic manifestations in this condition has been suggested by some reports. In most cases they are characterized by vague symptoms such as 'foggy mind', headache, fatigue, joint and muscle pain, leg or arm numbness even if more specific complaints have been described. NCGS has an immune-related background. Indeed there is a strong evidence that a selective activation of innate immunity may be the trigger for NCGS inflammatory response. The most commonly autoimmune disorders associated to NCGS are Hashimoto thyroiditis, dermatitis herpetiformis, psoriasis and rheumatologic diseases. The predominance of Hashimoto thyroiditis represents an interesting finding, since it has been indirectly confirmed by an Italian study, showing that autoimmune thyroid disease is a risk factor for the evolution towards NCGS in a group of patients with minimal duodenal inflammation. On these bases, an autoimmune stigma in NCGS is strongly supported; it could be a characteristic feature that could help the diagnosis and be simultaneously managed. A possible neurological involvement has been underlined by NCGS association with gluten ataxia, gluten neuropathy and gluten encephalopathy. NCGS patients may show even psychiatric diseases such as depression, anxiety and psychosis. Finally, a link with functional disorders (irritable bowel syndrome and fibromyalgia) is a topic under discussion. In conclusion, the novelty of this matter has generated an expansion of literature data with the unavoidable consequence that some

reports are often based on low levels of evidence. Therefore, only studies performed on large samples with the inclusion of control groups will be able to clearly establish whether the large information from the literature regarding extra-intestinal NCGS manifestations could be supported by evidence-based agreements.

Key words: Non celiac gluten sensitivity; Celiac disease; Gluten; Gluten ataxia; Autoimmunity; Gluten-related disorders; Thyroiditis; Extra-intestinal

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Core tip: Non celiac gluten sensitivity is an expanding field of investigation within gluten-related disorders. Similarly to celiac disease, it shows a systemic involvement, therefore several extra-intestinal manifestations have been hypothesized and investigated in many studies. They may involve many districts and have neurological/psychiatric, dermatological, rheumatologic and nutritional implications. Moreover, the possibility of association with other autoimmune diseases should not be underestimated. However, the large data amount from the literature often requires to be supported by evidence-based agreements.

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INTRODUCTION

Non celiac gluten sensitivity (NCGS) is a syndrome characterized by a set of symptoms related to the ingestion of gluten-containing food in subjects who are not affected by celiac disease (CD) or wheat allergy[1]. Despite it has been included in the spectrum of gluten related disorders, it shows a peculiar picture with some elements resembling CD, *i.e.*, immunological involvement and response to gluten free diet, and some features close to irritable bowel syndrome[2].

In detail, NCGS is distinguished by symptoms that typically take place soon after gluten ingestion, withdraw with gluten exclusion, and relapse following gluten challenge within hours or days. The "classical" clinical picture of NCGS is a combination of irritable bowel syndrome-like manifestations, such as abdominal pain, bloating, diarrhea or alterations in bowel habit with alternation of constipation and loose stools.

However, the possibility of systemic manifestations in this condition has been suggested by some reports. In most cases they are characterized by vague symptoms such as 'foggy mind', headache, fatigue, joint and muscle pain, leg or arm numbness even if more specific complaints have been described, such as dermatitis, (eczema or skin rash), depression, neurological symptoms and anemia[3-8]. Moreover, the possibility of association with other autoimmune diseases has been hypothesized. Indeed, similarly to CD, NCGS can be considered as an immune system-related disease and this aspect should be of relevance.

In conclusion, the spectrum of NCGS extra-intestinal manifestations is constantly expanding with new reports. Therefore, we aimed to summarize the main extra-intestinal manifestations of NCGS in a narrative review. In particular, in this review we focused on the associations supported by an evidence-based link more than single case reports, where it is difficult to differentiate a casual association from a real relationship. For this reason we searched in PubMed database in February 2018 using the following terms: gluten sensitivity, extra-intestinal, autoimmune, thyroid, neurology, psychiatry, rheumatology, skin, dermatology, nutrition, irritable bowel syndrome and fibromyalgia. In this way, 880 articles were found, and, as reported in the flow chart in Figure 1, we selected 86 studies for this review. Other studies which were not focused on NCGS or reporting an unclear definition of NCGS, or in which results about extra-intestinal manifestation were not listed have been excluded. Additionally, we graded the level of evidence on the association between NCGS and systemic manifestations using the Oxford consensus[9].

ASSOCIATION WITH AUTOIMMUNE DISEASES

On the base of convincing evidence, NCGS has an immune-related background. Indeed it has been demonstrated that a selective activation of innate immunity may be the trigger for NCGS inflammatory response[10,11]. It is unclear whether gliadin is the real responsible for the autoimmune event onset, since some other components of wheat, such as amylase-trypsin inhibitors or fermentable oligo-di-mono-saccharides and polyols (FODMAPs) have been invoked[12-14]. For this reason some Authors consider the term "non celiac wheat sensitivity" more appropriate than the current one[15].

CD, which is the most common and studied gluten-related disorder, is often associated to several other autoimmune diseases, such as type 1 diabetes, autoimmune thyroiditis or dermatitis herpetiformis[16]. For this reason it is conceivable that also patients with NCGS could show autoimmune disorders. In a cohort of 131 NCGS patients[17], the prevalence of autoimmune disease (29%) was found to be higher than in control group (4%, $P < 0.001$). Moreover, anti-nucleus antibody (ANA) positivity, a well-known marker of autoimmune setting, was present in the 46% of NCGS subjects, compared to the 2% of controls, and ANA positivity

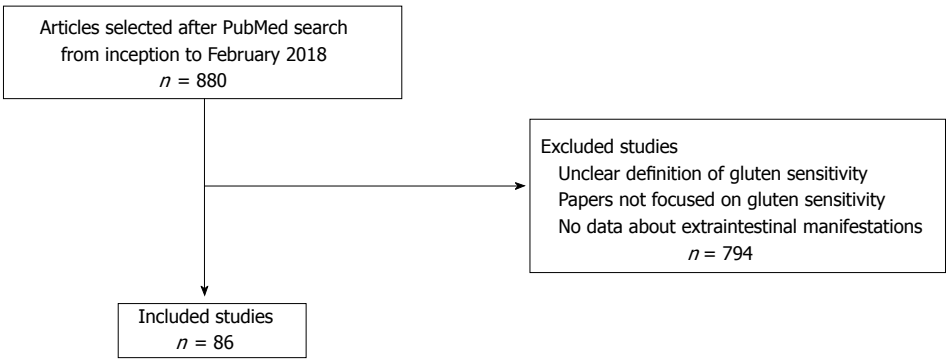

Figure 1 Flowchart summarizing the process of study selection.

correlated with DQ2/8 haplotypes. In detail, the most frequently reported NCGS-associated autoimmune disorder was Hashimoto thyroiditis (29 patients). Other diseases were psoriasis (4 cases), type 1 diabetes (4 cases), mixed connective tissue disease (1 case) and ankylosing spondylitis (1 case). The predominance of autoimmune thyroiditis represents an interesting finding, since it was indirectly confirmed by an Italian experience[18], showing that autoimmune thyroiditis is a risk factor for the evolution towards NCGS in a group of patients with minimal duodenal inflammation[19].

On these bases, an autoimmune stigma in NCGS is strongly supported; it could be a characteristic feature that could help the diagnosis and be simultaneously managed.

NEUROLOGIC AND PSYCHIATRIC MANIFESTATIONS

Recently, many studies explored the bond between the ingestion of gluten-containing food and the onset of neurologic and psychiatric disorders or symptoms such as ataxia, peripheral neuropathy, schizophrenia, autism, depression, anxiety, and hallucinations[20].

In patients with CD, a neurological involvement could be the only clinical manifestation of the disease. The production of autoantibodies directed against the tissue transglutaminase isoform 6 (expressed selectively in brain tissue) has been found in up to the 85% of these patients[21,22]. Anti-gliadin antibodies (AGA) frequently occur in such cases[21,22]. It is unclear whether the production of these antibodies takes place in the brain or in the gut mucosa, but these antibodies are considered to be the etiologic agent of neurological manifestations of CD. Finally, an inflammatory infiltrate of T lymphocytes resembling IELs in the white matter or in perivascular cuff of nerves is an important finding suggesting a specific pathogenetic mechanism of gluten-induced neuropathies[23].

Three main diseases have been described in the spectrum of gluten-related neurologic manifestations: gluten ataxia, gluten neuropathy and gluten encephalopathy[23].

Gluten ataxia has the strongest relationship with gluten-related disorders. It encompasses about the 20% of all causes of ataxia. This is mainly characterized by pure cerebellar ataxia and, rarely, by ataxia combined with myoclonus, palatal tremor, opsoclonus, or chorea. Gaze-evoked nystagmus and other ocular marks of cerebellar dysfunction are observed in about the 80%. All subjects show gait ataxia and most of them have limb ataxia[24]. A frequent finding at magnetic resonance imaging is cerebellar atrophy, secondary to necrosis of Purkinje cells[25]. Less than 10% of patients with gluten ataxia complain of gastrointestinal symptoms. A gluten free diet is able to reverse symptoms, however an early diagnosis significantly improves the prognosis, since gluten free diet may stop the loss of Purkinje cells. Therefore, a late diagnosis may be associated with an irreversible damage[26].

Gluten neuropathy is a form of peripheral neuronal damage, in which there is a serological evidence of CD positivity in the absence of alternative aetiologies. The most common type is a symmetrical sensorimotor axonal peripheral neuropathy, but other types have also been described (asymmetrical neuropathy, pure motor neuropathy or autonomic neuropathy)[27]. Gluten neuropathy occurs in the sixth decade and slowly progresses with a 9 year mean latency time between the diagnosis of neuropathy and that of CD. A third of patients shows duodenal inflammation on biopsy, however the presence or absence of enteropathy does not influence the effect of a gluten-free diet[28]. The most common histopathological feature of gluten neuropathy is lymphocyte infiltration of peri-neural vessels[29].

Gluten encephalopathy is a central nervous system disease characterized by focal abnormalities of the white matter (usually area of low perfusion) in presence of AGA or anti-transglutaminase 2 antibodies[30]. The most common symptom is migraine. It has been demonstrated that a gluten free diet improves the headaches and stops the progression of cerebral alterations detected at magnetic resonance imaging[31].

Some reports about the direct relationship between the above cited diseases and NCGS have been

published in the last years. Hadjivassiliou *et al*[32] have retrospectively evaluated 562 patients with gluten-related disorders (228 CD and 334 NCGS) and concomitant neurological involvement. In NCGS the most frequent disorder was peripheral neuropathy (54%) followed by ataxia (46%) and encephalopathy, while in CD, ataxia was the most frequent one (41%). In all cases a deep linkage with AGA positivity was recorded. Additionally, the severity of ataxia was similar in both conditions (CD and NCGS), while patients with CD exhibited more frequently severe forms of neuropathy. Rodrigo *et al*[33] found, in a cohort of 31 subjects with gluten ataxia, AGA positivity rate of 100%; this value was more similar to NCGS (89%) than CD (48%) and was associated to Marsh 1 duodenal histological picture. On the bases of such results, they concluded that gluten ataxia shows a strict affinity to NCGS more than CD.

Headache is a very frequent finding in NCGS. However, no study has so far analyzed in depth the nature of this association. The available data relies mainly on observational studies aiming to elucidate the prevalence of this condition, which ranges around the 25%[3-8,34,35]. However, the lack of case-control studies is a serious limitation to ascertain the reliability of the association. Moreover there are no studies investigating possible pathogenetic mechanisms.

The association with other neurologic diseases such as epilepsy[36], miopathy[37] and demyelinating disease[38], is anecdotal or based on a non conventional diagnosis of NCGS, therefore it is not possible to draw solid conclusions.

Among the psychiatric diseases, depression and anxiety have been hypothesized as systemic manifestations of NCGS. In an Australian study[39], a group of patients with established diagnosis of NCGS underwent a double blind crossover study with a placebo versus oral gluten supplementation after a gluten free diet. Results showed that gluten induced depression scale worsening when compared to placebo, while other symptoms (anxiety, curiosity and anger) were not influenced by the diet. However, the mechanism by which gluten may induce these changes is not yet clear. Depression is indeed a frequent finding in Western society, and it could be a distinctive mood tract of personality rather than an extra-intestinal manifestation of NCGS. However, in another study NCGS patients did not exhibit a tendency for general somatization. Additionally, personality and quality of life did not differ between NCGS and CD patients and were mostly similar to healthy controls[40].

Some authors have invoked a role of gluten for some psychiatric diseases like schizophrenia or bipolar disorder[41], but there are no studies exploring these entities in NCGS. On the other hand, some cases of "gluten psychosis" in patients with NCGS have been described[42]. In these patients, hallucinations, crying spells, relevant confusion, ataxia, severe anxiety and paranoid delirium occurred shortly after gluten ingestion and disappeared within one week of gluten free diet.

Finally, the relationship between autism and gluten is an hot topic. It has been shown that children with autism have more frequently IgG-AGA positivity than healthy children (24% *vs* 7%)[43], but currently there are no studies in which a solid diagnosis of NCGS has been achieved in autistic subjects. A gluten free diet is often proposed to these children in an empiric setting, since it has been demonstrated that it improves behavioral scores[44,45]. However, at present there are no evidence-based reasons to look for gluten sensitivity in autism and to advise an exclusion diet[46].

SKIN MANIFESTATIONS

The association between CD and skin diseases, in particular dermatitis herpetiformis, is well known[47]. Similarly to CD, the possibility of a skin involvement in the 18% of NCGS has been reported[4]. In the published case series[3-8], undefined dermatitis, rash and eczema were the most common skin manifestations in NCGS. The possibility of an association with skin autoimmune diseases such as psoriasis has been above mentioned[17]. A case report has shown that even dermatitis herpetiformis may occur[48].

Some reports have been mainly focused on the characteristics of skin lesions in NCGS from a dermatological point of view. In a series of 17 NCGS patients with skin lesions, the most common ones were very similar to dermatitis herpetiformis or subacute eczema (erythematous, excoriated papular-vesicular and extremely itchy)[49]. Some patients had also hyperkeratotic scaly lesions resembling psoriasis. The most common skin location was the extensor surfaces of upper limbs, in the 94%, alike dermatitis herpetiformis. The histological analysis showed complement C3 deposits at dermoepidermal junction in the 82%. Finally, in all patients a gluten free diet was able to lead to lesions disappearance within one month, much faster than in dermatitis herpetiformis.

Some Authors have claimed that an allergic sensitivity to food allergens other than gluten could underlie NCGS[50]. Indeed, an Italian study found that the 10% of NCGS patients suffered from nickel allergy with contact dermatitis and this prevalence was higher than in control group (5%, $P = 0.04$). However, NCGS subjects referred onset of dermatitis after wheat ingestion[51].

RHEUMATOLOGIC MANIFESTATIONS

As we already mentioned, NCGS shows the tendency to cluster autoimmune diseases. Some reports about its coexistence with rheumatologic diseases are available. The first evidence demonstrated that in a group of 30 subjects with ankylosing spondylitis, 11 had AGA positivity, while no patient in a control group exhibited this finding[52]. Isasi *et al*[53] reported 4 cases of axial spondyloarthritis (2 ankylosing spondylitis and 1

Table 1 Studies reporting the prevalence of people avoiding gluten-containing foods

Ref.	Country	Population	Sample size	Avoidance rate of gluten-based products
Tanpowpong *et al*[60], 2012	New Zealand	Pediatric	916	5.2%
Rubio-Tapia *et al*[61], 2013	United States	Pediatric	7798	0.7%
DiGiacomo *et al*[62], 2013	United States	National Health and Nutrition Examination Survey	7762	0.6%
Lis *et al*[63], 2014	Australia	Adults	910	41.2%
Golley *et al*[64], 2015	Australia	Adults	1184	10.6%
Mardini *et al*[65], 2015	United States	Pediatric	14701	1%
Aziz *et al*[59], 2014	United Kingdom	Adults	1002	3.7%
Van Gils *et al*[8], 2016	The Netherlands	Adults	785	6.2%
Carroccio *et al*[7], 2017	Italy	Adolescents	548	2.9%

psoriatic spondyloarthritis) with a microscopic enteritis picture at duodenal biopsy. They all underwent a gluten free diet, and in all cases an improvement or remission of back pain was reported, with a recrudescence after wheat challenge. The same result was recorded in another group of patients with systemic sclerosis, Raynaud's phenomenon, symmetric polyarthritis and Sjogren's syndrome[53].

However, despite such reports, the evidence for NCGS/rheumatologic association is weak, since case reports represent only a low level of evidence and case-control studies are necessary.

FIBROMYALGIA AND OTHER FUNCTIONAL DISORDERS

Fibromyalgia is a disease characterized by widespread pain, often accompanied by fatigue, memory problems, sleep disturbances, depression or irritable bowel syndrome[54]. In many case series, several NCGS patients complain of chronic muscle or joint pain, leg numbness, fatigue and headache[3-8], therefore it is possible that an underlying undiagnosed fibromyalgia could be present. Indeed, starting from some case reports demonstrating this association[55], further studies have analyzed in depth this relationship. In a Spanish series[56] of 246 fibromyalgia patients undergoing gluten free diet, 90 showed clinical symptom improvement. Additionally, Authors described the features of 20 out of such 90 patients. They had a mean duration of fibromyalgia of 12 years, and 17 had also gastrointestinal symptoms. Eighteen had a DQ2/8 haplotype and all showed an increase in duodenal IELs. After a mean gluten free diet period of 16.4 mo, 15 of them (75%) experienced a full remission of pain and in 8 of them gluten challenge led to symptom re-appearance. In another trial, gluten free diet was able to induce a decrease in some scales evaluating fibromyalgia symptoms[57]. On these bases, it is possible to hypothesize that the link between these two disorders is quite strong, but the role of microscopic enteritis in this setting should be tested in other controlled trials.

Fibromyalgia is frequently recognized as a functional disease. In this regard, NCGS has a tight bond with irritable bowel syndrome (IBS)[58]. Many patients with IBS often identify some foods that they believe to be more offending, and wheat is often invoked. Furthermore, a certain symptom overlap between NCGS and IBS-type symptoms exists[4,59]. For this reason, many patients tend to exclude gluten from their diet on their own, without medical advice, as summarized in Table 1[7,8,59-65]. The basic difference between the two conditions is that patients with NCGS assert that symptoms take place when they eat wheat so that they believe to have identified gluten as the culprit. Some experimental investigations have shown that gliadin can alter the integrity of the small intestinal mucosa, as shown by the appearance of epithelial leaks/gaps and widened inter-villous spaces detected by using confocal laser endomicroscopy[66]. Based on these assumptions, some clinical trials have demonstrated that a gluten free diet may lead to improvement of gastrointestinal symptoms in IBS, as reported in Table 2[5,67-73]. However it is not clear whether gluten is really the responsible for such symptoms. Indeed wheat contains FODMAPs as well, which are considered as a possible trigger for IBS itself, and FODMAP restriction demonstrated an improvement in IBS symptoms in up to the 74%[74]. Additionally, one trial underlined that subjects with self-reported NCGS (and IBS-like symptoms) had benefits by a low FODMAP diet despite they were still consuming a gluten free diet[75]. Based on these evidences, the link between IBS and NCGS seems to be strict even if quite nebulous. Is it possible that IBS and NCGS should be considered as the two sides of the same coin? Such fascinating question needs to be answered by well designed studies for this purpose.

NUTRITIONAL IMPAIRMENT IN NCGS

CD is often disclosed by nutritional impairments, such as vitamin D or iron deficiency, anemia or alterations in bone mineralization[76,77].

Anemia prevalence value ranges between 15% and 23% in NCGS[3,4]. Nevertheless, studies enclosing a control group are lacking, therefore it is not possible to establish which is the real relationship between anemia and NCGS. Additionally, folate deficiency has

Table 2 Main studies exploring the effect of gluten free diet in irritable bowel syndrome

Ref.	Country	Population	Outcome
Wahnschaffe *et al*[67], 2001	Germany	102 IBS-D	Stool frequency/bowel movement improved in DQ2-8 positive subjects
Aziz *et al*[68], 2016	United Kingdom	40 IBS-D	A 6-wk GFD reduced symptoms in 70%
Vazquez-Roque *et al*[69], 2013	United States	45 IBS-D	Stool frequency/bowel movement reduced in patients under GFD
Di Sabatino *et al*[5], 2015	Italy	59 IBS with self-diagnosis of NCGS	A challenge with 4 g/d of gluten worsened symptoms compared to placebo
Shahbazkhani *et al*[70], 2015	Iran	72 IBS	Worsening of intestinal symptoms with gluten compared to placebo
Zanwar *et al*[71], 2016	India	60 IBS	A 4-wk GFD improved a visual-analogue scale of symptoms
Elli *et al*[72], 2016	Italy	140 IBS with self-diagnosis of NCGS	Only the 14% showed a response to GFD as well as challenge test
Barmeyer *et al*[73], 2017	Germany	34 IBS	The 34% showed clinical improvement to GFD and continued for one year

GFD: Gluten free diet; IBS-D: Irritable bowel syndrome, diarrhea subtype; NCGS: Non celiac gluten sensitivity.

Table 3 Main extra-intestinal manifestations of non-celiac gluten sensitivity and associated disorders

Manifestations	Extra-intestinal manifestations	Level of evidence	Associated disorders	Level of evidence
General symptoms	Tiredness	4	Aphthous stomatitis	4
	Lack of wellbeing	4		
	Foggy mind	4		
	Joint or muscle pain	4		
	Arm/leg numbness	4		
Neurologic manifestations			Ataxia	3b
			Neuropathy	3b
			Encephalopathy	3b
			Epilepsy	4
			Miopathy	4
			Myelopathy	4
			Demyelinating disease	4
Psychiatric manifestations	Depression	1c	Bipolar disorder	4
	Anxiety	1c	Gluten psychosis	4
			Autism	2b
			Schizophrenia	4
Other autoimmune diseases and rheumatologic diseases			Psoriasis	2b
			Autoimmune thyroiditis	2b
			Rheumatoid arthritis	4
			Scleroderma	4
			Sjogren syndrome	4
			Raynaud phenomenon	4
Skin diseases			Dermatitis herpetiformis	2b
			Contact dermatitis	2b
			Rash and undetermined dermatitis	2b
Functional disorders			Fibromyalgia	1c
			Irritable bowel syndrome	1c
Nutritional imbalance	Anemia	4		
	Osteoporosis	2b		
Other			Interstitial cystitis	4
			Ingrown hairs	4
			Rhinitis, asthma	4
			Postural tachycardia syndrome	2b
			Oligo- or polymenorrhea	4

The level of evidence was expressed according to the Oxford consensus[85]

been reported in NCGS with solid evidence and it has been even described as a predictive factor for its development[18].

An Italian study illustrated that NCGS carries a risk of osteopenia similar to CD[78]. Low bone mineral density measured by Dual-energy X-ray absorptiometry was found in 28% of NCGS subjects, *vs* 6% of IBS as well as an influence of body mass index on mineralization was observed. This result has been explained by a lower calcium dietary intake (only 615 mg/d, while

recommended dose is 1000 mg/d).

This last observation may suggest that NCGS patients could experience an alteration in macro- and micronutrients intake due to dietary self-restrictions. Indeed Zingone *et al*[79] evaluated diet habits of 29 NCGS subjects and discovered that they ingested lower mean amounts of carbohydrates, proteins, fiber, and polyunsaturated fatty acids. Patients with NCGS reported avoiding fruit, vegetables, milk, and dairy products as well as snacks and mixed spices when compared to a control population.

NCGS is characterized by absent or minimal duodenal inflammation and, therefore, cannot be associated to nutrient deficiencies linked to malabsorption. However, an inflammatory status of duodenal mucosa, witnessed by increased expression of interferon gamma, may not be overlooked[33,80-82]. Finally, alterations in dietary pattern should not be underestimated. Gluten free diet itself can lead to an inadequate balance in macronutrients assumption[83-85].

CONCLUSION

Data from literature about extra-intestinal manifestations of NCGS strongly suggests that this condition could have a systemic involvement, similarly to CD. However, the novelty of this topic has generated an expansion of literature data with the unavoidable consequence that some reports are often based on low levels of evidence, as summarized in Table 3, with a grading of evidence according to the Oxford classification[9]. Therefore, only studies performed on large samples with the addition of control groups will be able to clearly establish whether the large information from the literature regarding extra-intestinal NCGS manifestations could be supported by evidence-based agreements.

REFERENCES

1 **Catassi C**, Bai JC, Bonaz B, Bouma G, Calabrò A, Carroccio A, Castillejo G, Ciacci C, Cristofori F, Dolinsek J, Francavilla R, Elli L, Green P, Holtmeier W, Koehler P, Koletzko S, Meinhold C, Sanders D, Schumann M, Schuppan D, Ullrich R, Vécsei A, Volta U, Zevallos V, Sapone A, Fasano A. Non-Celiac Gluten sensitivity: the new frontier of gluten related disorders. *Nutrients* 2013; **5**: 3839-3853 [PMID: 24077239 DOI: 10.3390/nu5103839]

2 **Sapone A**, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, Kaukinen K, Rostami K, Sanders DS, Schumann M, Ullrich R, Villalta D, Volta U, Catassi C, Fasano A. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med* 2012; **10**: 13 [PMID: 22313950 DOI: 10.1186/1741-7015-10-13]

3 **Volta U**, Tovoli F, Cicola R, Parisi C, Fabbri A, Piscaglia M, Fiorini E, Caio G. Serological tests in gluten sensitivity (nonceliac gluten intolerance). *J Clin Gastroenterol* 2012; **46**: 680-685 [PMID: 22138844 DOI: 10.1097/MCG.0b013e3182372541]

4 **Volta U**, Bardella MT, Calabrò A, Troncone R, Corazza GR; Study Group for Non-Celiac Gluten Sensitivity. An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity. *BMC Med* 2014; **12**: 85 [PMID: 24885375 DOI: 10.1186/1741-7015-12-85]

5 **Di Sabatino A**, Volta U, Salvatore C, Biancheri P, Caio G, De Giorgio R, Di Stefano M, Corazza GR. Small Amounts of Gluten in Subjects With Suspected Nonceliac Gluten Sensitivity: A Randomized, Double-Blind, Placebo-Controlled, Cross-Over Trial. *Clin Gastroenterol Hepatol* 2015; **13**: 1604-1612.e3 [PMID: 25701700 DOI: 10.1016/j.cgh.2015.01.029]

6 **Carroccio A**, Mansueto P, Iacono G, Soresi M, D'Alcamo A, Cavataio F, Brusca I, Florena AM, Ambrosiano G, Seidita A, Pirrone G, Rini GB. Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity. *Am J Gastroenterol* 2012; **107**: 1898-1906; quiz 1907 [PMID: 22825366 DOI: 10.1038/ajg.2012.236]

7 **Carroccio A**, Giambalvo O, Blasca F, Iacobucci R, D'Alcamo A, Mansueto P. Self-Reported Non-Celiac Wheat Sensitivity in High School Students: Demographic and Clinical Characteristics. *Nutrients* 2017; **9**: pii: E771 [PMID: 28753927 DOI: 10.3390/nu9070771]

8 **van Gils T**, Nijeboer P, IJssennagger CE, Sanders DS, Mulder CJ, Bouma G. Prevalence and Characterization of Self-Reported Gluten Sensitivity in The Netherlands. *Nutrients* 2016; **8**: pii: E714 [PMID: 27834802 DOI: 10.3390/nu8110714]

9 **Centre for Evidence-Based Medicine**. Oxford Centre for Evidence-based Medicine – Levels of Evidence (March 2009). Accessed March 17, 2018 Available from: URL: https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

10 **Caio G**, Riegler G, Patturelli M, Facchiano A, DE Magistris L, Sapone A. Pathophysiology of non-celiac gluten sensitivity: where are we now? *Minerva Gastroenterol Dietol* 2017; **63**: 16-21 [PMID: 27808487 DOI: 10.23736/S1121-421X.16.02346-1]

11 **Sapone A**, Lammers KM, Casolaro V, Cammarota M, Giuliano MT, De Rosa M, Stefanile R, Mazzarella G, Tolone C, Russo MI, Esposito P, Ferraraccio F, Cartenì M, Riegler G, de Magistris L, Fasano A. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med* 2011; **9**: 23 [PMID: 21392369 DOI: 10.1186/1741-7015-9-23]

12 **Zevallos VF**, Raker V, Tenzer S, Jimenez-Calvente C, Ashfaq-Khan M, Rüssel N, Pickert G, Schild H, Steinbrink K, Schuppan D. Nutritional Wheat Amylase-Trypsin Inhibitors Promote Intestinal Inflammation via Activation of Myeloid Cells. *Gastroenterology* 2017; **152**: 1100-1113.e12 [PMID: 27993525 DOI: 10.1053/j.gastro.2016.12.006]

13 **Skodje GI**, Sarna VK, Minelle IH, Rolfsen KL, Muir JG, Gibson PR, Veierød MB, Henriksen C, Lundin KEA. Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-Reported Non-Celiac Gluten Sensitivity. *Gastroenterology* 2018; **154**: 529-539.e2 [PMID: 29102613 DOI: 10.1053/j.gastro.2017.10.040]

14 **De Giorgio R**, Volta U, Gibson PR. Sensitivity to wheat, gluten and FODMAPs in IBS: facts or fiction? *Gut* 2016; **65**: 169-178 [PMID: 26078292 DOI: 10.1136/gutjnl-2015-309757]

15 **Carroccio A**, Rini G, Mansueto P. Non-celiac wheat sensitivity is a more appropriate label than non-celiac gluten sensitivity. *Gastroenterology* 2014; **146**: 320-321 [PMID: 24275240 DOI: 10.1053/j.gastro.2013.08.061]

16 **Leffler DA**, Green PH, Fasano A. Extraintestinal manifestations of coeliac disease. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 561-571 [PMID: 26260366 DOI: 10.1038/nrgastro.2015.131]

17 **Carroccio A**, D'Alcamo A, Cavataio F, Soresi M, Seidita A, Sciumè C, Geraci G, Iacono G, Mansueto P. High Proportions of People With Nonceliac Wheat Sensitivity Have Autoimmune Disease or Antinuclear Antibodies. *Gastroenterology* 2015; **149**: 596-603.e1 [PMID: 26026392 DOI: 10.1053/j.gastro.2015.05.040]

18 **Losurdo G**, Piscitelli D, Giangaspero A, Principi M, Buffelli F, Giorgio F, Montenegro L, Sorrentino C, Amoruso A, Ierardi E, Di Leo A. Evolution of nonspecific duodenal lymphocytosis over 2 years of follow-up. *World J Gastroenterol* 2015; **21**: 7545-7552 [PMID: 26140001 DOI: 10.3748/wjg.v21.i24.7545]

19 **Ierardi E**, Losurdo G, Iannone A, Piscitelli D, Amoruso A, Barone M, Principi M, Pisani A, Di Leo A. Lymphocytic duodenitis or microscopic enteritis and gluten-related conditions: what needs

to be explored? *Ann Gastroenterol* 2017; **30**: 380-392 [PMID: 28655974 DOI: 10.20524/aog.2017.0165]
20 **Jackson JR**, Eaton WW, Cascella NG, Fasano A, Kelly DL. Neurologic and psychiatric manifestations of celiac disease and gluten sensitivity. *Psychiatr Q* 2012; **83**: 91-102 [PMID: 21877216 DOI: 10.1007/s11126-011-9186-y]
21 **Hadjivassiliou M**, Aeschlimann P, Strigun A, Sanders DS, Woodroofe N, Aeschlimann D. Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase. *Ann Neurol* 2008; **64**: 332-343 [PMID: 18825674 DOI: 10.1002/ana.21450]
22 **Zis P**, Rao DG, Sarrigiannis PG, Aeschlimann P, Aeschlimann DP, Sanders D, Grünewald RA, Hadjivassiliou M. Transglutaminase 6 antibodies in gluten neuropathy. *Dig Liver Dis* 2017; **49**: 1196-1200 [PMID: 28886934 DOI: 10.1016/j.dld.2017.08.019]
23 **Hadjivassiliou M**, Sanders DS, Grünewald RA, Woodroofe N, Boscolo S, Aeschlimann D. Gluten sensitivity: from gut to brain. *Lancet Neurol* 2010; **9**: 318-330 [PMID: 20170845 DOI: 10.1016/S1474-4422(09)70290-X]
24 **Hadjivassiliou M**, Sanders DS, Woodroofe N, Williamson C, Grünewald RA. Gluten ataxia. *Cerebellum* 2008; **7**: 494-498 [PMID: 18787912 DOI: 10.1007/s12311-008-0052-x]
25 **Wilkinson ID**, Hadjivassiliou M, Dickson JM, Wallis L, Grünewald RA, Coley SC, Widjaja E, Griffiths PD. Cerebellar abnormalities on proton MR spectroscopy in gluten ataxia. *J Neurol Neurosurg Psychiatry* 2005; **76**: 1011-1013 [PMID: 15965215]
26 **Pellecchia MT**, Scala R, Perretti A, De Michele G, Santoro L, Filla A, Ciacci C, Barone P. Cerebellar ataxia associated with subclinical celiac disease responding to gluten-free diet. *Neurology* 1999; **53**: 1606-1608 [PMID: 10534283]
27 **Chin RL**, Sander HW, Brannagan TH, Green PH, Hays AP, Alaedini A, Latov N. Celiac neuropathy. *Neurology* 2003; **60**: 1581-1585 [PMID: 12771245]
28 **Hadjivassiliou M**, Kandler RH, Chattopadhyay AK, Davies-Jones AG, Jarratt JA, Sanders DS, Sharrack B, Grünewald RA. Dietary treatment of gluten neuropathy. *Muscle Nerve* 2006; **34**: 762-766 [PMID: 17013890]
29 **Hadjivassiliou M**, Grünewald RA, Kandler RH, Chattopadhyay AK, Jarratt JA, Sanders DS, Sharrack B, Wharton SB, Davies-Jones GA. Neuropathy associated with gluten sensitivity. *J Neurol Neurosurg Psychiatry* 2006; **77**: 1262-1266 [PMID: 16835287]
30 **Hadjivassiliou M**, Grünewald RA, Lawden M, Davies-Jones GA, Powell T, Smith CM. Headache and CNS white matter abnormalities associated with gluten sensitivity. *Neurology* 2001; **56**: 385-388 [PMID: 11171906]
31 **Serratrice J**, Disdier P, de Roux C, Christides C, Weiller PJ. Migraine and coeliac disease. *Headache* 1998; **38**: 627-628 [PMID: 11398309]
32 **Hadjivassiliou M**, Rao DG, Grìnewald RA, Aeschlimann DP, Sarrigiannis PG, Hoggard N, Aeschlimann P, Mooney PD, Sanders DS. Neurological Dysfunction in Coeliac Disease and Non-Coeliac Gluten Sensitivity. *Am J Gastroenterol* 2016; **111**: 561-567 [PMID: 26832652 DOI: 10.1038/ajg.2015.434]
33 **Rodrigo L**, Hernández-Lahoz C, Lauret E, Rodriguez-Peláez M, Soucek M, Ciccocioppo R, Kruzliak P. Gluten ataxia is better classified as non-celiac gluten sensitivity than as celiac disease: a comparative clinical study. *Immunol Res* 2016; **64**: 558-564 [PMID: 26676361 DOI: 10.1007/s12026-015-8750-1]
34 **Losurdo G**, Giorgio F, Piscitelli D, Montenegro L, Covelli C, Fiore MG, Giangaspero A, Iannone A, Principi M, Amoruso A, Barone M, Di Leo A, Ierardi E. May the assessment of baseline mucosal molecular pattern predict the development of gluten related disorders among microscopic enteritis? *World J Gastroenterol* 2016; **22**: 8017-8025 [PMID: 27672296 DOI: 10.3748/wjg.v22.i35.8017]
35 **Losurdo G**, Piscitelli D, Pezzuto F, Fortarezza F, Covelli C, Marra A, Iannone A, Amoruso A, Principi M, Ierardi E, Di Leo A. T Helper Lymphocyte and Mast Cell Immunohistochemical Pattern in Nonceliac Gluten Sensitivity. *Gastroenterol Res Pract* 2017; **2017**: 5023680 [PMID: 29362561 DOI: 10.1155/2017/5023680]
36 **Bruni O**, Dosi C, Luchetti A, Della Corte M, Riccioni A, Battaglia D, Ferri R. An unusual case of drug-resistant epilepsy in a child with non-celiac gluten sensitivity. *Seizure* 2014; **23**: 674-676 [PMID: 24813861 DOI: 10.1016/j.seizure.2014.04.005]
37 **Hadjivassiliou M**, Chattopadhyay AK, Grünewald RA, Jarratt JA, Kandler RH, Rao DG, Sanders DS, Wharton SB, Davies-Jones GA. Myopathy associated with gluten sensitivity. *Muscle Nerve* 2007; **35**: 443-450 [PMID: 17143894]
38 **Jacob S**, Zarei M, Kenton A, Allroggen H. Gluten sensitivity and neuromyelitis optica: two case reports. *J Neurol Neurosurg Psychiatry* 2005; **76**: 1028-1030 [PMID: 15965221]
39 **Peters SL**, Biesiekierski JR, Yelland GW, Muir JG, Gibson PR. Randomised clinical trial: gluten may cause depression in subjects with non-coeliac gluten sensitivity - an exploratory clinical study. *Aliment Pharmacol Ther* 2014; **39**: 1104-1112 [PMID: 24689456 DOI: 10.1111/apt.12730]
40 **Brottveit M**, Vandvik PO, Wojniusz S, Løvik A, Lundin KE, Boye B. Absence of somatization in non-coeliac gluten sensitivity. *Scand J Gastroenterol* 2012; **47**: 770-777 [PMID: 22519894 DOI: 10.3109/00365521.2012.679685]
41 **Casella G**, Pozzi R, Cigognetti M, Bachetti F, Torti G, Cadei M, Villanacci V, Baldini V, Bassotti G. Mood disorders and non-celiac gluten sensitivity. *Minerva Gastroenterol Dietol* 2017; **63**: 32-37 [PMID: 27647538 DOI: 10.23736/S1121-421X.16.02325-4]
42 **Lionetti E**, Leonardi S, Franzonello C, Mancardi M, Ruggieri M, Catassi C. Gluten Psychosis: Confirmation of a New Clinical Entity. *Nutrients* 2015; **7**: 5532-5539 [PMID: 26184290 DOI: 10.3390/nu7075235]
43 **Lau NM**, Green PH, Taylor AK, Hellberg D, Ajamian M, Tan CZ, Kosofsky BE, Higgins JJ, Rajadhyaksha AM, Alaedini A. Markers of Celiac Disease and Gluten Sensitivity in Children with Autism. *PLoS One* 2013; **8**: e66155 [PMID: 23823064 DOI: 10.1371/journal.pone.0066155]
44 **Ghalichi F**, Ghaemmaghami J, Malek A, Ostadrahimi A. Effect of gluten free diet on gastrointestinal and behavioral indices for children with autism spectrum disorders: a randomized clinical trial. *World J Pediatr* 2016; **12**: 436-442 [PMID: 27286693 DOI: 10.1007/s12519-016-0040-z]
45 **El-Rashidy O**, El-Baz F, El-Gendy Y, Khalaf R, Reda D, Saad K. Ketogenic diet versus gluten free casein free diet in autistic children: a case-control study. *Metab Brain Dis* 2017; **32**: 1935-1941 [PMID: 28808808 DOI: 10.1007/s11011-017-0088-z]
46 **Cruchet S**, Lucero Y, Cornejo V. Truths, Myths and Needs of Special Diets: Attention-Deficit/Hyperactivity Disorder, Autism, Non-Celiac Gluten Sensitivity, and Vegetarianism. *Ann Nutr Metab* 2016; **68** Suppl 1: 43-50 [PMID: 27356007 DOI: 10.1159/000445393]
47 **Collin P**, Salmi TT, Hervonen K, Kaukinen K, Reunala T. Dermatitis herpetiformis: a cutaneous manifestation of coeliac disease. *Ann Med* 2017; **49**: 23-31 [PMID: 27499257 DOI: 10.1080/07853890.2016.1222450]
48 **Philips C**, Paramaguru R, Indiran DA, Augustine P. Dermatitis Herpetiformis as the Initial Presentation of Primary Biliary Cholangitis in a Male with Gluten Sensitivity. *Cureus* 2017; **9**: e1247 [PMID: 28630805 DOI: 10.7759/cureus.1247]
49 **Bonciolini V**, Bianchi B, Del Bianco E, Verdelli A, Caproni M. Cutaneous Manifestations of Non-Celiac Gluten Sensitivity: Clinical Histological and Immunopathological Features. *Nutrients* 2015; **7**: 7798-7805 [PMID: 26389946 DOI: 10.3390/nu7095368]
50 **Mansueto P**, D'Alcamo A, Seidita A, Carroccio A. Food allergy in irritable bowel syndrome: The case of non-celiac wheat sensitivity. *World J Gastroenterol* 2015; **21**: 7089-7109 [PMID: 26109796 DOI: 10.3748/wjg.v21.i23.7089]
51 **D'Alcamo A**, Mansueto P, Soresi M, Iacobucci R, Blasca F, Geraci G, Cavataio F, Fayer F, Arini A, Di Stefano L, Iacono G, Bosco L, Carroccio A. Contact Dermatitis Due to Nickel Allergy in Patients Suffering from Non-Celiac Wheat Sensitivity. *Nutrients* 2017; **9**: pii: E103 [PMID: 28157173 DOI: 10.3390/nu9020103]
52 **Toğrol RE**, Nalbant S, Solmazgül E, Ozyurt M, Kaplan M, Kiralp MZ, Dinçer U, Sahan B. The significance of coeliac disease antibodies in patients with ankylosing spondylitis: a case-controlled

study. *J Int Med Res* 2009; **37**: 220-226 [PMID: 19215694 DOI: 10.1177/147323000903700127]
53 **Isasi C**, Tejerina E, Morán LM. Non-celiac gluten sensitivity and rheumatic diseases. *Reumatol Clin* 2016; **12**: 4-10 [PMID: 25956352 DOI: 10.1016/j.reuma.2015.03.001]
54 **Clauw DJ**. Fibromyalgia: a clinical review. *JAMA* 2014; **311**: 1547-1555 [PMID: 24737367 DOI: 10.1001/jama.2014.3266]
55 **Isasi C**, Tejerina E, Fernandez-Puga N, Serrano-Vela JI. Fibromyalgia and chronic fatigue syndrome caused by non-celiac gluten sensitivity. *Reumatol Clin* 2015; **11**: 56-57 [PMID: 25047419 DOI: 10.1016/j.reuma.2014.06.005]
56 **Isasi C,** Colmenero I, Casco F, Tejerina E, Fernandez N, Serrano-Vela JI, Castro MJ, Villa LF. Fibromyalgia and non-celiac gluten sensitivity: a description with remission of fibromyalgia. *Rheumatol Int* 2014; **34**: 1607-1612 [PMID: 247280274 DOI: 10.1007/s00296-014-2990-6]
57 **Slim M**, Calandre EP, Garcia-Leiva JM, Rico-Villademoros F, Molina-Barea R, Rodriguez-Lopez CM, Morillas-Arques P. The Effects of a Gluten-free Diet Versus a Hypocaloric Diet Among Patients With Fibromyalgia Experiencing Gluten Sensitivity-like Symptoms: A Pilot, Open-Label Randomized Clinical Trial. *J Clin Gastroenterol* 2017; **51**: 500-507 [PMID: 27548732 DOI: 10.1097/MCG.0000000000000651]
58 **Verdu EF**, Armstrong D, Murray JA. Between celiac disease and irritable bowel syndrome: the "no man's land" of gluten sensitivity. *Am J Gastroenterol* 2009; **104**: 1587-1594 [PMID: 19455131 DOI: 10.1038/ajg.2009.188]
59 **Aziz I**, Lewis NR, Hadjivassiliou M, Winfield SN, Rugg N, Kelsall A, Newrick L, Sanders DS. A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care. *Eur J Gastroenterol Hepatol* 2014; **26**: 33-39 [PMID: 24216570 DOI: 10.1097/01.meg.0000435546.87251.f7]
60 **Tanpowpong P**, Ingham TR, Lampshire PK, Kirchberg FF, Epton MJ, Crane J, Camargo CA Jr; New Zealand Asthma and Allergy Cohort Study Group. Coeliac disease and gluten avoidance in New Zealand children. *Arch Dis Child* 2012; **97**: 12-16 [PMID: 22075107 DOI: 10.1136/archdischild-2011-300248]
61 **Rubio-Tapia A**, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The prevalence of celiac disease in the United States. *Am J Gastroenterol* 2012; **107**: 1538-44; quiz 1537, 1545 [PMID: 22850429 DOI: 10.1038/ajg.2012.219]
62 **DiGiacomo DV**, Tennyson CA, Green PH, Demmer RT. Prevalence of gluten-free diet adherence among individuals without celiac disease in the USA: results from the Continuous National Health and Nutrition Examination Survey 2009-2010. *Scand J Gastroenterol* 2013; **48**: 921-925 [PMID: 23834276 DOI: 10.3109/00365521.2013.809598]
63 **Lis DM**, Stellingwerff T, Shing CM, Ahuja KD, Fell JW. Exploring the popularity, experiences, and beliefs surrounding gluten-free diets in nonceliac athletes. *Int J Sport Nutr Exerc Metab* 2015; **25**: 37-45 [PMID: 24901744 DOI: 10.1123/ijsnem.2013-0247]
64 **Golley S**, Corsini N, Topping D, Morell M, Mohr P. Motivations for avoiding wheat consumption in Australia: results from a population survey. *Public Health Nutr* 2015; **18**: 490-499 [PMID: 24739252 DOI: 10.1017/S1368980014000652]
65 **Mardini HE**, Westgate P, Grigorian AY. Racial Differences in the Prevalence of Celiac Disease in the US Population: National Health and Nutrition Examination Survey (NHANES) 2009-2012. *Dig Dis Sci* 2015; **60**: 1738-1742 [PMID: 25577269 DOI: 10.1007/s10620-014-3514-7]
66 **Fritscher-Ravens A**, Schuppan D, Ellrichmann M, Schoch S, Röcken C, Brasch J, Bethge J, Böttner M, Klose J, Milla PJ. Confocal endomicroscopy shows food-associated changes in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology* 2014; **147**: 1012-1020.e4 [PMID: 25083606 DOI: 10.1053/j.gastro.2014.07.046]
67 **Wahnschaffe U**, Ullrich R, Riecken EO, Schulzke JD. Celiac disease-like abnormalities in a subgroup of patients with irritable bowel syndrome. *Gastroenterology* 2001; **121**: 1329-1338 [PMID: 11729112]
68 **Aziz I**, Trott N, Briggs R, North JR, Hadjivassiliou M, Sanders DS. Efficacy of a Gluten-Free Diet in Subjects With Irritable Bowel Syndrome-Diarrhea Unaware of Their HLA-DQ2/8 Genotype. *Clin Gastroenterol Hepatol* 2016; **14**: 696-703.e1 [PMID: 26748221 DOI: 10.1016/j.cgh.2015.12.031]
69 **Vazquez-Roque MI**, Camilleri M, Smyrk T, Murray JA, Marietta E, O'Neill J, Carlson P, Lamsam J, Janzow D, Eckert D, Burton D, Zinsmeister AR. A controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function. *Gastroenterology* 2013; **144**: 903-911.e3 [PMID: 23357715 DOI: 10.1053/j.gastro.2013.01.049]
70 **Shahbazkhani B**, Sadeghi A, Malekzadeh R, Khatavi F, Etemadi M, Kalantri E, Rostami-Nejad M, Rostami K. Non-Celiac Gluten Sensitivity Has Narrowed the Spectrum of Irritable Bowel Syndrome: A Double-Blind Randomized Placebo-Controlled Trial. *Nutrients* 2015; **7**: 4542-4554 [PMID: 26056920 DOI: 10.3390/nu7064542]
71 **Zanwar VG**, Pawar SV, Gambhire PA, Jain SS, Surude RG, Shah VB, Contractor QQ, Rathi PM. Symptomatic improvement with gluten restriction in irritable bowel syndrome: a prospective, randomized, double blinded placebo controlled trial. *Intest Res* 2016; **14**: 343-350 [PMID: 27799885]
72 **Elli L**, Tomba C, Branchi F, Roncoroni L, Lombardo V, Bardella MT, Ferretti F, Conte D, Valiante F, Fini L, Forti E, Cannizzaro R, Maiero S, Londoni C, Lauri A, Fornaciari G, Lenoci N, Spagnuolo R, Basilisco G, Somalvico F, Borgatta B, Leandro G, Segato S, Barisani D, Morreale G, Buscarini E. Evidence for the Presence of Non-Celiac Gluten Sensitivity in Patients with Functional Gastrointestinal Symptoms: Results from a Multicenter Randomized Double-Blind Placebo-Controlled Gluten Challenge. *Nutrients* 2016; **8**: 84 [PMID: 26867199 DOI: 10.3390/nu8020084]
73 **Barmeyer C**, Schumann M, Meyer T, Zielinski C, Zuberbier T, Siegmund B, Schulzke JD, Daum S, Ullrich R. Long-term response to gluten-free diet as evidence for non-celiac wheat sensitivity in one third of patients with diarrhea-dominant and mixed-type irritable bowel syndrome. *Int J Colorectal Dis* 2017; **32**: 29-39 [PMID: 27695975 DOI: 10.1007/s00384-016-2663-x]
74 **Shepherd SJ**, Gibson PR. Fructose malabsorption and symptoms of irritable bowel syndrome: guidelines for effective dietary management. *J Am Diet Assoc* 2006; **106**: 1631-1639 [PMID: 17000196 DOI: 10.1016/j.jada.2006.07.010]
75 **Biesiekierski JR**, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates. *Gastroenterology* 2013; **145**: 320-8.e1-3 [PMID: 23648697 DOI: 10.1053/j.gastro.2013.04.051]
76 **Abenavoli L**, Delibasic M, Peta V, Turkulov V, De Lorenzo A, Medić-Stojanoska M. Nutritional profile of adult patients with celiac disease. *Eur Rev Med Pharmacol Sci* 2015; **19**: 4285-4292 [PMID: 26636515]
77 **García-Manzanares A**, Lucendo AJ. Nutritional and dietary aspects of celiac disease. *Nutr Clin Pract* 2011; **26**: 163-173 [PMID: 21447770 DOI: 10.1177/0884533611399773]
78 **Carroccio A**, Soresi M, D'Alcamo A, Sciumè C, Iacono G, Geraci G, Brusca I, Seidita A, Adragna F, Carta M, Mansueto P. Risk of low bone mineral density and low body mass index in patients with non-celiac wheat-sensitivity: a prospective observation study. *BMC Med* 2014; **12**: 230 [PMID: 25430806 DOI: 10.1186/s12916-014-0230-2]
79 **Zingone F**, Bartalini C, Siniscalchi M, Ruotolo M, Bucci C, Morra I, Iovino P, Ciacci C. Alterations in Diets of Patients With Nonceliac Gluten Sensitivity Compared With Healthy Individuals. *Clin Gastroenterol Hepatol* 2017; **15**: 63-68.e2 [PMID: 27552857 DOI: 10.1016/j.cgh.2016.08.017]
80 **Ierardi E**, Amoruso A, Giorgio F, Principi M, Losurdo G, Piscitelli D, Buffelli F, Fiore MG, Mongelli A, Castellaneta NM, Giangaspero A, De Francesco V, Montenegro L, Di Leo A. Mucosal molecular pattern of tissue transglutaminase and interferon gamma in suspected seronegative celiac disease at marsh

1 and 0 stages. *Saudi J Gastroenterol* 2015; **21**: 379-385 [PMID: 26655133 DOI: 10.4103/1319-3767.167189]
81 **Dinu M**, Macchia D, Pagliai G, Gori AM, Cesari F, Marcucci R, Sofi F, Casini A. Symptomatic efficacy of buckwheat products in Non-Celiac Gluten Sensitivity (NCGS). *Asia Pac J Clin Nutr* 2017; **26**: 630-636 [PMID: 28582812 DOI: 10.6133/apjcn.072016.07]
82 **Brottveit M**, Beitnes AC, Tollefsen S, Bratlie JE, Jahnsen FL, Johansen FE, Sollid LM, Lundin KE. Mucosal cytokine response after short-term gluten challenge in celiac disease and non-celiac gluten sensitivity. *Am J Gastroenterol* 2013; **108**: 842-850 [PMID: 23588237 DOI: 10.1038/ajg.2013.91]
83 **Vici G**, Belli L, Biondi M, Polzonetti V. Gluten free diet and nutrient deficiencies: A review. *Clin Nutr* 2016; **35**: 1236-1241 [PMID: 27211234 DOI: 10.1016/j.clnu.2016.05.002]
84 **Barone M**, Della Valle N, Rosania R, Facciorusso A, Trotta A, Cantatore FP, Falco S, Pignatiello S, Viggiani MT, Amoruso A, De Filippis R, Di Leo A, Francavilla R. A comparison of the nutritional status between adult celiac patients on a long-term, strictly gluten-free diet and healthy subjects. *Eur J Clin Nutr* 2016; **70**: 23-27 [PMID: 26173865 DOI: 10.1038/ejcn.2015.114]
85 **Shepherd SJ**, Gibson PR. Nutritional inadequacies of the gluten-free diet in both recently-diagnosed and long-term patients with coeliac disease. *J Hum Nutr Diet* 2013; **26**: 349-358 [PMID: 23198728 DOI: 10.1111/jhn.12018]

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ORIGINAL ARTICLE

Basic Study

Punctual mutations in *23S rRNA* gene of clarithromycin-resistant *Helicobacter pylori* in Colombian populations

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Abstract

AIM

To characterize punctual mutations in *23S rRNA* gene of clarithromycin-resistant *Helicobacter pylori* (*H. pylori*) and determine their association with therapeutic failure.

METHODS

PCR products of *23S rRNA* gene V domain of 74 *H. pylori* isolates; 34 resistant to clarithromycin (29 from a low-risk gastric cancer (GC) population: Tumaco-Colombia, and 5 from a high-risk population: Tuquerres-Colombia) and 40 from a susceptible population (28 from Tumaco and 12 from Túquerres) were sequenced using capillary electrophoresis. The concordance between mutations of V domain *23S rRNA* gene of *H. pylori* and therapeutic failure was determined using the *Kappa* coefficient and McNemar's test was performed to determine the relationship between *H. pylori* mutations

and clarithromycin resistance.

RESULTS

23S rRNA gene from *H. pylori* was amplified in 56/74 isolates, of which 25 were resistant to clarithromycin (20 from Tumaco and 5 from Túquerres, respectively). In 17 resistant isolates (13 from Tumaco and 4 from Túquerres) the following mutations were found: A1593T1, A1653G2, C1770T, C1954T1, and G1827C in isolates from Tumaco, and A2144G from Túquerres. The mutations T2183C, A2144G and C2196T in *H. pylori* isolates resistant to clarithromycin from Colombia are reported for the first time. No association between the *H. pylori* mutations and *in vitro* clarithromycin resistance was found. However, therapeutic failure of eradication treatment was associated with mutations of *23S rRNA* gene in clarithromycin-resistant *H. pylori* (κ = 0.71).

CONCLUSION

The therapeutic failure of eradication treatment in the two populations from Colombia was associated with mutations of the *23S rRNA* gene in clarithromycin-resistant *H. pylori*.

Key words: Clarithromycin; *In vitro* resistance; Point mutation; *Helicobacter pylori*; Gastric cancer; *23S rRNA*

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Core tip: Mutations in *23S rRNA* gene V domain of *Helicobacter pylori* (*H. pylori*) were studied in order to determine their association with therapeutic failure. In clarithromycin-resistant *H. pylori* isolated from individuals at high-risk of gastric cancer (GC) in Túquerres-Colombia and at low-risk of GC in Tumaco-Colombia, mutations A1593T1, A1653G2, C1770T, C1954T1, and G1827C in isolates from Tumaco, and A2144G from Túquerres were found. Mutations T2183C and C2196T from both cities were not associated with clarithromycin resistance. However, therapeutic failure of eradication treatment in the sampled Colombian populations was associated with mutations of *23S rRNA* gene in clarithromycin-resistant *H. pylori*.

Matta AJ, Zambrano DC, Pazos AJ. Punctual mutations in *23S rRNA* gene of clarithromycin-resistant *Helicobacter pylori* in Colombian populations. *World J Gastroenterol* 2018; 24(14): 1531-1539 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i14/1531.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i14.1531

INTRODUCTION

Eradication of *Helicobacter pylori* (*H. pylori*) from the gastric mucosa is the current treatment for conditions such as chronic gastritis, peptic ulcer, atrophic gastritis, dysplasia, and metaplasia[1]. The first line scheme for the eradication of *H. pylori* is triple therapy, which includes a proton pump inhibitor and two antibiotics such as amoxicillin and clarithromycin. This treatment aims to eradicate infection in at least 90% of patients. However, therapeutic failure is inherent and can be due to multiple factors (human and bacterial), including improper drug dose, short treatment duration, early treatment discontinuation, drug activity associated with the use of other substances, quick reinfection of successfully treated patients, and the presence of antibiotic-resistant strains[1-4]. Among the main causes of resistance to clarithromycin in *H. pylori* are mutations in the V domain of *23S rRNA* gene, this domain is the binding site for macrolide-type antibiotics. The most frequent mutations are A2143G (69.8%), A2142G (11.7%), and A2142C (2.6%). In addition, mutations A2115G, G2141A, C2147G, T2190C, C2195T, A2223G and C2694A have also been reported, but their role in resistance to clarithromycin is not yet clear[3].

In Latin America and worldwide, *H. pylori* resistance to antibiotics has been documented, with eradication being negatively affected by clarithromycin resistance[2]. In Colombia, resistance to this macrolide is estimated to be 17.2%[5]. Geographical conditions have also been documented to influence the risk of gastric cancer (GC). Coastal regions such as Tumaco have a low risk of GC, while Andean regions such as Túquerres have a high risk of GC. Hence, these geographical differences offer unique opportunities for the study of mutations of *23S rRNA* gene in *H. pylori*. This study characterized the mutations of *23S rRNA* gene V domain in *H. pylori* and their association with clarithromycin resistance and with therapeutic failure in patients from two Colombian populations (Tumaco and Túquerres) who were at different risk of developing GC.

MATERIALS AND METHODS

Subjects and samples

The subjects in this study included adult men and women with dyspepsia symptoms from Tumaco (n = 203) and from Túquerres (n = 206). Four gastric mucosal biopsies were obtained from each patient; two from the antrum and two from the gastric body, in order to isolate *H. pylori*, and determine *in vitro* susceptibility of the isolates to clarithromycin and amoxicillin using agar dilution and molecular biology procedures.

For *H. pylori* culture and genotyping, the gastric mucosa biopsies were preserved in 25% thioglycollate and glycerol. The biopsies were frozen in liquid nitrogen and later placed in dry ice and stored at -70 ℃ for analysis at the Microbiology Laboratory and Histopathology Laboratory of the Department of Pathology of the Universidad del Valle, in Cali, Colombia. This study was supported by the CIREH (Human Ethics Committee) of the Universidad del Valle. All study subjects signed an informed consent form.

After the antimicrobial susceptibility microbiological study, 74 *H. pylori* isolates were obtained, of which

34 (46%) were *in vitro* clarithromycin resistant and 40 (54%) were susceptible to the antibiotic. 39.2% (29/34) of the resistant isolates and 37.8% (30/42) of the susceptible isolates were taken from patients in Tumaco. In addition, the sequences of *23S rRNA* gene V domain of strains ATCC 43502 and ATCC 700392 were amplified and used as positive controls. DNA extraction was carried out by salting out[6] and susceptibility tests were performed using the agar dilution method[7].

Amplification of 23S rRNA gene V domain of H. pylori

The amplification of *23S rRNA* gene V domain of *H. pylori* by PCR was carried out using a thermal cycler (Swift MiniProTM, Esco, Cincinnati, OH, United States), and the following reagents were added to a 0.2 mL tube: buffer 1× (Buffer green 5× Promega®), $MgCl_2$ 1 μmol/L (Promega®), DMSO 10%, dNTPs 0.288 mmol/L (Promega®), 50 pmol/μL of each primer (starting position 1585, 5´-GATTGGAGGGAAGGCAAT-3´/3´-CTCCATAAGAGCCAAAGCCC-5´ final position 2247), 0.5 U of GoTaq DNA polymerase (Promega®); and 25 ng of *H. pylori* genomic DNA in a final volume of 50 μL. The thermal cycle consisted in an initial denaturation at 95 ℃/2 min, followed by 35 cycles [95 ℃/1 min, 54 ℃/1 min, 59 ℃/1 min and 72 ℃/1 min] and a final extension at 72 ℃/15 min[8].

The amplification fragments were detected by 2% agarose gel electrophoresis (Sigma®), stained with 1 μL of ethidium bromide (Invitrogen, Carlsbad, CA, United States) (0.5 μg/mL), with an EC-105 power source (Thermo Fisher Scientific Inc., Asheville, NC, United States), at 75 V for 60 min, using a horizontal chamber (Spectroline bio-o-visión®). The DNA bands were visualized in UV light (260/280 nm), using a transilluminator (Spectroline bio-o-visión®). The size of the amplified fragment was approximately 662 pb (expected fragment by *in silico* analysis)[8].

Sequencing and identification of mutations

The amplified fragments were sequenced in two directions (forward and reverse), using a genetic analyzer (ABI 3130 Applied Biosystem®) and the *Big Dye Terminator* methodology (Applied Biosystem®), following standardized conditions at Vanderbilt Genetic Institute Core Facilities, United States. The edition and alignment of the sequences was carried out using Bioedit software V 7.1.11® (Hall, 1999). Changes in sequences were matched by local alignment, with the reference sequence for *23S rRNA* gene, code GenBank: U27270.1[8].

Statistical analysis

For categorical variables, McNemar's Test was used for matching data, in order to identify significant differences between clarithromycin resistant and clarithromycin susceptible genotypes and the punctual mutations detected before treatment. The concordance correlation coefficient *Kappa* (*k*) was used to determine the concordance between the mutations of *23S rRNA* gene V domain and *in vitro* clarithromycin resistance such as the concordance of mutations of *23S rRNA* gene V domain with therapeutic failure in patients evaluated using the [^{13}C]-Urea breath test (UBT), 45 d after completing *H. pylori* eradication treatment. The anti-*H. pylori* treatment included omeprazole (Genfar®) 20 mg, clarithromycin (Genfar®) 500 mg, and amoxicillin (Genfar®) 1000 mg, for 14 d in accordance with the recommendations of the Maastricht Consensus[9]. Therapeutic failure was considered in patients with a positive UBT. All data were analyzed using statistical software SPSS version 15.0 for Windows. Statistical significance was estimated at $P < 0.05$.

Ethical considerations

This study was approved by the Institutional Committee for Human Ethics Revision (CIREH) of the Faculty of Health of the Universidad del Valle, regulated by Resolution 008430 of October 4/1993, issued by the Colombian Ministry of Health.

RESULTS

The prevalence of *H. pylori* infection, which was diagnosed by histopathology, was higher in the low-risk GC population from Tumaco (88.77%), than in the high-risk GC population from Túquerres (85.4%), without a statistically significant difference. However, the prevalence of *H. pylori* resistance to clarithromycin and amoxicillin was significantly higher in the low-risk GC population from Tumaco, than in the high-risk GC population from Túquerres (20.5%, 22.8%) *vs* (3.4%, 5.4%), respectively, $P < 0.05$. Efficacy of the anti-*H. pylori* treatment was similar in both populations. Of 169 infected and treated patients from Tumaco, 130 (76.9%) were cured, and of 165 infected and treated patients from Tuquerres, infection was resolved in 123 (74.6%).

PCR amplification of the 23S rRNA gene of H. pylori

The amplification and sequencing of a fragment of 662 bp (Figure 1) between nucleotides 1585 and 2247 of *23S rRNA* gene V domain of *H. pylori*, was carried out in 56 (76%) of the isolates, of which 39 (69.6%) were from Tumaco patients; of these, 20 (35.7%) were resistant and 19 (33.9%) were susceptible to clarithromycin under *in vitro* conditions. Five (8.9%) of the amplified isolates from Túquerres were resistant to clarithromycin and 12 (21.4%) were susceptible (Table 1).

Table 1, shows the number of *H. pylori* isolates at baseline, which were susceptible and resistant to clarithromycin *in vitro*. The total number of *H. pylori* isolates from both populations and those used to amplify *23S rRNA* gene V domain were evaluated; the number of *H. pylori* isolates amplified from both populations represents fragment amplification where possible. The total number of isolates is represented

Table 1 PCR frequencies of *23S rRNA* gene V domain from *Helicobacter pylori* according to the risk of gastric cancer *n* (%)

Helicobacter pylori isolates	Risk of gastric cancer		Total
	Low risk-Tumaco	High risk-Túquerres	
Evaluated			
Susceptible	28 (37.8)	12 (16.2)	40 (54)
Resistant	29 (39.2)	5 (6.8)	34 (46)
Total	57 (77)	17 (23)	74 (100)
Amplified			56 (76)
Susceptible	19 (33.93)	12 (21.43)	31 (55.4)
Resistant	20 (35.7)	5 (8.93)	25 (44.6)
Total	39 (69.6)	17 (30.4)	56 (100)

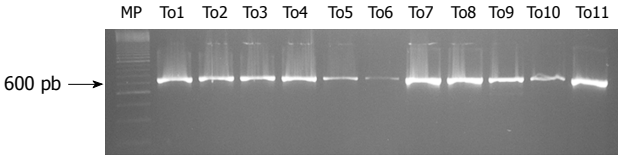

Figure 1 Electrophoretic pattern of PCR products of *23S rRNA* gene V domain in Colombian *Helicobacter pylori* isolates. Electrophoresis of PCR amplification products of *23S rRNA* gene V domain of *Helicobacter pylori* isolates was performed using 2% agarose gel. MP corresponds to the molecular weight marker of 100 bp; the arrow indicates the band corresponding to 600 bp; lanes To1 to To11, correspond to DNA of the isolates resistant to clarithromycin from the Colombian population with a low risk of gastric cancer (Tumaco).

by bold typeface.

Mutations in the 23S rRNA gene of H. pylori and resistance to clarithromycin

At least one mutation was identified in the sequences of 31 (55.3%) *H. pylori* isolates, with 17 (33.3%) resistant and 14 (25%) susceptible to clarithromycin. Of the resistant isolates, 13 (23.2%) were from Túmaco patients and 4 (7.1%) were from Túquerres patients. In addition, 9 (16.1%) of the resistant isolates did not show any mutations in their sequence; of these, 8 (14.3%) were isolated from Tumaco patients and 1 (1.8%) was isolated from Túquerres patients. The *Kappa* coefficients (κ = 0.17) and (κ = 0.23) for the low risk and high risk GC populations, respectively, suggest that there was no relationship between the presence of mutations and *in vitro* resistance to clarithromycin. Similarly, there was no association between the lack of mutations in *23S rRNA* gene and *in vitro* susceptibility to clarithromycin in both populations, $P > 0.05$ (Table 2).

Characterization of mutations in the 23S rRNA gene of H. pylori

Twenty different mutations were characterized in 33 sequences of *H. pylori* evaluated. Mutations T2183C and C2196T were present only in resistant isolates in both populations; the first mutation was observed in 2 isolates from the low risk GC population (Tumaco) and in 1 isolate from the high risk GC population (Túquerres). The second mutation was observed in 1 isolate in each population. Similarly, mutations A1593T, A1653G, C1770T, C1954T, and G1827C, were observed only in resistant isolates in Tumaco patients. Conversely, mutation A2144G was present only in 1 isolate from Túquerres (Tables 3 and 4).

Tables 3 and 4 show the changes in the sequences of *23S rRNA* gene V domain of *H. pylori* in high-risk and low-risk GC patients according to susceptibility or resistance to clarithromycin. Column MIC shows the minimum inhibitory concentration at μg/mL, which was evaluated using the agar dilution method. In Column mutations, the punctual changes in the nucleotides of *23S rRNA* gene observed in the sequence of each isolate are shown.

It was found that the mutations of *H. pylori* susceptible to clarithromycin were located in domain IV of *23S rRNA* gene, nucleotides 1562-1931, except for mutation G2221A which was located in domain V of an isolate susceptible to clarithromycin. In contrast, mutations in domain V, nucleotides 1932-2541, were mainly present in resistant isolates, except for changes C1770T, A1593T and G1827C, which were associated with mutations in domain IV (Table 5).

Mutations in the 23S rRNA gene and therapeutic failure of anti-H. pylori treatment

Although the mutations in isolates resistant to clarithromycin were observed mainly in *23S rRNA* gene V domain of *H. pylori*, no relationship was found between them and *in vitro* resistance to clarithromycin ($P > 0.05$, Tables 2-5). Punctual mutations in domain IV of the target gene were found in susceptible isolates (Table 5). However, the *Kappa* coefficient κ = 0.64 and κ = 0.69 shows that there was a good level of concordance between the mutations in *23S rRNA* gene and therapeutic failure in patients unsuccessfully treated, both in the high-risk and low-risk GC populations, respectively, and the two populations together, κ = 0.71, as shown by the positive UBT, which was performed 45 d after the end of *H. pylori* eradication treatment (Table 6).

DISCUSSION

Research on the prevalence of clarithromycin resistance and characterization of the mutations of *23S rRNA* gene, which may be associated with *in vitro* resistance in *H. pylori,* is scarce in Colombia. In general, research has focused on evaluating the frequency of mutations already

Table 2 Frequencies of mutations in *23S rRNA* gene of *Helicobacter pylori* according to susceptibility to clarithromycin and risk of gastric cancer *n* (%)

Susceptibility	Risk of gastric cancer							
	Low risk *n* = 39				High risk *n* = 17			
	Mutant		Non mutant		Mutant		Non mutant	
Resistant	13 (23.2)		8 (14.3)		4 (7.1)		1 (1.8)	
Susceptible	8 (14.3)		10 (17.8)		6 (10.7)		6 (10.7)	
Kappa-P	*k* = 0.17		*P* = 0.28		*k* = 0.23		*P* = 0.25	
Total	21	37.5	18	32.1	10	17.8	7	12.5

Table 3 Punctual mutations in *23S rRNA* gene of *Helicobacter pylori* from the population at low-risk of gastric cancer, according to susceptibility or resistance to clarithromycin

Resistant *n* = 13			Susceptible *n* = 8		
Patient ID	Mutations	MIC	Patient ID	Mutations	MIC
138	A1593G [1]T2183C	1	17	A1822G/G1827A/G1941A/T1831C	< 0.25
64	A1653G	2	94	T1645C	< 0.25
60		4			
4	A1739G [1]C1954T/G1695A	4	96	A1739G	< 0.25
65	A1739G [1]C2196T [1]G1827C	1	97	T1645C	< 0.25
42	A1822G/G1827A/T1831C	1	98	C1632T	< 0.25
102		2			
174		1			
88	C1632T	> 4	101	A1822G/G1827A/T1645C/T1831C	< 0.25
107	[1]C1770T	1	103	C1632T	< 0.25
38	T1645C	1	107	A1667G/T1668C	< 0.25
36		2			
6	[1]T2183C/A1593T/A1822G/G1827A/T1831C	4			
ATCC 700392	A1593G		ATCC 43504	A1667G/T1668C	

[1]Unique mutations of *Helicobacter pylori* resistant to clarithromycin. MIC: Minimum inhibitory concentration (μg/mL).

Table 4 Punctual mutations in *23S rRNA* gene of *Helicobacter pylori* from the population at high-risk of gastric cancer, according to susceptibility or resistance to clarithromycin

Resistant *n* = 4			Susceptible *n* = 6		
Patient ID	Mutations	MIC	Patient ID	Mutations	MIC
323	A1593G/A1822G/G1827A/T1645C/T1831C/[1]T2183C	1	351	A1822G/G1827A/T1831C	< 0.25
336	A1593G/[1]C2196T	2	377	A1822G/G1827A/G2221A/T1645C/T1831C	< 0.25
339	[1]A2144G/G1827A	4	394	A1593G	< 0.25
440	A1822G/G1827A/G2221A/T1831C	4	457	A1822G/G1827A/G2221A/T1831C	< 0.25
			467	A1739G/G1695A	< 0.25
			513	A1822G/G1827A/T1831C	< 0.25
ATCC 700392	A1593G		ATCC 43504	A1667G/T1668C	

[1]Unique mutations of *Helicobacter pylori* resistant to clarithromycin. MIC: Minimum inhibitory concentration (μg/mL).

reported and the most frequently observed mutations , such as mutations A2142G, A2143G y A2142C[3].

In Colombia, studies carried out in Risaralda, Quindío, and Cauca have reported frequencies between 1.85% and 7.3% for mutation A2142G, and between 2.2% and 2.46% for mutation A2143G in *H. pylori isolates* resistant to *in vitro* clarithromycin[10-12]. In our study, no *H. pylori* isolate which was resistant or susceptible to clarithromycin *in vitro* and exhibited these mutations was detected.

Among the mutations studied in *H. pylori* isolates resistant to clarithromycin was C2196T with a frequency of 0.05% (1/21) and 0.2% (1/5) in isolates from Tumaco and Túquerres patients, respectively. This change was reported in a study carried out in the Province of Guiyang (China), which found resistance of 30% (13/42) to *in vitro* clarithromycin, this study also reported mutation C2196T in a resistant and in a susceptible isolate, and mutation A2143G in susceptible isolates[13]. In contrast to this, mutation C2196T was found only in resistant isolates in our study, with a similar frequency. However, it was not linked to other mutations with such

Table 5 Position of mutations according to the domains of *23S rRNA* gene of *Helicobacter pylori* resistant or susceptible to clarithromycin

Domain-Region	Tumaco		Túquerres	
	Resistant position	Susceptible position	Resistant position	Susceptible position
Domain IV 1562-1931	C1770T	A1593G		A1593G
	A1593T	A1667G		A1667G
	G1827C	A1739G		A1739G
		A1822G		A1822G
		C1632T		C1632T
		G1695A		G1695A
		G1827A		G1827A
		G1941A		G1941A
		T1645C		T1645C
		T1668C		T1668C
		T1831C		T1831C
Domain V 1932-2541	C1954T	G2221A	C2196T	G2221A
	T2183C		T2183C	
	C2196T		A2144G	
	A1653G			
	C2196T			

Table 6 Concordance between mutations in *23S rRNA* gene and success or failure of anti-*Helicobacter pylori* treatment in the studied populations

Breath test [^{13}C]-urea	Population at risk of gastric cancer				Total	
	Low risk n = 39		High risk n = 17			
	Mutant	No mutant	Mutant	No mutant	Mutant	No mutant
Positive						
Therapeutic failure	18	3	8	1	26	4
Negative						
Therapeutic Success	3	15	2	6	5	21
Total	21	18	10	7	31	25
Kappa	k = 0.69		k = 0.64		k = 0.71	

resistance, but it is important to consider the proximity of a nucleotide to mutation C2195T, associated with resistance[3].

Mutation T2183C exhibited frequencies of 0.09 (2/21) and 0.2 (1/5) in resistant isolates from high-risk and low-risk GC patients from Túquerres and Tumaco, respectively. Similar results were reported in studies carried out in *H. pylori* isolates from Korean dyspepsia patients, where the frequency of this mutation was between 0.25 (1/4)[14] and 0.35 (5/14)[15]. Although this mutation is found in domain V and occurred only in isolates resistant to *in vitro* clarithromycin, some researchers believe that its relationship with clarithromycin resistance is not yet clear, as it may be found in isolates both resistant and susceptible to this drug[16,17]. However, its presence in isolates growing at MIC $\geqslant$ 1 μg/mL of clarithromycin, suggests its capability to inhibit the effect of the antibiotic, at least as reported in this study.

Mutation A2144G was found in an *H. pylori* isolate from Túquerres, with a frequency of 0.25 (1/4), which corroborates findings which suggest that the mutation is clearly associated with *in vitro* clarithromycin resistance[18-20]. It was found that the frequency in the sampled population in this study, is in line with the frequencies reported in other regions, 0.01 (1/73)[21] and 0.81(9/11)[20-23]. This mutation was first reported in *H. pylori* isolates resistant to clarithromycin in Colombia, which indicates that it may be associated with the inclusion of strains from high frequency countries such as South Korea (frequency of 0.57)[15]; Japan (frequency of 0.7)[24] and Turkey (frequency between 0.29 and 0.81[20,22].

The mutations associated with clarithromycin resistance in the *H. pylori* isolates described in this study (A2144G, C2196T, and T2183C), are located in *23S rRNA* gene V domain, as reported in the current literature[3]. Inhibition of the action of the macrolide may be due to spatial alterations in the V domain of *23S rRNA* gene, which inhibit the target, as seen in transversion mutations A2143G, A2142G, A2142C[3], A2144G[18,19,22], where a nitrogenous base with two H groups (Adenine) is changed for another with three H groups (Guanine and Cytosine), with the inherent spatial alteration of the molecular structure, a phenomenon similar in transitions C2196T and T2183C[17].

This study found that there was no concordance between the presence of punctual mutations of *H. pylori* and *in vitro* resistance to clarithromycin and no association between the absence of mutations

in the *23S rRNA* gene and *in vitro* susceptibility to clarithromycin in both populations. These findings and the absence of mutations in 36% of the isolates resistant to *in vitro* clarithromycin may be explained by the occurrence of mutations outside the amplified region, a fragment located between positions 1585-2224. Among the changes associated with clarithromycin resistance, which are located outside this fragment, are A2223G, C2694A[3], T2711C[21], T2288C[24], and T2289C[25], and these mutations may explain the discrepancy of the results on the presence of punctual mutations in the amplified region, the *in vitro* resistance to clarithromycin and the good level of concordance between punctual mutations in the *23S rRNA* gene of *H. pylori* with therapeutic failure in patients with unsuccessful eradication treatment. Clarithromycin resistance may be mediated by flow pumps that help *H. pylori* resist concentrations higher than 1 μg/mL of clarithromycin[23,26]. The presence of these mechanisms in *H. pylori* isolates in the high-risk and low-risk GC populations in Colombia was not evaluated in this study.

H. pylori resistance to clarithromycin is the main cause of failed eradication treatment; thus, the characterization of resistance is fundamental to validate gold standard methodology, such as the microbiological method of dilution in agar; however, this is a technically difficult and time-consuming method. It is worth mentioning that in our study, the sequencing method of the amplified *H. pylori* fragments of *23S rRNA* gene by PCR and the detection of their punctual mutations were consistent with the UBT, a method used to diagnose therapeutic failure in patients with unsuccessful treatment, (κ = 0.64, κ = 0.69), both for high-risk and low-risk GC populations (κ = 0.71). These results may be reproducible in future studies, improve *H. pylori* infection eradication regimens and and may be applicable in clinical practice in Colombia. However the UBT is used to evaluate the follow-up of *H. pylori* treatments and its effectiveness should be an additional test in clinical practice and in the programs and policies for the prevention of GC in Colombia.

Although two first-line antibiotics were used in the anti-*H. pylori* treatment regimen, the results of resistance mechanisms in *H. pylori* to amoxicillin were not reported in this study. It is important to emphasize that *H. pylori* resistance to clarithromycin is mainly due to mutations in *23S rRNA* gene V domain and is the main cause of first-line eradication treatment failure[2].

Other techniques that require less time for the identification of resistance include the E-test (sensibility of 45% and specificity of 95%) and DNA-based techniques, such as FISH (sensibility of 97% and specificity of 94%), PNA-FISH (sensibility of 80% and specificity of 93%), *Line Probe Test* (sensibility of 100% and specificity of 82.2%), and PCR (sensibility of 98% and specificity of 92%)[3], which require specific methods for each mutation (FISH; PNA-FISH, *Line Probe Test*) or sequencing of the amplified fragment (PCR). The efficiency of these tests is subject to knowledge of the mutations associated with clarithromycin resistance in *H. pylori* strains.

This study demonstrated that the resistant isolates from these two contrasting populations involved in the development of GC, mutations A2143G, A2142G, and A2142C, which are usually reported as the most frequent, were not found in the isolates evaluated. With regard to the design of these tests, the changes A2144G, T2183C and C2196T found in these populations should be considered for use in fast-diagnostic methods of clarithromycin resistance in clinical practice. These mutations associated with *H. pylori* resistance to clarithromycin are the first to be reported in Colombia.

It may be concluded that in *H. pylori* isolates resistant to clarithromycin in patients from both Colombian populations, no high-frequency mutation was observed in *23S rRNA* gene V domain, but there was high genotypic variation among the isolates.

No relationship between the mutations in *23S rRNA* gene V domain of *H. pylori* and *in vitro* resistance was found, contrary to that seen in other *H. pylori* non-mutant isolates resistant to clarithromycin, which may be explained by mutations outside the evaluated fragment or by the existence of flow pumps. However, the failure of eradication treatment in the Colombian populations in this study was associated with punctual mutations in *23S rRNA* gene of *H. pylori* resistant to clarithromycin.

In the Colombian populations studied, it was difficult to use a fast-resistance detection test for specific mutations, as information is scarce and the mutations reported exhibited a low frequency.

ARTICLE HIGHLIGHTS

Research background

Infection by *Helicobacter pylori* (*H. pylori*) is the leading risk factor for the development of gastric adenocarcinoma, especially in individuals infected with strains resistant to antibiotics used in primary treatment regimens. The eradication of *H. pylori* infection is a valid primary prevention strategy for gastric lesions, atrophy, and gastric cancer (GC). However, resistance of this microorganism to clarithromycin is associated with therapeutic failure and a major risk of GC in Colombia. Thus, although significant improvements in the efficacy of treatment regimens have been made, none of these regimens successfully eradicate the infection. A few studies have focused on the evaluation of clarithromycin-resistance mechanisms, particularly mutations of *23S rRNA* gene of the infecting strains in Colombia, which are associated with treatment failure and early subsequent prevention of GC.

Research motivation

Taking into account that GC prevention programs are focused on the eradication of *H. pylori*, it is important to know the specific treatment regimens for each country seeking to apply this strategy. In Colombia, the efficacy of standard triple therapy which includes clarithromycin, amoxicillin, and a proton pump inhibitor is currently being questioned. However, there are insufficient multicenter studies suggesting alternative regimens and basic studies on antibiotic resistance mechanisms in *H. pylori*. Mutations in *H. pylori 23S rRNA* gene V domain were studied to evaluate *in vitro* resistance to clarithromycin. This study identified mutations not documented in the current literature, which although are not associated with *in vitro* resistance to clarithromycin, they are

linked to the therapeutic failure of triple therapy. Punctual mutations in the Colombian strains could be useful in future studies focusing on diagnostic methods for antibiotic susceptibility and in the therapeutic efficacy of GC prevention schemes in Colombia.

Research objectives

In this study, the researchers characterized mutations in domain V of *23S rRNA* gene in clarithromycin-resistant *H. pylori* and determined their association with therapeutic failure in a high-risk gastric cancer population from Tuquerres, Colombia, and in a low-risk gastric cancer population from Tumaco, Colombia. A very interesting basic study clearly showed that therapeutic failure of eradication treatment in the sampled Colombian populations was associated with mutations of *23S rRNA* gene in clarithromycin-resistant *H. pylori*. Hopefully, these findings will help to further improve treatment success and may be applied in the future for the fast diagnosis of therapeutic failure. This study found no concordance between the presence of punctual mutations in *H. pylori* and *in vitro* resistance to clarithromycin and there was no association between the absence of mutations in the *23S rRNA* gene and *in vitro* susceptibility to clarithromycin in both populations. These findings and the absence of mutations in 36% of the isolates resistant to *in vitro* clarithromycin may be explained by the occurrence of mutations outside the amplified region, a fragment located between positions 1585-2224. Among the changes associated with clarithromycin resistance, which are located outside this fragment, are A2223G, C2694A T2711C, T2288C, and T2289C, mutations that may explain the discrepancy between the presence of punctual mutations in the amplified region and *in vitro* resistance to clarithromycin

Research methods

To achieve the objectives of this study, we used the capillary electrophoresis sequencing method of the amplified DNA fragments of the *H. pylori 23S rRNA* gene and the detection of its punctual mutations, which were concordant with the [^{13}C]-Urea breath test. This method was used in a novel way to diagnose the therapeutic failure of anti-*H. pylori* treatment *in vivo*. The [^{13}C]-Urea breath test was used during the follow-up period to evaluate the effectiveness of *H. pylori* treatments.

Research results

This study demonstrated that the resistant isolates from these two contrasting populations involved in the development of GC, mutations A2143G, A2142G, and A2142C, which are usually reported as the most frequent, were not found in the isolates evaluated. With regard to the design of tests, the changes A2144G, T2183C and C2196T found in these populations should be considered for use in fast-diagnostic methods of clarithromycin resistance in clinical practice.

These results are important in the definition of treatments for gastroduodenal diseases caused by *H. pylori*. They suggest that the failure of anti-*H. pylori* treatment is mainly due to mutations in *23S rRNA* gene V domain. The application of these findings could be complemented by studies on the genetics and virulence of the microorganism, as individuals with similar ancestry may not require anti-*H. pylori* treatment. In contrast individuals infected with strains of different evolutionary origins than their host, would benefit from additional studies on antibiotic susceptibility. These advances in basic studies tend to elucidate the African enigma, and indicate that human-*H. pylori* coevolution and virulence of the bacterium could explain the contrast in risk of disease observed in our study populations. These findings may contribute to the future identification of individuals at higher risk of GC and require antibiotic susceptibility studies prior to treatment of the infection and early GC prevention.

Research conclusions

In this investigation, mutations A2144G, C2196T and T2183C were observed in *23S rRNA* gene V domain of *H. pylori* resistant to clarithromycin and were associated with failure of eradication treatment. The mutations T2183C, A2144G and C2196T in *23S rRNA* gene V domain are reported for the first time in clarithromycin-resistant isolates of *H. pylori* in Colombia. This study demonstrated that the therapeutic failure of *H. pylori* eradication treatment in high and low risk GC populations from Colombia was associated with mutations of the *23S rRNA* gene of clarithromycin-resistant *H. pylori*. The sequencing method for the detection of punctual mutations of DNA amplified *23S rRNA* gene fragments is proposed to predict therapeutic failure induced by clarithromycin-resistant *H. pylori*. This new knowledge allows us to propose the design of a rapid detection test for *H. pylori* resistance to clarithromycin where mutations A2144G, T2183C and C2196T should be considered and can be applied in clinical practice to predict therapeutic failure of anti-*H. pylori* treatment.

Research perspectives

Following therapeutic failure, reinfection may occur in patients as well as medication with antagonistic drugs or others such as proton pump inhibitors, which allow the appearance of false positives. In this study, adherence to treatment and self-medication were taken into account during the follow-up period. Characterization of the mutations in the *23S rRNA* gene in a larger number of Colombian populations is required, in order to confirm the mutations associated with clarithromycin resistance in *H. pylori* and to determine, from multicenter studies, the optimal treatment regimen in Colombia. The molecular analysis of *23S rRNA* gene V domain of *H. pylori* and other candidate genes is required, in order predict therapeutic failure. It is possible to reproduce the method in future investigations using total DNA from gastric mucosa biopsies and validate the presence of mutations found in this study. The [^{13}C]-Urea breath test is recommended during follow-up to evaluate the effectiveness of anti-*H. pylori* treatment.

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REFERENCES

1 **Wu W**, Yang Y, Sun G. Recent Insights into Antibiotic Resistance in Helicobacter pylori Eradication. *Gastroenterol Res Pract* 2012; **2012**: 723183 [PMID: 22829809 DOI: 10.1155/2012/723183]
2 **Camargo MC**, García A, Riquelme A, Otero W, Camargo CA, Hernandez-García T, Candia R, Bruce MG, Rabkin CS. The problem of Helicobacter pylori resistance to antibiotics: a systematic review in Latin America. *Am J Gastroenterol* 2014; **109**: 485-495 [PMID: 24589670 DOI: 10.1038/ajg.2014.24]
3 **Thung I**, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, Valasek MA. Review article: the global emergence of Helicobacter pylori antibiotic resistance. *Aliment Pharmacol Ther* 2016; **43**: 514-533 [PMID: 26694080 DOI: 10.1111/apt.13497]
4 **Ghotaslou R**, Leylabadlo HE, Asl YM. Prevalence of antibiotic resistance in Helicobacter pylori: A recent literature review. *World J Methodol* 2015; **5**: 164-174 [PMID: 26413490 DOI: 10.5662/wjm.v5.i3.164]
5 **Trespalacios AA,** Otero W, Marcela M. Helicobacter pylori resistance to metronidazole, clarithromycin and amoxicillin in Colombian patients. *Rev Colomb Gastroenterol* 2010; **25**: 31-38
6 **Ho SA**, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR. Direct polymerase chain reaction test for detection of Helicobacter pylori in humans and animals. *J Clin Microbiol* 1991; **29**: 2543-2549 [PMID: 1723072]
7 **Bustamante-Rengifo JA**, Matta AJ, Pazos A, Bravo LE. In vitro effect of amoxicillin and clarithromycin on the 3' region of cagA gene in Helicobacter pylori isolates. *World J Gastroenterol* 2013; **19**: 6044-6054 [PMID: 24106405 DOI: 10.3748/wjg.v19.i36.6044]
8 **Taylor DE**, Ge Z, Purych D, Lo T, Hiratsuka K. Cloning and sequence analysis of two copies of a 23S rRNA gene from Helicobacter pylori and association of clarithromycin resistance with 23S rRNA mutations. *Antimicrob Agents Chemother* 1997;

41: 2621-2628 [PMID: 9420030]
9 **Malfertheiner P**, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM; European Helicobacter and Microbiota Study Group and Consensus panel. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; **66**: 6-30 [PMID: 27707777 DOI: 10.1136/gutjnl-2016-312288]
10 **Isaza MC,** Henao BJ, Alvarez A, Moncayo JI, Santacruz JJ, Meisel E, Salazar F, Giraldo D. Comparación de dos protocolos de erradicación de Helicobacter pylori. *Rev Médica Risaralda* 2007; **13**: 1-8
11 **Alvarez A**, Moncayo JI, Santacruz JJ, Corredor LF, Reinosa E, Martínez JW, Beltrán L. [Antimicrobial susceptibility of Helicobacter pylori strains isolated in Colombia]. *Rev Med Chil* 2009; **137**: 1309-1314 [PMID: 20011937]
12 **Acosta CP**, Hurtado FA, Trespalacios AA. [Determination of single nucleotide mutations in the 23S rRNA gene of Helicobacter pylori related to clarithromycin resistance in a population from Cauca, Colombia]. *Biomedica* 2014; **34** Suppl 1: 156-162 [PMID: 24968047 DOI: 10.7705/biomedica.v34i0.1649]
13 **Boyanova L**, Markovska R, Yordanov D, Gergova G, Mitov I. Clarithromycin Resistance Mutations in Helicobacter pylori in Association with Virulence Factors and Antibiotic Susceptibility of the Strains. *Microb Drug Resist* 2016; **22**: 227-232 [PMID: 26618567 DOI: 10.1089/mdr.2015.0199]
14 **Yoon KH**, Park SW, Lee SW, Kim BJ, Kim JG. Clarithromycin-based standard triple therapy can still be effective for Helicobacter pylori eradication in some parts of the Korea. *J Korean Med Sci* 2014; **29**: 1240-1246 [PMID: 25246742 DOI: 10.3346/jkms.2014.29.9.1240]
15 **Sung J**, Kim N, Park YH, Hwang YJ, Kwon S, Na G, Choi JY, Kang JB, Kim HR, Kim JW, Lee DH. Rifabutin-based Fourth and Fifth-line Rescue Therapy in Patients with for Helicobacter pylori Eradication Failure. *Korean J Gastroenterol* 2017; **69**: 109-118 [PMID: 28239079 DOI: 10.4166/kjg.2017.69.2.109]
16 **Hwang TJ**, Kim N, Kim HB, Lee BH, Nam RH, Park JH, Lee MK, Park YS, Lee DH, Jung HC, Song IS. Change in antibiotic resistance of Helicobacter pylori strains and the effect of A2143G point mutation of 23S rRNA on the eradication of H. pylori in a single center of Korea. *J Clin Gastroenterol* 2010; **44**: 536-543 [PMID: 20179610 DOI: 10.1097/MCG.0b013e3181d04592]
17 **Teh X**, Khosravi Y, Lee WC, Leow AH, Loke MF, Vadivelu J, Goh KL. Functional and molecular surveillance of Helicobacter pylori antibiotic resistance in Kuala Lumpur. *PLoS One* 2014; **9**: e101481 [PMID: 25003707 DOI: 10.1371/journal.pone.0101481]
18 **Momynaliev KT**, Selezneva OV, Kozlova AA, Vereshchagin VA, Il'ina EN, Govorun VM. [A2144G is the main mutation in the 23S rRNA gene of Helicobacter pylori associated with clarithromycin resistance]. *Genetika* 2005; **41**: 1338-1344 [PMID: 16316005]
19 **Sezgin O**, Aslan G, Altintaş E, Tezcan S, Serin MS, Emekdaş G. Detection of point mutations on 23S rRNA of Helicobacter pylori and resistance to clarithromycin with PCR-RFLP in gastric biopsy specimens in Mersin, Turkey. *Turk J Gastroenterol* 2008; **19**: 163-167 [PMID: 19115151]
20 **Caliskan R**, Tokman HB, Erzin Y, Saribas S, Yuksel P, Bolek BK, Sevuk EO, Demirci M, Yılmazli O, Akgul O, Kalayci F, Cakan H, Salih B, Bal K, Kocazeybek B. Antimicrobial resistance of Helicobacter pylori strains to five antibiotics, including levofloxacin, in Northwestern Turkey. *Rev Soc Bras Med Trop* 2015; **48**: 278-284 [PMID: 26108005 DOI: 10.1590/0037-8682-0027-2015]
21 **Toracchio S**, Aceto GM, Mariani-Costantini R, Battista P, Marzio L. Identification of a novel mutation affecting domain V of the 23S rRNA gene in Helicobacter pylori. *Helicobacter* 2004; **9**: 396-399 [PMID: 15361077 DOI: 10.1111/j.1083-4389.2004.00267.x]
22 **Tajbakhsh S,** Falahi J, Motamed N, Tabib SM, Bahador A, Gharibi S. Prevalence of A2143G and A2144G point mutations responsible for clarithromycin resistance among Helicobacter pylori strains in Bushehr, Iran. *Avicenna J Clin Microb Infec* 2016; **3**: e36521 [DOI: 10.17795/ajcmi-36521]
23 **Kim JM**, Kim JS, Jung HC, Kim N, Kim YJ, Song IS. Distribution of antibiotic MICs for Helicobacter pylori strains over a 16-year period in patients from Seoul, South Korea. *Antimicrob Agents Chemother* 2004; **48**: 4843-4847 [PMID: 15561865 DOI: 10.1128/AAC.48.12.4843-4847.2004]
24 **Rimbara E**, Noguchi N, Kijima H, Yamaguchi T, Kawai T, Sasatsu M. Mutations in the 23S rRNA gene of clarithromycin-resistant Helicobacter pylori from Japan. *Int J Antimicrob Agents* 2007; **30**: 250-254 [PMID: 17590317 DOI: 10.1016/j.ijantimicag.2007.04.009]
25 **Hao Q**, Li Y, Zhang ZJ, Liu Y, Gao H. New mutation points in 23S rRNA gene associated with Helicobacter pylori resistance to clarithromycin in northeast China. *World J Gastroenterol* 2004; **10**: 1075-1077 [PMID: 15052698 DOI: 10.3748/wjg.v10.i7.1075]
26 **Hirata K**, Suzuki H, Nishizawa T, Tsugawa H, Muraoka H, Saito Y, Matsuzaki J, Hibi T. Contribution of efflux pumps to clarithromycin resistance in Helicobacter pylori. *J Gastroenterol Hepatol* 2010; **25** Suppl 1: S75-S79 [PMID: 20586871 DOI: 10.1111/j.1440-1746.2009.06220.x]

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ORIGINAL ARTICLE

Retrospective Study

Post-polypectomy bleeding and thromboembolism risks associated with warfarin *vs* direct oral anticoagulants

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Author contributions: Yanagisawa N collected the clinical data and drafted the manuscript; Nagata N designed the study and is equally a first author; Shimbo T was responsible for statistical analysis; Yanagisawa N, Iida T, Hamada M and Kobayashi S performed data collection and are the main authors of the manuscript; Watanabe K and Akiyama J assisted with treatment; Akiyama J and Uemura N edited the manuscript; all authors read and approved the submitted version of the manuscript.

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Abstract

AIM

To verify the validity of the endoscopy guidelines for patients taking warfarin or direct oral anticoagulants (DOAC).

METHODS

We collected data from 218 patients receiving oral anticoagulants (73 DOAC users, 145 warfarin users) and 218 patients not receiving any antithrombotics (age- and sex-matched controls) who underwent polypectomy. (1) We evaluated post-polypectomy bleeding (PPB) risk in patients

receiving warfarin or DOAC compared with controls; (2) we assessed the risks of PPB and thromboembolism between three AC management methods: Discontinuing AC with heparin bridge (HPB) (endoscopy guideline recommendation), continuing AC, and discontinuing AC without HPB.

RESULTS

PPB rate was significantly higher in warfarin users and DOAC users compared with controls (13.7% and 13.7% *vs* 0.9%, $P < 0.001$), but was not significantly different between rivaroxaban (13.2%), dabigatran (11.1%), and apixaban (13.3%) users. Two thromboembolic events occurred in warfarin users, but none in DOAC users. Compared with the continuing anticoagulant group, the discontinuing anticoagulant with HPB group (guideline recommendation) had a higher PPB rate (10.8% *vs* 19.6%, $P = 0.087$). These findings were significantly evident in warfarin but not DOAC users. One thrombotic event occurred in the discontinuing anticoagulant with HPB group and the discontinuing anticoagulant without HPB group; none occurred in the continuing anticoagulant group.

CONCLUSION

PPB risk was similar between patients taking warfarin and DOAC. Thromboembolism was observed in warfarin users only. The guideline recommendations for HPB should be re-considered.

Key words: High-risk endoscopic procedures; Novel oral anticoagulants; Endoscopic guideline validation; Post-procedure gastrointestinal bleeding

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Core tip: First, we found that anticoagulant (AC) users were at higher risk of post-polypectomy bleeding (PPB) than controls. Second, PPB risk was similar between warfarin users and direct oral anticoagulant users, whereas thromboembolism risk was observed only in warfarin users. Third, PPB risk was not significantly different between rivaroxaban, dabigatran, and apixaban users. Fourth, the strategy of discontinuing AC with heparin bridge as recommended in the endoscopy guidelines showed a higher bleeding rate than continuing AC alone and had one thrombotic event, thus indicating that heparin bridge increased bleeding and may not prevent thromboembolism.

Yanagisawa N, Nagata N, Watanabe K, Iida T, Hamada M, Kobayashi S, Shimbo T, Akiyama J, Uemura N. Post-polypectomy bleeding and thromboembolism risks associated with warfarin *vs* direct oral anticoagulants. *World J Gastroenterol* 2018; 24(14): 1540-1549 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i14/1540.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i14.1540

INTRODUCTION

The number of oral anticoagulants (AC) used for prophylaxis or treatment of thromboembolic events is expected to increase as the population ages[1,2]. Along with this, the number of colonoscopic polypectomies, the most common high-risk endoscopic procedure, is also expected to increase in patients receiving AC[3-5]. Physicians are thus confronted with the issue of striking a balance between performing procedures with bleeding risk, such as polypectomy, and temporarily discontinuing AC agents to mitigate thromboembolic risk[4,6-8]. Among the AC agents commonly prescribed, warfarin requires careful and complex management because of its intricate pharmacodynamics and narrow therapeutic range[2,9], whereas direct oral anticoagulants (DOAC) offer easier management because of the rapid onset of anticoagulation and short half-lives[10]. However, whether post-polypectomy bleeding (PPB) or thromboembolic risk differs between warfarin and DOAC users remains unknown.

Several endoscopy guidelines recommend that warfarin be discontinued and replaced by heparin bridge (HPB) in patients at high thromboembolic risk during polypectomy[6-8]. In one study, DOAC were also stopped in one-third of patients who underwent HPB for a high-risk endoscopic procedure[11]. As yet however, the guideline recommendation on AC management for polypectomy has not been confirmed by a validation study. In addition, the situation is further complicated in the real-world clinical setting as some physicians may choose to continue the AC agent or to discontinue it without HPB in the peri-endoscopic period[11]. Previous data suggest that patients undergoing HPB are at higher risk of procedural-related bleeding than those not undergoing HPB or continuing their warfarin[12,13]. Therefore, continuing the AC strategy without HPB may be acceptable for polypectomy. However, there are currently no data available on the comparative risks of bleeding and thromboembolism between patients discontinuing AC with HPB, continuing AC, or discontinuing AC without HPB.

To address these gaps in our knowledge, in this study we first evaluated PPB risk in patients receiving warfarin or DOAC compared with patients not receiving any antithrombotics (controls). Second, we assessed the risks of PPB and thromboembolism between the three AC management methods mentioned above, discontinuing AC with HPB (guideline recommendation), continuing AC, and discontinuing AC without HPB.

MATERIALS AND METHODS

Study design, setting, and participants

We conducted a retrospective cohort study at the Department of Gastroenterology, National Center for Global Health and Medicine (NCGM), Japan. NCGM, with 900 beds, is the largest emergency hospital in the Tokyo

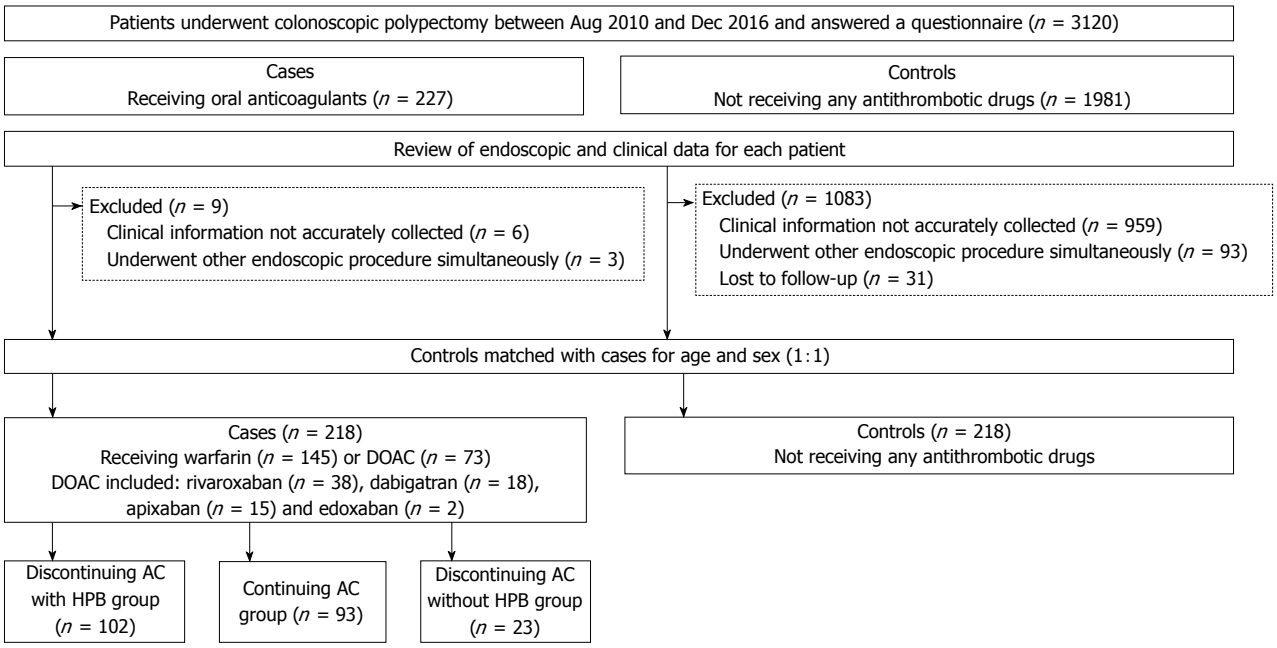

Figure 1 Patient selection and flow. AC: Anticoagulants; DOAC: Direct oral anticoagulants; HPB: Heparin bridge.

metropolitan area. We collected clinical and endoscopic data using an electronic medical database (MegaOak online imaging system, NEC, Japan) and an electronic endoscopic database (SolemioEndo, Olympus, Japan). Physicians or nurses input all findings immediately after clinical evaluation or endoscopy into the electronic medical and endoscopic reports. Staff also completed a detailed questionnaire that included patient background factors and medication information during a face-to-face interview with each patient at the endoscopy unit on the same day as pre-colonoscopy[14,15]. Patient selection and the study flow are shown in Figure 1. From the databases, we identified 5950 patients who underwent colonoscopic polypectomy at our institution between August 2010 and December 2016. Of these, 3120 provided responses to the questionnaire during the interview. We identified 227 patients receiving oral AC (cases) and 1981 patients not receiving any antithrombotics (controls). Then, we reviewed the clinical and endoscopic data for each patient and excluded the following patients: among cases, 6 patients whose clinical information could not be accurately collected and 3 patients who underwent polypectomy plus endoscopic submucosal dissection (ESD) simultaneously; among controls, 959 patients whose clinical information could not be accurately collected, 93 patients who underwent polypectomy plus another endoscopic procedure simultaneously, and 31 patients who were lost to follow-up. Then, controls (non-users of antithrombotics) were randomly selected from the cases (AC users) matched for decennial age and sex at a ratio of 1:1. Ultimately, data from a total of 436 patients (218 AC users and 218 controls) were analyzed.

This study was approved by the institutional review board of NCGM and patient consent was waived as this was a retrospective study (approval number 2176).

Patient characteristics

Using the electronic database and prospectively collected questionnaire results, we assessed the following factors: height, weight, body mass index (BMI), alcohol, smoking, 14 comorbidities or past history (diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, abnormal liver function, stroke, bleeding past history, chronic heart disease, vascular disease, acute coronary syndrome, pulmonary embolism, peripheral arterial disease, deep vein disease and advanced cancer), and medication [warfarin, rivaroxaban, dabigatran, apixaban, edoxaban, antiplatelet, and non-steroidal anti-inflammatory drugs (NSAIDs)]. We also evaluated laboratory data before colonoscopy [platelet count, prothrombin time-international normalized ratio (PT-INR), and creatinine clearance (Ccr)] and calculated the HAS-BLED[16] and CHA2DS2-VASc2[17] scores. During hospitalization, data were collected on the following AC management factors: HPB use, HPB duration, drug continuation/discontinuation, and use of reversal agent (vitamin K).

Endoscopic factors

After full bowel preparation, polypectomies were done with or without local injection of saline using a high-resolution colonoscope (CF260AI or CF260AZI, Olympus Co., Tokyo, Japan), snare (SnareMaster, Olympus Co.), and electrosurgical device (ERBE ICC-350, Somo Technology Inc., Tokyo, Japan or ESG-100, Olympus Co.). After polypectomy, patients routinely underwent prophylactic clipping. Number of polyps and polyp size were evaluated from data in the endoscopic database. Advanced ade-

noma was defined as adenoma ⩾ 1 cm with villous components (tubulovillous or villous) or high-grade or severe dysplasia[18].

AC management and heparin bridge

American, European, and Asian guidelines[6-8] recommend that patients discontinue AC and be bridged with heparin before polypectomy, and to confirm the validity of this strategy, we classified patients during the peri-endoscopic period into three main AC management groups: (1) Discontinuing AC with HPB (as recommended by the guidelines); (2) continuing AC alone (*i.e.*, without HPB) before endoscopy; and (3) discontinuing AC for > 24 h without HPB before endoscopy. Which of these strategies was adopted was at the discretion of the treating physician.

For HPB, patients received prophylactic unfractionated heparin infusion intravenously (because low-molecular-weight heparin is not covered by Japan's health care insurance system[8,19]), with the exception of 1 patient who received low-molecular-weight heparin because of heparin-induced thrombocytopenia.

In our institution, we carry out anticoagulant management during high-risk endoscopy in accordance with the Japanese Endoscopy Guidelines[8]; warfarin was stopped 3-5 d before endoscopy and DOAC was stopped 24-48 h after endoscopy. Heparin was administered after cessation of anticoagulants[8]. INR value before polypectomy was set at < 1.5 in warfarin users[8]. In these users, heparin was continued until INR was optimal after polypectomy. Because the guidelines do not recommend HPB for DOAC users[8], some DOAC users continued heparin for one day and others did not use heparin after polypectomy. The HPB period included the entire period before and after polypectomy.

Clinical outcomes

The main outcomes of interest were PPB within 30 d of polypectomy. PPB was defined as massive, continuous, or frequent hematochezia after polypectomy[20]. Not all patients underwent additional colonoscopy when PPB occurred, but those with unstable vital signs or in need of transfusion tended to undergo colonoscopy. Major bleeding was defined according to the International Society on Thrombosis and Haemostasis (ISTH) bleeding scale as (1) fatal bleeding; and/or (2) symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, or intramuscular with compartment syndrome; and/or (3) bleeding causing a fall in hemoglobin level of 20 g/L (1.24 mmol/L) or more, or leading to transfusion of two or more units of whole blood or red cells[21]. In addition, we defined late PPB as bleeding occurring more than 24 h after polypectomy and all other cases as early PPB[22]. We defined a thromboembolic event as the occurrence of acute coronary syndrome, stroke, transient ischemic attack, pulmonary embolism, deep vein thrombosis, or arterial thromboembolism. We also evaluated mortality at 30 d after polypectomy. Date and cause of death were ascertained from the electronic medical records and death certificates.

Statistical analysis

Pearson's chi-squared test or Fisher's exact test was used for categorical data to assess the difference in risk factors between subjects. Continuous data were compared with Mann-Whitney *U* test. Risk factors were examined by univariate and multivariate analysis. Odds ratios (OR) and 95% confidence intervals (CI) were estimated.

First, we compared baseline characteristics and clinical outcomes between AC users and controls. Second, we compared baseline characteristics and clinical outcomes between the following groups: discontinuing AC with HPB and continuing AC group alone and between discontinuing AC with HPB group and discontinuing AC without HPB group. These comparisons were also evaluated for the subgroups of warfarin and DOAC users.

Third, to determine the risk factors for PPB, we conducted univariate and multivariate analysis. In multivariate analysis, we developed multivariate models adjusting for propensity score for each strategy. Although there are four different propensity score methods-matching, stratification, inverse probability treatment weighting, and covariates adjustment[23,24]-we used propensity score as a covariate rather than perform a regression adjustment with all of the covariates (traditional covariate adjustment[25]), because many covariates were associated with a small number of bleeding outcomes in this study and we did not want to lose the observations of patients as typically occurs in matching. Propensity score as a covariate method allows for a large number of baseline variables to be included in the regression model, which are not adequately adjusted for when there are insufficient numbers of outcomes[23,24]. To estimate the propensity score, we employed a logistic regression model including potentially clinically important variables. Some of these were shown to differ ($P < 0.10$) between groups. We evaluated the area under the receiver operating characteristic (ROC) curve for each propensity score in each group.

A P value of < 0.05 was considered statistically significant. All statistical analyses were conducted using STATA version 14 software (StataCorp, College Station, TX, United States).

RESULTS

Baseline characteristics and outcomes of AC users and controls

There were some significant differences in baseline characteristics between AC users and controls (Table 1). In terms of outcomes, there were 32 patients with PPB and only 2 patients with major bleeding, both of whom were warfarin users and received HPB. Four patients had early PPB (bleeding within 24 h) and 28 with late PPB: 9 cases at day 2, 9 cases at day 3, 6 cases at day

Table 1 Baseline characteristics of oral anticoagulant users, warfarin users, direct oral anticoagulants users, and controls not taking any antithrombotic drugs (*n* = 436) *n* (%)

Factors	Controls (*n* = 218)	AC users (*n* = 218)	*P* value Control *vs* AC users	Warfarin users (*n* = 145)	*P* value Control *vs* warfarin users	DOAC users (*n* = 73)	*P* value Control *vs* DOAC users
Age ⩾ 75 yr	104 (47.1)	113 (51.8)	0.389	79 (54.5)	0.206	34 (46.6)	0.867
Male	157 (72.0)	157 (72.0)	1.000	103 (71.0)	0.839	54 (74.0)	0.746
BMI ⩾ 25	54 (24.8)	69 (31.7)	0.110	44 (30.3)	0.241	25 (34.2)	0.115
Drinker	119 (54.6)	131 (62.1)	0.115	77 (55.4)	0.881	54 (75.0)	0.002
Smoker	36 (16.5)	32 (14.8)	0.626	21 (14.6)	0.622	11 (15.3)	0.805
Laboratory data							
Platelet < 10×10^4 μL	6 (2.8)	5 (2.3)	1.000	3 (2.1)	1.000	2 (2.7)	1.000
Ccr < 30 mL/min	9 (4.1)	24 (11.0)	0.007	20 (13.8)	0.001	4 (5.48)	0.743
Comorbidities							
Diabetes mellitus	45 (20.6)	52 (23.9)	0.420	39 (26.9)	0.166	13 (17.8)	0.600
Hypertension	121 (55.5)	148 (67.9)	0.008	94 (64.8)	0.077	54 (74.0)	0.005
Dyslipidemia	74 (33.9)	102 (46.8)	0.006	67 (46.2)	0.019	35 (48.0)	0.037
Chronic kidney disease	49 (22.5)	37 (17.0)	0.149	32 (22.1)	0.927	5 (6.9)	0.003
Abnormal liver function	15 (6.9)	8 (3.7)	0.134	3 (2.1)	0.047	5 (6.9)	0.993
Stroke	10 (4.6)	47 (21.6)	< 0.001	29 (20.0)	< 0.001	18 (24.7)	< 0.001
Bleeding past history	21 (9.6)	13 (6.0)	0.153	10 (6.9)	0.361	3 (4.1)	0.217
Chronic heart failure	1 (0.5)	56 (25.7)	< 0.001	46 (31.7)	< 0.001	10 (13.7)	< 0.001
Vascular disease	6 (2.8)	56 (25.7)	< 0.001	49 (33.8)	< 0.001	7 (9.6)	0.014
Acute coronary syndrome	6 (2.8)	34 (15.6)	< 0.001	28 (19.3)	< 0.001	6 (8.2)	0.042
Pulmonary embolism	0 (0.0)	7 (3.2)	0.008	6 (4.1)	0.004	1 (1.4)	0.251
Peripheral arterial disease	0 (0.0)	7 (3.2)	0.008	6 (4.1)	0.004	1 (1.4)	0.251
Deep vein thrombosis	0 (0.0)	14 (6.4)	< 0.001	14 (9.7)	< 0.001	0	NA
Advanced carcinoma	7 (3.2)	33 (15.1)	< 0.001	21 (14.5)	< 0.001	12 (16.4)	< 0.001
Medications							
Antiplatelet	0 (0.0)	53 (24.3)	< 0.001	43 (30.0)	< 0.001	10 (13.7)	< 0.001
Low-dose aspirin	0 (0.0)	40 (18.4)	< 0.001	33 (22.8)	< 0.001	7 (9.6)	< 0.001
Thienopyridine[1]	0 (0.0)	5 (2.3)	0.025	5 (3.5)	0.006	0 (0.0)	NA
Other antiplatelets[2]	0 (0.0)	11 (5.1)	0.001	8 (5.5)	< 0.001	3 (4.1)	0.003
NSAIDs	21 (9.6)	7 (3.2)	0.006	3 (2.1)	0.004	4 (5.5)	0.341
Endoscopic factors							
Number of polyps	2.0 ± 1.4	8.3 ± 5.3	0.019	2.4 ± 1.8	0.063	2.5 ± 1.8	0.041
Number of polyps ⩾ 5	13 (6.0)	28 (12.8)	0.014	17 (11.7)	0.078	11 (15.1)	0.014
Polyp size	6.0 ± 3.3	6.3 ± 3.4	< 0.001	8.7 ± 5.9	< 0.001	7.4 ± 3.7	0.001
Polyp size ⩾ 10 mm	28 (12.8)	69 (31.7)	< 0.001	47 (32.4)	< 0.001	22 (30.1)	0.001
Advanced adenoma[3]	27 (12.4)	64 (29.4)	< 0.001	43 (29.7)	< 0.001	21 (28.8)	0.001

[1]Thienopyridine includes ticlopidine, clopidogrel, and prasugrel; [2]Other antiplatelets are antiplatelets other than low-dose aspirin and thienopyridine; [3]Advanced adenoma is adenoma ⩾ 1 cm with villous components (tubulovillous or villous) or high-grade or severe dysplasia. Values in parentheses are percentages. Values presented with a plus/minus sign are means ± SD. Bold type indicates statistical significance ($P < 0.05$). AC: Anticoagulant; DOAC: Direct oral anticoagulants; BMI: Body mass index; Ccr: Creatinine clearance; NSAIDs: Non-steroidal anti-inflammatory drugs.

4, 1 case at day 5, 2 cases at day 6, and 1 case at day 8. The 4 patients with early PPB were all warfarin users. Compared with controls, there were a significantly higher rate among AC users of PPB (13.7% *vs* 0.9%, $P < 0.001$; Figure 2). Adjusting for propensity score between groups, AC users had a significantly increased PPB risk (adjusted OR = 18.9, $P < 0.001$; Table 2). Two thromboembolic events occurred in AC users, but none in controls. Thromboembolism occurred in 2 warfarin users and no DOAC users. No mortality events were noted in either group.

Warfarin users vs DOAC users

In the subgroup analysis of warfarin users, there were some significant differences in baseline characteristics with controls (Table 1). In terms of outcomes, warfarin users had a significantly higher rate of PPB (13.7% *vs* 0.9%, $P < 0.001$; Figure 2); a significantly increased PPB risk when adjusting for propensity score (adjusted OR = 18.6, $P < 0.001$; Table 2). In the subgroup analysis of DOAC users, there were also some significant differences in baseline characteristics with controls (Table 1). As for outcomes, DOAC users had a significantly higher rate of PPB (13.8% *vs* 0.9%, $P < 0.001$; Figure 2); significantly increased PPB risk when adjusting for propensity score (adjusted OR = 17.8, $P = 0.001$; Table 2). PPB rates did not differ significantly between rivaroxaban, dabigatran, and apixaban users (Figure 2).

Differences in baseline characteristics and clinical outcomes between the three AC management strategies

Discontinuing AC with HPB (guideline recommendation) *vs* continuing AC: There were some significant differences in baseline characteristics between strategies (Supplementary Table 1). The discontinuing AC with HPB group showed a higher rate of PPB (19.6%

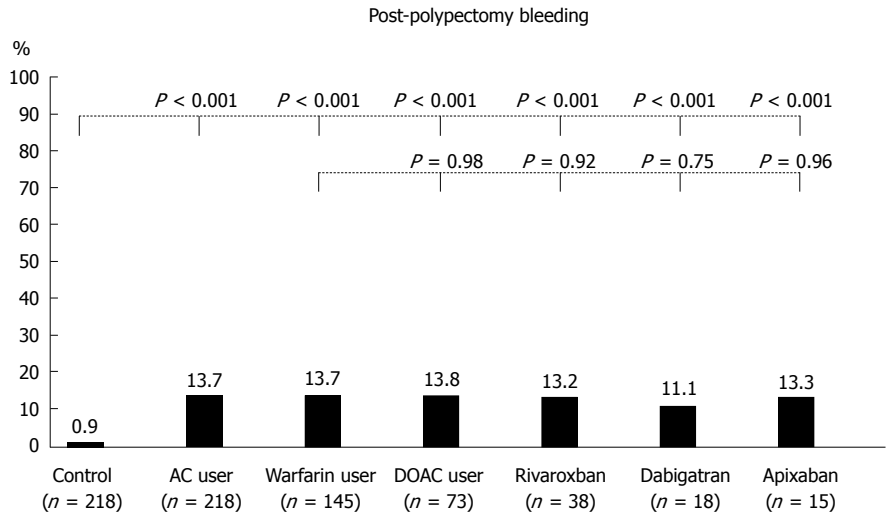

Figure 2 Thirty-day post-polypectomy bleeding in controls (*n* = 218), anticoagulants users (*n* = 218) and subgroups of warfarin (*n* = 145) and direct oral anticoagulants users [*n* = 73: rivaroxaban (*n* = 38), dabigatran (*n* = 18), and apixaban (*n* = 15)]. *P*-values for comparison of each group with controls and for comparison of direct oral anticoagulants users with warfarin users. AC: Anticoagulants; DOAC: Direct oral anticoagulants.

Table 2 Crude and adjusted odds ratios for post-polypectomy bleeding in controls (*n* = 218), anticoagulant users (*n* = 218), warfarin users (*n* = 145), and direct oral anticoagulants users (*n* = 73)

Subjects	Crude OR (95%CI)	*P* value	Propensity score-adjusted OR[1] (95%CI)	*P* value
Controls	1 (referent)		1 (referent)	
AC users	17.2 (4.1-73.1)	< 0.001	18.9 (4.2-85.5)	< 0.001
Warfarin users	17.3 (4.0-75.2)	< 0.001	18.6 (3.8-89.9)	< 0.001
DOAC users	17.1 (3.7-80.3)	< 0.001	17.8 (3.2-98.8)	0.001

[1]Propensity score estimations. Values in parentheses are percentages. Values presented with a plus/minus sign are means ± SD; bold type indicates statistical significance ($P < 0.05$). AC users *vs* controls: Logistic regression model included 17 factors that are potentially clinically important variables; area under the receiver operating characteristic (ROC) curve for propensity scores for AC users was 0.81 (95%CI: 0.77-0.85); Warfarin users *vs* controls: Logistic regression model included 18 factors that are potentially clinically important variables; area under the ROC curve for propensity scores for warfarin users was 0.83 (95%CI: 0.78-0.88); DOAC users *vs* controls: Logistic regression model included 14 factors that are potentially clinically important variables; area under the ROC curve for DOAC user propensity scores was 0.85 (95%CI: 0.80-0.90). NA: Not applicable; AC: Anticoagulants; DOAC: Direct oral anticoagulants; HPB: Heparin bridge; OR: Odds ratio.

vs 10.8%, $P = 0.087$; Figure 3A); a higher PPB risk when adjusting for propensity score (adjusted OR = 2.2, $P = 0.069$; Table 3).

In the warfarin subgroups, the discontinuing warfarin with HPB group showed a significantly higher rate of PPB (21.7% *vs* 4.7%, $P = 0.013$; Figure 3B); increased PPB risk on multivariate analysis (Table 3). In the subgroup of DOAC users, there were no significant differences between the two groups in PPB risk (Figure 3C), and multivariate models adjusted for propensity score also revealed no significant difference (Table 3).

Discontinuing AC with HPB (guideline recommendation) *vs* discontinuing AC without HPB: The discontinuing AC with HPB group showed a significantly higher rate of PPB (19.6% *vs* 0.0%, $P = 0.020$; Figure 3A); increased PPB risk on univariate analysis (OR = 7.7, $P = 0.023$; Table 3).

In the warfarin subgroups, the discontinuing AC with HPB group had a significantly higher rate of PPB (21.7% *vs* 0%, $P = 0.025$; Figure 3B); increased PPB risk on univariate analysis (OR = 7.2, $P = 0.033$; Table 3). In the DOAC subgroups, there were no significant differences in PPB risk between the two subgroups (Table 3).

Association of rate of PPB with HPB duration and INR value at endoscopy

The rate of PPB increased significantly with longer duration of HPB ($P = 0.015$ for trend; Figure 4). This trend was also found in warfarin and DOAC users (Figure 4). Rate of PPB was 18.7% for INR < 1.5, 0% for INR 1.5-1.9, 25% for INR 2.0-2.4, and 0% for INR > 2.5. INR value at pre-endoscopy did not predict PPB ($P = 0.431$ for trend; Supplementary Figure 1).

DISCUSSION

The four main findings of the study are as follows: (1) AC users were at higher risk of PPB than controls; (2) PPB risk was similar between warfarin users and DOAC users, whereas thromboembolism risk was observed only in warfarin users; (3) PPB risk was not significantly different

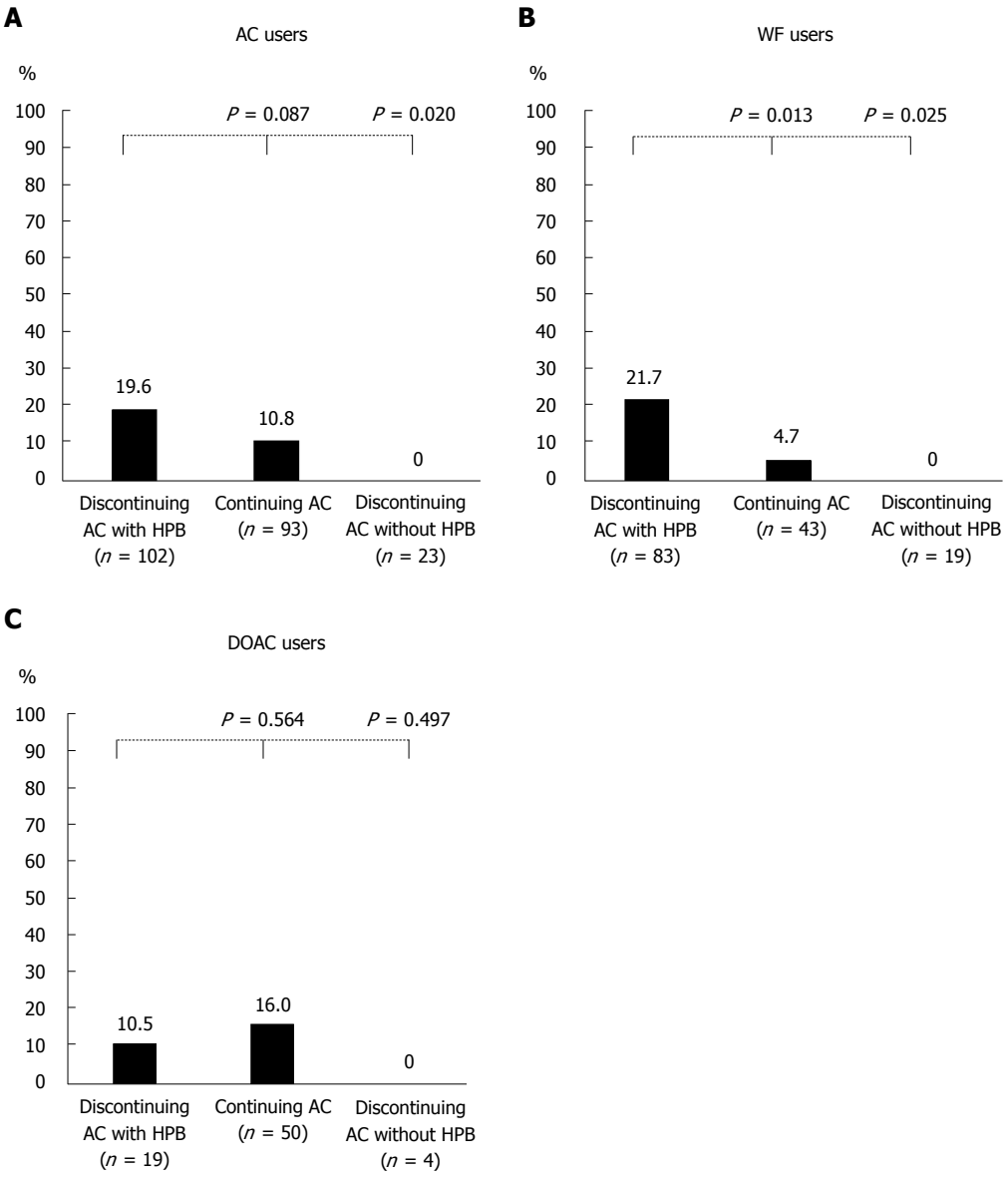

Figure 3 Post-polypectomy bleeding according to the three main anticoagulants management strategies in anticoagulants (A), warfarin (B), and direct oral anticoagulants (C) users. For the 218 patients, 102 patients (46.8%) in the discontinuing anticoagulants with heparin bridge group, 93 (42.7%) in the continuing anticoagulants group, and 23 (10.6%) in the discontinuing anticoagulants without heparin bridge group. AC: Anticoagulants; DOAC: Direct oral anticoagulants; HPB: Heparin bridge.

between rivaroxaban, dabigatran, and apixaban users; and (4) the recommended strategy of discontinuing AC with HPB showed a higher bleeding rate than continuing AC alone and had one thrombotic event, indicating that HPB increased bleeding and may not prevent thromboembolism. These findings were significantly evident in warfarin users compared with DOAC users.

In agreement with past studies, our AC users had a significantly higher OR for PPB than did controls (adjusted OR = 18.9). Witt *et al*[26] reported that PPB occurred more often in AC users than non-AC users (adjusted OR = 11.6). Hui *et al*[27] demonstrated that warfarin use was an independent risk factor for PPB (adjusted OR = 13.4). The ORs in these studies were lower than ours because their control subjects included antiplatelet users.

We revealed for the first time in this study that PPB risk was similar between warfarin and DOAC users compared with controls. A meta-analysis study indicated a higher risk of non-procedural-related bleeding in DOAC users than in warfarin users[28]. Thus, bleeding risk might be different between procedure-related and non-procedure-related bleeding. Only limited data are available on differences in post-endoscopic bleeding between DOAC and warfarin users. In this study, we found that 14% of DOAC and warfarin users had PPB. In agreement with this, Nagata *et al*[29] showed that 14% of DOAC users had PPB and 16.9% of warfarin users had PPB (P = 0.324). However, post-polypectomy-related bleeding differ according to site of the bleed in the upper or lower GI tract, because upper GI polypectomy-related bleeding was higher in warfarin users than in DOAC users (P = 0.06)[29].

Several endoscopy guidelines recommend that AC be discontinued with HPB[6-8]. However, in our study, following

Table 3 Crude and adjusted odds ratios for post-polypectomy bleeding in anticoagulant users (*n* = 218), warfarin users (*n* = 145), and direct oral anticoagulants users (*n* = 73)

AC management during peri-endoscopic period	Crude OR (95%CI)	*P* value	Propensity score-adjusted OR[1] (95%CI)	*P* value
AC users				
Discontinuing AC with HPB *vs* continuing AC	2.0 (0.9-4.6)	0.091	2.2 (0.9-5.2)	0.069
Discontinuing AC with HPB *vs* discontinuing AC without HPB	7.7 (1.3-Inf)	0.023	NA	NA
Warfarin users				
Discontinuing warfarin with HPB *vs* continuing warfarin	5.7 (1.3-25.8)	0.024	4.7 (1.0-22.1)	0.049
Discontinuing warfarin with HPB *vs* discontinuing warfarin without HPB	7.2 (1.1-Inf)	0.033	NA	NA
DOAC users				
Discontinuing DOAC with HPB *vs* continuing DOAC	0.6 (0.1-3.2)	0.567	0.7 (0.1-4.5)	0.664
Discontinuing DOAC with HPB *vs* discontinuing DOAC without HPB	0.5 (0.4-Inf)	1.000	NA	NA

[1]Propensity score estimations. Values in parentheses are percentages. Values presented with a plus/minus sign are means ± SD; bold type indicates statistical significance ($P < 0.05$). Continuing AC group *vs* standard group: Logistic regression model included 8 factors that are potentially clinically important variables; area under the ROC curve for propensity scores for the continuing AC group was 0.71 (95%CI: 0.63-0.79); standard group *vs* continuing warfarin group: Logistic regression model included 6 factors that are potentially clinically important variables; area under the ROC curve for propensity scores for the continuing warfarin group was 0.63 (95%CI: 0.53-0.73); standard group *vs* continuing DOAC group: Logistic regression model included 6 factors that are potentially clinically important variables; area under the ROC curve for propensity scores for the continuing DOAC group was 0.90 (95%CI: 0.82-0.98). NA: Not applicable; AC: Anticoagulants; CI: Confidential interval; DOAC: Direct oral anticoagulants; HPB: Heparin bridge; Inf: Infinity; OR: Odds ratio.

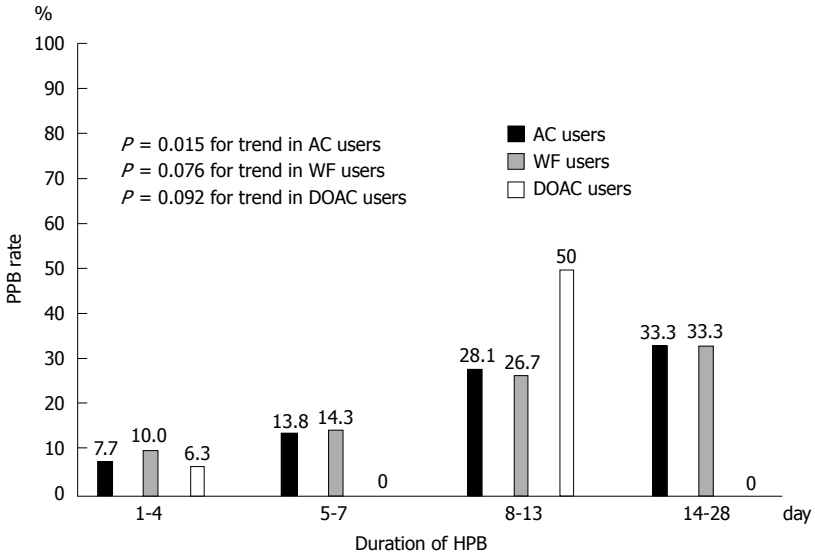

Figure 4 Association of post-polypectomy bleeding rate with duration of heparin bridge in anticoagulants, warfarin, and direct oral anticoagulants users. AC: Anticoagulants; WF: Warfarin; DOAC: Direct oral anticoagulants; HPB: Heparin bridge; PPB: Post-polypectomy bleeding.

this guideline strategy showed a higher bleeding risk and longer hospital stay compared with the continuing AC strategy, and one thrombotic event occurred with the guideline strategy and none in the continuing AC strategy. These findings suggest that continuing oral AC might be acceptable for polypectomy.

Consistent with our results, a meta-analysis[30] showed that HPB was associated with a higher rate of PPB and did not prevent thromboembolism. A randomized study[13] found that post-procedural bleeding risk was higher in patients with HPB than in those without it, and thromboembolic risk was similar in both groups. Taken together, the evidence suggests that the recommendation of several endoscopic guidelines[6-8] should be re-evaluated.

It is not clear why following the guideline strategy was associated with increased PPB risk in warfarin users but not DOAC users. One possible explanation is that in warfarin users, it takes several days for the anticoagulant effect to be sufficient, whereas onset is rapid with DOAC and therapeutic anticoagulation is achieved in a few hours[31]. The criterion for discontinuing heparin in warfarin users is that INR reaches the effective range, but the time to reach this range varies among patients. Therefore, heparin may need to be used for a long time after the procedure; the time is much shorter in DOAC users. Also, simultaneously administering warfarin and heparin (double anticoagulation effect) can increase bleeding risk. From these considerations, GI bleeding risk is high when HPB is performed in warfarin users compared with DOAC users. These prior findings, together with ours here, suggest that warfarin should be switched to DOAC before high-risk endoscopic procedures are performed.

One of the strengths of our study was the analysis of detailed clinical and endoscopic data that was collected and that we could adjust for propensity score by including these factors in the multivariate models. Another was that we identified a difference in clinical outcomes between the three main AC management strategies investigated. We also recognize limitations. First, this was a retrospective study conducted at a single site. Second, the AC users were heterogeneous and included those with atrial fibrillation, valvular disease, or with low or high thromboembolic risk. Third, we have no data on subcutaneous heparin because intravenous heparin is used in Japan. However, a previous study reported a similar incidence of major bleeding between patients treated with subcutaneous unfractionated heparin and those treated with intravenous unfractionated heparin (OR 0.91).

In conclusion, patients receiving oral AC had higher risks of bleeding after colonoscopic polypectomy compared with patients not receiving any antithrombotics. PPB risk was similar between warfarin and DOAC users, whereas thromboembolism risk was observed in warfarin users only. HPB increased bleeding risk, and may not prevent thromboembolism and therefore the current guideline recommendation should be re-considered. Continuing oral AC may be acceptable for polypectomy.

ARTICLE HIGHLIGHTS

Research background

The number of oral anticoagulants (AC) used increases as the population ages, and the number of colonoscopic polypectomies is expected to increase in patients receiving AC.

Research motivation

Whether post-polypectomy bleeding (PPB) or thromboembolic risk differs between warfarin and direct oral anticoagulant (DOAC) users remains unknown.

Research objectives

We evaluated PPB risk in patients receiving warfarin or DOAC compared with patients not receiving any antithrombotics (controls). We also assessed the risks of PPB and thromboembolism between the three AC management methods mentioned above, discontinuing AC with heparin bridge (guideline recommendation), continuing AC, and discontinuing AC without heparin bridge.

Research methods

We conducted a retrospective cohort study and collected data from 218 patients receiving oral anticoagulants (73 DOAC users, 145 warfarin users) and 218 patients not receiving any antithrombotics (age- and sex-matched controls) who underwent polypectomy.

Research results

PPB rate was significantly higher in both warfarin users and DOAC users compared with controls. Two thromboembolic events occurred in warfarin users, but none in DOAC users. Compared with the continuing anticoagulant group, the discontinuing anticoagulant with heparin bridge group (guideline recommendation) had a higher PPB rate. One thrombotic event occurred in the discontinuing anticoagulant with heparin bridge group and the discontinuing anticoagulant without heparin bridge group; none occurred in the continuing anticoagulant group.

Research conclusions

Patients receiving oral anticoagulant had higher risks of bleeding after colonoscopic polypectomy compared with patients not receiving any antithrombotics. PPB risk was similar between warfarin and DOAC users, whereas thromboembolism risk was observed in warfarin users only. Heparin bridge increased bleeding risk, and may not prevent thromboembolism.

Research perspectives

The current guideline recommendation for heparin bridge should be re-considered, and continuing oral anticoagulant may be acceptable for polypectomy.

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REFERENCES

1 **Mozaffarian D**, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation* 2015; **131**: e29-322 [PMID: 25520374 DOI: 10.1161/CIR.0000000000000152]

2 **Humbert X**, Roule V, Chequel M, Fedrizzi S, Brionne M, Lelong-Boulouard V, Milliez P, Alexandre J. Non-vitamin K oral anticoagulant treatment in elderly patients with atrial fibrillation and coronary heart disease. *Int J Cardiol* 2016; **222**: 1079-1083 [PMID: 27514627 DOI: 10.1016/j.ijcard.2016.07.212]

3 **Abraham NS**, Castillo DL. Novel anticoagulants: bleeding risk and management strategies. *Curr Opin Gastroenterol* 2013; **29**: 676-683 [PMID: 24100724 DOI: 10.1097/MOG.0b013e328365d415]

4 **Baron TH**, Kamath PS, McBane RD. Management of antithrombotic therapy in patients undergoing invasive procedures. *N Engl J Med* 2013; **368**: 2113-2124 [PMID: 23718166 DOI: 10.1056/NEJMra1206531]

5 **Kwok A**, Faigel DO. Management of anticoagulation before and after gastrointestinal endoscopy. *Am J Gastroenterol* 2009; **104**: 3085-3097; quiz 3098 [PMID: 19672250 DOI: 10.1038/ajg.2009.469]

6 **Veitch AM**, Vanbiervliet G, Gershlick AH, Boustiere C, Baglin TP, Smith LA, Radaelli F, Knight E, Gralnek IM, Hassan C, Dumonceau JM. Endoscopy in patients on antiplatelet or anticoagulant therapy, including direct oral anticoagulants: British Society of Gastroenterology (BSG) and European Society of Gastrointestinal Endoscopy (ESGE) guidelines. *Endoscopy* 2016; **48**: 385-402 [PMID: 26890676 DOI: 10.1055/s-0042-102652]

7 **ASGE Standards of Practice Committee.**, Acosta RD, Abraham NS, Chandrasekhara V, Chathadi KV, Early DS, Eloubeidi MA, Evans JA, Faulx AL, Fisher DA, Fonkalsrud L, Hwang JH, Khashab MA, Lightdale JR, Muthusamy VR, Pasha SF, Saltzman JR, Shaukat A, Shergill AK, Wang A, Cash BD, DeWitt JM. The management of antithrombotic agents for patients undergoing GI endoscopy. *Gastrointest Endosc* 2016; **83**: 3-16 [PMID: 26621548 DOI: 10.1016/j.gie.2015.09.035]

8 **Fujimoto K**, Fujishiro M, Kato M, Higuchi K, Iwakiri R, Sakamoto C, Uchiyama S, Kashiwagi A, Ogawa H, Murakami K, Mine T, Yoshino J, Kinoshita Y, Ichinose M, Matsui T; Japan Gastroenterological Endoscopy Society. Guidelines for gastroenterological endoscopy in patients undergoing antithrombotic treatment. *Dig Endosc* 2014; **26**: 1-14 [PMID: 24215155 DOI: 10.1111/den.12183]

9 **Phillips KW**, Ansell J. Outpatient management of oral vitamin K antagonist therapy: defining and measuring high-quality management. *Expert Rev Cardiovasc Ther* 2008; **6**: 57-70 [PMID:

18095907 DOI: 10.1586/14779072.6.1.57]
10 **Burnett AE**, Mahan CE, Vazquez SR, Oertel LB, Garcia DA, Ansell J. Guidance for the practical management of the direct oral anticoagulants (DOACs) in VTE treatment. *J Thromb Thrombolysis* 2016; **41**: 206-232 [PMID: 26780747 DOI: 10.1007/s11239-015-1310-7]
11 **Heublein V**, Pannach S, Daschkow K, Tittl L, Beyer-Westendorf J. Gastrointestinal endoscopy in patients receiving novel direct oral anticoagulants: results from the prospective Dresden NOAC registry. *J Gastroenterol* 2018; **53**: 236-246 [PMID: 28493007 DOI: 10.1007/s00535-017-1346-x]
12 **Li HK**, Chen FC, Rea RF, Asirvatham SJ, Powell BD, Friedman PA, Shen WK, Brady PA, Bradley DJ, Lee HC, Hodge DO, Slusser JP, Hayes DL, Cha YM. No increased bleeding events with continuation of oral anticoagulation therapy for patients undergoing cardiac device procedure. *Pacing Clin Electrophysiol* 2011; **34**: 868-874 [PMID: 21410724 DOI: 10.1111/j.1540-8159.2011.03049.x]
13 **Douketis JD**, Spyropoulos AC, Kaatz S, Becker RC, Caprini JA, Dunn AS, Garcia DA, Jacobson A, Jaffer AK, Kong DF, Schulman S, Turpie AG, Hasselblad V, Ortel TL; BRIDGE Investigators. Perioperative Bridging Anticoagulation in Patients with Atrial Fibrillation. *N Engl J Med* 2015; **373**: 823-833 [PMID: 26095867 DOI: 10.1056/NEJMoa1501035]
14 **Nagata N**, Niikura R, Aoki T, Shimbo T, Kishida Y, Sekine K, Tanaka S, Okubo H, Watanabe K, Sakurai T, Yokoi C, Akiyama J, Yanase M, Mizokami M, Uemura N. Lower GI bleeding risk of nonsteroidal anti-inflammatory drugs and antiplatelet drug use alone and the effect of combined therapy. *Gastrointest Endosc* 2014; **80**: 1124-1131 [PMID: 25088922 DOI: 10.1016/j.gie.2014.06.039]
15 **Nagata N**, Niikura R, Aoki T, Shimbo T, Sekine K, Okubo H, Watanabe K, Sakurai T, Yokoi C, Yanase M, Akiyama J, Uemura N. Association between colonic diverticulosis and bowel symptoms: A case-control study of 1629 Asian patients. *J Gastroenterol Hepatol* 2015; **30**: 1252-1259 [PMID: 25777157 DOI: 10.1111/jgh.12941]
16 **Pisters R**, Lane DA, Nieuwlaat R, de Vos CB, Crijns HJ, Lip GY. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. *Chest* 2010; **138**: 1093-1100 [PMID: 20299623 DOI: 10.1378/chest.10-0134]
17 **Lip GY**, Nieuwlaat R, Pisters R, Lane DA, Crijns HJ. Refining clinical risk stratification for predicting stroke and thromboembolism in atrial fibrillation using a novel risk factor-based approach: the euro heart survey on atrial fibrillation. *Chest* 2010; **137**: 263-272 [PMID: 19762550 DOI: 10.1378/chest.09-1584]
18 **Brenner H**, Hoffmeister M, Stegmaier C, Brenner G, Altenhofen L, Haug U. Risk of progression of advanced adenomas to colorectal cancer by age and sex: estimates based on 840,149 screening colonoscopies. *Gut* 2007; **56**: 1585-1589 [PMID: 17591622]
19 **Ono K**, Hidaka H, Koyama Y, Ishii K, Taguchi S, Kosaka M, Okazaki N, Tanimoto W, Katayama A. Effects of heparin bridging anticoagulation on perioperative bleeding and thromboembolic risks in patients undergoing abdominal malignancy surgery. *J Anesth* 2016; **30**: 723-726 [PMID: 27206420 DOI: 10.1007/s00540-016-2187-0]
20 **Feagins LA**, Iqbal R, Harford WV, Halai A, Cryer BL, Dunbar KB, Davila RE, Spechler SJ. Low rate of postpolypectomy bleeding among patients who continue thienopyridine therapy during colonoscopy. *Clin Gastroenterol Hepatol* 2013; **11**: 1325-1332 [PMID: 23403011 DOI: 10.1016/j.cgh.2013.02.003]
21 **Schulman S**, Kearon C; Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost* 2005; **3**: 692-694 [PMID: 15842354]
22 **Park SK**, Seo JY, Lee MG, Yang HJ, Jung YS, Choi KY, Kim H, Kim HO, Jung KU, Chun HK, Park DI. Prospective analysis of delayed colorectal post-polypectomy bleeding. *Surg Endosc* 2018; **17**: Epub ahead of print [PMID: 29344790 DOI: 10.1007/s00464-018-6048-9]
23 **Austin PC**. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behav Res* 2011; **46**: 399-424 [PMID: 21818162 DOI: 10.1080/00273171.2011.568786]
24 **Kurth T**, Walker AM, Glynn RJ, Chan KA, Gaziano JM, Berger K, Robins JM. Results of multivariable logistic regression, propensity matching, propensity adjustment, and propensity-based weighting under conditions of nonuniform effect. *Am J Epidemiol* 2006; **163**: 262-270 [PMID: 16371515]
25 **Elze MC**, Gregson J, Baber U, Williamson E, Sartori S, Mehran R, Nichols M, Stone GW, Pocock SJ. Comparison of Propensity Score Methods and Covariate Adjustment: Evaluation in 4 Cardiovascular Studies. *J Am Coll Cardiol* 2017; **69**: 345-357 [PMID: 28104076]
26 **Witt DM**, Delate T, McCool KH, Dowd MB, Clark NP, Crowther MA, Garcia DA, Ageno W, Dentali F, Hylek EM, Rector WG; WARPED Consortium. Incidence and predictors of bleeding or thrombosis after polypectomy in patients receiving and not receiving anticoagulation therapy. *J Thromb Haemost* 2009; **7**: 1982-1989 [PMID: 19719825 DOI: 10.1111/j.1538-7836.2009.03598.x]
27 **Hui AJ**, Wong RM, Ching JY, Hung LC, Chung SC, Sung JJ. Risk of colonoscopic polypectomy bleeding with anticoagulants and antiplatelet agents: analysis of 1657 cases. *Gastrointest Endosc* 2004; **59**: 44-48 [PMID: 14722546]
28 **Holster IL**, Valkhoff VE, Kuipers EJ, Tjwa ET. New oral anticoagulants increase risk for gastrointestinal bleeding: a systematic review and meta-analysis. *Gastroenterology* 2013; **145**: 105-112.e15 [PMID: 23470618 DOI: 10.1053/j.gastro.2013.02.041]
29 **Nagata N**, Yasunaga H, Matsui H, Fushimi K, Watanabe K, Akiyama J, Uemura N, Niikura R. Therapeutic endoscopy-related GI bleeding and thromboembolic events in patients using warfarin or direct oral anticoagulants: results from a large nationwide database analysis. *Gut* 2017; pii: gutjnl-2017-313999 [PMID: 28874418]
30 **Jaruvongvanich V**, Assavapongpaiboon B, Wijarnpreecha K, Ungprasert P. Heparin-bridging therapy and risk of post-polypectomy bleeding: Meta-analysis of data reported by Japanese colonoscopists. *Dig Endosc* 2017; **29**: 743-748 [PMID: 28370508 DOI: 10.1111/den.12882]
31 **Desai J**, Granger CB, Weitz JI, Aisenberg J. Novel oral anticoagulants in gastroenterology practice. *Gastrointest Endosc* 2013; **78**: 227-239 [PMID: 23725876 DOI: 10.1016/j.gie.2013.04.179]

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ORIGINAL ARTICLE

Randomized Controlled Trial

Maintenance for healed erosive esophagitis: Phase Ⅲ comparison of vonoprazan with lansoprazole

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Abstract

AIM

To compare vonoprazan 10 and 20 mg *vs* lansoprazole 15 mg as maintenance therapy in healed erosive esophagitis (EE).

METHODS

A total of 607 patients aged ⩾ 20 years, with endoscopically-confirmed healed EE following 8 wk of treatment with vonoprazan 20 mg once daily, were randomized 1:1:1 to receive lansoprazole 15 mg (*n* = 201), vonoprazan 10 mg (*n* = 202), or vonoprazan 20 mg (*n* = 204), once daily. The primary endpoint of the study was the rate of endoscopically-confirmed EE recurrence during a 24-wk maintenance period. The secondary endpoint was the EE recurrence rate at Week 12 during maintenance treatment. Additional efficacy endpoints included the incidence of heartburn and acid reflux, and the EE healing rate 4 wk after the initiation of maintenance treatment. Safety endpoints comprised adverse events (AEs), vital signs, electrocardiogram findings, clinical laboratory results, serum gastrin and pepsinogen Ⅰ/Ⅱ levels, and gastric mucosa histopathology results.

RESULTS

Rates of EE recurrence during the 24-wk maintenance period were 16.8%, 5.1%, and 2.0% with lansoprazole 15 mg, vonoprazan 10 mg, and vonoprazan 20 mg, respectively. Vonoprazan was shown to be non-inferior to lansoprazole 15 mg ($P < 0.0001$ for both doses). In a *post-hoc* analysis, EE recurrence at Week 24 was significantly reduced with vonoprazan at both the 10 mg and the 20 mg dose *vs* lansoprazole 15 mg (5.1% *vs* 16.8%, $P = 0.0002$, and 2.0% *vs* 16.8%, $P < 0.0001$, respectively); by contrast, the EE recurrence rate did not differ significantly between the two doses of vonoprazan ($P = 0.1090$). The safety profiles of vonoprazan 10 and 20 mg were similar to that of lansoprazole 15 mg in patients with healed EE. Treatment-related AEs were reported in 11.4%, 10.4%, and 10.3% of patients in the lansoprazole 15 mg, vonoprazan 10 mg, and vonoprazan 20 mg arms, respectively.

CONCLUSION

Our findings confirm the non-inferiority of vonoprazan 10 and 20 mg to lansoprazole 15 mg as maintenance therapy for patients with healed EE.

Key words: Gastroesophageal reflux disease; Erosive esophagitis; Lansoprazole; Potassium-competitive acid blockers; Vonoprazan; Maintenance therapy

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Core tip: Proton pump inhibitors (PPIs), including lansoprazole, are widely used to maintain healing of erosive esophagitis (EE) in patients with gastroesophageal reflux disease; however, symptoms of reflux persist in significant numbers of patients treated with PPIs. We compared two doses of the novel potassium-competitive acid blocker vonoprazan (10 and 20 mg once daily) with lansoprazole at its approved dose of 15 mg once daily as maintenance therapy for healed EE in 607 Japanese patients. Vonoprazan was shown to be non-inferior to lansoprazole 15 mg at both investigated doses, while demonstrating a similar safety profile.

Ashida K, Iwakiri K, Hiramatsu N, Sakurai Y, Hori T, Kudou K, Nishimura A, Umegaki E. Maintenance for healed erosive esophagitis: Phase Ⅲ comparison of vonoprazan with lansoprazole. *World J Gastroenterol* 2018; 24(14): 1550-1561 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i14/1550.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i14.1550

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common gastric acid-related disorder that is characterized by heartburn and/or acid regurgitation caused by the reflux of gastric contents[1]. The spectrum of GERD ranges from non-erosive to erosive or complicated disease (ulcer, columnar metaplasia, and stricture), each of which is thought likely to progress if either left untreated or not treated adequately[2]. The main goals for the clinical management of GERD consist of symptom relief, healing of erosive esophagitis (EE), prevention of recurrences and complications, and overall improvement of patients' quality of life[1,3].

Owing to their superior ability to inhibit gastric acid secretion compared with H_2 receptor antagonists (H_2RAs), proton pump inhibitors (PPIs) remain the mainstay of long-term therapy for GERD[1,3-5]. However, resolution of GERD symptoms with PPIs appears to have a less predictable outcome than esophageal mucosal inflammation[4-6], with reflux symptoms persisting in up to 60% of patients treated with PPIs in randomized controlled clinical trials[7] and observational studies[5]. Proposed underlying mechanisms for PPI failure include drug- and patient-related factors, such as low bioavailability, nocturnal acid breakthrough, rapid metabolism (CYP2C19 extensive metabolizer genotype), and poor compliance with the prescribed regimen[6]. The slow cumulative onset of PPI action at therapeutic doses may also be a contributing factor[8-10]. These limitations have led to a renewed interest in alternative treatment modalities for the management of patients with GERD[1,4].

Discovered and developed by Takeda Pharmaceutical Company Limited, Japan, vonoprazan fumarate (TAK-438) belongs to a novel class of acid suppressants known as potassium-competitive acid blockers (P-CABs)[11]. Like PPIs, vonoprazan inhibits gastric H^+, K^+-ATPase,

an enzyme that catalyzes the final step in the acid secretion pathway. However, unlike PPIs, vonoprazan inhibits the enzyme in a K^+-competitive and reversible manner[12], with its inhibitory effects (pKa 9.4) on gastric acid secretion largely unaffected by ambient pH, as it accumulates in parietal cells under both acidic and resting conditions[12,13]. In animal studies, vonoprazan produced more potent and sustained suppression of gastric acid secretion than lansoprazole[11-14]. In healthy volunteers, single doses of vonoprazan 1-120 mg were well tolerated, and produced rapid, prolonged, and dose-related suppression of 24-h gastric acid secretion[15]. In another study in healthy volunteers, these effects were maintained with multiple dosing (10-40 mg once daily) over 7 d, and were also dose-related[16].

Lansoprazole 30 mg once daily is the recommended dosage for healing EE, while its step-down dose of 15 mg once daily is recommended for the maintenance treatment of healed EE, providing well-balanced efficacy and safety over the long term[17]. The current study aimed to demonstrate that vonoprazan 20 mg and its step-down dose of 10 mg once daily were non-inferior to lansoprazole 15 mg once daily in preventing EE recurrence during a 24-wk maintenance period in Japanese patients who achieve EE healing after 2, 4, or 8 wk treatment with vonoprazan 20 mg.

MATERIALS AND METHODS

Study design

This was a multicenter, randomized, double-blind, parallel-group, phase Ⅲ clinical study, which was designed and conducted to demonstrate the non-inferiority of vonoprazan 20 and 10 mg to lansoprazole 15 mg as maintenance therapy in Japanese patients with healed EE. During the initial treatment period, patients with EE Los Angeles (LA) Classification grades A to D received vonoprazan 20 mg once daily for up to 8 wk. All patients in whom endoscopic healing of EE was confirmed 2, 4, or 8 wk after the start of the study medication were immediately stratified by baseline endoscopic LA Classification grade (A/B or C/D), and subsequently randomized in a 1:1:1 ratio to receive maintenance therapy with vonoprazan 10 mg, vonoprazan 20 mg, or lansoprazole 15 mg given once daily after breakfast for 24 wk. All patients in whom endoscopic healing of EE was not confirmed at Week 8 completed the study without entering the maintenance phase. All patients in whom EE recurrence was endoscopically confirmed during maintenance treatment were withdrawn from the study and handled as 'completed cases'.

Registered at ClinicalTrials.gov with the identifier NCT01459367, the study was conducted at 55 sites in Japan between November 2011 and March 2013. The study protocol was reviewed and approved by the Institutional Review Board at each study site, and was conducted in accordance with the Declaration of Helsinki, the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Harmonized Tripartite Guideline for Good Clinical Practice, and Japanese regulatory requirements. All patients provided written informed consent prior to undergoing any study procedures.

Patients

Male or female outpatients aged ⩾ 20 years, who presented with endoscopically-confirmed healed EE (no mucosal breaks) after up to 8 wk of treatment with vonoprazan 20 mg once daily, entered the maintenance phase of the study. Main exclusion criteria included: esophageal complications (*e.g.,* eosinophilic esophagitis, esophageal varices, scleroderma, infection, esophageal stenosis); acute upper gastrointestinal bleeding; gastric or duodenal ulcer characterized by mucosal defects; hypersecretion disorders, such as Zollinger-Ellison syndrome; serious neurologic, cardiovascular, pulmonary, hepatic [alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2.5 × the upper limit of normal (ULN)], renal (serum creatinine > 2 mg/dL), metabolic, gastrointestinal, urologic, endocrinologic, or hematologic disorders; need for surgery; history of drug (including alcohol) abuse; HIV or hepatitis; history of malignancy; and pregnancy or lactation in females. Any sexually active female of childbearing potential was required to use adequate contraceptive measures. Excluded concomitant medications included PPIs, H_2RAs, muscarinic M_3 receptor antagonists, gastrointestinal motility stimulants, anticholinergic drugs, prostaglandins, acid suppressants, anti-gastrin drugs, mucosal protective agents, *H. pylori* eradication therapies, atazanavir sulfate, and any other investigational drug. As the exclusion of non-steroidal anti-inflammatory drugs (NSAIDs) would have been difficult for patients eligible for inclusion in this study, their use was permitted; however, changes to NSAID regimens during the study were prohibited.

Treatment, randomization, and blinding

Patients were randomized to treatment groups in a 1:1:1 ratio according to a computer-generated randomization schedule prepared by independent randomization personnel. The independent randomization personnel managed the randomization process, and stored the randomization schedule in a secure area. The randomization schedule incorporated LA Classification grades as a stratification factor (A/B or C/D), to ensure that treatment groups were balanced with respect to disease severity. A double-dummy method, using matched vonoprazan placebo tablets and lansoprazole placebo capsules, was employed to ensure that the double-blind conditions were maintained throughout the study.

Procedures

Maintenance treatment was initiated on the day of randomization. Clinic visits were scheduled at Weeks

4, 12, and 24, or upon early withdrawal from the study (discontinuation/recurrence). Endoscopic examinations were performed at Weeks 12 and 24. A central adjudication committee (CAC), composed of independent experts, was established to perform standardized and consistent reviews of endoscopic EE grading by investigators, while all decisions about patient eligibility and withdrawal owing to EE recurrence were made by the investigators, irrespective of the CAC's assessment. Safety assessments were conducted at Weeks 4, 12, and 24. Histopathologic examinations of the gastric mucosa were performed at the start of treatment (baseline) and at Week 24 for subjects enrolled at designated study sites only. All biopsy specimens were full mucosal layer samples taken from the greater curvature of the upper gastric corpus during endoscopic procedures. Samples were fixed in 20% neutral buffered formalin and embedded in paraffin. Five slices were taken from each paraffin block, and were stained with hematoxylin and eosin, Grimelius, chromogranin, synaptophysin, and Ki-67 (MIB-1). For the CYP2C19 genotyping, a single 2 mL blood sample was collected at Week 4, and was analyzed to obtain information on genotypes that affect the pharmacokinetics of lansoprazole. G681A (*2) and G636A (*3) of CYP2C19 were detected using an Invader® assay. Both the histopathologic testing and CYP2C19 genotyping were carried out by Mitsubishi Chemical Medience Corporation, Tokyo, Japan. The gastric mucosa histopathology findings reported by the company were reviewed by an independent assessment committee, which assessed specimens for distribution patterns of Grimelius-positive cells, chromogranin A-positive cells, synaptophysin-positive cells, and Ki-67-positive cells. Treatment compliance was assessed in all patients on the basis of returned tablet/capsule counts at each study site visit.

Although no evidence has been reported of vonoprazan-associated liver function test abnormalities[18], drug-related hepatic changes have previously been reported with another member of the P-CAB drug class[19]. Liver function abnormalities (ALT or AST > 3 × ULN, or total bilirubin > 2 × ULN in two consecutive measurements) were therefore classified as special-interest adverse events (SIAEs) in the present study, and were monitored throughout.

The primary study endpoint was the rate of recurrence of endoscopically-confirmed EE at Week 24 of the maintenance period. The secondary endpoint was the rate of EE recurrence at Week 12 of the maintenance period. Safety endpoints included adverse events (AEs), vital signs, electrocardiogram (ECG) findings, clinical laboratory test values (hematology, serum chemistry, and urinalysis), serum gastrin and pepsinogen Ⅰ/Ⅱ levels, and gastric mucosa histopathologic findings.

Statistical analyses

A double-blind, controlled study of lansoprazole as maintenance therapy for patients with healed EE reported EE recurrence rates of 30% and 14% with lansoprazole 15 mg and 30 mg, respectively, over 24 wk[20]. It was therefore assumed that the endoscopic EE recurrence rate with vonoprazan 20 mg in the present study would be 14%, while the EE recurrence rate with vonoprazan 10 mg would be 22% - that is, halfway between the rates observed with lansoprazole 15 mg and 30 mg in the study mentioned above. It was assumed that the EE recurrence rate with lansoprazole 15 mg would again be 30%. Based on these assumptions, a sample size of 148 patients per treatment group would provide > 90% power to confirm the non-inferiority of the two vonoprazan doses to lansoprazole, with respect to the EE recurrence rate at Week 24, with a non-inferiority margin of 10% utilizing a two-sided 95% confidence interval (CI). Assuming a dropout rate of 15% during maintenance therapy, 174 randomized patients would be required for each treatment group. We therefore set the randomization target at 200 patients per treatment group, to enable evaluation of the long-term safety of vonoprazan in a sufficient number of patients.

For the primary endpoint of EE recurrence rate at Week 24 of maintenance treatment, frequency, point estimates, and corresponding 95%CIs were calculated by treatment group for the full analysis set (FAS), defined as all randomized patients who received at least one dose of study drug during the maintenance period. Vonoprazan 10 mg and 20 mg were evaluated for non-inferiority to lansoprazole 15 mg using the Farrington and Manning test[21] with a non-inferiority margin of 10%. The same analyses were performed for the secondary endpoint.

AEs (including their frequency, severity, investigator-assessed causality, and seriousness) and concomitant medications were monitored throughout the study. Treatment-emergent adverse events (TEAEs) were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 16.0. All TEAEs were summarized descriptively by treatment group, time of onset, and severity, and were categorized by System Organ Class and Preferred Term. All drug-related TEAEs were summarized by severity, while TEAEs leading to study discontinuation and serious TEAEs were summarized by treatment group.

The statistical methods of this study were prepared and conducted by Kentarou Kudou of Takeda Pharmaceutical Company Limited, and were reviewed and approved by Takamasa Hashimoto of Takeda Pharmaceutical Company Limited, Osaka, Japan.

RESULTS

Patients

In total, 737 patients signed the informed consent form. Of these 737 patients, 627 were enrolled into the treatment phase, with 611 patients completing up to 8 wk treatment for EE with vonoprazan 20 mg. Of the 611 who completed treatment, 607, who represented both the FAS and the safety analysis set (SAS), were

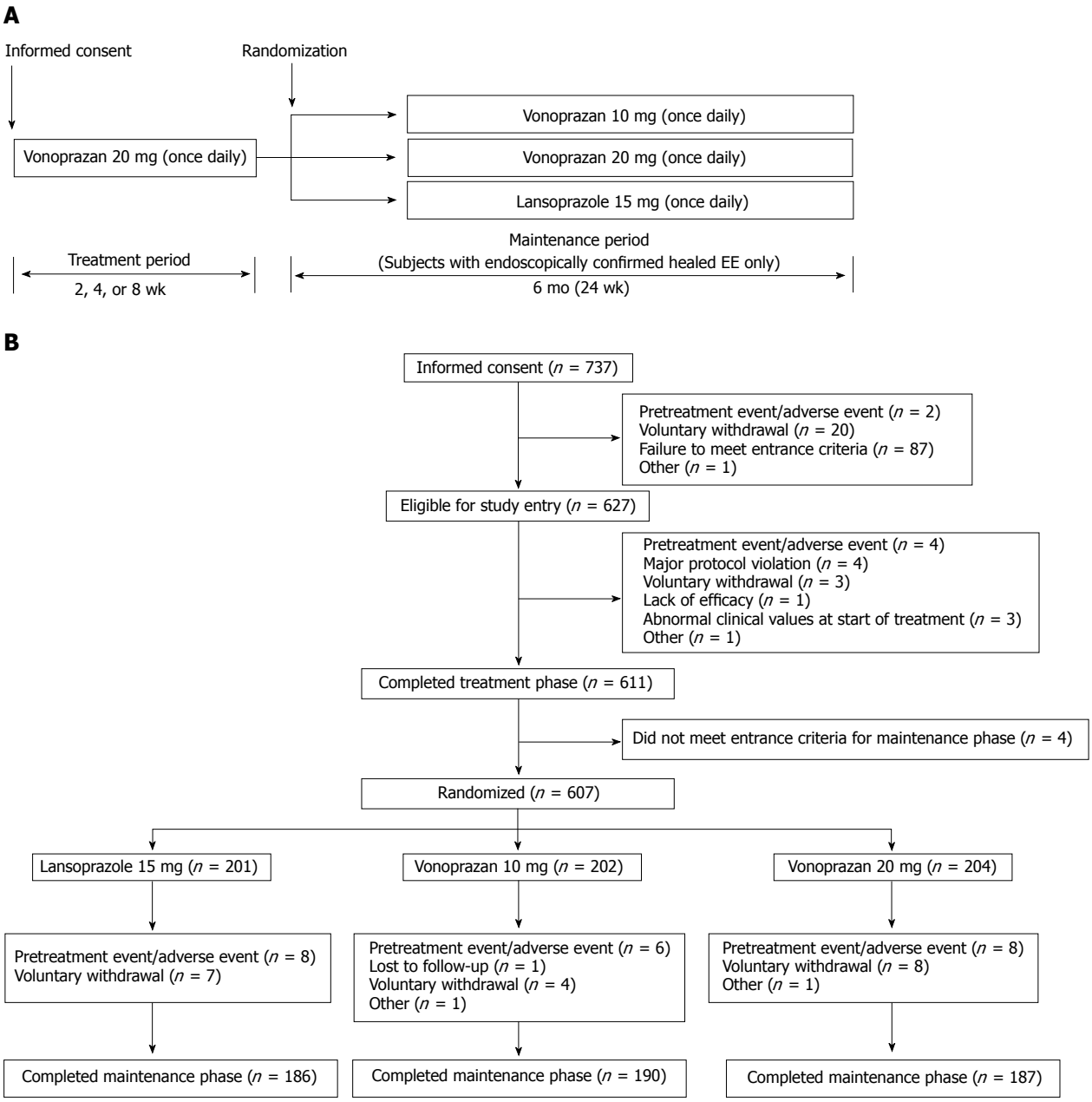

Figure 1 Study design (A) and patient disposition (B). EE: Erosive esophagitis.

randomized to maintenance therapy with lansoprazole 15 mg (n = 201), vonoprazan 10 mg (n = 202), or vonoprazan 20 mg (n = 204) (Figure 1). Five hundred sixty-three patients (92.8%) completed maintenance treatment. The main reasons for premature study discontinuation were pretreatment events/AEs (n = 22) and voluntary withdrawals (n = 19). The first informed consent form was signed on 21 November 2011, and the last follow-up visit took place on 7 March 2013.

The three maintenance groups were well matched in terms of demographic and other baseline characteristics (Table 1), and had similar baseline EE severities and medical histories. The mean treatment compliance rate was > 97% in each treatment group.

Efficacy

The rate of EE recurrence at 24 wk of maintenance therapy (primary endpoint) was 16.8%, 5.1%, and 2.0% with lansoprazole 15 mg, vonoprazan 10 mg, and vonoprazan 20 mg, respectively. Point estimates of differences in EE recurrence between the maintenance treatment groups and 95%CIs are shown in Table 2. Vonoprazan 10 mg and 20 mg were both found to be non-inferior to lansoprazole 15 mg in the FAS (both $P < 0.0001$), with the upper limits of 95%CIs for the differences between vonoprazan 10 mg or 20 mg and lansoprazole 15 mg being < 0, thus indicating a statistically significant difference. In a *post-hoc* analysis performed using the Fisher exact test, a statistically significant difference in the rate of EE recurrence was demonstrated between vonoprazan 10 mg or 20 mg and lansoprazole 15 mg ($P = 0.0002$ and $P < 0.0001$, respectively, *vs* lansoprazole 15 mg), but not between the two vonoprazan doses ($P = 0.1090$).

Table 1 Demographic and other baseline characteristics in the randomized set (n = 607)[1]

Characteristic	LPZ 15 mg (n = 201)	VPZ 10 mg (n = 202)	VPZ 20 mg (n = 204)
Age, yr	57.8 ± 12.9	55.5 ± 13.8	56.8 ± 13.6
Gender, male	140 (69.7)	160 (79.2)	160 (78.4)
Height, cm	163.5 ± 10.2	165.5 ± 9.3	165.6 ± 9.3
Weight, kg	67.0 ± 13.4	68.2 ± 12.3	69.0 ± 13.1
Erosive esophagitis grade, investigator-assessed			
LA Grade A/B	160 (79.6)	162 (80.2)	161 (78.9)
LA Grade C/D	41 (20.4)	40 (19.8)	43 (21.1)
Esophageal hiatal hernia			
⩾ 2 cm	31 (15.4)	45 (22.3)	46 (22.5)
< 2 cm	105 (52.2)	100 (49.5)	113 (55.4)
None	65 (32.3)	57 (28.2)	44 (21.6)
H. pylori infection status			
Positive	29 (14.4)	37 (18.3)	23 (11.3)
Negative	172 (85.6)	165 (81.7)	181 (88.7)
CYP2C19 genotype			
Extensive metabolizers	162 (80.6)	169 (84.1)	169 (83.3)
Poor metabolizers	39 (19.4)	32 (15.9)	34 (16.7)

[1]Values expressed as mean ± SD, or n (%). LA: Los Angeles; LPZ: Lansoprazole; SD: Standard deviation; VPZ: Vonoprazan.

Table 2 Recurrence rate of erosive esophagitis: Intergroup differences and non-inferiority test

Endpoint	LPZ 15 mg	VPZ 10 mg	VPZ 20 mg
Week 24 (primary endpoint)[1]	16.8% (33/196)	5.1% (10/197)	2.0% (4/201)
Week 12 (secondary endpoint)[1]	12.2% (24/196)	2.5% (5/197)	1.0% (2/201)
Comparison	Difference and 95%CI (%)	Non-inferiority, P value	Fisher exact test, P value[2]
Week 24 (primary endpoint)			
VPZ 10 mg *vs* LPZ 15 mg	-11.8 [-17.83, -5.69]	< 0.0001	0.0002
VPZ 20 mg *vs* LPZ 15 mg	-14.8 [-20.43, -9.26]	< 0.0001	< 0.0001
VPZ 10 mg *vs* VPZ 20 mg	-3.1 [-6.71, 0.54]	N/A	0.1090
Week 12 (secondary endpoint)			
VPZ 10 mg *vs* LPZ 15 mg	-9.7 [-14.80, -4.62]	< 0.0001	N/A
VPZ 20 mg *vs* LPZ 15 mg	-11.2 [-16.04, -6.46]	< 0.0001	N/A
VPZ 10 mg *vs* VPZ 20 mg	-1.5 [-4.13, 1.05]	N/A	N/A

[1]Values expressed as percentages with number of subjects in parentheses; [2]*Post hoc* analysis. CI: Confidence interval; LPZ: Lansoprazole; VPZ: Vonoprazan; N/A: Not applicable.

The intergroup differences in EE recurrence rate at Week 12 of the maintenance period (secondary endpoint) are shown in Table 2. Vonoprazan 10 mg and 20 mg were both shown to be non-inferior to lansoprazole 15 mg in the FAS; the upper limits of 95%CIs for the differences between vonoprazan 10 mg or 20 mg and lansoprazole 15 mg were < 0, thus consistently indicating a statistical difference.

Subgroup analyses were conducted on the EE recurrence rates during the 24-wk maintenance period according to age, sex, smoking classification, disease severity, extent of CYP2C19 metabolism, and *H. pylori* infection status. Post-hoc analyses confirmed that the differences in recurrence rates following treatment with vonoprazan 10 mg or 20 mg versus lansoprazole 15 mg were significant among: patients who were: aged < 65 years; of either sex; never smokers; had any LA classification grade; CYP2C19 extensive metabolizers; or *H. pylori*-negative (Table 3).

Safety

The incidence of TEAEs during the 24-wk maintenance period was comparable between the maintenance treatment groups (Table 4). All-cause TEAEs during maintenance therapy were reported in 51.2%, 54.0%, and 58.8% of patients treated with lansoprazole 15 mg, vonoprazan 10 mg, and vonoprazan 20 mg, respectively. Nasopharyngitis was the most commonly reported TEAE in each treatment group (13.9%, 16.8%, and 13.2%, respectively; 14.7% of patients overall). The only other TEAE occurring in > 5% of patients in any treatment group was diarrhea, which was reported in 5.5% of those treated with lansoprazole 15 mg. TEAEs were mostly mild in severity. The incidence of drug-related TEAEs was 11.4%, 10.4%, and 10.3% with lansoprazole 15 mg, vonoprazan 10 mg and vonoprazan 20 mg, respectively. Very few serious TEAEs were reported with lansoprazole 15 mg, vonoprazan 10 mg, or vonoprazan 20 mg (4, 5, and 4

Table 3 Recurrence rate of erosive esophagitis within 24 wk: sub-group analysis according to baseline characteristics

	LPZ 15 mg	VPZ 10 mg			VPZ 20 mg		
	Estimate (%)[1]	Estimate (%)[1]	Difference[2] and 95%CI (%)	Fisher exact test, *P* value[3]	Estimate (%)[1]	Difference[2] and 95%CI (%)	Fisher exact test, *P* value[3]
Age (yr)							
< 65	14.4 (19/132)	4.3 (6/139)	-10.1 [-16.95, -3.20]	0.0056	0.0 (0/136)	-14.4 [-20.38, -8.41]	< 0.0001
⩾ 65 to < 75	21.7 (10/46)	7.0 (3/43)	-14.8 [-28.91, -0.62]	0.0711	7.0 (3/43)	-14.8 [-28.91, -0.62]	0.0711
⩾ 75	22.2 (4/18)	6.7 (1/15)	-15.6 [-38.54, 7.43]	0.3457	4.5 (1/22)	-17.7 [-38.76, 3.41]	0.1554
Sex							
Male	13.9 (19/137)	6.3 (10/159)	-7.6 [-14.49, -0.67]	0.0321	1.3 (2/159)	-12.6 [-18.65, -6.57]	< 0.0001
Female	23.7 (14/59)	0.0 (0/38)	-23.7 [-34.58, -12.87]	0.0007	4.8 (2/42)	-19.0 [-31.59, -6.35]	0.0120
Smoking classification							
Never smoked	22.4 (17/76)	1.9 (1/54)	-20.5 [-30.55, -10.48]	0.0006	5.0 (3/60)	-17.4 [-28.24, -6.50]	0.0062
Current smoker	20.0 (8/40)	6.6 (4/61)	-13.4 [-27.31, 0.42]	0.0588	0.0 (0/57)	-20.0 [-32.40, -7.60]	0.0005
Ex-smoker	10.0 (8/80)	6.1 (5/82)	-3.9 [-12.27, 4.47]	0.3998	1.2 (1/84)	-8.8 [-15.78, -1.84]	0.0160
Erosive esophagitis grade[4]							
LA Grade A/B	11.0 (17/155)	3.1 (5/159)	-7.8 [-13.44, -2.21]	0.0075	1.3 (2/158)	-9.7 [-14.92, -4.48]	0.0002
LA Grade C/D	39.0 (16/41)	13.2 (5/38)	-25.9 [-44.26, -7.47]	0.0114	4.7 (2/43)	-34.4 [-50.58, -18.17]	0.0001
CYP2C19 genotype							
Extensive metabolizers	19.6 (31/158)	5.4 (9/166)	-14.2 [-21.28, -7.11]	0.0001	1.8 (3/168)	-17.8 [-24.34, -11.33]	< 0.0001
Poor metabolizers	5.3 (2/38)	3.2 (1/31)	-2.0 [-11.48, 7.40]	1.0000	3.0 (1/33)	-2.2 [-11.43, 6.97]	1.0000
H. pylori infection status							
Positive	3.7 (1/27)	2.7 (1/37)	-1.0 [-9.84, 7.83]	1.0000	0.0 (0/27)	-3.7 [-10.83, 3.42]	1.0000
Negative	18.9 (32/169)	5.6 (9/160)	-13.3 [-20.21, -6.41]	0.0003	2.2 (4/179)	-16.7 [-22.99. -10.41]	< 0.0001

[1]Data expressed as percentages with number of subjects in parentheses; [2]Calculated for difference between VPZ group and LPZ 15 mg group; [3]*Post hoc* analysis; [4]LA Classification Grade of erosive esophagitis by principal investigator at baseline. CI: Confidence interval; LA: Los Angeles; LPZ: Lansoprazole; VPZ: Vonoprazan.

Table 4 Summary of treatment-emergent adverse events during maintenance treatment *n* (%)

	LPZ 15 mg (*n* = 201)		VPZ 10 mg (*n* = 202)		VPZ 20 mg (*n* = 204)	
	Events	Patients	Events	Patients	Events	Patients
Any TEAE	166	103 (51.2)	220	109 (54.0)	212	120 (58.8)
Drug-related TEAE	30	23 (11.4)	26	21 (10.4)	23	21 (10.3)
TEAE leading to study discontinuation	10	8 (4.0)	5	5 (2.5)	8	8 (3.9)
Any serious TEAE	4	4 (2.0)	5	5 (2.5)	4	4 (2.0)
Death	0	0 (0.0)	0	0 (0.0)	0	0 (0.0)
TEAEs reported in ⩾ 2% of patients in any group, irrespective of causal relationship to study medication, during maintenance treatment.						
TEAE (preferred term)	LPZ 15 mg		VPZ 10 mg		VPZ 20 mg	
Nasopharyngitis	28 (13.9)		34 (16.8)		27 (13.2)	
Diarrhea	11 (5.5)		6 (3.0)		5 (2.5)	
Upper respiratory tract inflammation	3 (1.5)		8 (4.0)		4 (2.0)	
Elevated blood creatinine phosphokinase	2 (1.0)		4 (2.0)		6 (2.9)	
Elevated blood triglycerides	6 (3.0)		1 (0.5)		5 (2.5)	
Fall	1 (0.5)		8 (4.0)		2 (1.0)	
Gastroenteritis	1 (0.5)		5 (2.5)		5 (2.5)	
Back pain	1 (0.5)		3 (1.5)		5 (2.5)	
Constipation	4 (2.0)		2 (1.0)		3 (1.5)	
Elevated ALT[1]	1 (0.5)		3 (1.5)		4 (2.0)	
Contusion	1 (0.5)		5 (2.5)		2 (1.0)	
Seasonal allergy	2 (1.0)		4 (2.0)		2 (1.0)	
Bronchitis	2 (1.0)		5 (2.5)		0 (0.0)	
Dizziness	1 (0.5)		4 (2.0)		2 (1.0)	
Abnormal liver function test[2]	1 (0.5)		2 (1.0)		4 (2.0)	
Abnormal hepatic function[2]	1 (0.5)		0 (0.0)		4 (2.0)	
Periodontitis	0 (0.0)		4 (2.0)		1 (0.5)	

[1]Recorded as a special-interest adverse event (SIAE) if ALT > 3 × the upper limit of normal (ULN); [2]Recorded as a SIAE if total bilirubin > 2 × ULN in two consecutive measurements. ALT: Alanine aminotransferase; LPZ: Lansoprazole; TEAE: Treatment-emergent adverse event; VPZ: Vonoprazan.

TEAEs, respectively); of the TEAEs reported, one case of atrial fibrillation and abnormal liver function test [elevated ALT and AST (303 U/L and 228 U/L, respectively)] in the vonoprazan 20 mg group were considered to be possibly related to the study drug. The abnormal liver function test was reported in a patient with a prior history of alcoholic hepatic steatosis, and led to his premature withdrawal from the study. As no specific cause was identified, a possible causal relationship with the study drug could not be ruled out.

With regard to SIAEs, one case each of abnormal liver function test [elevated ALT (179 IU/L) and AST (209 IU/L) owing to fenofibrate treatment for dyslipidemia and elevated ALT (137 IU/L), which was not associated with any symptoms and was considered possibly related to the study medication] were reported in the lansoprazole 15 mg group, while two cases of abnormal liver function test were reported in the vonoprazan 10 mg group [elevated ALT (467 IU/L) and AST (571 IU/L) in one patient, which were considered possibly related to the study medication; and elevated ALT (326 IU/L) and AST (127 IU/L) that occurred in a patient with concurrent hepatic steatosis and were considered unrelated to the study drug]. In the vonoprazan 20 mg group, elevated ALT (86 IU/L) and AST (47 IU/L) were reported at the final study visit in a patient with concurrent hyperlipidemia and hepatic steatosis. Having completed the study, the patient began to receive lansoprazole as maintenance treatment for EE. Four weeks after the patient had completed the study, a further ALT elevation (139 IU/L) was reported, which qualified as a SIAE. Two days later, dark urine and itching were reported. The patient's condition remained unresolved 2 mo later but, owing to the invasive nature of blood sampling, the investigator decided that further follow-up was unnecessary, and that the patient should receive routine medical care and further treatment as required. As the initial ALT and AST elevations had occurred during the maintenance period of the study, the possibility of a causal relationship with the study medication could not be ruled out. Also in the vonoprazan 20 mg group, elevated ALT (138 IU/L, which was considered to have been caused by pre-existing hepatic steatosis) was reported in one patient, and two cases of abnormal liver function test were noted; the first in a patient with ALT elevated to 161 IU/L following the consumption of a large quantity of alcohol, and the second being the case that is described above as a serious TEAE. All the SIAEs were considered resolved or resolving, with the exception of the case of abnormal hepatic function in the vonoprazan 20 mg group. This patient was followed up with routine medical care and treated as required.

Mean levels of serum gastrin, pepsinogen Ⅰ, and pepsinogen Ⅱ increased in all three groups after the start of maintenance therapy; as shown in Figure 2, the increases were greatest with vonoprazan 20 mg and least with lansoprazole 15 mg. Histopathologic examinations showed that the observed increases in serum gastrin were not associated with clinically significant effects on the gastric mucosa. Similar slight increases in the number and density of Grimelius-positive cells were observed from baseline to Week 24 in all treatment groups (Table 5), leading to increased ratios of Grimelius-positive cells to epithelial cells. No clinically significant treatment-related changes were noted in gastric mucosal cell density, or in the percentage and density of chromogranin A-, synaptophysin-, and Ki-67-positive cells (Table 5).

No clinically significant changes were observed in clinical laboratory test values, vital signs, or ECG findings in any group during maintenance treatment.

DISCUSSION

The findings of this study demonstrate the non-inferiority of once-daily maintenance therapy with vonoprazan 10 mg or 20 mg to lansoprazole 15 mg for the prevention of EE recurrence in Japanese patients with healed EE. The upper limits of 95%CI for the differences in EE recurrence rate between vonoprazan 10 mg or 20 mg and lansoprazole 15 mg at 24 wk of maintenance treatment were below 0, indicating a statistically significant difference.

The prevalence of EE has increased in Japan over the past few decades, owing to factors such as the adoption of a westernized lifestyle, the aging of the population, and the decreasing incidence of *H. pylori* infection[22]. Moreover, endoscopic EE remission rates after healing following PPI treatment have been shown to be markedly lower in patients with more severe (LA grades C/D) *vs* milder disease[23]. In the current study, recurrence rates in patients with baseline LA grade C/D EE were significantly reduced with vonoprazan 10 mg (13.2%) and 20 mg (4.7%) *vs* lansoprazole 15 mg (39.0%) (P = 0.0114 and P = 0.0001, respectively). In addition, treatment with both vonoprazan 10 mg and 20 mg reduced recurrence rates compared with lansoprazole 15 mg among CYP2C19 extensive metabolizers (5.4% and 1.8%, respectively, *vs* 19.6%). These findings support the hypothesis that vonoprazan provides clinical benefits through potent and sustained gastric suppression in difficult-to-treat EE subgroups with more severe disease, as well as in those with milder disease.

The doses of vonoprazan and lansoprazole selected for evaluation in this study were consistent with the doses of acid suppressants commonly used for the maintenance of healed EE. PPIs are well-established in this indication, typically being approved for administration at either the same or half the dose approved for the healing of EE[24-26]. As vonoprazan is an acid suppressant, we decided to evaluate both the clinically recommended dose for EE healing and half that dose as maintenance regimens in this study. Our group previously carried out a phase Ⅱ dose-ranging study of vonoprazan in 732 Japanese patients with EE[27]. Vonoprazan, administered at once-daily doses of 5-40 mg, was found to be non-inferior to lansoprazole 30 mg once daily with respect to the rate of endoscopically-confirmed EE healing after 4 wk of treatment. Moreover, the rate of EE healing in patients with LA grade C/D EE was > 95% with vonoprazan doses of ⩾ 20 mg, *vs* 87% with lansoprazole 30 mg. The safety profile of vonoprazan at all administered doses was similar to that of lansoprazole 30 mg. On

Table 5 Histopathology of gastric mucosa: neuroendocrine cell density (/mm^2)

	LPZ 15 mg		VPZ 10 mg		VPZ 20 mg	
	n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
Epithelial cells (× 10^3)						
Baseline	28	1.58 (0.4831)	29	1.82 (0.3188)	28	1.74 (0.3943)
Week 24	24	1.63 (0.2689)	26	1.71 (0.4304)	28	1.54 (0.4744)
Grimelius-positive cells (× 10^2)						
Baseline	28	0.716 (0.3997)	29	0.705 (0.5562)	28	0.656 (0.3778)
Week 24	24	1.06 (0.2676)	26	1.07 (0.3858)	28	0.943 (0.4260)
Chromogranin A-positive cells (× 10^2)						
Baseline	28	1.35 (0.6625)	29	1.25 (0.7250)	28	1.35 (0.7073)
Week 24	24	1.35 (0.2962)	26	1.31 (0.4595)	28	1.20 (0.5041)
Synaptophysin-positive cells (× 10^2)						
Baseline	28	1.73 (0.7005)	29	1.73 (0.8123)	28	1.83 (0.9076)
Week 24	24	1.58 (0.3716)	26	1.55 (0.4490)	28	1.45 (0.6173)
Ki-67-positive cells (× 10^2)						
Baseline	28	1.44 (0.8192)	29	1.10 (0.6624)	28	1.32 (0.5513)
Week 24	24	1.14 (0.5037)	26	1.09 (0.4075)	28	1.05 (0.4853)

LPZ: Lansoprazole; SD: Standard deviation; VPZ: Vonoprazan.

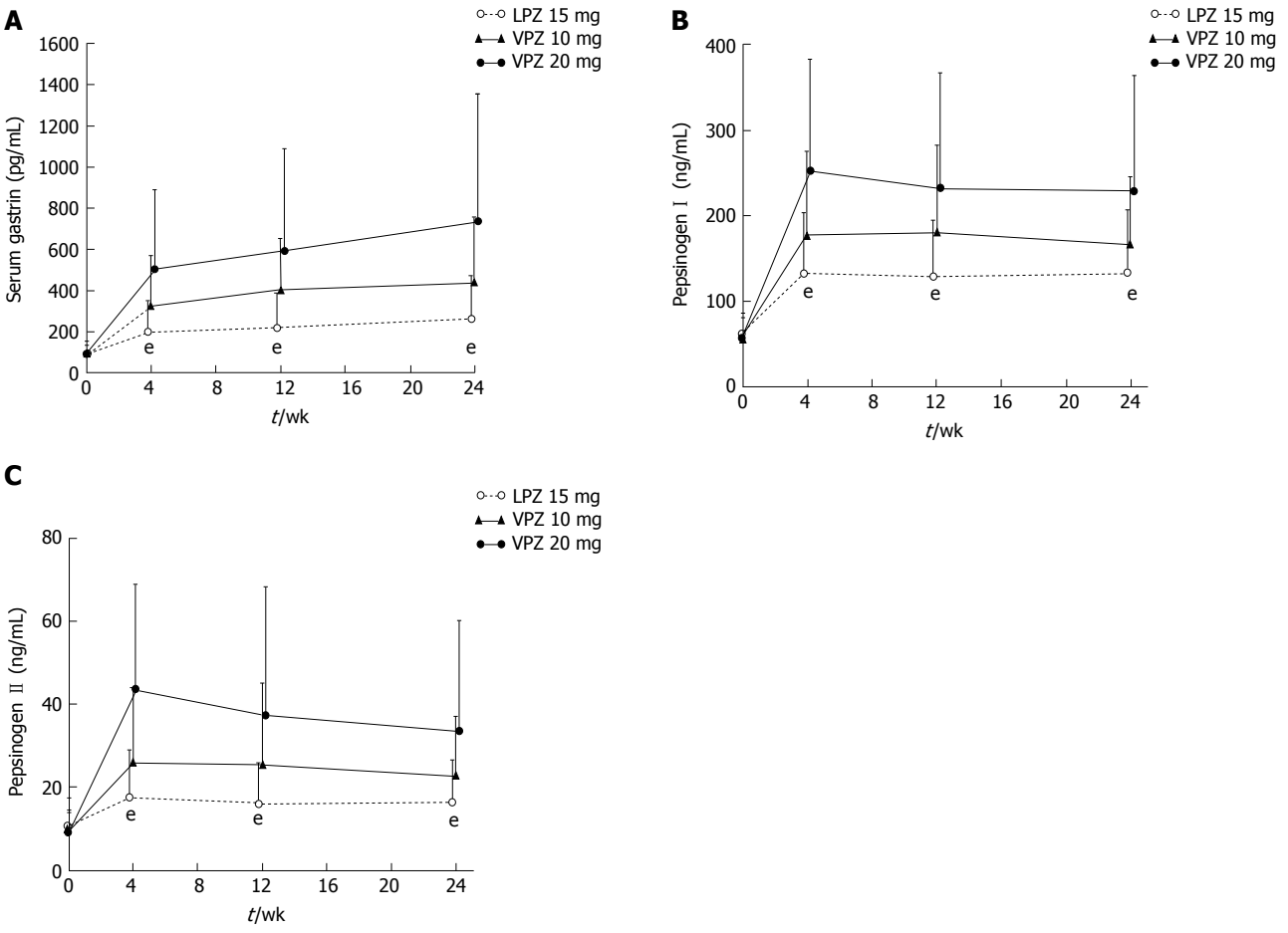

Figure 2 Time course of serum gastrin, pepsinogen Ⅰ, and pepsinogen Ⅱ concentrations. Data expressed as arithmetic mean ± SD. $^e P < 0.0001$ for VPZ 10 mg or 20 mg *vs* LPZ 15 mg. LPZ: Lansoprazole; SD: Standard deviation; VPZ: Vonoprazan.

the basis of these findings, 20 mg once daily was established as the clinically recommended dose of vonoprazan for the treatment of EE[27]. Therefore, the doses of vonoprazan evaluated as maintenance therapy in the present study were 20 and 10 mg once daily - representing the clinically recommended dose for the treatment of EE and half that dose. Lansoprazole was evaluated at the 15 mg dose that is approved for the maintenance of healed EE[26].

Vonoprazan 10 and 20 mg demonstrated similar safety profiles to lansoprazole 15 mg during the 24-wk maintenance period. All three investigated maintenance

regimens were well tolerated overall, with only a small number of TEAE-related withdrawals reported in each group. No new safety signals were identified for vonoprazan during the study. The increase in serum gastrin that we observed was not associated with clinically significant effects on the gastric mucosa. This, as well as the observed increases in pepsinogen Ⅰ and Ⅱ, were likely a negative feedback effect caused by the increase in intragastric pH that resulted from treatment with lansoprazole or vonoprazan. Histopathology of the gastric mucosa revealed no notable effects of the study drugs on neuroendocrine cells between baseline and Week 24, although the study was too short to rule out the possibility of clinically significant histopathologic changes occurring in the gastric mucosa over the long term. Thus, longer-term studies (> 1 year) are required to monitor any potential effects of vonoprazan on gastric mucosa.

This study was limited by its relatively short duration; nevertheless, the findings reported in this paper build on those from prior studies by our group, which investigated the efficacy and safety of vonoprazan in patients with acid-related disorders. In addition to the aforementioned phase Ⅱ dose-ranging study, which demonstrated the non-inferiority of vonoprazan 5-40 mg once daily to lansoprazole 30 mg once daily in terms of rates of EE healing over 4 wk[27], a recent phase Ⅲ trial confirmed the non-inferiority of vonoprazan 20 mg to lansoprazole 30 mg in the same indication within an 8-wk period[18]. Vonoprazan was found to be highly effective even among CYP2C19 extensive metabolizers and patients with baseline EE of LA Classification grade C/D. Other studies have also shown promising results with vonoprazan in the treatment of gastric or duodenal ulcers[28], and in the prevention of recurrent ulcers of these types in patients receiving low-dose aspirin or NSAIDs (ClinicalTrials.gov. identifiers NCT01452763, NCT01456247, NCT01452750, and NCT01456260).

While the primary objective of the present study was to verify the non-inferiority of vonoprazan to lansoprazole, the two-sided 95%CI for the difference between each vonoprazan group and the lansoprazole group were calculated as pre-planned for the primary analysis. A *post-hoc* Fisher's exact test was also performed as a sensitivity analysis to further support the results of the primary assessment using the CIs. These analyses confirmed that vonoprazan provided more consistent maintenance of EE healing at doses of 10 mg and 20 mg than lansoprazole 15 mg, even among CYP2C19 extensive metabolizers and patients with LA grade C/D EE. These findings suggest that vonoprazan may represent a viable alternative to PPIs in maintaining EE healing, with two doses being available for physicians to choose from.

In conclusion, this phase Ⅲ trial confirmed the non-inferiority of vonoprazan 10 mg and 20 mg to lansoprazole 15 mg once daily in preventing EE recurrence during 24 wk of maintenance treatment in Japanese patients. The safety profile of vonoprazan at the administered doses was similar to that of lansoprazole 15 mg.

ARTICLE HIGHLIGHTS

Research background

Proton-pump inhibitors (PPIs) such as lansoprazole are widely accepted as the treatment of choice for acid-related disorders, including erosive esophagitis (EE). Nevertheless, agents of this class are associated with notable shortcomings, which include: significant inter-individual variability in the time to onset of action; reduced night-time efficacy in preventing acid regurgitation, leading to nocturnal acid breakthrough; and differences in plasma concentrations and acid-inhibitory effects in extensive versus poor CYP2C19 metabolizers.

Vonoprazan fumarate (TAK-438) belongs to a relatively new class of acid suppressants known as potassium-competitive acid blockers (P-CABs), which, by virtue of their novel mechanism of action, offer a number of potential advantages over PPIs in the treatment of acid-related disorders. In animal studies, vonoprazan provided more potent and sustained suppression of gastric acid secretion than lansoprazole, while studies in healthy human volunteers demonstrated rapid, sustained, and dose-related suppression of 24-h gastric acid secretion. The present study adds to these earlier findings by confirming that vonoprazan is non-inferior to lansoprazole in preventing EE recurrence in Japanese patients with healed EE.

Research motivation

As a result of the increasingly widespread adoption of a westernized lifestyle and the general aging of the population, EE is now the most common acid-related disorder in Japan. Typical symptoms of EE include heartburn, acid reflux, difficulty swallowing, and sore throat, which can negatively impact patients' quality of life. In Japan, as elsewhere, PPIs remain the mainstay of treatment for EE and other acid-related disorders; however, in view of the limitations of PPIs mentioned above, there is a need for new treatment modalities that offer greater efficacy and more consistent outcomes. Any treatments that improve outcomes in EE may also be beneficial in gastroesophageal reflux disease, duodenal ulcer, and other acid-related disorders, and could become the focus of a new area of research.

Research objectives

The main objective of the research described in this paper was to demonstrate that the efficacy of vonoprazan in preventing EE recurrence is comparable to that of lansoprazole at its established maintenance dose. This objective was realized, with the results obtained confirming that vonoprazan, at doses of 10 and 20 mg once daily, is non-inferior to lansoprazole 15 mg once daily as maintenance therapy for healed EE. In addition, the safety profile of vonoprazan was shown to be similar to that of lansoprazole at the doses investigated. These findings suggest that vonoprazan may be a viable alternative to PPIs in the maintenance of EE healing, and provide a basis for future clinical trials to establish the optimal positioning of this new agent in the treatment of acid-related disorders.

Research methods

To establish the non-inferiority of vonoprazan 10 and 20 mg to lansoprazole 15 mg as maintenance therapy in Japanese patients with endoscopically-confirmed healed EE, we designed and conducted a multicenter, double-blind, randomized, phase Ⅲ clinical trial. Eligible patients received vonoprazan 10 or 20 mg, or lansoprazole 15 mg, once daily for 24 wk. The primary and secondary endpoints were the rate of EE recurrence at Weeks 24 and 12, respectively; safety outcomes were also evaluated. Based on EE recurrence rates in previous studies, it was calculated that 174 patients per treatment group would be required to provide > 90% power to confirm the non-inferiority of vonoprazan 10 and 20 mg to lansoprazole 15 mg.

Research results

We found that vonoprazan, administered at a dose of 10 or 20 mg once daily, is non-inferior to lansoprazole 15 mg once daily in maintaining EE healing in Japanese patients over a period of 24 wk, and demonstrates a comparable safety profile. Post-hoc analyses also confirmed that both doses of vonoprazan

investigated provide more consistent EE healing than lansoprazole, even in patients who are CYP2C19 extensive metabolizers and those with severe (Los Angeles grade C/D) EE. These results add to our previous findings that vonoprazan 5-40 mg once daily is non-inferior to lansoprazole 30 mg once daily in terms of EE healing rates over a 4-wk period, and that vonoprazan 20 mg once daily is non-inferior to lansoprazole 30 mg once daily in terms of 8-wk EE healing rates. As the maintenance period in this study was relatively short, further studies are needed to establish the long-term efficacy and safety characteristics of vonoprazan in the maintenance of EE healing.

Research conclusions

To our knowledge, this study is the first to confirm that vonoprazan is non-inferior to lansoprazole once daily in maintaining EE healing in Japanese patients. Importantly, it is also the first to show that vonoprazan is more consistent in maintaining EE healing, even in extensive CYP2C19 metabolizers and patients with more severe disease. These findings appear to confirm that the novel mechanism of action of vonoprazan is associated with advantages versus PPIs in the treatment of acid-related disorders, and suggest that vonoprazan could be an important new addition to the range of treatment options available to clinicians.

Research perspectives

This study confirms that vonoprazan demonstrates efficacy comparable with that of lansoprazole not only in healing EE, but also in maintaining the healing of EE over 24 wk. Future research should focus on evaluating the longer-term efficacy and safety of vonoprazan in this indication. In addition to randomized controlled trials, observational studies should be undertaken to gather valuable real-life data and inform decisions regarding the optimal positioning of vonoprazan in the management of EE.

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REFERENCES

1 **Maradey-Romero C**, Fass R. New and future drug development for gastroesophageal reflux disease. *J Neurogastroenterol Motil* 2014; **20**: 6-16 [PMID: 24466441 DOI: 10.5056/jnm.2014.20.1.6]
2 **Pace F**, Tonini M, Pallotta S, Molteni P, Porro GB. Systematic review: maintenance treatment of gastro-oesophageal reflux disease with proton pump inhibitors taken 'on-demand'. *Aliment Pharmacol Ther* 2007; **26**: 195-204 [PMID: 17593065 DOI: 10.1111/j.1365-2036.2007.03381.x]
3 **Hershcovici T**, Fass R. Gastro-oesophageal reflux disease: beyond proton pump inhibitor therapy. *Drugs* 2011; **71**: 2381-2389 [PMID: 22117130 DOI: 10.2165/11597300-000000000-00000]
4 **Fass R**. Alternative therapeutic approaches to chronic proton pump inhibitor treatment. *Clin Gastroenterol Hepatol* 2012; **10**: 338-345; quiz e39-40 [PMID: 22178462 DOI: 10.1016/j.cgh.2011.12.020]
5 **Hungin AP**, Hill C, Molloy-Bland M, Raghunath A. Systematic review: Patterns of proton pump inhibitor use and adherence in gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2012; **10**: 109-116 [PMID: 21782770 DOI: 10.1016/j.cgh.2011.07.008]
6 **Fass R**, Shapiro M, Dekel R, Sewell J. Systematic review: proton-pump inhibitor failure in gastro-oesophageal reflux disease--where next? *Aliment Pharmacol Ther* 2005; **22**: 79-94 [PMID: 16011666 DOI: 10.1111/j.1365-2036.2005.02531.x]
7 **El-Serag H**, Becher A, Jones R. Systematic review: persistent reflux symptoms on proton pump inhibitor therapy in primary care and community studies. *Aliment Pharmacol Ther* 2010; **32**: 720-737 [PMID: 20662774 DOI: 10.1111/j.1365-2036.2010.04406.x]
8 **Andersson K**, Carlsson E. Potassium-competitive acid blockade: a new therapeutic strategy in acid-related diseases. *Pharmacol Ther* 2005; **108**: 294-307 [PMID: 16000224 DOI: 10.1016/j.pharmthera.2005.05.005]
9 **Cederberg C**, Lind T, Röhss K, Olbe L. Comparison of once-daily intravenous and oral omeprazole on pentagastrin-stimulated acid secretion in duodenal ulcer patients. *Digestion* 1992; **53**: 171-178 [PMID: 1291405 DOI: 10.1159/000200992]
10 **Dammann HG**, Burkhardt F. Pantoprazole versus omeprazole: influence on meal-stimulated gastric acid secretion. *Eur J Gastroenterol Hepatol* 1999; **11**: 1277-1282 [PMID: 10563540]
11 **Shin JM**, Inatomi N, Munson K, Strugatsky D, Tokhtaeva E, Vagin O, Sachs G. Characterization of a novel potassium-competitive acid blocker of the gastric H,K-ATPase, 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine monofumarate (TAK-438). *J Pharmacol Exp Ther* 2011; **339**: 412-420 [PMID: 21828261 DOI: 10.1124/jpet.111.185314]
12 **Hori Y**, Imanishi A, Matsukawa J, Tsukimi Y, Nishida H, Arikawa Y, Hirase K, Kajino M, Inatomi N. 1-[5-(2-Fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine monofumarate (TAK-438), a novel and potent potassium-competitive acid blocker for the treatment of acid-related diseases. *J Pharmacol Exp Ther* 2010; **335**: 231-238 [PMID: 20624992 DOI: 10.1124/jpet.110.170274]
13 **Matsukawa J**, Hori Y, Nishida H, Kajino M, Inatomi N. A comparative study on the modes of action of TAK-438, a novel potassium-competitive acid blocker, and lansoprazole in primary cultured rabbit gastric glands. *Biochem Pharmacol* 2011; **81**: 1145-1151 [PMID: 21371447 DOI: 10.1016/j.bcp.2011.02.009]
14 **Hori Y**, Matsukawa J, Takeuchi T, Nishida H, Kajino M, Inatomi N. A study comparing the antisecretory effect of TAK-438, a novel potassium-competitive acid blocker, with lansoprazole in animals. *J Pharmacol Exp Ther* 2011; **337**: 797-804 [PMID: 21411494 DOI: 10.1124/jpet.111.179556]
15 **Sakurai Y**, Nishimura A, Kennedy G, Hibberd M, Jenkins R, Okamoto H, Yoneyama T, Jenkins H, Ashida K, Irie S, Täubel J. Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Single Rising TAK-438 (Vonoprazan) Doses in Healthy Male Japanese/non-Japanese Subjects. *Clin Transl Gastroenterol* 2015; **6**: e94 [PMID: 26111126 DOI: 10.1038/ctg.2015.18]
16 **Jenkins H**, Sakurai Y, Nishimura A, Okamoto H, Hibberd M, Jenkins R, Yoneyama T, Ashida K, Ogama Y, Warrington S. Randomised clinical trial: safety, tolerability, pharmacokinetics and pharmacodynamics of repeated doses of TAK-438 (vonoprazan), a novel potassium-competitive acid blocker, in healthy male subjects. *Aliment Pharmacol Ther* 2015; **41**: 636-648 [PMID: 25707624 DOI: 10.1111/apt.13121]
17 **Lauritsen K**, Devière J, Bigard MA, Bayerdörffer E, Mózsik G, Murray F, Kristjánsdóttir S, Savarino V, Vetvik K, De Freitas D, Orive V, Rodrigo L, Fried M, Morris J, Schneider H, Eklund S, Larkö A. Esomeprazole 20 mg and lansoprazole 15 mg in maintaining healed reflux oesophagitis: Metropole study results. *Aliment Pharmacol Ther* 2003; **17** Suppl 1: 24; discussion 25-24; discussion 27 [PMID: 12614304 DOI: 10.1046/j.1365-2036.17.s1.7.x]
18 **Ashida K**, Sakurai Y, Hori T, Kudou K, Nishimura A, Hiramatsu N, Umegaki E, Iwakiri K. Randomised clinical trial: vonoprazan, a novel potassium-competitive acid blocker, vs. lansoprazole for the healing of erosive oesophagitis. *Aliment Pharmacol Ther* 2016; **43**: 240-251 [PMID: 26559637 DOI: 10.1111/apt.13461]

19 **Kahrilas PJ**, Dent J, Lauritsen K, Malfertheiner P, Denison H, Franzén S, Hasselgren G. A randomized, comparative study of three doses of AZD0865 and esomeprazole for healing of reflux esophagitis. *Clin Gastroenterol Hepatol* 2007; **5**: 1385-1391 [PMID: 17950677 DOI: 10.1016/j.cgh.2007.08.014]
20 **Endo M**, Sugihara K. [Long-term maintenance treatment of reflux esophagitis resistant to H2-RA with PPI (lansoprazole)]. *Nihon Rinsho* 2000; **58**: 1865-1870 [PMID: 11004817]
21 **Farrington CP**, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. *Stat Med* 1990; **9**: 1447-1454 [PMID: 2281232 DOI: 10.1002/sim.4780091208]
22 **Fujiwara Y**, Arakawa T. Epidemiology and clinical characteristics of GERD in the Japanese population. *J Gastroenterol* 2009; **44**: 518-534 [PMID: 19365600 DOI: 10.1007/s00535-009-0047-5]
23 **Higuchi K**, Joh T, Nakada K, Haruma K. Is proton pump inhibitor therapy for reflux esophagitis sufficient?: a large real-world survey of Japanese patients. *Intern Med* 2013; **52**: 1447-1454 [PMID: 23812190 DOI: 10.2169/internalmedicine.52.0349]
24 **AstraZeneca Company**. NEXIUM prescribing information. Accessed July 12, 2017 Available from: URL: http://www.info.pmda.go.jp/go/pack/2329028F1023_1_28/
25 **Eisai Company Limited**. Pariet prescribing information. Accessed July 12, 2017 Available from: URL: http://www.info.pmda.go.jp/go/pack/2329029M1027_1_09/
26 **Takeda Pharmaceutical Company Limited**. Takepron prescribing information. Accessed July 12, 2017 Available from: URL: http://www.info.pmda.go.jp/go/pack/2329023M1020_1_23/
27 **Ashida K**, Sakurai Y, Nishimura A, Kudou K, Hiramatsu N, Umegaki E, Iwakiri K, Chiba T. Randomised clinical trial: a dose-ranging study of vonoprazan, a novel potassium-competitive acid blocker, vs. lansoprazole for the treatment of erosive oesophagitis. *Aliment Pharmacol Ther* 2015; **42**: 685-695 [PMID: 26201312 DOI: 10.1111/apt.13331]
28 **Miwa H**, Uedo N, Watari J, Mori Y, Sakurai Y, Takanami Y, Nishimura A, Tatsumi T, Sakaki N. Randomised clinical trial: efficacy and safety of vonoprazan vs. lansoprazole in patients with gastric or duodenal ulcers - results from two phase 3, non-inferiority randomised controlled trials. *Aliment Pharmacol Ther* 2017; **45**: 240-252 [PMID: 27891632 DOI: 10.1111/apt.13876]

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META-ANALYSIS

Application of enhanced recovery after gastric cancer surgery: An updated meta-analysis

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Abstract

AIM

To provide an updated assessment of the safety and efficacy of enhanced recovery after surgery (ERAS) protocols in elective gastric cancer (GC) surgery.

METHODS

PubMed, Medline, EMBASE, World Health Organization International Trial Register, and Cochrane Library were searched up to June 2017 for all available randomized controlled trials (RCTs) comparing ERAS protocols and standard care (SC) in GC surgery. Thirteen RCTs, with a total of 1092 participants, were analyzed in this study, of whom 545 underwent ERAS protocols and 547 received SC treatment.

RESULTS

No significant difference was observed between ERAS and control groups regarding total complications (P = 0.88), mortality (P = 0.50) and reoperation (P = 0.49).

The incidence of pulmonary infection was significantly reduced (P = 0.03) following gastrectomy. However, the readmission rate after GC surgery nearly tripled under ERAS (P = 0.009). ERAS protocols significantly decreased the length of postoperative hospital stay (P < 0.00001) and medical costs (P < 0.00001), and accelerated bowel function recovery, as measured by earlier time to the first flatus (P = 0.0004) and the first defecation (P < 0.0001). Moreover, ERAS protocols were associated with a lower level of serum inflammatory response, higher serum albumin, and superior short-term quality of life (QOL).

CONCLUSION

Collectively, ERAS results in accelerated convalescence, reduction of surgical stress and medical costs, improved nutritional status, and better QOL for GC patients. However, high-quality multicenter RCTs with large samples and long-term follow-up are needed to more precisely evaluate ERAS in radical gastrectomy.

Key words: Enhanced recovery after surgery; Safety; Gastric cancer; Efficacy; Meta-analysis

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Core tip: Enhanced recovery after surgery (ERAS) has emerged as an optimal perioperative strategy for improving clinical outcomes in gastric cancer surgery. However, numerous controversies exist with regard to ERAS practice after gastrectomy. To our knowledge, this study is the largest meta-analysis of randomized controlled trials to date, incorporating 1092 participants, of whom 545 received ERAS protocols and 547 received standard care, to assess the role of ERAS for radical gastrectomy. Our review clarified that ERAS results in accelerated convalescence, reduction of surgical stress and medical costs, improved nutritional status, and better quality of life for gastric cancer patients.

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INTRODUCTION

Enhanced recovery after surgery (ERAS), or fast-track surgery program, which was pioneered by Kehlet and Wilmore in the late 1990s, intends to attenuate surgical stress and accelerate postoperative functional recovery[1,2]. ERAS protocols involve a series of perioperative evidence-based interventions, the core elements of which include preoperative short fasting and carbohydrate-loaded fluids, intraoperative epidural anesthesia, minimally invasive procedures and fluid restriction, postoperative pain management, nutritional care and early ambulation[3-5]. Multimodal optimizing perioperative procedures were explored initially in the setting of elective colorectal resections, resulting in a significant reduction in overall hospital stay from 8-12 d to 2-5 d under the standard discharge criteria for conventional care[6,7]. Since then, ERAS concepts have become widely recognized and applied gradually to clinical practice. Currently, accumulating evidence highlights that the implementation of ERAS protocols in multiple surgical disciplines significantly reduces morbidity and mortality, while improving clinical outcomes without compromising patient safety[8-10].

Gastric cancer (GC) remains a major health problem in China and worldwide, and radical gastrectomy remains the most likely approach to cure GC. However, conventional perioperative care is associated with a high risk of morbidity after radical surgery, ranging from 12.5% to 39%[11-13]. Moreover, due to malnutrition of patients with gastric neoplasms and chronic comorbidities, perioperative mortality can reach up to 8.8%[14]. Postoperative complications result in prolonged inflammatory response, which is considered to have a negative influence not only on the overall survival (OS) but also on the disease-specific mortality of patients undergoing gastrectomy, even if the carcinoma is radically resected[15].

Given the strong evidence and recommendations for colorectal cancer, the application of ERAS protocols for gastrectomy procedures has been investigated in several studies[16-19]. ERAS principles combined with laparoscopic treatment for GC lead to satisfactory clinical outcomes[20-22], even in elderly patients[23,24]. Several meta-analyses have revealed that ERAS pathways in GC patients reduce the duration of hospital stay and medical costs without significantly increasing complications and hospital readmission[25-28], and the ERAS Society issued consensus guidelines for perioperative care after elective gastrectomy for GC in 2014[29].

However, there still remain numerous controversies, limitations and difficulties in ERAS practice after gastrectomy. Following the recent publication of two related high-level randomized controlled trials (RCTs)[22,30], we conducted an updated systematic review and meta-analysis to thoroughly assess the safety and efficacy of ERAS application in GC patients.

MATERIALS AND METHODS

Literature search

A comprehensive literature search in PubMed, Medline, EMBASE, World Health Organization International Trial Registry platform, and Cochrane Library was performed, until June 2017, independently to identify all available publications comparing the ERAS program with standard perioperative care (SC) for GC patients undergoing gastrectomy. The medical subject heading (MeSH) terms and free text terms searched

for, individually and in combination, were as follows: "fast track surgery" OR "accelerated rehabilitation" OR "enhanced recovery" OR "ERAS" OR "multimodal perioperative care" AND "gastric cancer" OR "stomach carcinoma" OR "gastrectomy" OR "gastric resection." This search strategy was able to identify all potential publications involving humans, without language restriction. Reference lists of all eligible articles were also scrutinized to identify any other related studies. Furthermore, bibliographies of systematic reviews or meta-analyses on this issue were hand-searched for additional articles that the electronic retrieval failed to capture.

Inclusion and exclusion criteria

The inclusion criteria for this study were: (1) evaluation of ERAS in comparison with traditional SC; (2) RCTs; (3) detailed patient data and outcomes available; (4) ERAS protocols composed of at least eight elements from consensus guidelines[29]; and (5) follow-up for at least 14 d after discharge. When more than one study reporting the same patient cohort was included in several publications, only the most recent or complete study was included.

The exclusion criteria were as follows: (1) non-comparative studies; (2) case-controlled trials, cohort studies, or retrospective studies; (3) application of less than eight items of ERAS; (4) no follow-up after discharge; and (5) other documentations that did not meet the inclusion criteria.

Study selection and data extraction

Following identification of citations from all potentially eligible studies, two investigators independently retrieved the full-text articles according to the inclusion criteria. Any discrepancies or divergences concerning inclusion were settled through discussion with a third reviewer until consensus was reached.

Data were extracted using a double-extraction method from each eligible study by the two investigators. Outcomes included morbidity, mortality, rates of readmission and reoperation, length of postoperative hospital stay (POHS), duration of flatus and defecation, medical costs, and postoperative inflammatory response and nutritional status, such as determined by serum C-reactive protein (CRP), interleukin-6 (IL-6) and serum albumin (ALB) concentrations.

Assessment of risk of bias

Another two investigators separately assessed the quality of identified RCTs using the criteria addressed in the Cochrane Collaboration[31]. The evaluation indices contained several aspects across randomization, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other bias. Risk of bias in each domain listed was graded as "high risk," "low risk," or "unclear."

Statistical analysis

Statistical analysis was performed using the software package Review Manager Version 5.3.3 (Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and STATA version 12 (Stata Corp LP, College Station, TX, United States). Pooled risk ratio (RR) with 95% confidence interval (CI) was utilized to analyze dichotomous data, while continuous data were analyzed as mean differences (MDs) with 95%CIs. Heterogeneity was evaluated using the chi-square test, for which $P < 0.1$ was considered statistically significant. The I^2 value was used to quantify the impact of heterogeneity on each analysis. If the test of heterogeneity was statistically significant, the random-effects model was used; otherwise, a fixed-effects model was used. When the study did not report specific values for mean and standard deviation (SD), these were estimated using median and range based on the methods previously described[32]. In short, the median was used as a substitute for the mean. When the sample size was greater than 70, SD was estimated as range/6, and when the sample size was 15-69, SD was calculated as range/4. In the case where the interquartile range (IQR) was available, the range was estimated to be the median ± IQR.

RESULTS

Included studies

The flow chart for the selection of literature according to the predefined retrieval strategies is shown in Figure 1. Ten studies[21-24,30,33-37] published between 2010 and 2017 met the inclusion criteria. Two studies[24,34] consisted of four groups comparing ERAS protocols and SC in laparoscopic or open radical gastrectomy, respectively, for stomach cancer, while another[23] comprised four groups comparing ERAS protocols and SC in adults (aged 45-74 years) or elderly individuals (aged 75-89 years) undergoing open gastrectomy for GC. These three studies were considered to be six independent studies with reference to previous reports[26,28]. Consequently, 13 RCTs from these 10 studies were included in the current systematic review and meta-analysis.

Characteristics and methodological quality

The main characteristics of the included studies are detailed in Table 1. All studies were from a single center involving a total of 1092 participants, of whom 545 underwent the ERAS protocol and 547 received SC treatment. The sample size ranged from 41 to 256, and four studies contained more than 100 patients[22,23,30,33]. Table 2 lists the relevant elements involved in these studies regarding the implementation of ERAS pathways based on the consensus conducted in RCTs. Surgical procedures for GC with curative intent involved proximal gastrectomy, distal gastrectomy, and total gastrectomy. These included studies were implemented predominantly in Asia (China, South

Table 1 Main characteristics of the included studies

Study	Year	Sample size		Age in yr		Sex, male/female		Approach	Neoadjuvant chemotherapy	Follow-up (d)
		ERAS	SC	ERAS	SC	ERAS	SC			
Abdikarim *et al*[21]	2015	30	31	63 ± 12	62 ± 11	21/9	20/11	Lap	No	30
Bu *et al*[23]-Adult	2015	64	64	62.4 ± 7.8	63.0 ± 7.4	31/33	35/29	Open	No	30
Bu *et al*[23]-Elderly	2015	64	64	80.1 ± 4.0	79.6 ± 3.5	37/27	40/24	Open	No	30
Chen Hu *et al*[34]-Lap	2012	19	22	59 (49-71)	62.5 (45-72)	10/9	10/12	Lap	No	28
Chen Hu *et al*[34]-Open	2012	21	20	62.5 (45-72)	64.5 (49-75)	9/12	12/8	Open	No	28
Feng *et al*[33]	2013	59	60	55.0 ± 11.4	55.8 ± 10.1	41/18	44/16	Open	No	28
Kim *et al*[35]	2012	22	22	52.6 ± 11.6	57.5 ± 14.5	13/9	15/7	Lap	-	14
Liu *et al*[36]	2010	33	30	60.7 ± 9.7	61.9 ± 8.3	18/15	16/14	Open	No	30
Liu *et al*[24]-Lap	2016	21	21	69.2 ± 5.1	70.3 ± 5.8	10/11	12/9	Lap	No	30
Liu *et al*[24]-Open	2016	21	21	67.8 ± 3.9	68.6 ± 4.9	9/12	11/10	Open	No	30
Mingjie *et al*[22]	2017	73	76	61 (40-75)	63 (35-75)	48/25	50/26	Lap	No	30
Tanaka *et al*[30]	2017	73	69	68 (29-85)	67 (44-85)	49/24	49/20	Lap/Open	No	30
Wang *et al*[37]	2010	45	47	58.8 ± 9.7	56.9 ± 9.1	32/13	29/18	Open	No	28

ERAS: Enhanced recovery after surgery; Lap: laparoscopic surgery; Open: Open surgery; SC: Standard care.

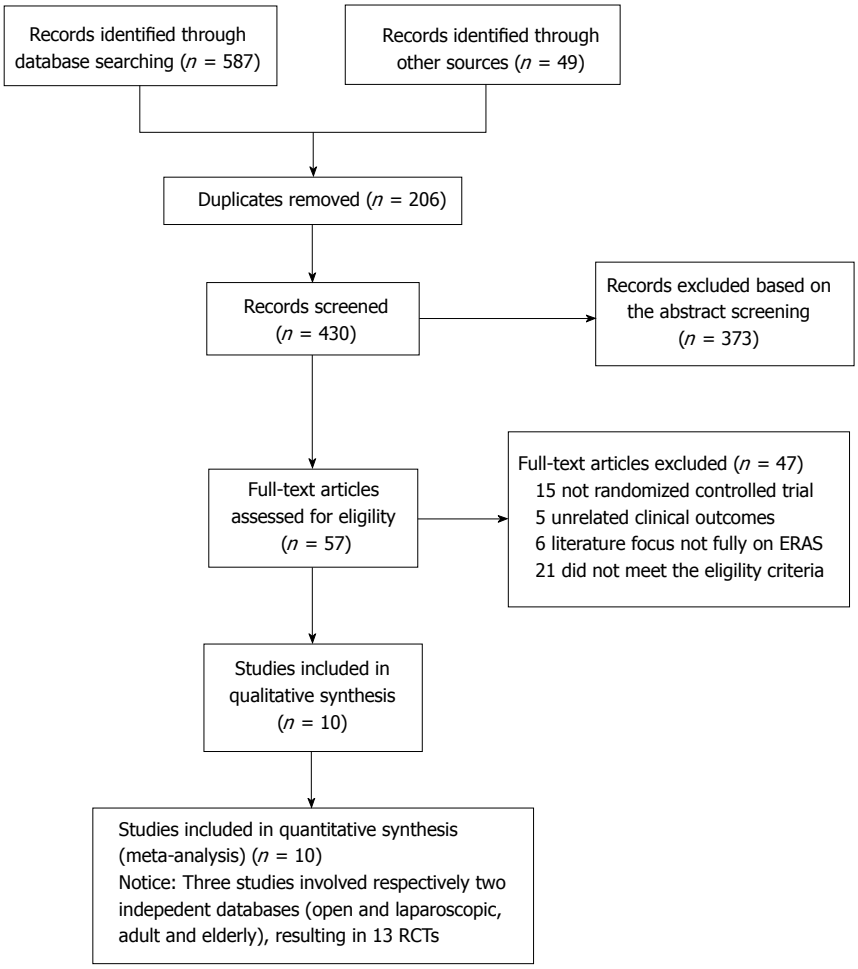


Figure 1 Study flow diagram: Enhanced recovery after surgery in gastric cancer. ERAS: Enhanced recovery after surgery; RCTs: Randomized controlled trials.

Korea, and Japan). Assessment of the risk of bias across all included studies is presented in Figure 2, most of which were of moderate quality. Blinding was the main risk of bias among these RCTs, as it was not easy to comply with double blinding in such procedural trials.

Postoperative morbidity and short-term mortality

Total complications: No significant difference was demonstrated between ERAS and the control group in the 13 RCTs regarding the incidence of total complications following gastrectomy (RR: 1.03, 95%CI: 0.73-1.44, P = 0.88) (Figure 3 and Table 3), but there

Table 2 Elements of enhanced recovery after surgery protocol applied in the included studies

Study	Year	No bowel preparation	Carbohydrate loading	No routine use of abdominal drainage	Fluid restriction	Pain management	Early mobilization	Early feeding	Others	No. of ERAS elements
Abdikarim *et al*[21]	2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	11
Bu *et al*[23]	2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	14
Chen Hu *et al*[34]	2012	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13
Feng *et al*[33]	2013	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Kim *et al*[35]	2012	Yes	Yes	-	-	Yes	Yes	Yes	Yes	10
Liu *et al*[36]	2010	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	12
Liu *et al*[24]	2016	Yes	Yes	-	Yes	Yes	Yes	Yes	Yes	11
Mingjie *et al*[22]	2017	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	13
Tanaka *et al*[30]	2017	Yes	Yes	Yes	-	Yes	Yes	Yes	Yes	22
Wang *et al*[37]	2010	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	14

ERAS: Enhanced recovery after surgery.

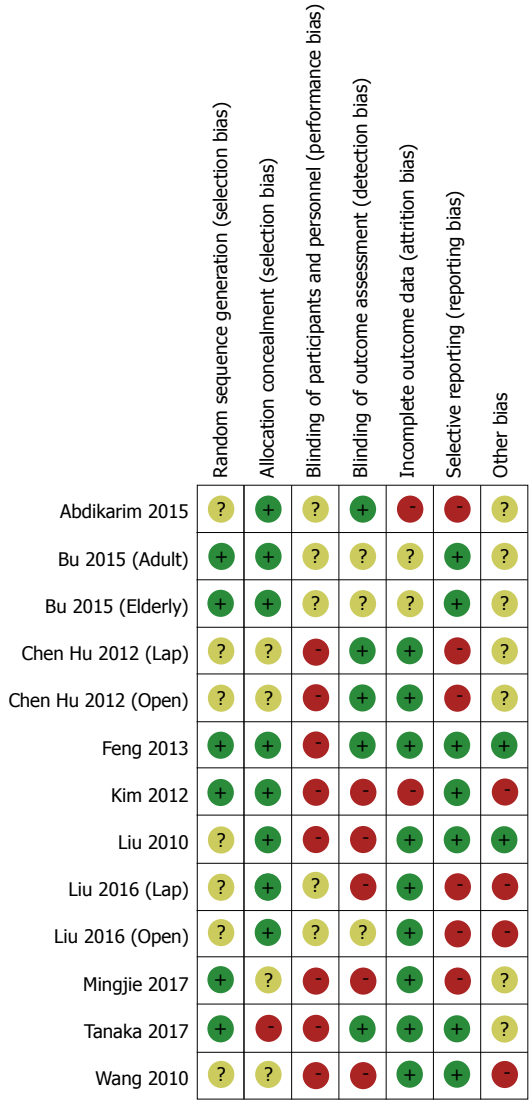


Figure 2 Risk of bias summary: Review of authors' judgments concerning each risk-of-bias item for each included study.

was significant heterogeneity among these studies (χ^2 = 47.12, I^2 = 75%, P < 0.00001). In five RCTs reporting a laparoscopic approach for GC[21,22,24,34,35], no significant difference in postoperative morbidity was found between the ERAS and SC groups (RR: 1.44, 95%CI: 0.93-2.23, P = 0.10), and no heterogeneity was observed (χ^2 = 2.18, P = 0.70; I^2 = 0). Similarly, in the open surgery RCTs[23,24,33,34,36,37], ERAS pathways did not increase the surgical complications (RR: 1.05, 95%CI: 0.68-1.63, P = 0.81), and significant heterogeneity was observed (χ^2 = 31.10, P < 0.0001; I^2 = 81%). However, three RCTs in the elderly[23,24] demonstrated that the incidence of complications was significantly higher in the ERAS arm than in the SC arm (RR: 1.45, 95%CI: 1.23-1.70, P < 0.00001), and no heterogeneity was found in the elderly (χ^2 = 1.51, P = 0.47; I^2 = 0).

Anastomotic leak: Ten RCTs[21-23,30,33,34,36,37] (964 patients) provided data on anastomotic leaks, whereby 2.3% (11/481 patients) in the ERAS group and 1.7% (8/483) in the SC group had an anastomotic leak. Pooling the results indicated that ERAS did not increase the incidence of anastomotic leaks compared with conventional care (RR: 1.36, 95%CI: 0.54-3.45, P = 0.51) (Figure 3), and heterogeneity was excluded among these trials (χ^2 = 2.35, P = 0.50; I^2 = 0).

Ileus: Twelve RCTs[21-24,30,33,34,36,37] (1048 patients) provided data regarding ileus: 3.3% (17/523 patients) in the ERAS group, and 1.9% (10/525) in the SC group had ileus. Pooling the results indicated that ERAS did not increase ileus compared with SC (RR: 1.62, 95%CI: 0.75-3.52, P = 0.22) (Figure 3), and no heterogeneity was observed among these trials (χ^2 = 5.76, P = 0.57; I^2 = 0).

Incision infection: Eleven RCTs[21-24,30,33,34,36,37] (1007 patients) reported incision infection, amounting to 2.8% (14/504 patients) in the ERAS group and 3.6% (18/503) in the SC group. Pooling the results indicated that ERAS did not increase incision infection compared with conventional care (RR: 0.79, 95%CI: 0.39-1.60, P = 0.52) (Figure 3), and there was no heterogeneity among these studies (χ^2 = 4.52, P = 0.87; I^2 = 0).

Urinary tract infection: Nine RCTs[23,24,33-37] (699 patients) provided data regarding urinary tract infection, which was observed in 2.6% (9/350 patients) in the

Table 3 Evaluation of the complications or outcomes in enhanced recovery after surgery *vs* standard care groups in the included studies

Subgroup	Studies, *n*	Participants, *n*	Statistical method	Effect estimate	Heterogeneity	
					I^2	*P* value
Total complications	13	1092	Risk ratio (M-H, random, 95%CI)	1.03 [0.73, 1.44]	75%	< 0.00001
Anastomotic leak	10	964	Risk ratio (M-H, random, 95%CI)	1.36 [0.54, 3.45]	0	0.50
Ileus	12	1048	Risk ratio (M-H, random, 95%CI)	1.62 [0.75, 3.52]	0	0.57
Incision infection	11	1007	Risk ratio (M-H, random, 95%CI)	0.79 [0.39, 1.60]	0	0.87
Urinary tract infection	9	699	Risk ratio (M-H, random, 95%CI)	0.53 [0.26, 1.08]	0	0.99
Pulmonary infection	9	775	Risk ratio (M-H, random, 95%CI)	0.52 [0.28, 0.94]	0	0.99
Postoperative hospital stay	13	1092	Mean difference (IV, random, 95%CI)	-1.65 [-2.09, -1.21]	89%	< 0.00001
Duration of first flatus	11	882	Mean difference (IV, random, 95%CI)	-12.70 [-19.71, -5.69]	92%	< 0.00001
Duration of first defecation	4	471	Mean difference (IV, random, 95%CI)	-28.07 [-41.48, -14.67]	90%	< 0.00001
Medical costs	10	819	Mean difference (IV, random, 95%CI)	-0.50 [-0.69, -0.30]	85%	< 0.00001
CRP						
POD1	8	514	Mean difference (IV, random, 95%CI)	-14.81 [-21.42, -8.21]	72%	0.0007
POD4	6	378	Mean difference (IV, random, 95%CI)	-19.81 [-29.64, -9.98]	64%	0.02
POD7	5	258	Mean difference (IV, random, 95%CI)	-21.36 [-28.81, -13.91]	74%	0.004
IL-6						
POD1	4	239	Mean difference (IV, random, 95%CI)	-61.22 [-114.58, -7.86]	99%	< 0.00001
POD4	3	147	Mean difference (IV, random, 95%CI)	-31.50 [-55.63, -7.38]	96%	< 0.00001
POD7	3	176	Mean difference (IV, random, 95%CI)	-26.62 [-34.23, -19.01]	89%	0.0001
ALB						
POD1	2	84	Mean difference (IV, random, 95%CI)	0.24 [-0.89, 1.36]	0	0.79
POD4	4	166	Mean difference (IV, random, 95%CI)	3.27 [2.24, 4.30]	23%	0.27
POD7	4	166	Mean difference (IV, random, 95%CI)	5.68 [3.31, 8.05]	83%	0.0005
Readmission	8	777	Risk ratio (M-H, Fixed, 95%CI)	2.86 [1.31, 6.24]	0	0.92
Reoperation	3	517	Risk ratio (M-H, Fixed, 95%CI)	0.62 [0.17, 2.35]	33%	0.22
Quality of life	2	136	Std. mean difference (IV, Fixed, 95%CI)	-0.46 [-0.80, -0.12]	36%	0.21

ALB: Serum albumin; CRP: C-reactive protein; IL-6: Interleukin-6; IV: Inverse Variance; M-H: Mantel-Haenszel; POD: Postoperative day.

ERAS group and 5.4% (19/349) in the SC group. Pooling the results indicated that ERAS did not increase urinary tract infection compared with conventional care (RR: 0.53, 95%CI: 0.26-1.08, $P = 0.08$) (Figure 3), and heterogeneity was excluded among these studies ($\chi^2 = 1.61$, $P = 0.99$; $I^2 = 0$).

Pulmonary infection: Nine RCTs[23,24,30,33,34,37] (775 patients) reported pulmonary infection, which affected 3.4% (13/387 patients) in the ERAS group and 7.2% (28/388) in the SC group. Pooling the results indicated that ERAS decreased significantly the incidence of pulmonary infection compared with conventional care (RR: 0.52, 95%CI: 0.28-0.94, $P = 0.03$) (Figure 3), and there was no heterogeneity among these studies ($\chi^2 = 1.09$, $P = 0.99$; $I^2 = 0$).

Short-term mortality

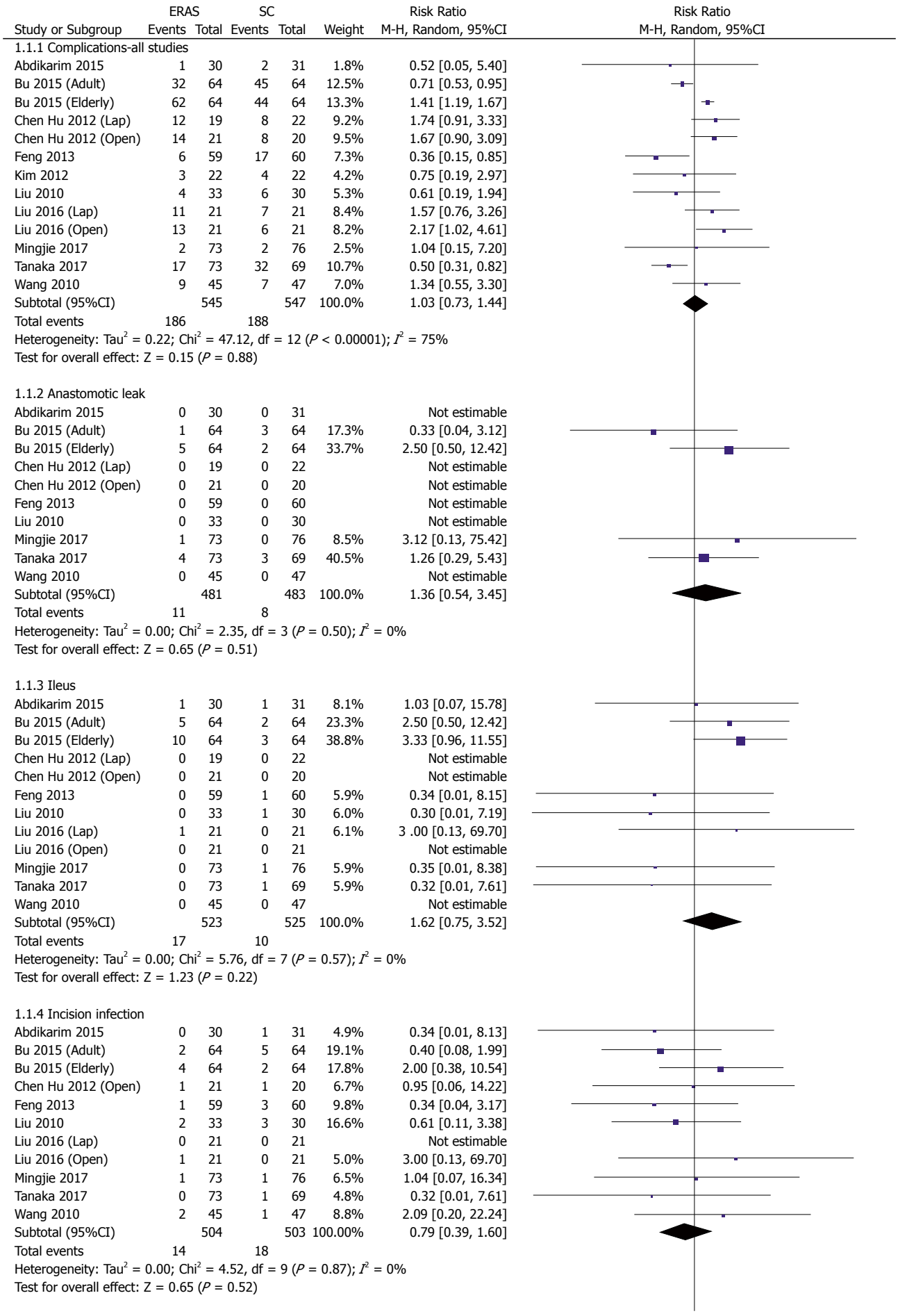
All studies reported short-term mortality after GC surgery; one patient (1/64) died of severe abdominal cavity infection in the elderly group[23]. No cases of death associated with surgery occurred in other studies during short-term follow-up. Pooling the results suggested that ERAS did not increase mortality compared with conventional care (RR: 3.0, 95%CI: 0.12-72.29, $P = 0.50$) (Figure 4).

Length of postoperative hospital stay

All included RCTs (1092 patients) reported POHS. Ten of these studies reported a significant reduction of POHS in the ERAS group, and three reported no significant difference. The elderly group in Bu's report[23], the laparoscopic group in Chen Hu's study[34], and the open group of Liu's report[24] demonstrated that patients receiving rapid rehabilitation care had POHS similar to that of the traditional care protocol. Meta-analysis revealed a significant reduction in POHS by 1.65 d with the application of the ERAS schemes compared with traditional perioperative care in pooled analysis (MD: -1.65, 95%CI: -2.09 to -1.21, $P < 0.00001$) (Figure 5), and the heterogeneity was significant among these studies ($\chi^2 = 105.17$, $P < 0.00001$; $I^2 = 89\%$). Laparoscopic surgery combined with ERAS[21,22,24,34,35] markedly reduced POHS compared with laparoscopic surgery alone (MD: -1.49, 95%CI: -2.25 to -0.74, $P < 0.0001$), and the heterogeneity was significant ($\chi^2 = 18.21$, $P = 0.001$; $I^2 = 78\%$). Similarly, there was a significant reduction in POHS observed in open surgery with ERAS[23,24,33,34,36,37] compared with open surgery alone (MD: -1.89, 95%CI: -2.69 to -1.09, $P < 0.00001$), and the heterogeneity was also significant ($\chi^2 = 61.54$, $P < 0.00001$; $I^2 = 90\%$).

Duration of intestinal function recovery

Eleven RCTs[23,24,30,33-37] (882 patients) analyzed the duration of first flatus. Recovery of gut function was earlier in ERAS groups, as shown by shorter duration of the first flatus and first defecation. The MD for duration of first flatus was -12.70 (95%CI: -19.71 to -5.69, $P = 0.0004$), but the heterogeneity was significant among


ERAS
SC
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95%CI
M-H, Random, 95%CI
1.1.1 Complications-all studies
Abdikarim 2015 1 30 2 31 1.8% 0.52 [0.05, 5.40]
Bu 2015 (Adult) 32 64 45 64 12.5% 0.71 [0.53, 0.95]
Bu 2015 (Elderly) 62 64 44 64 13.3% 1.41 [1.19, 1.67]
Chen Hu 2012 (Lap) 12 19 8 22 9.2% 1.74 [0.91, 3.33]
Chen Hu 2012 (Open) 14 21 8 20 9.5% 1.67 [0.90, 3.09]
Feng 2013 6 59 17 60 7.3% 0.36 [0.15, 0.85]
Kim 2012 3 22 4 22 4.2% 0.75 [0.19, 2.97]
Liu 2010 4 33 6 30 5.3% 0.61 [0.19, 1.94]
Liu 2016 (Lap) 11 21 7 21 8.4% 1.57 [0.76, 3.26]
Liu 2016 (Open) 13 21 6 21 8.2% 2.17 [1.02, 4.61]
Mingjie 2017 2 73 2 76 2.5% 1.04 [0.15, 7.20]
Tanaka 2017 17 73 32 69 10.7% 0.50 [0.31, 0.82]
Wang 2010 9 45 7 47 7.0% 1.34 [0.55, 3.30]
Subtotal (95%CI) 545 547 100.0% 1.03 [0.73, 1.44]
Total events 186 188
Heterogeneity: Tau² = 0.22; Chi² = 47.12, df = 12 (P < 0.00001); I² = 75%
Test for overall effect: Z = 0.15 (P = 0.88)
1.1.2 Anastomotic leak
Abdikarim 2015 0 30 0 31 Not estimable
Bu 2015 (Adult) 1 64 3 64 17.3% 0.33 [0.04, 3.12]
Bu 2015 (Elderly) 5 64 2 64 33.7% 2.50 [0.50, 12.42]
Chen Hu 2012 (Lap) 0 19 0 22 Not estimable
Chen Hu 2012 (Open) 0 21 0 20 Not estimable
Feng 2013 0 59 0 60 Not estimable
Liu 2010 0 33 0 30 Not estimable
Mingjie 2017 1 73 0 76 8.5% 3.12 [0.13, 75.42]
Tanaka 2017 4 73 3 69 40.5% 1.26 [0.29, 5.43]
Wang 2010 0 45 0 47 Not estimable
Subtotal (95%CI) 481 483 100.0% 1.36 [0.54, 3.45]
Total events 11 8
Heterogeneity: Tau² = 0.00; Chi² = 2.35, df = 3 (P = 0.50); I² = 0%
Test for overall effect: Z = 0.65 (P = 0.51)
1.1.3 Ileus
Abdikarim 2015 1 30 1 31 8.1% 1.03 [0.07, 15.78]
Bu 2015 (Adult) 5 64 2 64 23.3% 2.50 [0.50, 12.42]
Bu 2015 (Elderly) 10 64 3 64 38.8% 3.33 [0.96, 11.55]
Chen Hu 2012 (Lap) 0 19 0 22 Not estimable
Chen Hu 2012 (Open) 0 21 0 20 Not estimable
Feng 2013 0 59 1 60 5.9% 0.34 [0.01, 8.15]
Liu 2010 0 33 1 30 6.0% 0.30 [0.01, 7.19]
Liu 2016 (Lap) 1 21 0 21 6.1% 3 .00 [0.13, 69.70]
Liu 2016 (Open) 0 21 0 21 Not estimable
Mingjie 2017 0 73 1 76 5.9% 0.35 [0.01, 8.38]
Tanaka 2017 0 73 1 69 5.9% 0.32 [0.01, 7.61]
Wang 2010 0 45 0 47 Not estimable
Subtotal (95%CI) 523 525 100.0% 1.62 [0.75, 3.52]
Total events 17 10
Heterogeneity: Tau² = 0.00; Chi² = 5.76, df = 7 (P = 0.57); I² = 0%
Test for overall effect: Z = 1.23 (P = 0.22)
1.1.4 Incision infection
Abdikarim 2015 0 30 1 31 4.9% 0.34 [0.01, 8.13]
Bu 2015 (Adult) 2 64 5 64 19.1% 0.40 [0.08, 1.99]
Bu 2015 (Elderly) 4 64 2 64 17.8% 2.00 [0.38, 10.54]
Chen Hu 2012 (Open) 1 21 1 20 6.7% 0.95 [0.06, 14.22]
Feng 2013 1 59 3 60 9.8% 0.34 [0.04, 3.17]
Liu 2010 2 33 3 30 16.6% 0.61 [0.11, 3.38]
Liu 2016 (Lap) 0 21 0 21 Not estimable
Liu 2016 (Open) 1 21 0 21 5.0% 3.00 [0.13, 69.70]
Mingjie 2017 1 73 1 76 6.5% 1.04 [0.07, 16.34]
Tanaka 2017 0 73 1 69 4.8% 0.32 [0.01, 7.61]
Wang 2010 2 45 1 47 8.8% 2.09 [0.20, 22.24]
Subtotal (95%CI) 504 503 100.00% 0.79 [0.39, 1.60]
Total events 14 18
Heterogeneity: Tau² = 0.00; Chi² = 4.52, df = 9 (P = 0.87); I² = 0%
Test for overall effect: Z = 0.65 (P = 0.52)

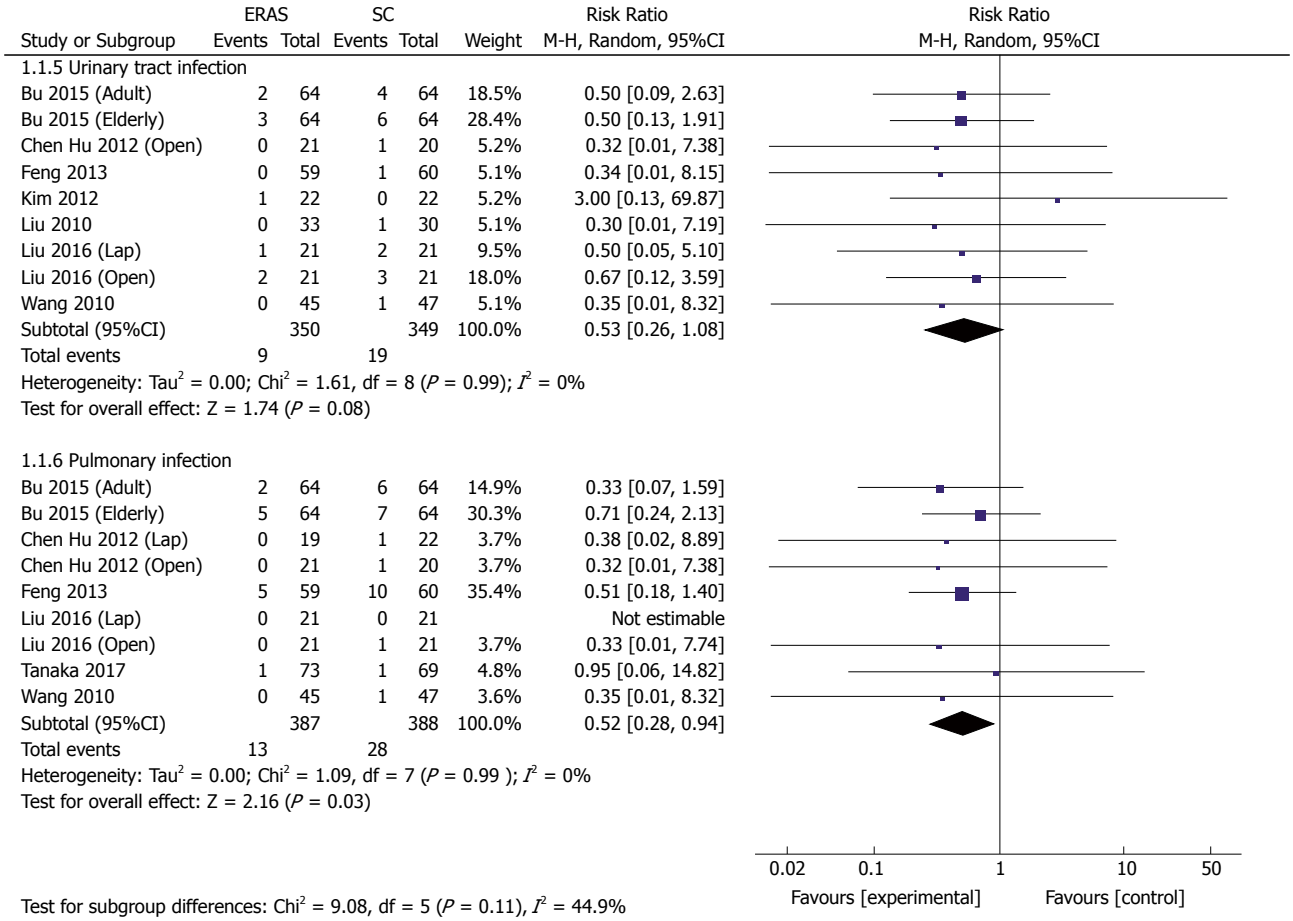


Figure 3 Forest plot evaluating the relative risk of surgical complications: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; SC: Standard care.

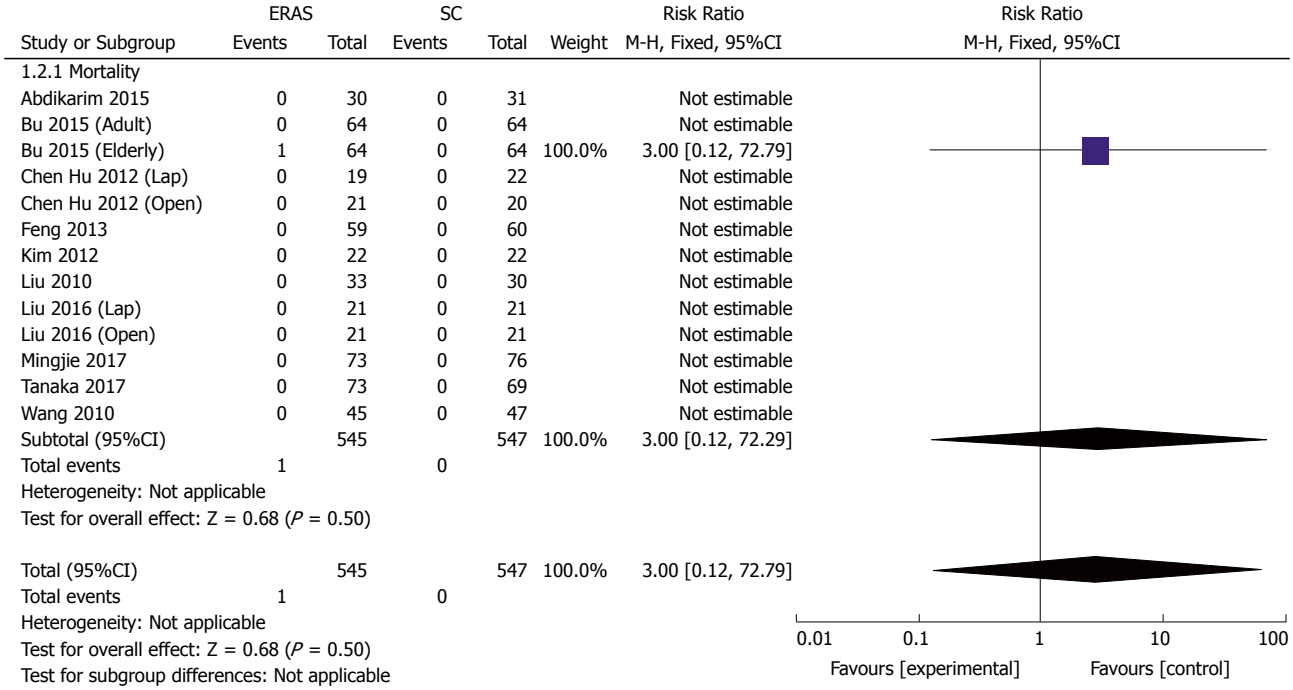


Figure 4 Forest plot evaluating the relative risk of short-term mortality: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; SC: Standard care.

these studies (χ^2 = 119.74, I^2 = 92%, P < 0.0001) (Figure 6). In the patients undergoing laparoscopic gastrectomy[24,34,35], the duration of the first flatus of patients in the ERAS group was 7.20 h less than

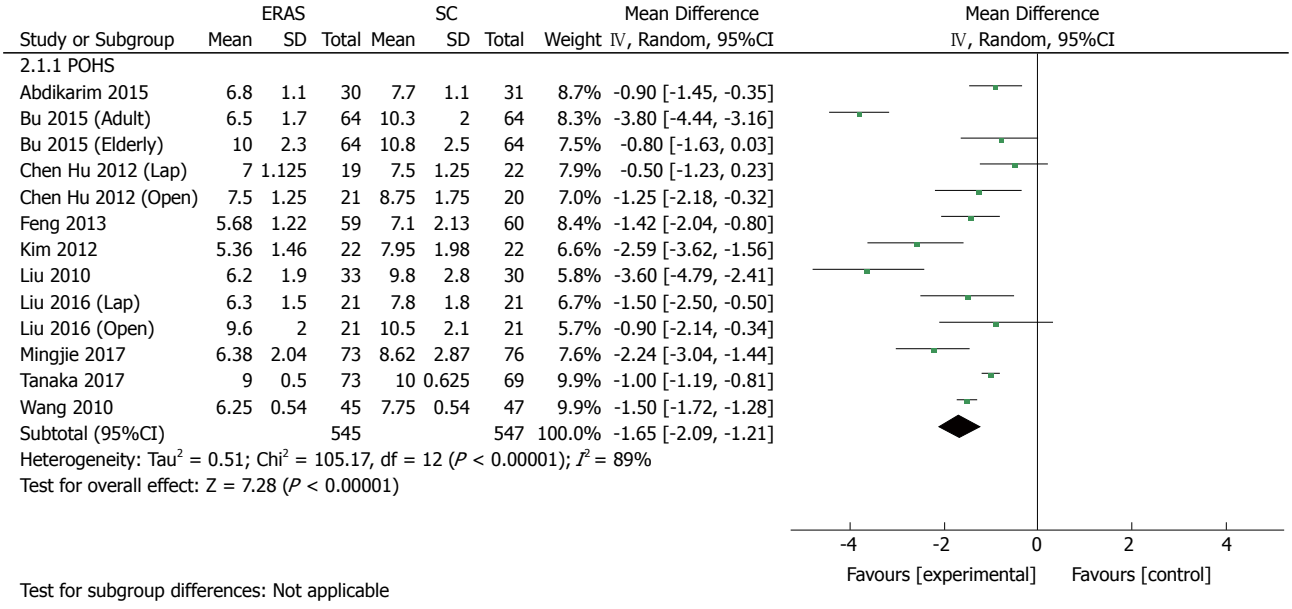


Figure 5 Forest plot evaluating the length of postoperative hospital stay: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; SC: Standard care.

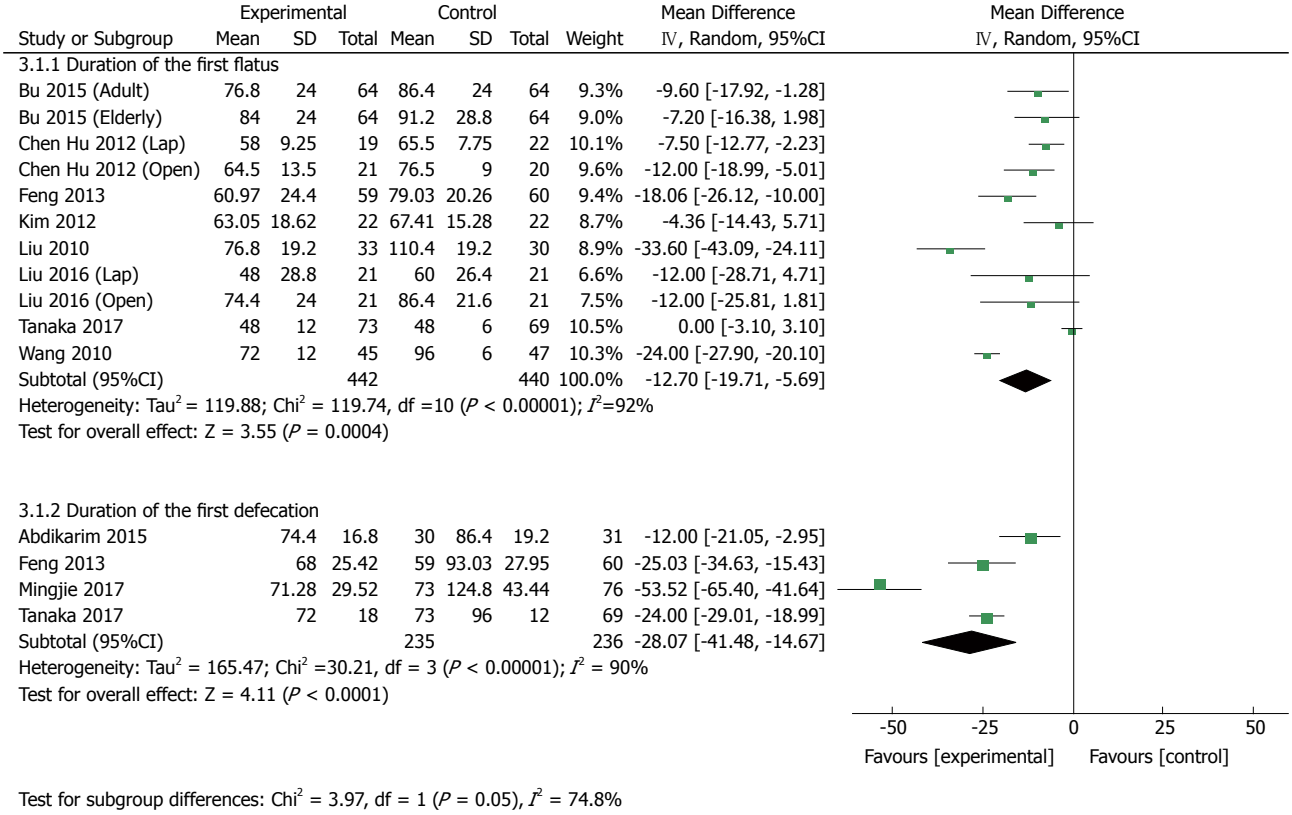


Figure 6 Forest plot evaluating the duration of intestinal function recovery: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; SC: Standard care.

that in the control group (MD: -7.20, 95%CI: -11.70 to -2.70, P = 0.002), and there was no heterogeneity among these studies (χ^2 = 0.64, P = 0.73; I^2 = 0). Similarly, the first flatus was significantly earlier in the ERAS group than in the SC group (MD: -14.47, 95%CI: -23.61 to -5.33, P = 0.002) among patients undergoing open surgery[23,24,33,34,36,37], but the heterogeneity was significant (χ^2 = 116.69, P < 0.00001; I^2 = 94%). Four RCTs[21,22,30,33] (471 patients) reported the duration of first defecation. The MD was -28.07 (95%CI: -41.48 to -14.67, P < 0.0001) (Figure 6), and there was significant heterogeneity among the studies (χ^2 = 30.21, P < 0.00001; I^2 = 90%).

Medical costs

Ten RCTs[23,24,30,33-35,37] (819 patients) provided data

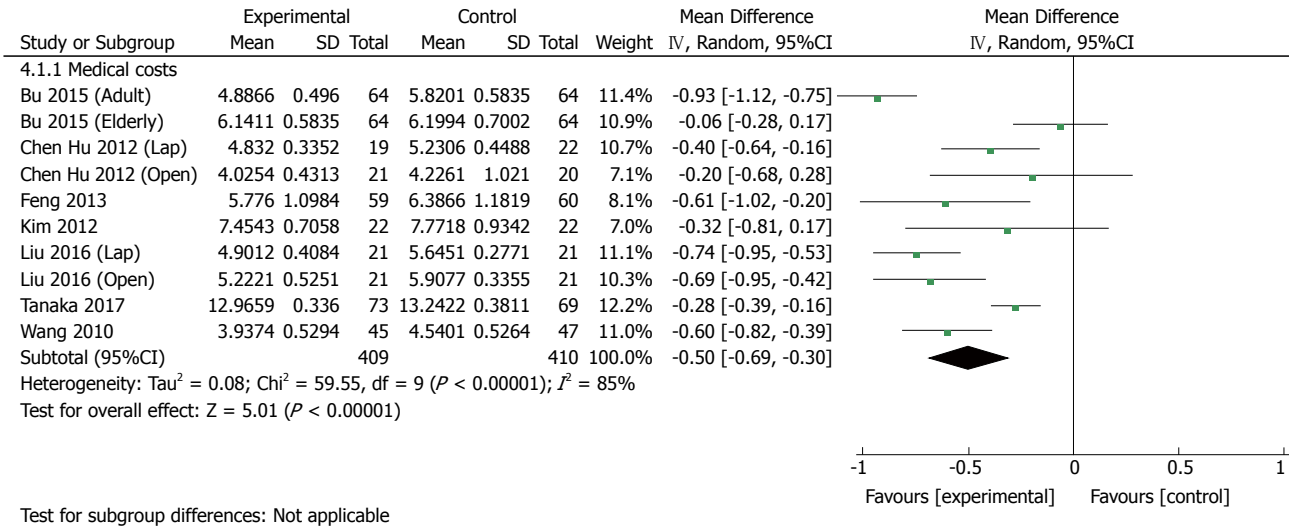


Figure 7 Forest plot evaluating the difference in total medical costs: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; SC: Standard care.

regarding medical costs. The costs of hospitalization were reported in US dollars (USD) in one trial[37], Japanese yen in one trial[30], and Chinese renminbi (RMB) in six trials. All of the medical care expenses were converted to USD (http://www.xe.com) by use of the exchange rates of the aforementioned currencies on June 28, 2017. The medical costs were significantly lower with ERAS than with traditional care (MD: -5000 USD, 95%CI: -6900 to -3000, $P < 0.00001$) (Figure 7), and there was significant heterogeneity among trials by using the random-effects model ($\chi^2 = 59.55$, $P < 0.00001$; $I^2 = 85\%$). In laparoscopic groups[24,34,35], ERAS significantly decreased the medical costs compared with traditional care (MD: -5200 USD, 95%CI: -8000 to -2500, $P = 0.0002$), and the heterogeneity was significant ($\chi^2 = 5.58$, $P = 0.06$; $I^2 = 64\%$). Similarly, there was a significant reduction in medical costs in open surgery with ERAS[23,24,33,34,37] compared with open surgery alone (MD: -5300, 95%CI: -8300 to -2300, $P = 0.0005$), and significant heterogeneity was observed ($\chi^2 = 37.63$, $P < 0.00001$; $I^2 = 87\%$).

Readmission

Eight RCTs[21,23,30,34-37] (777 patients) reported data concerning the readmission rate after discharge, whereby 5.6% (22/390) from ERAS groups and 1.8% (7/387) from SC groups had to be readmitted. A higher readmission rate was perceived in the ERAS group than in the control group (RR: 2.86, 95%CI: 1.31-6.24, $P = 0.009$) (Figure 8). There was no significant heterogeneity observed among these studies ($\chi^2 = 1.44$, $P = 0.92$; $I^2 = 0$). However, sensitivity analysis showed no significant difference in readmission (RR: 2.17, 95%CI: 0.77-6.14, $P = 0.14$) when excluding the elderly group in Bu's study[23], and no heterogeneity was observed ($\chi^2 = 0.85$, $P = 0.93$; $I^2 = 0$).

Reoperation

Three RCTs[23,30,36] (517 patients) reported reoperation rates after discharge. Two patients (0.8%) in ERAS groups and four patients (1.6%) in the conventional protocol groups had to undergo reoperation because of serious complications including abdominal infection, intraabdominal bleeding, and pancreatic fistula. There was no statistical difference in the rate of reoperation between the two groups (RR: 0.62, 95%CI: 0.17-2.35, $P = 0.49$) (Figure 8). Heterogeneity among these studies remained moderate ($\chi^2 = 3.01$, $P = 0.22$; $I^2 = 33\%$).

Inflammatory response indicators and nutritional status

Eight RCTs[22,24,34-37] (514 patients) and four RCTs[24,36,37] (239 patients) reported CRP and IL-6 levels after gastrectomy, respectively. As markers of surgical stress-associated response, levels of CRP and IL-6 were significantly elevated after surgery. Compared with patients in the conventional care group, a milder acute-phase response was detected in the ERAS group after gastrectomy. The pooled MD using a random-effects model for serum CRP was -14.81 (95%CI: -21.42 to -8.21, $P < 0.0001$), -19.81 (95%CI: -29.64 to -9.98, $P < 0.0001$), and -21.36 (95%CI: -28.81 to -13.91, $P < 0.00001$) on days 1, 4 and 7 after surgery, respectively (Figure 9), and significant heterogeneity was observed among these studies ($I^2 = 72\%$, 64%, and 74% on day 1, 4 and 7 after surgery, respectively). The level of pooled MD for IL-6 was -61.22 (95%CI: -114.58 to -7.86, $P = 0.02$), -31.50 (95%CI: -55.63 to -7.38, $P = 0.01$) and -26.62 (95%CI: -34.23 to -19.01, $P < 0.0001$) on days 1, 4 and 7 after surgery, respectively (Figure 10), and there was a high degree of heterogeneity among these studies ($I^2 = 99\%$, 96% and 89% on day 1, 4 and 7 after surgery, respectively).

Four RCTs[24,32] reported serum ALB. In general, ALB concentration dropped significantly compared with preoperative parameters. On postoperative day (POD) 1, there was no significant difference regarding the level of ALB between the ERAS and conventional care groups (MD 0.24, 95%CI: -0.89 to 1.36, $P = 0.68$) (Figure 11). On

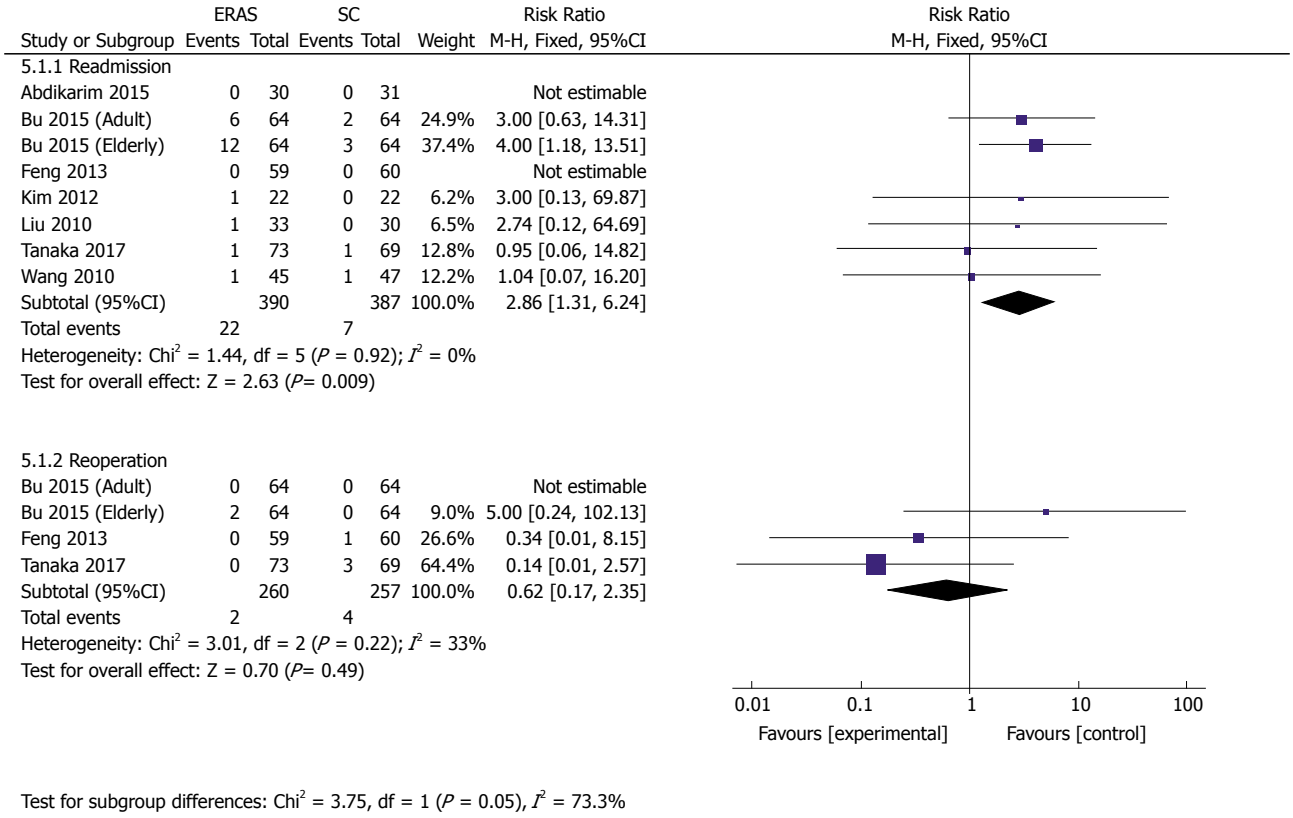


Figure 8 Forest plot evaluating the incidence of readmission and reoperation within 30 d: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; SC: Standard care.

PODs 4 and 7, the level of ALB was higher in the ERAS group than in the control group (MD: 3.27, 95%CI: 2.24-4.30, $P < 0.00001$; MD: 5.68, 95%CI: 3.31-8.05, $P < 0.00001$, respectively). Mild heterogeneity was detected on POD 4 ($\chi^2 = 3.90$, $P = 0.27$; $I^2 = 23\%$). However, there was significant heterogeneity in the outcomes on POD 7 ($\chi^2 = 17.54$, $P = 0.0005$; $I^2 = 83\%$) (Figure 11).

Quality of life

Health-related QOL was reported in two trials[35,37]. One trial checked health-related QOL with the European Organization for Research and Treatment of Cancer quality-of-life questionnaire C-30 and STO-22 at 14 d after discharge[35], while the other measured the QOL score using questionnaires at the time of discharge[37]. A significant superiority was found in the fast-track surgery protocol group compared with the conventional care program group in terms of short-term QOL using the fixed-effects model. The pooled standardized MD was -0.46 (95%CI: -0.80 to -0.12, $P = 0.008$) (Figure 12), and there was a mild degree of heterogeneity in the outcomes ($\chi^2 = 1.56$, $P = 0.21$; $I^2 = 36\%$).

Publication bias

Potential publication bias was appraised graphically by using funnel plots, Begg's test and Egger's test. No obvious asymmetry was revealed by visual indication of the Begg's funnel plot for postoperative total complications including all studies (Figure 13), and Begg's test and Egger's test indicated no significant bias was associated with publication for this meta-analysis ($P = 0.55$ and $P = 0.435$, respectively).

DISCUSSION

ERAS protocols have been gradually accepted as being able to optimize clinical outcomes, value and experience for patients with GC[22-29]. The present study is the largest meta-analysis to date, incorporating 13 RCTs enrolling 1092 participants, of whom 545 received ERAS protocols and 547 received SC for GC. Our results demonstrated that the optimized multimodal strategies significantly expedite bowel function recovery, shorten the length of POHS and reduce medical costs, and that ERAS pathways maintain comparable total complications, reoperation rates and mortality rates. The present analysis indicates that the implementation of ERAS approaches accelerates recovery, and is feasible and safe for patients with GC undergoing radical gastrectomy.

The core mechanism of ERAS is that multimodal interventions may lead to a major reduction in the undesirable sequelae of surgical injury, and stress-free surgery is the key goal of ERAS[1]. Robust evidence suggested that ERAS played an important role in attenuating the surgical stress response and accelerating the return to baseline in colorectal cancer surgery[38,39], which was afforded eloquent proof in GC surgery. The inflammatory

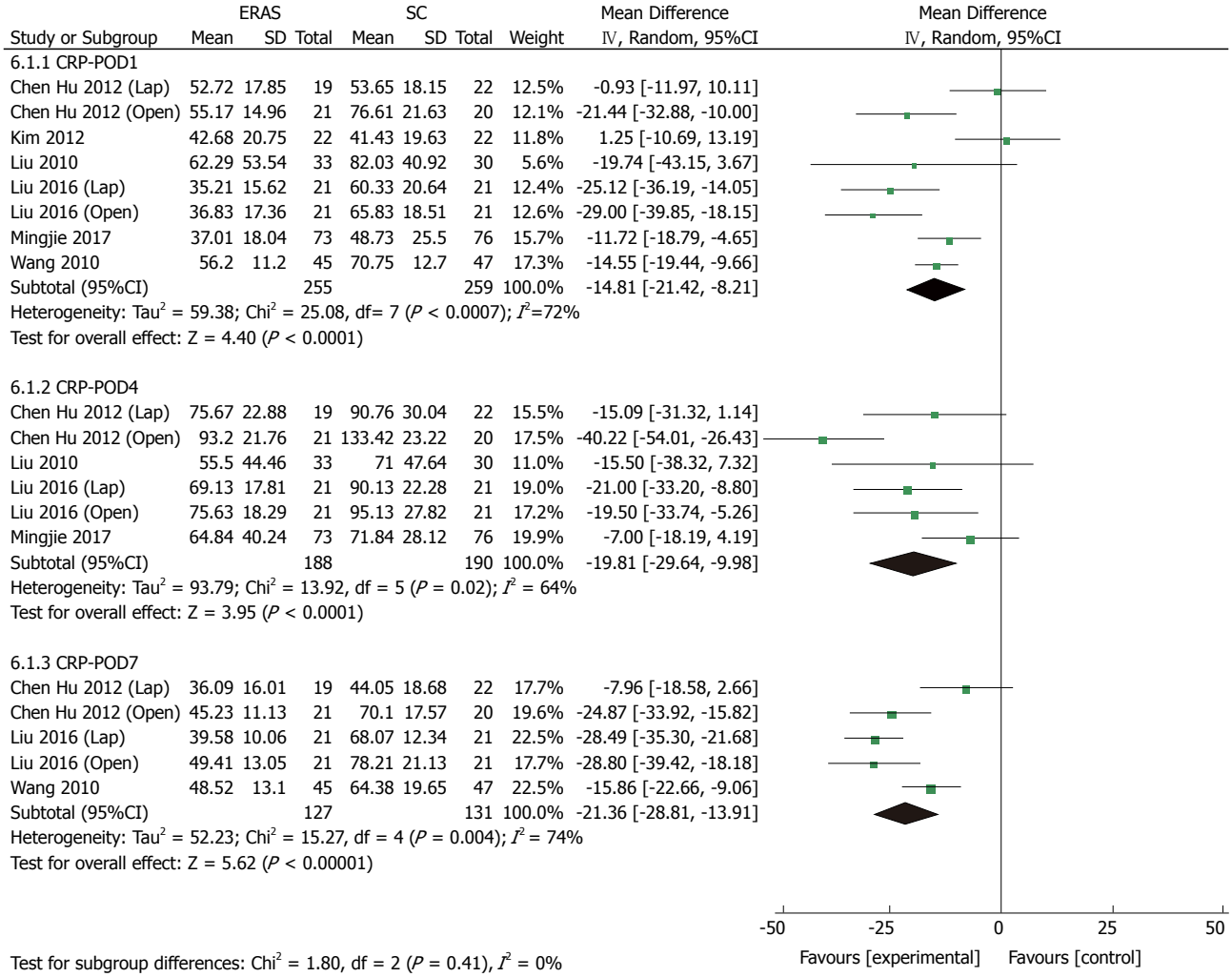


Figure 9 Forest plot evaluating the postoperative level of C-reactive protein: Enhanced recovery after surgery *vs* standard care. CRP: C-reactive protein; ERAS: Enhanced recovery after surgery; SC: Standard care.

factors, such as CRP, IL-6 and tumor necrosis factor α, are related to the extent of tissue injury caused by surgery[40,41]. In the present study, the ERAS approaches significantly reduced the concentration of CRP and IL-6 in comparison with SC on days 1, 4 and 7 after gastrectomy for GC, which was consistent with accelerated recovery. More importantly, our study suggests that the level of serum ALB after surgery in ERAS patients was significantly higher and steadier than that in SC patients, which fully demonstrates that the ERAS program could serve to improve the nutritional status of patients with GC. Good nutritional status and rapid rehabilitation after surgery allow patients to receive early postoperative multimodality therapy, including chemotherapy, thereby potentially improving their oncological outcome.

The main characteristic of ERAS is faster post-operative recovery and early discharge. However, it is noteworthy that this accelerated recovery does not come at the cost of increased medical expense. In our study, 10 RCTs reported data on medical costs and identified a mean reduction of 5000 USD in the ERAS group. If the trials with mean and imputed SD were excluded, medical expenses would be reduced by 5300 USD. Therefore, implementation of ERAS appears to have an advantage when combining clinical efficacy and cost effectiveness, which is consistent with previous reports[42,43].

More importantly, our study shows that ERAS pathways increased the readmission rate for GC patients after gastrectomy, a radically different result from previous meta-analyses[25-27]. However, sensitivity analysis, excluding the elderly patients in Bu's study[23], indicated that there was no significant difference in readmission rates between ERAS and SC groups. To date, the evidence on the application of ERAS procedures in elderly patients with GC, especially if older than 75 years, is sparse. Only two RCTs have reported ERAS care in elderly patients with GC to date, and the age criterion for inclusion was inconsistent. Liu *et al*[24] confirmed that the use of ERAS in elderly patients (60-80 years) was safe and feasible, effectively reducing the stress response, speeding up the recovery of intestinal function, and improving postoperative nutritional status without increasing the complications. However, Bu *et al*[23] showed that

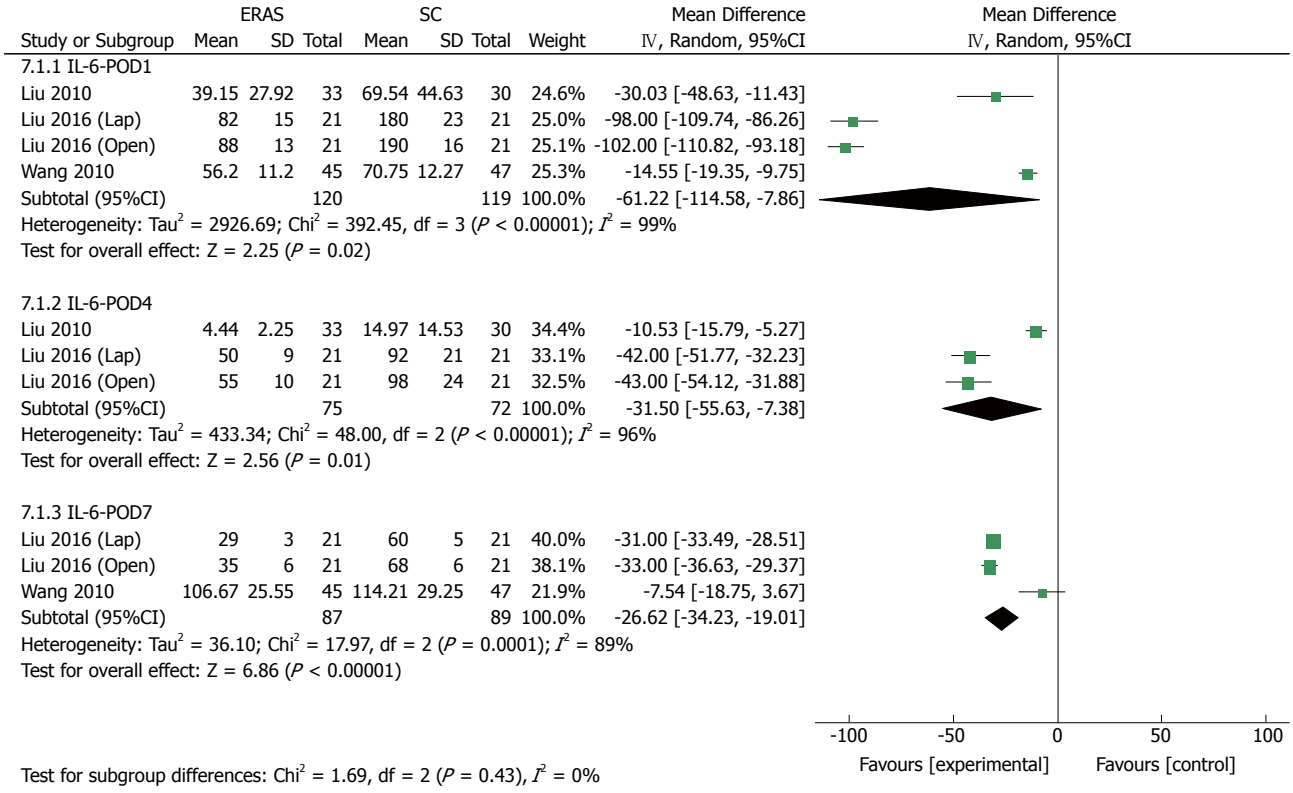


Figure 10 Forest plot evaluating the postoperative level of IL-6: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; IL: Interleukin; SC: Standard care.

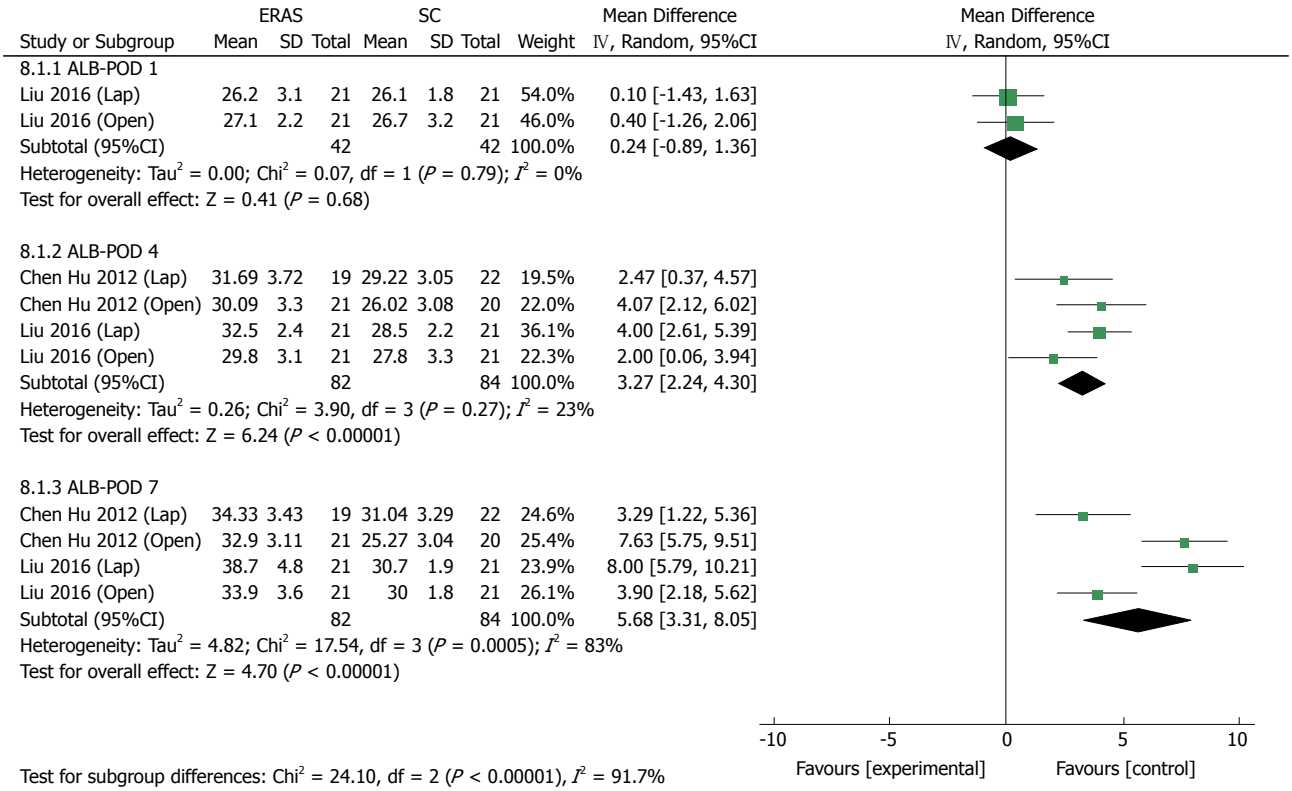


Figure 11 Forest plot evaluating the postoperative level of serum albumin: Enhanced recovery after surgery *vs* standard care. ALB: Albumin; ERAS: Enhanced recovery after surgery; SC: Standard care.

implementation of the multimodal procedure in older patients (75-89 years) undergoing distal or total gastrectomy increased significantly the incidence of nausea and vomiting, gastric retention and ileus, as well as the readmission rate, in comparison with the SC group. These inconsistent results may be due to

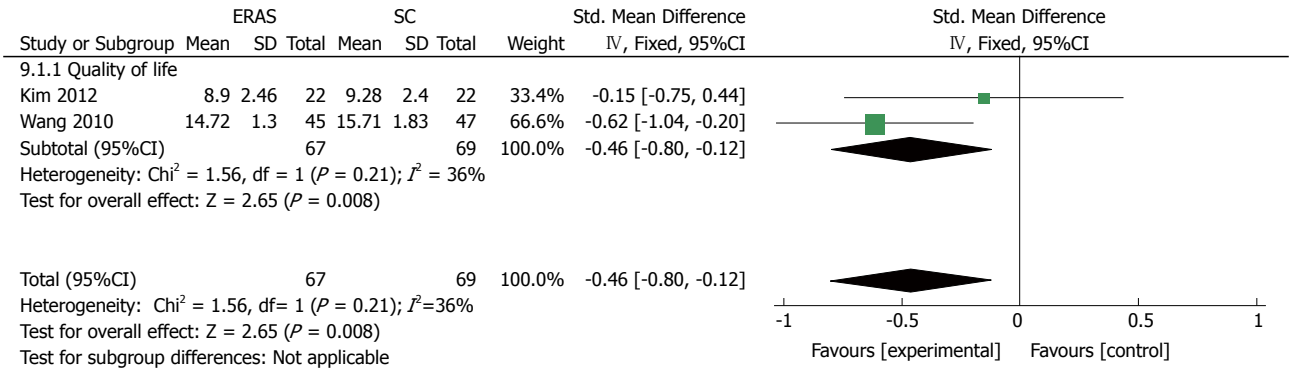


Figure 12 Forest plot evaluating health-related quality of life: Enhanced recovery after surgery *vs* standard care. ERAS: Enhanced recovery after surgery; SC: Standard care.

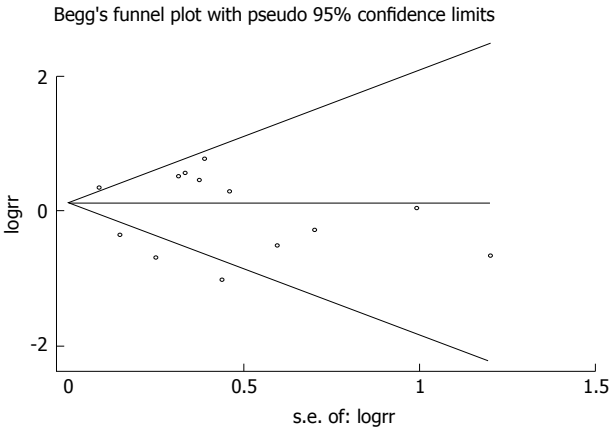


Figure 13 Begg's funnel plot to explore publication bias of all the included studies.

inclusion of age criterion, surgical type, and element selection.

Gerontal patients often experience underlying comorbidities and low physiological reserve, usually resulting in a high incidence of complications and delayed convalescence. Therefore, tailored perioperative care should be conducted in such a specific patient population. It was reported that a high degree of ERAS compliance was associated with fewer complications and shorter hospital stay[44,45]. Feroci *et al*[46] reported that male sex, advanced age (> 75 years), and American Society of Anesthesiologists' score of grade 3 and above were correlated with lower compliance to enhanced recovery with specific reference to early removal of the urethral catheter, early oral feeding, and early ambulation in patients undergoing colorectal surgery. In our study, protocol compliance was only mentioned in studies by Feng *et al*[33] and Liu *et al*[24]. Whether the compliance of elderly GC patients with ERAS regimens affects the outcomes remains to be further investigated, although several studies have indicated that ERAS in colorectal surgery was safe and feasible, with postoperative outcomes similar to those of the younger group[47-49].

In our meta-analysis, two RCTs provided QOL data at the time of discharge[37] or 14 d after discharge[35], whereby ERAS approaches showed significant superiority in QOL over SC groups. However, many investigators prefer postoperative recovery to assess the efficacy of ERAS, which begins at the time of surgery and is complete only when the patient returns (recovers) to their baseline function or to population norms[50]. Therefore, functional status and QOL attracts more interest.

The introduction of laparoscopic surgery has dramatically lessened the impact of surgical traumas on patients and accelerated their recovery. In the past 2 decades, minimally invasive surgery and the implementation of ERAS have been considered two major revolutions in elective major abdominal surgery, both intending to minimize the surgical stress and improve patient outcomes[51]. Meta-analyses of RCTs in laparoscopic colorectal surgery have demonstrated that application of the ERAS approaches is associated with fewer complications, faster recovery of bowel function and shorter hospitalization, without increased readmissions[52,53]. Laparoscopic surgery has been recommended in the guidelines for enhanced recovery after gastrectomy[29]. In this study, we observed that laparoscopic surgery combined with ERAS markedly reduced POHS and medical costs, and speeded up the return of intestinal function in patients with GC; however, laparoscopic surgery with ERAS did not increase total complications compared with laparoscopic surgery alone.

There are undoubtedly several limitations in the present study. First, several included RCTs were smaller in size, although the total sample size of the study was greater than 1000, and a multicenter trial was lacking. Second, among the included studies there was considerable heterogeneity. No remarkable heterogeneity was found with regard to the incidence of complications (including anastomotic leaks, ileus, incision infection, urinary tract infection, and pulmonary infection), rates of readmission and reoperation, and postoperative serum ALB level (POD 1 and POD 4) and QOL. However, there was significant heterogeneity for overall complications, POHS, intestinal function recovery, medical costs, and inflammatory response indicators (I^2 = 64%-99%). This substantial heterogeneity may be attributable to the clinical heterogeneity, including technical status of each institution, inclusion criteria,

surgical approach, inconsistent evaluation of the outcomes, and ERAS elements used. Third, most studies excluded patients receiving neoadjuvant chemotherapy, which may increase the potential bias to a certain extent.

In conclusion, this updated meta-analysis and systematic review provides a comprehensive assessment of ERAS following gastrectomy, and demonstrates that ERAS protocols lead to accelerated recovery, reduction of surgical stress and medical costs, improved nutritional status, and better health-related QOL for GC patients. However, it appears to be associated with increased readmission rates. Further high-quality, large-sample, multicenter RCTs with long-term follow-up are needed to more precisely evaluate ERAS pathways in GC surgery.

ARTICLE HIGHLIGHTS

Research background

Enhanced recovery after surgery (ERAS) has emerged as an optimal perioperative strategy for improving clinical outcomes in elective gastric cancer (GC) surgery. However, numerous controversies exist with regard to ERAS practice after radical gastrectomy.

Research motivation

Accumulating studies highlight that implementation of ERAS protocols reduces overall hospital stay, morbidity and mortality significantly, without compromising patient safety in multiple surgical disciplines. However, the safety and feasibility of applying ERAS in its current form in radical gastrectomy still remains to be proven by performing an updated meta-analysis.

Research objectives

This meta-analysis aims to provide an updated assessment of the safety and efficacy of ERAS protocols in GC surgery.

Research methods

A comprehensive literature search in PubMed, Medline, EMBASE, World Health Organization International Trial Registry platform, and Cochrane Library until June 2017 was performed independently to identify all available randomized controlled trials (RCTs) comparing the ERAS program with standard perioperative care (SC) in GC surgery. Non-comparative studies, case-controlled trials, cohort studies, retrospective studies, items of ERAS applied being less than four, and no follow-up after discharge were excluded.

Research results

Thirteen RCTs, with a total of 1092 participants, were analyzed in this study, of whom 545 underwent ERAS protocols and 547 received SC treatment. ERAS protocols significantly decreased the length of postoperative hospital stay and medical costs, and accelerated bowel function recovery. Moreover, ERAS protocols were associated with a lower level of serum inflammatory response, higher serum albumin, and superior short-term quality of life. There were no significant differences regarding the incidence of total complications, mortality and reoperation following gastrectomy. However, the readmission rate after GC surgery nearly tripled under ERAS.

Research conclusions

ERAS results in accelerated convalescence, reduction of surgical stress and medical costs, improved nutritional status, and better quality of life for GC patients, but increased the readmission rate. Furthermore, the significant heterogeneity of some results is a major limitation of this study. ERAS investigators need to proceed with caution as far as ERAS is concerned beyond colorectal cancer surgery.

Research perspectives

This study provides an updated assessment of ERAS in GC surgery and is expected to provide guidance and reference for clinical practice, and also to provide high-level evidence for evidence-based medicine. High-quality multicenter RCTs with large samples and long-term follow-up are needed to more precisely evaluate ERAS in radical gastrectomy.

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REFERENCES

1 **Kehlet H**. Multimodal approach to control postoperative pathophysiology and rehabilitation. *Br J Anaesth* 1997; **78**: 606-617 [PMID: 9175983]
2 **Wilmore DW**, Kehlet H. Management of patients in fast track surgery. *BMJ* 2001; **322**: 473-476 [PMID: 11222424]
3 **Lassen K**, Soop M, Nygren J, Cox PB, Hendry PO, Spies C, von Meyenfeldt MF, Fearon KC, Revhaug A, Norderval S, Ljungqvist O, Lobo DN, Dejong CH; Enhanced Recovery After Surgery (ERAS) Group. Consensus review of optimal perioperative care in colorectal surgery: Enhanced Recovery After Surgery (ERAS) Group recommendations. *Arch Surg* 2009; **144**: 961-969 [PMID: 19841366 DOI: 10.1001/archsurg.2009.170]
4 **Ramírez JM**, Blasco JA, Roig JV, Maeso-Martínez S, Casal JE, Esteban F, Lic DC; Spanish working group on fast track surgery. Enhanced recovery in colorectal surgery: a multicentre study. *BMC Surg* 2011; **11**: 9 [PMID: 21489315 DOI: 10.1186/1471-2482-11-9]
5 **Kehlet H**. Fast-track colonic surgery: status and perspectives. *Recent Results Cancer Res* 2005; **165**: 8-13 [PMID: 15865015]
6 **Kehlet H**. Fast-track colorectal surgery. *Lancet* 2008; **371**: 791-793 [PMID: 18328911 DOI: 10.1016/S0140-6736(08)60357-8]
7 **Kehlet H**, Dahl JB. Anaesthesia, surgery, and challenges in postoperative recovery. *Lancet* 2003; **362**: 1921-1928 [PMID: 14667752 DOI: 10.1016/S0140-6736(03)14966-5]
8 **Faucheron JL**, Trilling B. Laparoscopy in combination with fast-track management is the best surgical strategy in patients undergoing colorectal resection for cancer. *Tech Coloproctol* 2015; **19**: 379-380 [PMID: 25995151 DOI: 10.1007/s10151-015-1309-2]
9 **Muallem MZ**, Dimitrova D, Pietzner K, Richter R, Feldheiser A, Scharfe I, Schmeil I, Hösl TM, Mustea A, Wimberger P, Burges A, Kimmig R, Sehouli J. Implementation of Enhanced Recovery After Surgery (ERAS) Pathways in Gynecologic Oncology. A NOGGO-AGO* survey of 144 Gynecological Departments in Germany. *Anticancer Res* 2016; **36**: 4227-4232 [PMID: 27466536]
10 **Auyong DB**, Allen CJ, Pahang JA, Clabeaux JJ, MacDonald KM, Hanson NA. Reduced Length of Hospitalization in Primary Total Knee Arthroplasty Patients Using an Updated Enhanced Recovery After Orthopedic Surgery (ERAS) Pathway. *J Arthroplasty* 2015; **30**: 1705-1709 [PMID: 26024988 DOI: 10.1016/j.arth.2015.05.007]
11 **Bösing NM**, Goretzki PE, Röher HD. Gastric cancer: which patients benefit from systematic lymphadenectomy? *Eur J Surg Oncol* 2000; **26**: 498-505 [PMID: 11016473 DOI: 10.1053/ejso.1999.0930]
12 **Sano T**, Sasako M, Yamamoto S, Nashimoto A, Kurita A, Hiratsuka M, Tsujinaka T, Kinoshita T, Arai K, Yamamura Y, Okajima K. Gastric cancer surgery: morbidity and mortality results from a prospective randomized controlled trial comparing D2 and extended para-aortic lymphadenectomy--Japan Clinical Oncology Group study 9501. *J Clin Oncol* 2004; **22**: 2767-2773 [PMID: 15199090 DOI: 10.1200/JCO.2004.10.184]
13 **Kim HH**, Han SU, Kim MC, Hyung WJ, Kim W, Lee HJ, Ryu SW, Cho GS, Song KY, Ryu SY. Long-term results of laparoscopic gastrectomy for gastric cancer: a large-scale case-control and case-matched Korean multicenter study. *J Clin Oncol* 2014; **32**: 627-633 [PMID: 24470012 DOI: 10.1200/JCO.2013.48.8551]
14 **Damhuis RA**, Wijnhoven BP, Plaisier PW, Kirkels WJ, Kranse R, van Lanschot JJ. Comparison of 30-day, 90-day and in-hospital

postoperative mortality for eight different cancer types. *Br J Surg* 2012; **99**: 1149-1154 [PMID: 22718521 DOI: 10.1002/bjs.8813]
15 **Kubota T**, Hiki N, Sano T, Nomura S, Nunobe S, Kumagai K, Aikou S, Watanabe R, Kosuga T, Yamaguchi T. Prognostic significance of complications after curative surgery for gastric cancer. *Ann Surg Oncol* 2014; **21**: 891-898 [PMID: 24254205 DOI: 10.1245/s10434-013-3384-9]
16 **Grantcharov TP**, Kehlet H. Laparoscopic gastric surgery in an enhanced recovery programme. *Br J Surg* 2010; **97**: 1547-1551 [PMID: 20665480 DOI: 10.1002/bjs.7184]
17 **Yamada T**, Hayashi T, Cho H, Yoshikawa T, Taniguchi H, Fukushima R, Tsuburaya A. Usefulness of enhanced recovery after surgery protocol as compared with conventional perioperative care in gastric surgery. *Gastric Cancer* 2012; **15**: 34-41 [PMID: 21573918 DOI: 10.1007/s10120-011-0057-x]
18 **Yamada T**, Hayashi T, Aoyama T, Shirai J, Fujikawa H, Cho H, Yoshikawa T, Rino Y, Masuda M, Taniguchi H, Fukushima R, Tsuburaya A. Feasibility of enhanced recovery after surgery in gastric surgery: a retrospective study. *BMC Surg* 2014; **14**: 41 [PMID: 25001198 DOI: 10.1186/1471-2482-14-41]
19 **Liu XX**, Pan HF, Jiang ZW, Zhang S, Wang ZM, Chen P, Zhao Y, Wang G, Zhao K, Li JS. "Fast-track" and "Minimally Invasive" Surgery for Gastric Cancer. *Chin Med J* (Engl) 2016; **129**: 2294-2300 [PMID: 27647187 DOI: 10.4103/0366-6999.190659]
20 **Fang F**, Gao J, Bi X, Han F, Wang HJ. Effect and clinical significance of fast-track surgery combined with laparoscopic radical gastrectomy on the plasma level of vascular endothelial growth factor in gastric antrum cancer. *Springerplus* 2016; **5**: 50 [PMID: 26835230 DOI: 10.1186/s40064-016-1699-2]
21 **Abdikarim I**, Cao XY, Li SZ, Zhao YQ, Taupyk Y, Wang Q. Enhanced recovery after surgery with laparoscopic radical gastrectomy for stomach carcinomas. *World J Gastroenterol* 2015; **21**: 13339-13344 [PMID: 26715818 DOI: 10.3748/wjg.v21.i47.13339]
22 **Mingjie X**, Luyao Z, Ze T, YinQuan Z, Quan W. Laparoscopic Radical Gastrectomy for Resectable Advanced Gastric Cancer Within Enhanced Recovery Programs: A Prospective Randomized Controlled Trial. *J Laparoendosc Adv Surg Tech A* 2017; **27**: 959-964 [PMID: 27875094 DOI: 10.1089/lap.2016.0057]
23 **Bu J**, Li N, Huang X, He S, Wen J, Wu X. Feasibility of Fast-Track Surgery in Elderly Patients with Gastric Cancer. *J Gastrointest Surg* 2015; **19**: 1391-1398 [PMID: 25943912 DOI: 10.1007/s11605-015-2839-7]
24 **Liu G**, Jian F, Wang X, Chen L. Fast-track surgery protocol in elderly patients undergoing laparoscopic radical gastrectomy for gastric cancer: a randomized controlled trial. *Onco Targets Ther* 2016; **9**: 3345-3351 [PMID: 27330314 DOI: 10.2147/OTT.S107443]
25 **Chen ZX**, Liu AH, Cen Y. Fast-track program *vs* traditional care in surgery for gastric cancer. *World J Gastroenterol* 2014; **20**: 578-583 [PMID: 24574728 DOI: 10.3748/wjg.v20.i2.578]
26 **Beamish AJ**, Chan DS, Blake PA, Karran A, Lewis WG. Systematic review and meta-analysis of enhanced recovery programmes in gastric cancer surgery. *Int J Surg* 2015; **19**: 46-54 [PMID: 26004350 DOI: 10.1016/j.ijsu.2015.05.021]
27 **Chen S**, Zou Z, Chen F, Huang Z, Li G. A meta-analysis of fast track surgery for patients with gastric cancer undergoing gastrectomy. *Ann R Coll Surg Engl* 2015; **97**: 3-10 [PMID: 25519256 DOI: 10.1308/rcsann.2015.97.1.3]
28 **Ding J**, Sun B, Song P, Liu S, Chen H, Feng M, Guan W. The application of enhanced recovery after surgery (ERAS)/fast-track surgery in gastrectomy for gastric cancer: a systematic review and meta-analysis. *Oncotarget* 2017; **8**: 75699-75711 [PMID: 29088903 DOI: 10.18632/oncotarget.18581]
29 **Mortensen K**, Nilsson M, Slim K, Schäfer M, Mariette C, Braga M, Carli F, Demartines N, Griffin SM, Lassen K; Enhanced Recovery After Surgery (ERAS®) Group. Consensus guidelines for enhanced recovery after gastrectomy: Enhanced Recovery After Surgery (ERAS®) Society recommendations. *Br J Surg* 2014; **101**: 1209-1229 [PMID: 25047143 DOI: 10.1002/bjs.9582]
30 **Tanaka R**, Lee SW, Kawai M, Tashiro K, Kawashima S, Kagota S, Honda K, Uchiyama K. Protocol for enhanced recovery after surgery improves short-term outcomes for patients with gastric cancer: a randomized clinical trial. *Gastric Cancer* 2017; **20**: 861-871 [PMID: 28062937 DOI: 10.1007/s10120-016-0686-1]
31 **Higgins JP**, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**: d5928 [PMID: 22008217 DOI: 10.1136/bmj.d5928]
32 **Hozo SP**, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005; **5**: 13 [PMID: 15840177 DOI: 10.1186/1471-2288-5-13]
33 **Feng F**, Ji G, Li JP, Li XH, Shi H, Zhao ZW, Wu GS, Liu XN, Zhao QC. Fast-track surgery could improve postoperative recovery in radical total gastrectomy patients. *World J Gastroenterol* 2013; **19**: 3642-3648 [PMID: 23801867 DOI: 10.3748/wjg.v19.i23.3642]
34 **Chen Hu J**, Xin Jiang L, Cai L, Tao Zheng H, Yuan Hu S, Bing Chen H, Chang Wu G, Fei Zhang Y, Chuan Lv Z. Preliminary experience of fast-track surgery combined with laparoscopy-assisted radical distal gastrectomy for gastric cancer. *J Gastrointest Surg* 2012; **16**: 1830-1839 [PMID: 22854954 DOI: 10.1007/s11605-012-1969-4]
35 **Kim JW**, Kim WS, Cheong JH, Hyung WJ, Choi SH, Noh SH. Safety and efficacy of fast-track surgery in laparoscopic distal gastrectomy for gastric cancer: a randomized clinical trial. *World J Surg* 2012; **36**: 2879-2887 [PMID: 22941233 DOI: 10.1007/s00268-012-1741-7]
36 **Liu XX**, Jiang ZW, Wang ZM, Li JS. Multimodal optimization of surgical care shows beneficial outcome in gastrectomy surgery. *JPEN J Parenter Enteral Nutr* 2010; **34**: 313-321 [PMID: 20467014 DOI: 10.1177/0148607110362583]
37 **Wang D**, Kong Y, Zhong B, Zhou X, Zhou Y. Fast-track surgery improves postoperative recovery in patients with gastric cancer: a randomized comparison with conventional postoperative care. *J Gastrointest Surg* 2010; **14**: 620-627 [PMID: 20108171 DOI: 10.1007/s11605-009-1139-5]
38 **Ren L**, Zhu D, Wei Y, Pan X, Liang L, Xu J, Zhong Y, Xue Z, Jin L, Zhan S, Niu W, Qin X, Wu Z, Wu Z. Enhanced Recovery After Surgery (ERAS) program attenuates stress and accelerates recovery in patients after radical resection for colorectal cancer: a prospective randomized controlled trial. *World J Surg* 2012; **36**: 407-414 [PMID: 22102090 DOI: 10.1007/s00268-011-1348-4]
39 **Carli F**. Physiologic considerations of Enhanced Recovery After Surgery (ERAS) programs: implications of the stress response. *Can J Anaesth* 2015; **62**: 110-119 [PMID: 25501695 DOI: 10.1007/s12630-014-0264-0]
40 **Feng J**, Li K, Li L, Wang X, Huang M, Yang J, Hu Y. The effects of fast-track surgery on inflammation and immunity in patients undergoing colorectal surgery. *Int J Colorectal Dis* 2016; **31**: 1675-1682 [PMID: 27517680 DOI: 10.1007/s00384-016-2630-6]
41 **Mari G**, Crippa J, Costanzi A, Mazzola M, Rossi M, Maggioni D. ERAS Protocol Reduces IL-6 Secretion in Colorectal Laparoscopic Surgery: Results From a Randomized Clinical Trial. *Surg Laparosc Endosc Percutan Tech* 2016; **26**: 444-448 [PMID: 27783027 DOI: 10.1097/SLE.0000000000000324]
42 **Stowers MD**, Lemanu DP, Hill AG. Health economics in Enhanced Recovery After Surgery programs. *Can J Anaesth* 2015; **62**: 219-230 [PMID: 25391739 DOI: 10.1007/s12630-014-0272-0]
43 **Nelson G**, Kiyang LN, Chuck A, Thanh NX, Gramlich LM. Cost impact analysis of Enhanced Recovery After Surgery program implementation in Alberta colon cancer patients. *Curr Oncol* 2016; **23**: e221-e227 [PMID: 27330358 DOI: 10.3747/co.23.2980]
44 **ERAS Compliance Group.** The Impact of Enhanced Recovery Protocol Compliance on Elective Colorectal Cancer Resection: Results From an International Registry. *Ann Surg* 2015; **261**: 1153-1159 [PMID: 25671587 DOI: 10.1097/SLA.0000000000001029]

45 **Alcántara-Moral M**, Serra-Aracil X, Gil-Egea MJ, Frasson M, Flor-Lorente B, Garcia-Granero E; E.B.S.Q.-C on behalf of the collaborative Group of Coloproctology Section of The Spanish Association of Surgeons. Observational cross-sectional study of compliance with the fast track protocol in elective surgery for colon cancer in Spain. *Int J Colorectal Dis* 2014; **29**: 477-483 [PMID: 24435243 DOI: 10.1007/s00384-013-1825-3]

46 **Feroci F**, Lenzi E, Baraghini M, Garzi A, Vannucchi A, Cantafio S, Scatizzi M. Fast-track surgery in real life: how patient factors influence outcomes and compliance with an enhanced recovery clinical pathway after colorectal surgery. *Surg Laparosc Endosc Percutan Tech* 2013; **23**: 259-265 [PMID: 23751989 DOI: 10.1097/SLE.0b013e31828ba16f]

47 **Bardram L**, Funch-Jensen P, Kehlet H. Rapid rehabilitation in elderly patients after laparoscopic colonic resection. *Br J Surg* 2000; **87**: 1540-1545 [PMID: 11091243 DOI: 10.1046/j.1365-2168.2000.01559.x]

48 **Pawa N**, Cathcart PL, Arulampalam TH, Tutton MG, Motson RW. Enhanced recovery program following colorectal resection in the elderly patient. *World J Surg* 2012; **36**: 415-423 [PMID: 22146943 DOI: 10.1007/s00268-011-1328-8]

49 **Baek SJ**, Kim SH, Kim SY, Shin JW, Kwak JM, Kim J. The safety of a "fast-track" program after laparoscopic colorectal surgery is comparable in older patients as in younger patients. *Surg Endosc* 2013; **27**: 1225-1232 [PMID: 23232990 DOI: 10.1007/s00464-012-2579-7]

50 **Neville A**, Lee L, Antonescu I, Mayo NE, Vassiliou MC, Fried GM, Feldman LS. Systematic review of outcomes used to evaluate enhanced recovery after surgery. *Br J Surg* 2014; **101**: 159-170 [PMID: 24469616 DOI: 10.1002/bjs.9324]

51 **Feldman LS**, Delaney CP. Laparoscopy plus enhanced recovery: optimizing the benefits of MIS through SAGES 'SMART' program. *Surg Endosc* 2014; **28**: 1403-1406 [PMID: 24651892 DOI: 10.1007/s00464-013-3415-4]

52 **Lei QC**, Wang XY, Zheng HZ, Xia XF, Bi JC, Gao XJ, Li N. Laparoscopic Versus Open Colorectal Resection Within Fast Track Programs: An Update Meta-Analysis Based on Randomized Controlled Trials. *J Clin Med Res* 2015; **7**: 594-601 [PMID: 26124904 DOI: 10.14740/jocmr2177w]

53 **Zhao JH**, Sun JX, Huang XZ, Gao P, Chen XW, Song YX, Liu J, Cai CZ, Xu HM, Wang ZN. Meta-analysis of the laparoscopic versus open colorectal surgery within fast track surgery. *Int J Colorectal Dis* 2016; **31**: 613-622 [PMID: 26732262 DOI: 10.1007/s00384-015-2493-2]

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LETTERS TO THE EDITOR

Should hot biopsy forceps be abandoned for polypectomy of diminutive colorectal polyps?

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Abstract

Standardized approach to polypectomy of diminutive colorectal polyps (DCPs) is lacking since cold biopsy forceps have been associated with high levels of recurrence, hot biopsy forceps are considered inadequate and risky and cold snaring is currently under investigation for its efficacy and safety. This has led to confusion and a gap in clinical practice. This article discusses the usefulness and contemporary practical applicability of hot biopsy forceps and provides well-intentioned criticism of the new European guidelines for the treatment of DCPs. Diminutive colorectal polyps are a source of frustration for the endoscopist since their small size is accompanied by a considerable risk of premalignant neoplasia and a small but non-negligible risk of advanced neoplasia and even cancer. Since the proportion of diminutive colorectal polyps is substantial and exceeds that of larger polyps, their effective removal poses a considerable workload and a therapeutic challenge. During the last decade, the introduction of cold snaring to routine endoscopy practice has attempted to overcome the use of prior techniques, such as hot biopsy forceps. It is important to recognize that with the exception of endoscopic methods that are obviously unsafe and inadequate to serve their purpose, all other interventional endoscopic methods are operator-dependent in the sense that specific expertise and training are obligatory for the success of any therapeutic intervention. Since relevant publications on hot biopsy forceps are still in favor of its careful use, as it has not yet demonstrated inferiority compared with newer techniques, it would be prudent

for any medical practitioner to evaluate the available tools and judge any new proposed technique based on the evidence before it is adopted.

Key words: Hot forceps; Polypectomy; Endoscopy; Colon neoplasia; Diminutive polyps

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Core tip: Selection of the appropriate endoscopic method for the removal of diminutive colorectal polyps (DCPs), according to the prospective prevention of colorectal cancer, is still a debatable topic. The new recommendation released by ESGE (European Society of Gastrointestinal Endoscopy, 2017) concerning the use of hot biopsy forceps (HBF) is expected to create a shift in daily clinical practice since this technique is still popular and viable for the removal of DCPs. In this letter, the authors request reconsideration of this policy in response to published data referring on the efficacy and safety of HBF and recommend a more cautious approach and transition to prevent the premature acceptance of alternative techniques.

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TO THE EDITOR

In a recent article[1], European Society of Gastrointestinal Endoscopy has released guidelines for colorectal polypectomy, which include a strong recommendation against the use of hot biopsy forceps (HBF) based on the GRADE system of clinical evidence. The release of guidelines by professional medical societies is acknowledged by the medical community as policy that functions as a deterrent to specific practices. With respect to that notion, the abandonment of a useful technique such HBF, which for many decades, has contributed to the polypectomy of diminutive colorectal polyps (DCPs), should be considered in an appropriate conscientious and judicious manner.

The reasons for the negative criticism are based on the following: (1) unacceptably high risks of adverse events (AEs); (2) inadequate tissue sampling for histopathology (ITSH); and (3) high incomplete resection rates (IRR). The studies cited in support of the recommendation are 4 human studies (1 RCT non-blinded with a small number of patients[2], one anecdotal report[3] and 2 observational studies[4,5]), 3 of which have already been determined to be of low quality, and 2 animal studies[6,7] (Table 1). The overall quality of evidence was graded as high. Actually, apart from the methodological quality of the individual studies and the questionable generalizability, these studies are heterogeneous in terms of ITSH and IRR. Moreover, all studies are consistent with respect to the absence of perforations, and the few bleeding episodes (0.36%) in one of the studies occured in patients taking antiplatelets[5].

Table 1 List of articles presented in support of European Society of Gastrointestinal Endoscopy guidelines

Ref.	Study design Intervention	No of polyps and patients	Level of evidence
Paspatis *et al*[2], 2005	Randomised trial Bipolar electro-coagulation *vs* HBF	38 *vs* 37 rectal DCPs among 50 patients	High quality
Peluso *et al*[3], 1991	Anecdotal report HBF	62 DCPs among 39 patients	Low quality
Yasar *et al*[4], 2015	Observational study HBF *vs* JBF	237 DCPs among 179 patients	Low quality
Weston *et al*[5], 1995	Observational study HBF *vs* CBF	1964 DCPs among 687 patients	Low quality
Savides *et al*[6], 1995	Animal study Canine model	231 biopsies in 16 right colotomies of 8 mongrel dogs	Not rated in Grade system
Metz *et al*[7], 2013	Animal study Porcine model	82 artificial polyps, sized 5-8 mm	Not rated in Grade system

JBF: Jumbo biopsy forceps; CBF: Cold biopsy forceps; DCPs: Diminutive colorectal polyps; HBF: Hot biopsy forceps.

HBF is considered an alternative method for the removal of DCPs (≤ 5 mm). According to different surveys, it seems that HBF is still a viable option that is preferred by 30%-50% of endoscopists[8-10]. The two studies, with the largest number of patients and polyps[11,12] showed no complications. The study by Wadas *et al*[13], which reports a 0.38% major bleeding rate and a 0.05% perforation rate, refers to a questionnaire-type survey from an era (1988) when the HBF technique was not standardized. Even this perforation rate is lower than the reported 0.15% for therapeutic colonoscopies[14]. The rate of AEs is also lower compared with that for snare polypectomies (3.3 *vs* 4.5/1000), and AEs are more likely to occur when low- volume endoscopists use HBF than when high-volume endoscopists (> 300 polypectomies/year) use the technique[15].

HBF has been reported to have a 17% IRR when white coagulum is present[16] and a variable rate of ITSH that ranges from 0.19%-13%-26.7% in studies with different mean polyp sizes[11,17,18]. It is acknowledged that a significant predictor of histological misinterpretation is decreasing polyp size with a cut off limit of 2

mm. It is important to mention that even in studies with high reported rates of cautery artifacts[4], the results showed that histological diagnosis could indeed have been reached in all specimens.

The new rival of HBF, namely, the cold snare polypectomy (CSP), has thus far presented disparate results for IRR at 3.4%-40%, retrieval failure at 1%-13%, and bleeding rates of 1.2%-20% for DCPs[19-24]. In the sole non-blinded RCT, in which HBF and CSP are directly compared, the IRR in the ITT analysis was 29.9% for CSP, which is still unacceptably high. However, the bleeding rates were statistically insignificant at 8.1% *vs* 8.8% for HBF and CSP, respectively, and no perforations were observed in either study arm[25].

In conclusion, it seems that available evidence is not adequate to exclude hot biopsy forceps from the routine endoscopy practice. We either need more prospective studies exhibiting beneficial comparisons with new techniques or we need to focus on proper utilization of HBF by more experienced endoscopists.

REFERENCES

1 **Ferlitsch M**, Moss A, Hassan C, Bhandari P, Dumonceau JM, Paspatis G, Jover R, Langner C, Bronzwaer M, Nalankilli K, Fockens P, Hazzan R, Gralnek IM, Gschwantler M, Waldmann E, Jeschek P, Penz D, Heresbach D, Moons L, Lemmers A, Paraskeva K, Pohl J, Ponchon T, Regula J, Repici A, Rutter MD, Burgess NG, Bourke MJ. Colorectal polypectomy and endoscopic mucosal resection (EMR): European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2017; **49**: 270-297 [PMID: 28212588 DOI: 10.1055/s-0043-102569]

2 **Paspatis GA**, Vardas E, Charoniti I, Papanikolaou N, Barbatzas C, Zois E. Bipolar electrocoagulation vs conventional monopolar hot biopsy forceps in the endoscopic treatment of diminutive rectal adenomas. *Colorectal Dis* 2005; **7**: 138-142 [PMID: 15720350 DOI: 10.1111/j.1463-1318.2004.00725.x]

3 **Peluso F**, Goldner F. Follow-up of hot biopsy forceps treatment of diminutive colonic polyps. *Gastrointest Endosc* 1991; **37**: 604-606 [PMID: 1756918]

4 **Yasar B**, Kayadibi H, Abut E, Benek D, Kochan K, Gonen C. The histological quality and adequacy of diminutive colorectal polyps resected using jumbo versus hot biopsy forceps. *Dig Dis Sci* 2015; **60**: 217-225 [PMID: 25112723 DOI: 10.1007/s10620-014-3320-2]

5 **Weston AP**, Campbell DR. Diminutive colonic polyps: histopathology, spatial distribution, concomitant significant lesions, and treatment complications. *Am J Gastroenterol* 1995; **90**: 24-28 [PMID: 7801943]

6 **Savides TJ**, See JA, Jensen DM, Jutabha R, Machicado GA, Hirabayashi K. Randomized controlled study of injury in the canine right colon from simultaneous biopsy and coagulation with different hot biopsy forceps. *Gastrointest Endosc* 1995; **42**: 573-578 [PMID: 8674930 DOI: 10.1016/S0016-5107(95)70013-7]

7 **Metz AJ**, Moss A, McLeod D, Tran K, Godfrey C, Chandra A, Bourke MJ. A blinded comparison of the safety and efficacy of hot biopsy forceps electrocauterization and conventional snare polypectomy for diminutive colonic polypectomy in a porcine model. *Gastrointest Endosc* 2013; **77**: 484-490 [PMID: 23199650 DOI: 10.1016/j.gie.2012.09.014]

8 **Din S**, Ball AJ, Taylor E, Rutter M, Riley SA, Johal S. Polypectomy practices of sub-centimeter polyps in the English Bowel Cancer Screening Programme. *Surg Endosc* 2015; **29**: 3224-3230 [PMID: 25591413 DOI: 10.1007/s00464-015-4064-6]

9 **Singh N**, Harrison M, Rex DK. A survey of colonoscopic polypectomy practices among clinical gastroenterologists. *Gastrointest Endosc* 2004; **60**: 414-418 [PMID: 15332033 DOI: 10.1016/S0016-5107(04)01808-5]

10 **Carter D**, Beer-Gabel M, Zbar A, Avidan B, Bardan E. A survey of colonoscopic polypectomy practice amongst Israeli gastroenterologists. *Ann Gastroenterol* 2013; **26**: 135-140 [PMID: 24714780]

11 **Waye JD**, Lewis BS, Frankel A, Geller SA. Small colon polyps. *Am J Gastroenterol* 1988; **83**: 120-122 [PMID: 3341333]

12 **Mann NS**, Mann SK, Alam I. The safety of hot biopsy forceps in the removal of small colonic polyps. *Digestion* 1999; **60**: 74-76 [PMID: 9892802 DOI: 10.1159/000007592]

13 **Wadas DD**, Sanowski RA. Complications of the hot biopsy forceps technique. *Gastrointest Endosc* 1988; **34**: 32-37 [PMID: 3258260 DOI: 10.1016/S0016-5107(88)71226-2]

14 **Panteris V**, Haringsma J, Kuipers EJ. Colonoscopy perforation rate, mechanisms and outcome: from diagnostic to therapeutic colonoscopy. *Endoscopy* 2009; **41**: 941-951 [PMID: 19866393 DOI: 10.1055/s-0029-1215179]

15 **Chukmaitov A**, Bradley CJ, Dahman B, Siangphoe U, BouHaidar D, Warren JL. Polypectomy techniques, endoscopist characteristics, and serious gastrointestinal adverse events. *J Surg Oncol* 2014; **110**: 207-213 [PMID: 24706376 DOI: 10.1002/jso.23615.Epub]

16 **Vanagunas A**, Jacob P, Vakil N. Adequacy of "hot biopsy" for the treatment of diminutive polyps: a prospective randomized trial. *Am J Gastroenterol* 1989; **84**: 383-385 [PMID: 2648816]

17 **Goldstein NS**, Watts JC, Neill JS, Vogel LM, Barkel D, Kadro O, Priest S, Klein S. The effect of electrothermal cautery-assisted resection of diminutive colonic polyps on histopathologic diagnosis. *Am J Clin Pathol* 2001; **115**: 356-361 [PMID: 11242791 DOI: 10.1309/0KPE-1RG6-KA78-R49Y]

18 **Mönkemüller KE**, Fry LC, Jones BH, Wells C, Mikolaenko I, Eloubeidi M. Histological quality of polyps resected using the cold versus hot biopsy technique. *Endoscopy* 2004; **36**: 432-436 [PMID: 15100953 DOI: 10.1055/s-2004-814321]

19 **Horiuchi A**, Hosoi K, Kajiyama M, Tanaka N, Sano K, Graham DY. Prospective, randomized comparison of 2 methods of cold snare polypectomy for small colorectal polyps. *Gastrointest Endosc* 2015; **82**: 686-692 [PMID: 25922251 DOI: 10.1016/j.gie.2015.02.012]

20 **Komeda Y**, Suzuki N, Sarah M, Thomas-Gibson S, Vance M, Fraser C, Patel K, Saunders BP. Factors associated with failed polyp retrieval at screening colonoscopy. *Gastrointest Endosc* 2013; **77**: 395-400 [PMID: 23211749 DOI: 10.1016/j.gie.2012.10.007]

21 **Repici A**, Hassan C, Vitetta E, Ferrara E, Manes G, Gullotti G, Princiotta A, Dulbecco P, Gaffuri N, Bettoni E, Pagano N, Rando G, Strangio G, Carlino A, Romeo F, de Paula Pessoa Ferreira D, Zullo A, Ridola L, Malesci A. Safety of cold polypectomy for <10mm polyps at colonoscopy: a prospective multicenter study. *Endoscopy* 2012; **44**: 27-31 [PMID: 22125197 DOI: 10.1055/s-0031-1291387]

22 **Takeuchi Y**, Yamashina T, Matsuura N, Ito T, Fujii M, Nagai K, Matsui F, Akasaka T, Hanaoka N, Higashino K, Iishi H, Ishihara R, Thorlacius H, Uedo N. Feasibility of cold snare polypectomy in Japan: A pilot study. *World J Gastrointest Endosc* 2015; **7**: 1250-1256 [PMID: 26634041 DOI: 10.4253/wjge.v7.i17.1250]

23 **Lee CK**, Shim JJ, Jang JY. Cold snare polypectomy vs. Cold forceps polypectomy using double-biopsy technique for removal of diminutive colorectal polyps: a prospective randomized study. *Am J Gastroenterol* 2013; **108**: 1593-1600 [PMID: 24042189 DOI: 10.1038/ajg.2013.302]

24 **Matsuura N**, Takeuchi Y, Yamashina T, Ito T, Aoi K, Nagai K, Kanesaka T, Matsui F, Fujii M, Akasaka T, Hanaoka N, Higashino K, Tomita Y, Ito Y, Ishihara R, Iishi H, Uedo N. Incomplete resection rate of cold snare polypectomy: a prospective single-arm observational study. *Endoscopy* 2017; **49**: 251-257 [PMID:

28192823 DOI: 10.1055/s-0043-100215]

25 **Komeda Y**, Kashida H, Sakurai T, Tribonias G, Okamoto K, Kono M, Yamada M, Adachi T, Mine H, Nagai T, Asakuma Y, Hagiwara S, Matsui S, Watanabe T, Kitano M, Chikugo T, Chiba Y, Kudo M. Removal of diminutive colorectal polyps: A prospective randomized clinical trial between cold snare polypectomy and hot forceps biopsy. *World J Gastroenterol* 2017; **23**: 328-335 [PMID: 28127206 DOI: 10.3748/wjg.v23.i2.328]

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