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REVIEW

Liver transplantation and alcoholic liver disease: History, controversies, and considerations

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Abstract

Alcohol consumption accounts for 3.8% of annual global mortality worldwide, and the majority of these deaths are due to alcoholic liver disease (ALD), mainly alcoholic cirrhosis. ALD is one of the most common indications for liver transplantation (LT). However, it remains a complicated topic on both medical and ethical grounds, as it is seen by many as a "self-inflicted disease". One of the strongest ethical arguments against LT for ALD is the probability of relapse. However, ALD remains a common indication for LT worldwide. For a patient to be placed on an LT waiting list, 6 mo of abstinence must have been achieved for most LT centers. However, this "6-mo rule" is an arbitrary threshold and has never been shown to affect survival, sobriety, or other outcomes. Recent studies have shown similar survival rates among individuals who undergo LT for ALD and those who undergo LT for other chronic causes of end-stage liver disease. There are specific factors that should be addressed when evaluating LT patients with ALD because these patients commonly have a high prevalence of multisystem alcohol-related changes. Risk factors for relapse include the presence of anxiety or depressive disorders, short pre-LT duration of

sobriety, and lack of social support. Identification of risk factors and strengthening of the social support system may decrease relapse among these patients. Family counseling for LT candidates is highly encouraged to prevent alcohol consumption relapse. Relapse has been associated with unique histopathological changes, graft damage, graft loss, and even decreased survival in some studies. Research has demonstrated the importance of a multidisciplinary evaluation of LT candidates. Complete abstinence should be attempted to overcome addiction issues and to allow spontaneous liver recovery. Abstinence is the cornerstone of ALD therapy. Psychotherapies, including 12-step facilitation therapy, cognitive-behavioral therapy, and motivational enhancement therapy, help support abstinence. Nutritional therapy helps to reverse muscle wasting, weight loss, vitamin deficiencies, and trace element deficiencies associated with ALD. For muscular recovery, supervised physical activity has been shown to lead to a gain in muscle mass and improvement of functional activity. Early LT for acute alcoholic hepatitis has been the subject of recent clinical studies, with encouraging results in highly selected patients. The survival rates after LT for ALD are comparable to those of patients who underwent LT for other indications. Patients that undergo LT for ALD and survive over 5 years have a higher risk of cardiorespiratory disease, cerebrovascular events, and *de novo* malignancy.

Key words: Alcoholic liver disease; Alcoholic hepatitis; Alcoholic cirrhosis; Alcoholism; Liver transplantation; Alcoholic recurrence; Controversies; Alcoholic abstinence; Relapse; Selection criteria

Core tip: Alcohol consumption accounts for 3.8% of annual global mortality worldwide. Cirrhosis is a common complication of alcoholic liver disease (ALD) and when end-stage liver disease is reached, the only chance of survival is liver transplantation (LT). There are controversies and ethical dilemmas associated with LT for ALD. This study reviews the history and controversies and considers the development of, indications for, and outcomes of LT in ALD, including severe acute alcoholic hepatitis. Relapse, therapeutic options, and outcomes are emphasized.

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INTRODUCTION

Liver transplantation (LT) is the treatment of choice for patients with liver failure in end-stage liver disease, and it is their only chance of survival. As patients on LT waiting lists outnumber the number of LTs performed, and due to the high waiting list mortality rate, prioritization of individuals who are most likely to die without LT is needed. Access based on who is most likely to benefit from organ donation requires a legal, fair and ethical basis for the allocation[1-3].

Alcohol consumption accounts for 3.8% of global mortality and 4.6% of disability-adjusted life-years (DALYs) lost due to premature death[4]. Among the various harmful effects of alcohol, alcoholic liver disease (ALD) induces a wide spectrum of liver abnormalities, including simple steatosis, alcoholic hepatitis (AH) or steatohepatitis, progressive fibrosis, and ultimately alcoholic cirrhosis (AC) and/or hepatocellular carcinoma (HCC)[5].

One of the common causes of chronic liver disease, for which LT is potentially lifesaving, is ALD. This has been highly controversial from the beginning. The ever-increasing shortage of organs has accentuated the low priority given to patients with ALD, which is considered a "self-inflicted" condition. However, by improving the long-term survival rates (thereby making them similar to those for LT patients with other indications) and recognizing that alcoholism is a primary disease, ALD has become one of the most common indications for LT in Europe and North America, a situation thought unfathomable 30 years ago. Unfortunately, there are still many issues with the use of LT for ALD.

LT for ALD used to be associated with many ethical dilemmas, but this has recently changed as alcoholism is now understood to be a chronic, recurrent neurological disease process with a clear biological basis. Alcoholism is no longer primarily viewed to be a result of moral weakness and self-destructive behavior, but an addiction and dependence that should be considered from other perspectives[5].

ALD progression is dependent on patient characteristics, as well as drinking patterns. Abstinence is fundamental to the treatment of all forms of ALD, and the alcohol consumption relapse rate is lower than that expected in alcoholics. The consequences of excessive drinking after LT range from asymptomatic biochemical and histological abnormalities to graft failure and death.

HISTORICAL PERSPECTIVE, CONTROVERSIES, AND CONSIDERATIONS

History

LT for ALD has been a controversial situation from the beginning because of the ever-increasing demand for donor organs and the inadequate rate of organ donation, combined with the concern that patients with alcoholism might relapse, thereby damaging the transplanted liver. There was an apprehension that the outcome of LT in these patients may not be the same as for LT patients with other indications.

Of the first ten patients who underwent LT, performed

by Starzl (before the advent of cyclosporine), nine did not survive the first 4 mo. This poor initial outcome was attributed to excessive alcohol consumption causing significant extrahepatic organ damage (such as pancreatitis, cardiomyopathy, cerebral dysfunction, and poor nutritional status). This was probably due to the selection of critically ill patients who were too sick to improve even with LT, and ALD was then considered a predictor of poor LT outcomes compared to other indications[6,7]. In 1984, Scharschmidt reported on the experience of four transplant centers that had performed 540 LTs in the United States and Western Europe. The 3-year survival rate for the 20 patients who underwent LT after 1980 was 20%; non-AC cirrhosis was associated with an impressive 42% survival rate[8].

In the United Kingdom, the University of Cambridge Department of Surgery at Addenbrooke's Hospital and the Liver Unit at King's College Hospital, London, started the Liver Transplant program in a joint collaborative endeavor in 1968[9], involving Roy Calne and Roger Williams. They have stated that patients with AC are seldom suitable for transplantation since they are often malnourished and particularly prone to precipitous clinical deterioration often provoked by infections, and there are often doubts as to their ability to control their drinking again after the transplant[9].

Between 1968 and 1987, 325 LTs were carried out, eight of which were for AC. Active alcoholism was a specific contraindication. All of them died before 25 wk, except for the last two, who died at 48 wk (around the time of publication of the report on the 325 patients); only one returned to drinking alcohol[10].

The United States National Institutes of Health (NIH) Consensus Conference on Liver Transplantation in 1983 concluded that ALD is an appropriate indication for LT, provided that the patient is judged likely to abstain from alcohol after LT[6]. Following this, there was an increase in the number of LTs being performed for ALD. However, the conference attendees still considered and predicted that ALD would be a marginal indication for LT.

The first positive data published on the survival rate of ALD patients after LT, in comparison to patients with other indications, were reported in 1988[11]. Starzl reported a 73% 1-year survival rate among 41 patients when cyclosporine was used as the main immunosuppressive drug[12], and only 3% of these patients had relapsed to alcoholism. This was a convincing argument in favor of LT for ALD.

In 1991, the US Health Care Financing Administration identified ALD as one of the seven conditions for which it approved payment for LT, but it recommended a "significant" period of abstinence before patients with alcoholism underwent the procedure, as well as the availability of a reasonable social support system. Beresford *et al*[12] proposed a selection method for identifying alcoholic patients who were suitable for LT. Furthermore, Lucey *et al*[13] reported on a multidisciplinary collaboration of transplant hepatologists, surgeons, and psychiatrists that identified psychosocial predictors of long-term sobriety and compliance after LT among patients with alcoholism.

The appropriateness of LT for ALD was confirmed in European and American centers in the early 90s, with 1-year survival rates being 66%-96%. There was increasing evidence that most ALD patients selected for LT have similar, if not better, survival rates compared to those who undergo LT for other indications (1-year survival rate of 86%)[14-18].The NIH workshop in 1996 on LT for ALD patients concluded that LT provides good outcomes for alcoholic patients and that relapse rates after LT were lower if the patients had successfully completed a conventional alcohol rehabilitation program prior to LT[7].

The European Liver Transplant Registry (ELTR)[19] accumulated information on LTs from 1968-2015 based on a progressive increase of cases. Among all the LT cases, the ELTR reported a rate of cases involving cirrhosis of 46%-55%, a rate of cases involving AC of 9%-44%, and a rate of cases involving AC + hepatitis C virus (HCV) infection of 2%-3%. In this period, viral cases increased from 8% to 27%, primary biliary cirrhosis cases decreased from 29% to 7%, and autoimmune hepatitis cases decreased from 10% to 5%[19]. There was a progressive increase in the number of indications for LT in the US after 2002, and the number of donors is much smaller than the number of patients who require LT.

Controversies

Patient selection for LT has always been a demanding responsibility for transplantation professionals. Less than 4% of AC patients were placed on an LT waiting list in the United States in 2007. In the United States, the majority of candidates with end-stage ALD who are eligible for referral for LT are not being referred. Kotlyar *et al*[20] analyzed data on alcohol abuse and dependence in the United States and found that the potential number of patients with ALD and decompensated AC who could be candidates for LT was 100000 patients/year. However, of these, only 10% (10000) were referred, and of these, 3673 were on the LT waiting list and 1200 underwent LT in 2018. Thus, every year, 4% of patients with decompensated AC were on the waiting list and 1.2% underwent LT. This pattern of referral may lead to as many as 12,000 deaths/year. There are multiple reasons for poor referral of these patients and these reasons occur at all levels[20].

LT for ALD still generates controversy because of both the perception that the patient's liver disease was self-inflicted and concerns related to relapse after LT. The general public, and even some practicing physicians outside the transplant community, view individuals with alcoholism as lower priorities for LT. Many specialists considered it unacceptable to "waste" grafts on individuals with alcoholism who were responsible for the harm caused to their liver, as they still consider alcoholism to be a bad habit, and there is a general reluctance to provide LT for these patients. Particularly in

the past, the naysayers believed that excessive alcohol consumption had multisystem organ consequences that precluded good surgery outcomes, that relapse-induced redevelopment of liver disease would occur, and that patients were unlikely to withstand the psychological issues caused by such a serious operation, resulting in poor compliance. However, neuroscience has shown that alcoholism is a chronic relapsing medical disease of the brain, and not a bad behavior. Ethical principles recommend active treatment of these patients, without discrimination[21-25].

LT provides the patients with a physiologically functioning liver and reverses the complications of end-stage liver disease, improving survival and quality of life, but it does not treat the underlying alcoholism and alcohol dependence, leading to the potential for relapse. The probability of long-term sobriety becomes robust only after 5 years of sustained abstinence[26-28].

Pre-LT alcohol abstinence represents one of the hottest and most controversial open questions on this topic. Many transplant centers use the criterion of 6 mo of abstinence to determine whether ALD patients should receive livers, known as the "6-mo rule". This rule has two purposes: to allow the patient's liver a chance to recover and to reduce the risk of alcohol consumption relapse. Six months is an arbitrary threshold and this period has never been shown to affect survival after LT, although there is a weak association between sobriety and LT outcome[29-32].

The rule was established in 1997, when the United Network for Organ Sharing (UNOS) had a meeting to discuss the criteria for placing adult ALD patients in need of a new liver on LT waiting lists. The recent guidelines of the American Association for the Study of Liver Diseases, European Association for the Study of the Liver, UNOS, and French Consensus Conference state that 6 mo of zero alcohol consumption before LT should no longer be an absolute rule or a defining factor to determine whether a patient is accepted as an LT candidate[29,33-36].

There is no firm consensus on the appropriate minimum duration of alcohol abstinence or on what constitutes good psychosocial criteria for placing patients on LT waiting lists. Physicians in the transplant community perceive selected patients with end-stage ALD as good candidates. An interval of sobriety prior to LT is very desirable from a medical point of view. Abstinence may markedly improve liver function[37].

ALD was the underlying etiology in the majority of patients who were removed from an LT waiting list following recompensation. There were only two independent predictors among ALD patients of recompensation/removal from the LT waiting list: A model for end-stage liver disease (MELD) score < 20 and serum albumin $\geqslant$ 32 g/L. The probability of recompensation was 70% when both factors were present when the patient was placed on the LT waiting list. Thus, it seems advisable for ALD patients to undergo a period of observation to check whether they have both favorable factors before embarking on living donor LT[38].

Patients with ALD were placed on an LT waiting list based on the Ohio Solid Organ Transplantation Consortium (OSOTC) exception criteria. These criteria allow patients with low to medium risk of relapse to undergo LT after only 1-3 mo of abstinence. The results showed that the LT rate and short- and long-term post-LT survival are comparable between these patients and the general population of United States patients with AC who underwent LT[39].

Considerations

Compared to the traditional criteria for assessing risk of relapse, a careful selection process with more flexibility to evaluate eligibility on a case-by-case basis can lead to similar survival rates after LT. With regard to alcoholism, a mandatory period of abstinence is a poor predictor of relapse. Pre-LT abstinence does not reliably predict post-LT abstinence or compliance[37].

When considering whether to add patients to an LT waiting list, 3 mo of alcohol abstinence may be better than 6 mo. Patients with a lack of social support, active smoking, psychotic or personality disorders, or a pattern of nonadherence should be added to the waiting list only with reservation. Those who have a diagnosis of alcohol abuse, as opposed to alcohol dependence, may make better LT candidates. Patients who have regular addiction treatment appointments with a psychiatrist or psychologist also seem to do more favorably[20].

Alcohol addiction is considered a big problem after LT, while moderate alcohol consumption is underestimated by both patients and their healthcare providers. Sometimes there is even a recommendation to carefully assess the alcohol consumption of patients on LT waiting lists who have non-AC cirrhosis[40,41].

Alcohol consumption can complicate a patient's health after LT, and moderate consumption is important because it can aggravate metabolic syndrome and non-alcoholic fatty liver disease, which often develop, irrespective of alcohol consumption, as the side effect of post-LT immunosuppressive agents. This immunosuppressive treatment can lead to a risk of de novo malignancy that is 2-7-fold higher than usual after adjusting for age and gender, and 5- and 10-year incidence rates are estimated to be 10%-14.6% and 20%-32%, respectively[42,43]. Moderate alcohol consumption can have a negative effect on LT as it increases the risk of liver fibrosis, mainly in women, even if alcohol consumption is < 12 g/d[40-43].

Medical law experts are repeatedly reminded, albeit from a different point of view than those associated with medical practice, that US constitutional law prohibits discrimination against subgroups and differentiating individuals as being either worthy or unworthy of life. The exclusion of non-sober ALD patients from LT waiting lists discriminates against them and violates this United States constitutional law[44].

The fact that patients with acute liver failure after ecstasy consumption and patients with acute hepatitis

B virus (HBV) infections due to careless sexual practices have full access to LT waiting lists raises the question as to why patients with severe acute AH or acute-on-chronic liver failure should be treated any differently[44]. The lack of pre-LT abstinence should not be considered as a justification for denying the legal right of patients with advanced ALD to have access to LT waiting lists[45]. The sidelining of patients with severe AC who, after complete evaluation, are otherwise considered to be candidates for LT must be avoided[46]. There are no moral or ethical arguments that could justify the exclusion of very ill patients with ALD from potentially lifesaving LT, as exclusion could be considered a death sentence for these patients[20,47].

ALD diagnoses are often made in the later stages of the disease because patients often remain in primary care for a long time, managing their alcoholism, and ALD diagnosis only occurs when hepatic manifestations belatedly raise clinical suspicion. Poor patient awareness, misinformation among the referring clinicians, delayed alcohol cessation intervention and counseling, premature and overconfident attribution of liver disease to another etiology (*e.g.*, HBV or HCV) are just some of the factors that limit effective management of AC[48,49].

In the end, alcoholism has to be accepted as a disease that, in some cases, has a genetic background[50]. As alcoholism is a life-long disease, it is to be expected that it should persist after LT[51].

LT FOR ALD

ALD is a worldwide health problem, resulting in high morbidity and mortality, not only due to the effects of alcohol on the liver, but because of the risk it poses to the health of other organs and the increased risk of accidents and violence-related deaths[52]. One type of ALD is AC, which is a leading indication for LT in the Unites States and Europe, accounting for approximately 15% and 20% of LT cases, respectively[53-55]. A recent analysis of three US databases (the National Health and Nutrition Examination Survey, HealthCore, and UNOS) showed that the proportion of patients on the LT waiting list or the proportion who have undergone LT due to cirrhosis secondary to HCV infection is declining, while the proportion on the list or who have undergone LT due to non-alcoholic fatty liver disease or ALD is increasing[56,57]. These findings are probably due to the advent of highly effective and well-tolerated treatments for HCV infection, which, up until now, was the main indication for LT[52].

PRE-LT EVALUATION OF PATIENTS WITH ALD

Comorbidities

In general, the indications and contraindications for LT in patients with AC are the same as those for patients with cirrhosis of any etiology[52]. There are, however, specific factors that should be addressed when evaluating AC patients for LT, given that they have a high prevalence of multisystemic alcohol-related changes. These comorbidities can be neurological (dementia, peripheral neuropathy, and vertigo), cardiological (cardiomyopathy, hypertension, and chronic renal disease), hematological (chronic anemia), gastrointestinal (chronic pancreatitis, diarrhea, and malnutrition), musculoskeletal (sarcopenia and osteoporosis), or psychiatric (including tobacco and illicit substance use)[57-61]. For example, malnutrition is present in about two-thirds of patients with cirrhosis on the LT waiting lists and negatively impacts survival, quality of life, and the patient's ability to cope with surgery or infections. When alcohol is an etiologic factor underlying cirrhosis, the prevalence of malnutrition is higher[62,63]. The incidence of comorbidities in AC has recently been reviewed, and a high comorbidity rate was found [hazard ratio (HR) for any comorbidity: 3.74; 95%CI: 3.56-3.94], including for non-cancer comorbidities (HR for any non-cancer comorbidity: 4.33; 95%CI: 4.06-4.62), but with the exception of acute myocardial infarction. The presence of these comorbidities must be carefully evaluated before LT, as they may negatively impact LT outcomes[57,64].

Alcohol consumption progresses over the years, and alcohol and its metabolites are toxic *per se*. However, there are other mechanisms of action regarding the negative health effects of alcohol consumption. By increasing gut permeability and exposing Kupffer cells to Gram-negative intestinal bacteria, alcohol induces cytokine production and a systemic inflammatory response[65].

An additional challenge of LT for AC is the need for lifelong post-LT follow-up, taking into consideration the comorbidities that may not be fully resolved or may return (along with alcohol consumption relapse) in the post-LT period. It is not surprising that cardiovascular illnesses and de novo malignancies are significantly overrepresented in AC patients who underwent LT[53]. With the recent advances in hepatitis C treatment and lack of HCV-related LTs, long-term follow-up of LT recipients will often entail more challenging circumstances involving patients who underwent LT for non-alcoholic steatohepatitis or ALD.

Neurological comorbidities

Chronic heavy intake of alcohol is a well-established cause of brain atrophy and dementia[54]. Patients may present with mild-to-moderate short- or long-term memory issues or more severe manifestations. There may be deficits in attention, concentration, learning, abstract reasoning, and motor skills. Hepatic encephalopathy can prevent a proper neurological evaluation of alcohol-induced brain damage prior to LT. These clinical manifestations may or may not be fully reversible after alcohol cessation. After LT, neurological improvement in AC patients with encephalopathy is not as good as for patients with cirrhosis of a different etiology[37].

Wernicke's encephalopathy is an alcohol-related syndrome characterized by ataxia, ophthalmoplegia, and

confusion, often with associated nystagmus, peripheral neuropathy, cerebellar signs, and hypotension. There is impaired short-term memory loss and emotional lability. Wernicke-Korsakoff's syndrome can develop after Wernicke's encephalopathy, characterized by anterograde and retrograde amnesia and confabulation. Wernicke-Korsakoff's syndrome is caused by a chronic thiamine deficiency, resulting in damage to the thalamic nuclei, mammillary bodies, brainstem, and cerebellar structures.

Alcohol can cause a polyneuropathy that can involve paresthesia, numbness, weakness, and chronic pain. Other neurologic conditions associated with chronic alcohol consumption are headache (cluster and migraine), neurocardiogenic (vasovagal and vasodepressor) syncope, compromised olfactory function, sleep disturbances, and peripheral vertigo. A small proportion of patients (< 1%) may develop midline cerebellar degeneration with ataxia. Seizures occur frequently upon alcohol withdrawal[66].

Cardiovascular comorbidities

The most common complications of ALD are cardiomyopathy, hypertension, and supraventricular arrhythmias. Alcoholic cardiomyopathy is the most common type of non-ischemic cardiomyopathy in Western countries (approximately 45% of cases). When being evaluated for surgery, many patients with ALD are found to have asymptomatic cardiac involvement, and are at risk of adverse short- and long-term outcomes. These findings may be confused with the findings associated with cirrhotic cardiomyopathy[53]. The clinical manifestations of ALD are similar to other causes of cardiac failure.

Abstinence can result in improvement in some cases. There are findings of beneficial cardiovascular effects with moderate alcohol consumption, but, when alcohol consumption is excessive, it results in hypertension. It has been shown that reducing alcohol dose-dependently decreases blood pressure, especially in heavy drinkers, and hypertension disappears with ⩽ 2 doses/d[37]. Chronic alcoholism is associated with a higher risk of cardiovascular mortality due to its epidemiological associations with known risk factors (smoking, age > 50 years, dyslipidemia, obesity, and hypertension).

The most common arrhythmias related to alcohol consumption are atrial fibrillation and supraventricular tachycardia, which commonly occur during acute intoxication and withdrawal. Cardiomyopathy can also induce ventricular arrhythmias.

Gastrointestinal comorbidities

Acute and chronic alcohol consumption cause mucosal inflammation, impairment of gut motility, sphincteric dysfunction, increased acid output, and damage to the small intestinal mucosa; these issues can occur directly due to a toxic effect or indirectly due to bacterial overgrowth and an impaired immune response[67].

Disregarding cirrhosis-related varices, Mallory-Weiss tears are a major cause of gastrointestinal bleeding, and a history of alcohol use can be found in > 40% of cases[68]. Diffuse esophageal spasm is also more frequent in individuals with alcoholism.

Alcoholic gastropathy (submucosal hemorrhages) typically involves abdominal pain, nausea, and vomiting.

Alcoholism accounts for about one-third of all cases of pancreatitis. The risk of pancreatitis in patients with alcohol dependence is approximately 4-fold higher than that in the general population, and it increases according to dose. Chronic pancreatitis develops in 10% of alcohol addicts after 6-12 years of 80 g daily alcohol intake[69]. Individuals with recurrent acute episodes of pancreatitis may develop chronic pancreatitis that can aggravate malnutrition.

Hematopoietic system comorbidities

The anemia that is commonly seen in patients with chronic alcohol problems can be multifactorial. Blood loss can cause anemia due to iron deficiency, which can occur due to the gastrointestinal diseases mentioned above. Dietary folate deficiency can cause megaloblastic anemia. Alcohol also has a direct toxic effect on the bone marrow, which can lead to sideroblastic anemia that resolves after abstinence. Alcohol also suppresses megakaryocyte production causing thrombocytopenia, which rapidly resolves about a week after cessation of alcohol intake. Lastly, alcohol interferes with platelet and white blood cell function, increasing the risks of bleeding and particular infections[66].

Malignancies

Previous alcohol abuse was shown to be associated with a 3-fold increased risk of post-LT *de novo* tumors. The mean duration until diagnosis has been reported to range between 3 and 5 years after LT[37]. Chronic alcohol use increases the risk of head and neck cancer, squamous cell carcinoma of the esophagus, and breast, prostate, pancreas, cervix, lung, and colon cancer. The risk is further potentiated by concomitant smoking and remains elevated despite alcohol abstinence, so screening should be considered after LT[66].

Infectious diseases

It has been demonstrated that both chronic alcohol consumption and moderate acute drinking can modulate the function of cells of the innate immune system, such as monocytes, macrophages, and dendritic cells. Alcohol is associated with increased intestinal permeability to endotoxins, altered proportions of monocyte population subsets, and altered cytokine profiles. These changes subside after 14 d of abstinence[70]. Alcoholism is associated with increased frequency and severity of infections, such as epidural abscesses, tuberculosis, meningitis, pneumonia, tick-borne fever, and others.

Psychiatric comorbidities

Psychiatric illnesses are commonly associated with alcoholism. The psychiatric and social issues associated with alcoholism can be more severe than the direct

medical effects. Anxiety and other mood disorders are found in at least a third of patients with alcoholism and multiple drug use is also prevalent. This is considered to be a bidirectional relationship. Alcohol may be used as a "medication" to relieve symptoms and, on the other hand, chronic use may lead to the development and/or worsening of these symptoms, either by compromising social skills or through the direct effect of alcohol on the brain[71]. Alcohol-related behavioral issues can cause other health issues, including domestic abuse injuries, other violence-related trauma, motor vehicle accidents, and burns.

Other diseases

There are many other conditions associated with alcohol consumption, including rhabdomyolysis, osteonecrosis (avascular necrosis), IgA nephropathy, and porphyria cutanea tarda.

ALD, HCC, AND SURVEILLANCE

In AC patients, mainly in those who were drinking > 80 g of alcohol daily, there was a positive association between the amount of alcohol intake and the risk of HCC (HR: 4.5), and this increased by 22% for those who drank 6 alcoholic units/day. In countries where there is heavy alcohol consumption, the cumulative risk of HCC is increased by 5-7-fold[72-74]. Several recent studies have established that the underlying etiology of liver disease determines the cumulative risk of HCC, and patients with viral, fatty liver, or autoimmune cirrhosis have a higher risk than those with AC[75-77].

The HCC surveillance recommendations for AC patients are similar to those for other cirrhotic patients (*i.e.*, periodic 6-mo ultrasound screening), with no specific recommendations. In alcoholic patients, the risk factors are age, metabolic syndrome, and the severity of the underlying liver disease[78]. In a surveillance study of 450 AC patients [Child-Turcotte-Pugh (CTP) classes A and B], Mancebo *et al*[79] found that 62 patients developed HCC, with an annual incidence of 2.6%. The risk was independently associated with age (> 55 years) and platelet count (< 125000/mm^3). The annual incidence was 0.3% in patients without risk factors, 2.6% in patients with one risk factor, and 4.8% in patients with two risk factors (P < 0.0001). Genomic analysis of alcohol-related HCC has demonstrated the presence of mutations in genes that modulate the HCC pathway, which helps to better define the risk classes and to adapt strategies for HCC surveillance.

NUTRITIONAL EVALUATION IN ALD

Poor nutritional status in patients with liver diseases is common, occurring in 20%-90% of cases. The main outcome is loss of muscle mass and fat, which is associated with the etiology of liver disease[80-83]. In ALD, there is marked loss of weight and muscle mass, with deficiencies of macronutrients and micronutrients, which can adversely affect the body composition of AC patients[84,85].

High daily alcohol intake can end up ensuring caloric maintenance, despite the fact that excessive alcohol consumption can inhibit hunger and compromise the palate, stimulating the search for ultra-processed foods, which also compromise the body composition of this population[86].

The change in muscle mass in quantity and/or function characterizes a clinical condition called sarcopenia. In some cases, no weight loss is observed. However, a discrepancy between the percentages of lean and fat mass may occur, which determines the diagnosis of sarcopenic obesity[86]. In sarcopenic obesity, there are mitochondrial and bioenergetic dysfunctions. The neurological consequences of alcohol that generate fatigue and asthenia make it difficult to determine whether changes in the skeletal muscles are the direct effects of ethanol and/or ALD.

After LT, some of the complications of cirrhosis disappear, but this does not occur easily with sarcopenia; on the contrary, the complications may be aggravated by the use of post-LT immunosuppressants[87,88]. Clinical improvement is not directly proportional to muscle function and muscle turnover but, in ALD, sarcopenia worsens the prognosis. The mechanisms involved are still unclear[89].

Nutritional assessment in patients with liver diseases has limitations due to difficulties with reproducibility and the lack of a gold standard. The current most accurate assessment involves the use of several methods that may be complementary[90]. The classic anthropometric assessment involving the assessment of body mass index (BMI), arm circumference, arm muscle circumference (BMC), and tricipital skinfold is a low-cost, universally used nutritional assessment. However, it has poor reproducibility (regarding inter- and intra-observer assessment) and it does not accurately measure muscle and fat mass in cases of cirrhosis involving ascites and edema[91].

The Subjective Global Assessment (SGA) is a low-cost, easy to apply method. However, it has flaws in implementation. It is based on body weight and subjective assessment and involves data that are self-reported by the participant (or guardian), with no accurate quantification of muscle mass[92-96]. The Patient-Generated Subjective Global Assessment (PG-SGA) is being used routinely, with early nutritional risk being identified more accurately, taking into account the disease staging and the patient's drug regimen; however, this method has only been validated for cancer patients[97].

The determination of the function of cirrhotic muscle mass using dynamometry is low cost and reproducible. However, it may not reflect the patient's actual nutritional status, especially in ALD patients, as it is based on the principle of muscular contractility and the use of alcohol

(depending on the amount of alcohol and duration of abstinence) may compromise these results. In AC patients with encephalopathy, it is not possible to apply this method because it is based on the principle of hand-grip strength[90,97-99].

Methods for quantifying lean mass, such as bioelectrical impedance analysis (BIA), dual energy X-ray absorptiometry (DEXA), and impedance plethysmography, are reproducible and objective. However, BIA and plethysmography may be inaccurate in cases of "body asymmetry" (*e.g.*, involving ascites and edema) and DEXA is a potential radiation-related risk factor (especially if it is routinely used) and is expensive, making it difficult to use in clinical practice[100-102].

Imaging methods, such as computed tomography (CT) and magnetic resonance imaging (MRI), can be used to quantify skeletal muscle mass, but they are high-cost methods[103,104]. The skeletal muscle area determined from a single CT or MRI section involving the third or fourth lumbar vertebra has been shown to reflect the muscle mass of the entire body[103]. Using these methods, patients with cirrhosis have lower muscle and fat mass compared to controls[87,105]. AC patients have been included in several studies but etiology-specific data have not yet been reported. Ultrasound assessments of lean muscle mass exhibit variability across observers, and analyzing body portions only provides an estimate of whole-body measurements and does not allow assessment of muscle functionality[87,104,105].

The above-mentioned methods all have limitations because they are based on the body composition model, valuing only lean muscle and fat mass. In 2000, Ellis proposed the use of a cellular composite multicompartment model of body composition[106]. This evaluates cellular functionality and can aid in an objective, reproducible, and serial way in the determination of cellular composition and functionality based on the use of the BIA phase angle (PA). PA is based on measurement of electrical resistance (R) and reactance (Xc) and it reflects the structure and functionality of the cell membrane[107].

Studies involving different populations have identified PA cutoff points that differentiate the pathophysiology in question from a lack of the pathophysiological condition. ALD patients have different cellular characteristics than patients with other conditions. This is because ethanol induces autophagic mechanisms (adaptive cellular responses to eliminate damaged organelles) and the production of cytotoxic cellular proteins (due to changes in homeostasis, protein synthesis, and autophagic proteolysis) that result in the loss of skeletal muscle[108]. Factors that contribute to cellular damage in ALD patients include hyperammonemia (due to cirrhosis), endocrine abnormalities (such as hypogonadism), and intestinal dysbiosis.

PA assessment of cirrhosis prognosis has a sensitivity of 68.9% and a specificity of 70.0%, indicating that it is a good prognostic marker. Assessing PA to investigate different cirrhosis stages is useful, as a lower PA indicates a worse disease stage[90,109-112]. In the follow-up of pre- and post-LT patients, Deutrich *et al*[113] observed that changes in PA were the only measurable changes that correlated with the improvement in the clinical condition. Two studies evaluating cirrhotic patients identified the same cutoff point for PA (5.4°), where those who were below this value had a poor prognosis[90,114]. Recently, a pilot study of cirrhotic patients identified a cutoff of 4.9°[115], which is different from the previous study findings. This demonstrates the need for cohort studies with greater robustness in order to determine a reliable cutoff point that can indicate the prognosis/clinical condition of cirrhotic patients.

For Baumgartner *et al*[116], the PA is an indicator for the diagnosis of metabolic, physiological, nutritional, and hydration disorders that could be applied to any living creature. In an experimental study of rats with carbon tetrachloride (CCl_4)-induced cirrhosis, PA was determined before and after induction of cirrhosis, being reduced in cirrhotic animals. It was also observed that a decrease in fatty acids (FA) accompanied the worsening of the cirrhosis, measured by cellular damage using the thiobarbituric acid reactive substances (TBARS) technique, compared to controls[117].

In different liver diseases, PA can provide different relevant information. For example, in chronically infected HCV patients, PA is a good predictor of advanced fibrosis; each degree of decrease in FA increases the risk of advanced fibrosis 4-fold. In HCV patients undergoing antiviral treatment, the reduction in PA is associated with an increase in the adverse effects of the therapy[118,119].

PA also serves as a basis for bioelectrical impedance vector analysis (BIVA), a slightly more complex evaluation, which provides information on the body composition (cellularity) and cell hydration state, independently of the alteration in body composition. BIVA is of great importance in cases of edema and ascites that make identifying nutritional compromise difficult[120,121].

There have been no studies of PA and/or BIVA in patients with ALD alone. There is a need to develop this research area further to understand cellular functioning in ALD patients in order to develop preventive and curative strategies regarding nutritional and other clinical issues, improving quality of life and post-LT outcomes.

FUNCTIONAL LIMITATIONS IN CIRRHOTIC PATIENTS

Cirrhosis leads to systemic and metabolic alterations that compromise pulmonary, renal, encephalic, cardiac, and metabolic functions, and complications such as ascites, encephalopathy, jaundice, and sarcopenia, which increase morbidity and mortality and compromise quality of life[122,123].

Metabolic changes associated with cirrhotic malnutrition are frequent, negatively affect the musculoskeletal system, and directly interfere with physical fitness[124,125]. The deficiency in protein synthesis leads to persistent cachexia, which limits the physiological integrity of the

Table 1 Liver transplantation survival rates reported by the European Liver Transplant Registry

n	Etiology	1 yr	5 yr	10 yr
		%		
15019	AC	86	73	59
1790	AC + HCV	85	69	54
6507	Acute liver failure	70	64	58
10753	HCV	80	65	53
4187	HBV	83	74	68
9122	Cirrhosis + HCC	83	62	49
9114	Cholestasis	87	78	70
1892	AIH	85	76	67
468	Hemochromatosis	76	66	53

AC: Alcoholic cirrhosis; HCV: Hepatitis C virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; AIH: Autoimmune hepatitis.

muscular system and impairs its functioning, with loss of muscle strength and worsening of quality of life. Protein filaments of actin and myosin undergo adaptive processes and lose their contractile components, with muscular hypotrophy. Concomitantly, there is a change in lipid metabolism that influences the biochemical composition of lipoproteins. In cirrhotic patients, there is insufficient glycogen due to limitations in hepatic synthesis, which increases the use of amino acids as an energy source and causes an acceleration in the decomposition of skeletal muscle to release amino acids, which results in a loss of muscle mass. These changes limit muscle functioning and negatively affect the performance of daily activities[126].

A possible explanation for the reduction in function may be related to the loss of muscle mass in this population, but it may also be due to a decrease in the mitochondrial oxidative capacity and/or number of mitochondria in the muscle tissue. The adenosine triphosphate (ATP), phosphocreatine, and total magnesium (Mg^{2+}) levels are decreased in cirrhotic skeletal muscle. This concept was demonstrated by Jacobsen *et al*[127], who found higher rates of mRNA and mitochondrial ATP in patients with CTP cirrhosis compared to those with CTP-B and CTP-C scores (Table 1).

Sarcopenia is associated with age, but it is also present in chronic neoplastic diseases and leads to a decrease in functional capacity and an increased risk of mortality[128]. Severe muscle depletion or sarcopenia is defined as a decrease in muscle mass, strength, and function. Sarcopenic dysfunctions are predictors of morbidity and mortality in cirrhotic patients[129].

Abdominal cross-sectional studies [involving lumbar segments 3 and 4 (L3-L4)], including those that involve CT or MRI, represent the gold standard for quantifying skeletal muscle mass. They involve detailed objective assessment for the identification of sarcopenia and assist in improving the nutritional/metabolic outcomes of patients with cirrhosis[130]. Muscle tissue loss may be an important factor for muscle dysfunction and worsening of quality of life. However, it is necessary to specifically measure muscle dysfunction with precise methods, such as isokinetic dynamometry, palmar grip dynamometry, and manovacuometry (which measures respiratory muscle strength)[131].

Montano-Loza *et al*[130], using measurements based on abdominal L3-L4 CT images, evaluated the impact of sarcopenia on cirrhosis in patients on a waiting list for LT. They found a 6-mo survival rate of 71% in sarcopenic patients and 90% in non-sarcopenic patients. The frequency of sepsis and death was significantly higher in the sarcopenic patients[130].

Regarding functional evaluation, the 6-min walk test (6MWT) is an accessible, easy, cheap, and reproducible assessment. In cirrhotic patients, 6MWT performance < 400 m is an independent predictor of mortality. Based on a receiver operating characteristic (ROC) curve analysis, 6MWT had a greater sensitivity and specificity for mortality compared to maximal oxygen consumption and respiratory muscle strength[132].

The muscular dysfunctions in patients with cirrhosis may be influenced by the etiology of the disease. Patients with AC may present with alcoholic myopathy. Galant *et al*[133] showed that AC patients had lower muscle strength and poorer 6MWT performance and quality of life compared to cirrhosis patients with HBV or HCV. Physical fitness is a marker of mortality due to cirrhosis in AC patients, with patients with a peak oxygen uptake (VO_2) < 14 mL/kg having a lower survival rate over 3 years compared to those with superior results.

A multidisciplinary intervention for muscular recovery in patients with cirrhosis, involving nutritional supplementation and supervised physical activity, led to a gain in muscular mass and improvement in functional activity, directly impacting quality of life and preparation for LT (Figure 1)[134].

MANAGEMENT OF ALCOHOL ADDICTION BEFORE LT

The patient's history of alcohol and other substance use, such as tobacco, opioids and illicit/recreational drugs must be thoroughly evaluated[52]. Psychiatrists, psychologists, social workers and dependency specialists are essential in the evaluation of these patients. The information collected will help the multidisciplinary team to determine whether a transplant should be performed in these patients, as well as to establish a therapeutic plan before and after the procedure[52,135,136]. There is evidence that the work of such teams in transplant centers reduces the rates of recurring alcoholism and mortality after LT compared to patients referred for outpatient treatment[55,60]. Simple standardized questionnaires, such as CAGE and Alcohol Use Disorders Identification Test (AUDIT), can be used in clinical practice to track the chronic and excessive use of alcohol in transplant patients, including those with cirrhosis of other etiologies[52,137,138]. An additional tool for the psychosocial assessment of transplant candidates is the Stanford Integrated Psychosocial Assessment for Transplantation. Its strengths include standardization

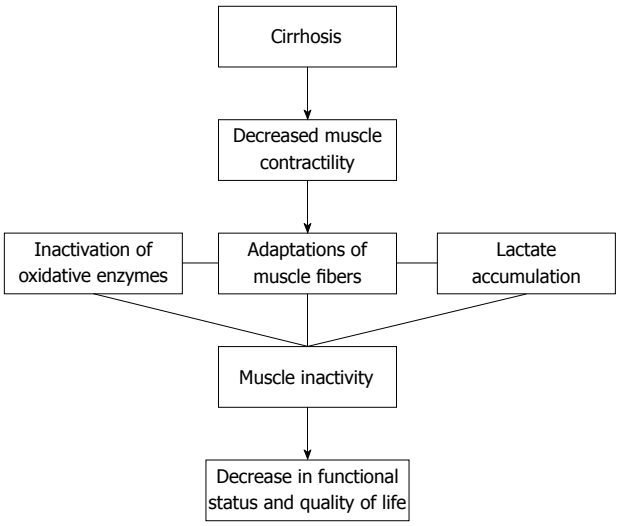

Figure 1 Flow chart demonstrating the consequences of cirrhosis regarding muscular adaptation and functional repercussions[134].

of the evaluation process and the ability to identify individuals at risk of negative psychosocial events after transplantation in order to develop interventions aimed at improving the patient's pre-LT conditions. This instrument also allows evaluation of the psychosocial factors that best predict patient compliance and graft survival. Eighteen risk factors are divided into four domains, including patient readiness, social support, psychological stability and substance use. Based on an index composed of all the domains, patients are classified as excellent, good, minimally acceptable, high risk or poor transplant candidates[52,139].

A constant concern of health professionals is the patient's possible return to alcohol use after LT or the relapse of an ALD patient before transplantation[40]. To date, however, no standardized definition of recurrence has been formulated. Consequently, the rates found in the literature vary widely, due both to the different definitions of the term and different follow-up times. A 2015 review[17] indicated rates from 3% (ingestion > 60 g/d) to 43.70% (any consumption). It seems important, then, to distinguish between patients who occasionally drink small amounts of alcohol from those who regularly drink moderate amounts from those who continuously drink large amounts. Thus, recurrence can be distinguished from alcoholism[136]. Nevertheless, it requires emphasis that patients who underwent LT for ALD are obligated to stay abstinent. Unfortunately, this is an extremely difficult goal to achieve consistently.

Predictive factors of recurrence

In 1997, an American consensus suggested that patients with AC should have a minimum abstinence period of 6 mo before being included on the LT waiting list, the so-called "6-mo rule." The rationale of this recommendation was to evaluate the improvement of liver function, commonly observed after three to 6 mo of sobriety[140,141]. This abstinence period also serves as a predictor of post-transplant recurrence. As a result, major guidelines recommend this rule[55,135]. The logic is that the longer the period of sobriety, the lower the risk of returning to alcohol use. However, rules based on specific sobriety times, particularly those of short duration, are not consistent with what is known about the evolution of alcohol use disorder or predictions of future abstinence[142,143]. Although each month of sobriety increases the likelihood that the patient will not resume drinking, patients abstinent for 6 mo have only a slightly better risk reduction than those with 4 or 5 mo of sobriety[60]. Therefore, rigid adherence to the "6-mo rule" could result in unnecessary delays in listing patients who would otherwise be good candidates for transplantation, especially if we consider that this abstinence period is a weak predictor of post-transplant alcohol consumption[52]. For this reason, other predictors of recurrence after LT should be identified besides a specific period of sobriety. A 2015 review[21] highlighted an association between the following factors and recurrence: psychiatric co-morbidity, poor social support, multiple treatment failures, illicit drug use, a family history of alcoholism, medical non-compliance and the continued use of alcohol despite its consequences. It is up to the multidisciplinary team to investigate the presence of these factors and, if appropriate, adopt the best strategies for circumventing them. However, other factors suggest a lower risk of recurrence, such as: the patient's recognition that alcoholism is a disease, family support, employment, having a permanent residence, the ability to perform activities that replace daily drinking, and participation in rehabilitation programs[21,52].

LT SURGICAL ISSUES IN ALD

At present, there is no evidence for the association between ALD and increased incidence of portal vein or arterial thrombosis pre- or post-LT. The type of LT technique used is based on the experience of the transplant center, and there are no recommendations based on cirrhosis etiology. The techniques include the piggyback technique (preservation of the vena cava with lateral-lateral cava-cava anastomosis or suprahepatic cava-hepatic veins terminal-terminal anastomosis) and conventional vena cava-cava terminal-terminal anastomosis (without preservation of the recipient's vena cava).

NEED FOR SURGERY AFTER LT

Causal associations have been established between alcohol consumption and cancers of the oral cavity, pharynx, larynx, esophagus, liver, colon, rectum, and, in women, breast; associations are also suspected for cancers of the pancreas and lung[26,37,136,144,145]. Evidence suggests that the effect of alcohol is modulated by polymorphisms in genes encoding enzymes for ethanol metabolism, folate metabolism, and DNA repair. The mechanisms by which alcohol consumption exerts a

carcinogenic effect have not been completely defined[145].

The higher risk of malignancies for patients with AC should be considered in the routine assessment of these patients[37]. Surveillance protocols for earlier detection of *de novo* malignancy are needed to improve long-term post-LT outcomes[144]. Appropriate surgical treatment is the best option for curing solid organ malignant neoplasms, such as skin, esophageal, lung, digestive, or head and neck cancer. Proper resection of these lesions can contribute to increased survival when the disease is diagnosed in the early stages. Radiotherapy, chemotherapy, or immunotherapy are indicated for palliative care in the advanced stages of each specific neoplastic disease.

ACUTE AH

Background

AH is a distinct severe form of steatohepatitis that occurs in patients with alcoholism. It can present as acute or chronic liver failure associated with a rapid decline in liver synthetic function, and a consequent increase in mortality[146,147]. The mortality rate in patients with severe AH is 30%-50% at 3 mo, often despite supportive medical care. The amount of alcohol intake that puts an individual at risk of AH is not known, but generally, most AH patients have a history of heavy alcohol use (> 100 g/d) for decades[35,146-149].

The pathogenic pathways that lead to the development of AH are complex and involve oxidative stress, gut dysbiosis, and dysregulation of the innate and adaptive immune system, with injury to parenchymal cells and activation of hepatic stellate cells[146].

Incidence

The annual incidence of AH remains largely unknown. Concerning its prevalence, a large study of systematic biopsies in 1604 alcoholic patients, symptomatic or not, showed the prevalence of AH to be 20%[149]. In symptomatic patients, including those with decompensated liver disease, the prevalence of AH is not well known, partly because most centers rely on clinical criteria and do not consider transjugular liver biopsy as a routine practice in the management of patients with decompensated ALD[35].

History

Patients with AH are often aged 40-50 years, with most patients presenting before the age of 60 years. Patients with AH typically have a history of daily heavy alcohol use (> 100 g/d) for > 20 years[150].

Clinical features and diagnosis

The characteristic clinical features of AH are malaise, anorexia, fever, jaundice, tender hepatomegaly, signs of malnutrition, and complications such as ascites or variceal bleeding[146,151]. Progressive jaundice is the main presenting feature of symptomatic cases. Patients with severe AH and/or underlying AC may exhibit signs of hepatic encephalopathy, may develop hepatorenal syndrome, and are prone to developing bacterial infections[147]. Serum aminotransferases are moderately elevated (typically < 300 IU/L and rarely > 500 IU/L), with aspartate transaminase (AST) > alanine transaminase (ALT) and often > 2:1, which is rarely seen in other forms of liver disease[152-154]. Patients with AH typically have elevated serum bilirubin and γ-glutamyltransferase (GGT) and leukocytosis with a predominance of neutrophils. Depending upon the severity, serum albumin may be decreased, and the international normalized ratio (INR) may be elevated[35].

Imaging tests and liver biopsy

Abdominal imaging (ultrasound, CT, and MRI scans) in patients with AH may suggest fatty changes in the liver, evidence of underlying AC, or ascites. Transjugular liver biopsy is recommended, as about 30% of patients diagnosed with AH can be misdiagnosed when the diagnosis is based only on clinical parameters[155]. Histologic findings in liver biopsies from patients with AH include steatosis (typically micro- or macrovesicular steatosis, but in some cases alcoholic foamy degeneration is seen); hepatocellular ballooning with cytoplasmic rarefaction, Mallory-Denk bodies; neutrophil or lymphocyte infiltration; cholestasis and bile duct proliferation; and fibrosis with a perivenular, perisinusoidal, and/or pericellular distribution[156,157].

Determining disease severity

Several models have been proposed to determine the severity of AH and predict early death 1-2 mo after hospitalization[35]. The Maddrey discriminant function (DF) and the MELD score are the most commonly used scores to help identify patients who are more likely to benefit from pharmacotherapy. Other validated scores include the Glasgow Alcoholic Hepatitis score, ABIC score (which includes Age, serum Bilirubin, International Normalized Ratio, and serum Creatinine), and Lille score (which is used to determine whether a patient is responding to treatment)[35,158,159].

Maddrey DF: The Maddrey DF (also known as the Maddrey score) was the first score to be developed and remains the most widely used. It is calculated as follows[160,161]:

DF = {4.6 × [prothrombin time (s) - control prothrombin time (s)]} + [serum bilirubin (mg/dL)]

In the absence of treatment, the 1-mo spontaneous survival of patients with a DF ⩾ 32 has fluctuated between 50% and 65%[162,163]. Those with lower scores have low short-term mortality rates and do not appear to benefit from glucocorticoids[164].

MELD score: The MELD score is a statistical model developed to predict survival in patients with cirrhosis that has also been used to predict mortality in patients

hospitalized for AH[165,166]. The score ranges from 6 to 40 and is based on serum bilirubin, creatinine, and INR.

In one report, a MELD score > 11 performed as well as the DF in predicting 30-d mortality[165]. The sensitivity and specificity of MELD for predicting 30-d mortality was 86% and 81%, respectively, and 86% and 48%, respectively, for the DF. In a second study, a MELD score ⩾ 21 had a sensitivity of 75% and a specificity of 75% for predicting 90-day mortality[167]. In addition, an increase in the MELD score ⩾ 2 points in the first week of hospitalization may independently predict in-hospital mortality[166].

Lille score: The Lille score is a method to determine whether patients with AH are responding to glucocorticoid therapy. It combines six variables: age, renal insufficiency (creatinine > 1.3 mg/dL or creatinine clearance < 40 mL/min), albumin, prothrombin time, bilirubin, and change in bilirubin at day 7 (bilirubin at day 7 - bilirubin at day 0)[165]. The Lille model guides treatment decisions, with a score > 0.45 suggesting that a patient is not responding to glucocorticoids and predicting a mortality rate of 75% at 6 mo[158]. Based on the Lille score, corticosteroid treatment can be stopped in those with no improvement after a week of therapy[168].

General management

Patients with AH require general supportive care, including support for alcohol abstinence, prevention and treatment of alcohol withdrawal symptoms, fluid management, nutritional support (enteral feeding is preferred over intravenous nutrition), correction of nutritional deficiencies, infection surveillance, prophylaxis against gastric mucosal bleeding, and discontinuation of nonselective beta blockers in patients with severe AH (in addition, beta blockers, if indicated, should not be started in patients with AH until after they have recovered)[164,168,169].

Infections are frequent and difficult to diagnose in AH patients as they often fulfil the criteria for systemic inflammatory response syndrome (SIRS) at admission, which reflects either the inflammatory state associated with the AH episode or an ongoing bacterial infection. Systematic body fluid sampling and close clinical monitoring are advised for early detection of infection. In the absence of scientific evidence, criteria for initiating empirical antibiotic administration, although widely used, remain debated. In patients with severe AH, infection screening at admission is particularly warranted because a quarter of them have infections at admission[170].

Mild to moderate AH

Patients with mild to moderate AH (Maddrey DF < 32) and without corticosteroid treatment have only a 10% mortality rate at 28 d. Supportive management is therefore adequate for such patients[171]. The mainstay of treatment for patients with mild to moderate AH is abstinence from alcohol. In addition, general supportive care (*e.g.*, nutritional support and hydration) should be provided, but pharmacological treatment with glucocorticoids is not recommended as it does not appear to be beneficial in patients with mild to moderate AH. Pentoxifylline has only been studied in patients with severe AH and not in patients with mild to moderate AH[164].

Severe AH

In addition to general supportive care, pharmacological treatment is indicated for patients with severe HA (Maddrey DF ⩾ 32). Most guidelines recommend treating patients with severe AH with prednisolone at 40 mg/d for 4 wk (then tapering the dose over 2-4 wk, or stopping prednisolone treatment, depending on the clinical situation), provided there are no contraindications for its use (*e.g.*, active bacterial or fungal infection or chronic hepatitis C or B)[35,172]. Steroids have a potent immunosuppressant effect, suppressing two pro-inflammatory transcription factors: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1). This results in lower levels of tumor necrosis factor (TNF)-α and interleukin (IL)-8. In a randomized trial [STeroids Or Pentoxifylline for Alcoholic Hepatitis (STOPAH)] involving 1103 patients with severe AH, prednisolone showed a trend toward improving survival (odds ratio: 0.72; 95%CI: 0.52-1.01)[173].

Pentoxifylline is an oral phosphodiesterase inhibitor that also inhibits the production of TNFα, which is increased in patients with AH, among other cytokines. The role of pentoxifylline in the treatment of AH remains uncertain because questions remain regarding its efficacy[173-175]. Pentoxifylline may be an alternative in patients who are at risk of sepsis or failing to follow up after discharge (thereby making tapering off prednisolone unlikely, which could result in serious adverse effects). Pentoxifylline is given as a 400 mg dose three times/day (or 400 mg once/day in patients with a creatinine clearance < 30 mL/min)[164]. The largest meta-analysis published on this topic supports the observation that pentoxifylline decreases the risk of acute kidney injury and suggests that pentoxifylline improves the mortality rate compared with placebo but not compared to glucocorticoids[174]. The American Association for the Study of Liver Diseases and the European Association for the Study of the Liver recommend pentoxifylline for patients who cannot receive glucocorticoids[35,172].

A multicenter randomized placebo-controlled trial of prednisolone, pentoxifylline, and combination therapy has been completed. It showed that 4-week treatment with combination therapy compared with prednisolone alone did not improve the 6-mo survival rate[176].

Prognosis

Mortality rates among patients who do not receive pharmacological therapy (*e.g.*, prednisolone) for AH are variable. In patients with severe AH, short-term mortality rates are high (approximately 25%-45% at 1 mo)[160,177-179], whereas patients with mild to moderate

AH have lower short-term mortality rates (< 10% at 1-3 mo)[180,181]. Multiple risk factors for increased mortality in patients with AH have been identified and some have been incorporated into prognostic models. These include older age, acute kidney injury, elevated bilirubin levels, elevated INR, leukocytosis, alcohol consumption > 120 g/d, infection (sepsis, spontaneous bacterial peritonitis, pneumonia, and other infections), hepatic encephalopathy, upper gastrointestinal bleeding, bilirubin to GGT ratio of > 1, and meeting the criteria for SIRS[164].

Long-term outcomes after initial hospitalization for AH

An important determinant of the outcome among patients with AH is whether they continue to drink alcohol. In a case series involving 87 patients with AH who survived their index hospitalization, the overall estimated 5-year survival rate was 32%. However, among those who abstained from alcohol, the estimated survival rate was 75%, whereas for those who relapsed and continued to consume alcohol it was 27% and 21%, respectively[182].

LT IN AH

AH is a clinical syndrome associated with hepatic impairment and systemic inflammatory response that is observed in alcoholic patients (most of them cirrhotic). It is characterized by progressive jaundice, mild to moderate elevation of liver enzymes, coagulopathy and hepatic encephalopathy[52,155]. In its severe form, mortality is 30% to 50% at 3 mo and up to 70% at 6 mo, especially when associated with renal impairment[147,176]. Most transplant centers adopt the "6-mo rule" before listing patients with ALD. However, patients with AH that do not respond to clinical treatments cannot wait 6 mo to be placed on an LT waiting list, considering the short-term mortality rate, which can be as high as 50%[183]. Thus, the lack of salvage treatment for these patients is the basis for considering immediate LT.

In a case-control study, Mathurin *et al*[184] selected 26 severe AH patients who had a favorable psychosocial profile and did not respond to standard treatment for early transplantation. This group of patients was compared to a matched group of 26 patients with severe AH who received standard treatment. The cumulative 6-mo survival rate of early transplant recipients was dramatically higher than controls (77% *vs* 23%, $P < 0.001$). The greatest benefits were observed within the first month after transplantation and were maintained during two years of follow-up (HR: 6.08; $P = 0.004$). Recurrence was reported in 12% of the cases. Singal *et al*[185] analyzed the UNOS database and identified 59 patients between 2004-2010 who underwent LT due to an AH diagnosis. The survival of grafts and AH transplant patients was compared with matched AC LT patients. Five-year graft and patient survival of AH and AC patients were 75% and 73% ($P = 0.97$) and 80% and 78% ($P = 0.90$), respectively. At New York's Mount Sinai Hospital, 94 patients with severe AH who did not respond to medical therapy were evaluated for early LT. Overall, nine (9.6%) candidates with favorable psychosocial profiles underwent early LT, comprising 3% of all adult LT during the study period. The 6-mo survival rate was higher among those receiving early LT than matched controls (89% *vs* 11%, $P < 0.001$). Eight recipients were still alive at a median of 735 d, with one alcohol relapse[186]. Most recently, the John Hopkins Hospital group published their trial of early LT in severe AH[187]. Seventeen patients with severe AH who were transplanted early were compared with 26 AC patients with ⩾ 6 mo of abstinence who were transplanted during the same period. The 6-mo survival was 100% and 89% for AH and alcoholic liver cirrhosis patients, respectively ($P = 0.27$). Alcohol relapse was similar in both groups: 23.5% and 29.2%, respectively ($P > 0.99$). Harmful drinking was higher among AH than cirrhotic patients, despite a lack of statistical significance (23.5% *vs* 11.5%, respectively; $P = 0.42$). A systematic review of 11 studies[188] concluded that survival and recurrence rates are similar in early-transplanted severe AH patients and AC transplant patients. Thus, despite the evidence that transplantation in patients with severe AH is feasible and presents good results in a well selected subgroup of patients, there is still a long way to go before considering this type of treatment as standard for this population[189,190].

POST-LT OUTCOMES IN ALD PATIENTS

Relapse

LT can cure liver disease, but not the underlying alcohol use disorder[135]. Therefore, the transplantation team should be alert to possible alcohol consumption relapse after LT. Although self-reported alcohol use is commonly of little value, biomarkers can be a helpful replacement. For instance, metabolites of alcohol, such as ethyl glucuronide, can reveal alcohol use up to 3-4 d after the last drink[191]. However, due to its high sensitivity, it can yield false-positive results when medications that contain alcohol or hand sanitizers that contain small amounts of ethanol are used[192]. Measuring ethyl glucuronide in hair samples can detect longer-term alcohol use[193].

A prospective study[146] following 208 ALD LT patients for up to 9 years found that 113 (54%) did not relapse. Among those who did ($n = 95$), four alcohol consumption patterns were identified: 1) the majority ($n = 55$; 28.6%) consumed small amounts infrequently; 2) others ($n = 13$; 6.4%) began by drinking moderate amounts early on, but reduced consumption over time; 3) some ($n = 15$; 7.9%) began drinking later and in increasing amounts; and 4) a minority ($n = 12$; 5.8%) resumed drinking shortly after LT and in increasing amounts. Patients in groups 2 and 4 (who started drinking early) were more likely to present with steatohepatitis (according to hepatic biopsy) and LT rejection, and all of those who died from ALD recurrence were in these groups. The researchers identified several pre-LT factors associated with relapse: An established diagnosis of alcohol dependence, a short sobriety period, a family history of alco-

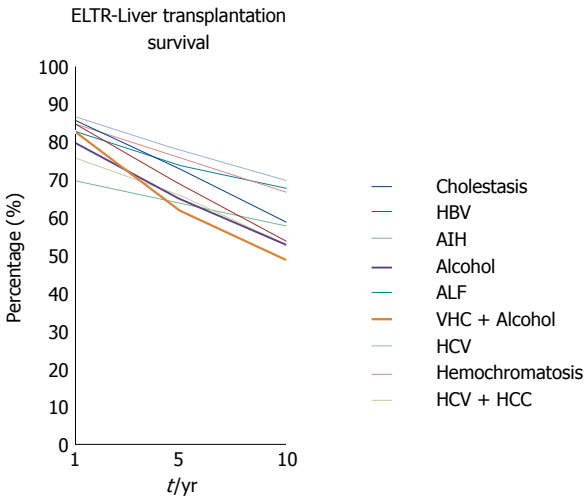

Figure 2 Survival of liver transplantation patients in Europe: 1, 5, and 10 years after liver transplantation. ELTR: European Liver Transplant Registry; HBV: Hepatitis B virus; AIH: Autoimmune hepatitis; ALF: Acute liver failure; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma.

holism, and the use of other substances[194].

A French multicenter retrospective study[147] analyzed the outcomes of 712 AC LT patients over approximately 9 years of follow up. At the end of the study, 128 patients (18%) had severe relapse (defined as a mean daily alcohol consumption > 20 g in women and > 30 g in men) for at least 6 mo. Of these, 41 (32%) developed AC on average 5 years after LT and 4 years after drinking again. A higher risk of relapse was observed in younger patients and those with a shorter sobriety period. Lastly, survival was lower in each time period in patients who developed recurrent AC compared to those who did not[195].

The results of these studies emphasize the importance of assessing alcohol use after LT and, if identified, taking measures to avoid the negative consequences.

Survival

Patient survival rates after LT for AC based on data from different parts of the world have been reported to be 81%-92%, 78%-86%, and 73%-86% at 1, 3, and 5 years, respectively[144].There are slight differences with the survival rates of ALD LT patients: 93.1%, 87.4%, and 82% at 1, 3, and 5 years, respectively; these results were mostly obtained from small case series in localized geographic regions and regions with particular ethnic characteristics, such as Australia[196].

Survival rates after LT for ALD are comparable to those for patients who underwent LT for other causes[190]. The ELTR evaluated 121,546 patients who underwent LT in 1968-2015 in Europe, and the study found that the survival rates for AC patients (n = 22648) at 1, 5 and 10 years after LT were 86%, 73%, and 59%, respectively. These rates were greater than those observed for patients with HCV (80%, 65%, and 53%), HCC (83%, 62%, and 49%), acute liver failure (70%, 64%, and 58%), and hemochromatosis (76%, 66%, and 53%); similar to those observed for patients with AC plus HCV or HBV (85%, 69%, and 54%); and a little lower than those observed for patients with cholestasis (87%, 78%, and 70%), autoimmune hepatitis (85%, 76%, and 67%), and HBV (83%, 74%, and 68%) (Figure 2)[197,198].

However, this somewhat equivalent survival among AC LT patients compared to other LT patient did not persist beyond 5 years due to the increased risk among AC LT patients of cardiorespiratory disease, cerebrovascular events, and *de novo* malignancy. Although all LT recipients are at greater risk of *de novo* malignancy, the incidence rate is significantly higher in patients with ALD, particularly regarding oropharyngeal and lung cancers, which may be related to substance abuse and smoking history[197]. Nearly 40% of ALD LT recipients resume smoking soon after LT[26]. Therefore, pre- and post-LT follow-up efforts regarding ALD patients should be focused not only on alcohol consumption relapse, but also on treating and avoiding other modifiable risk factors such as tobacco smoking. Pre-LT psychiatric and psychosocial evaluation and post-LT follow-up with physicians, psychiatrists, and addiction specialists are important for dealing with these problems[26].

Recent studies have indicated that resumption of alcohol abuse following LT leads to significantly reduced survival rates. Patients who resumed heavy drinking have been reported to have 5- and 10-year survival rates of 69.5% and 20.1%, respectively, compared to 90.3% and 81.5%, respectively, in abstinent patients[7,190,199].

In Australia, 16% of patients who underwent LT for ALD fulfilled criteria for harmful relapse and 21% experienced any form of alcohol consumption relapse. Harmful relapse was associated with increased mortality. Based on a multivariate analysis, only two factors were independently associated with harmful relapse: Lack of prior participation in an alcohol rehabilitation program and single versus married status[196]. Younger women dependent on alcohol shortly before LT are at greatest risk of relapse[199].

Among patients who underwent LT for AC, there is improvement in the quality of life, mood, and cognitive functioning, with no difference compared to patients who underwent LT for non-AC etiologies. LT patients were able to return to society and lead active and prolific lives, irrespective of the indication for LT[190].

The key factor determining the outcome of LT for AC is intensive lifelong medical and psychological care. Post-LT surveillance might be much more important than pre-LT selection[37].

CONCLUSION

Alcohol is largely consumed worldwide, causing many diseases in several organs and systems. For patients with ALD, when end-stage liver disease is reached, the only chance of survival is LT. There are controversies and ethical dilemmas associated with the indication of LT for ALD. Accurate stratification of potential LT candidates should be performed to identify those most likely to

remain abstinent after LT. The survival of patients who underwent LT for AC is comparable to that of patients who underwent LT for other non-AC etiologies. Psychiatrists, psychologists, social workers and dependency specialists, along with the transplantation team, are essential in the post-LT follow-up of these patients. AC patients who underwent LT are most likely to develop cardiovascular illnesses and malignancies 5 years after LT; it is also imperative that these patients stop smoking.

The two most important words related to ALD are addiction and abstinence. The first represents hell in the life of the alcoholic and refers to the most difficult pathways of degradation and death. It can be circumvented in a complex and time-consuming process, often with relatively little success that, when obtained, should be preserved with the greatest possible effort of the patient and the surrounding supporters, in a constant struggle. The second represents redemption, achieved with much effort and persistence, and it must be preserved at all costs, constantly and permanently, always glimpsing the future of physical and emotional recovery. The sequelae may disappear and recovery, when possible, may be complete. Abstinence is the solid foundation on which recovery is based. However, it is fragile as well, and requires constant vigilance from the patient and the supporters, not to return to hell.

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REVIEW

Current clinical management of gastrointestinal stromal tumor

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Abstract

Gastrointestinal stromal tumors (GISTs) are the most common malignant subepithelial lesions (SELs) of the gastrointestinal tract. They originate from the interstitial cells of Cajal located within the muscle layer and are characterized by over-expression of the tyrosine kinase receptor KIT. Pathologically, diagnosis of a GIST relies on morphology and immunohistochemistry [KIT and/or discovered on gastrointestinal stromal tumor 1 (DOG1) is generally positive]. The prognosis of this disease is associated with the tumor size and mitotic index. The standard treatment of a GIST without metastasis is surgical resection. A GIST with metastasis is usually only treated by tyrosine kinase inhibitors without radical cure; thus, early diagnosis is the only way to improve its prognosis. However, a GIST is usually detected as a SEL during endoscopy, and many benign and malignant conditions may manifest as SELs. Conventional endoscopic biopsy is difficult for tumors without ulceration. Most SELs have therefore been managed without a histological diagnosis. However, a favorable prognosis of a GIST is associated with early histological diagnosis and R0 resection. Endoscopic ultrasonography (EUS) and EUS-guided fine needle aspiration (EUS-FNA) are critical for an accurate diagnosis of SELs. EUS-FNA is safe and effective in enabling an early histological diagnosis and adequate treatment. This review outlines the current evidence for the diagnosis and management of GISTs, with an emphasis on early management of small SELs.

Key words: Gastrointestinal stromal tumor; Endoscopic ultrasonography-guided fine needle aspiration; Endoscopic ultrasonography; Diagnosis; Therapy

Core tip: Potentially malignant gastrointestinal stromal tumors are the most common subepithelial lesions (SELs) of the gastrointestinal tract. SELs include a broader range of differential diagnoses from benign to malignant lesions.

The possibility of having a malignant lesion may cause anxiety and discomfort in patients and gastroenterologists. Early and accurate diagnosis of SELs using endoscopic ultrasonography (EUS) and/or EUS-guided fine needle aspiration is vital to guide selection of early appropriate management.

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common malignant subepithelial lesions (SELs) of the gastrointestinal tract in the daily clinical setting[1,2]. GISTs are thought to originate from the interstitial cells of Cajal, which are the pacemaker cells of gastrointestinal movement[3]. GISTs are largely caused by oncogenic mutations in the tyrosine kinase receptor KIT[4] and/or platelet-derived growth factor receptor-α (PDGFR-α)[5]. Approximately 10% to 30% of GISTs have a malignant clinical course[1,6,7]. Additionally, it has been reported that not only large GISTs with a high mitotic index frequently exhibit a malignant clinical course, but also small GISTs with a low mitotic index rarely show a malignant course with metastasis. Thus, a GIST is considered to be a potentially malignant tumor. GISTs are not classified as either benign or malignant but are rather stratified by their clinical risk of malignancy: Very low, low, intermediate, or high[7]. Mietinenn reported that the metastatic risk of GISTs increases according to the tumor size irrespective of the mitotic count[6] (Figure 1). Surgical resection is the primary approach to management of localized GISTs[8]. Despite complete resection, postoperative recurrence occurs in at least half of all patients with GISTs[2,9]. Although tyrosine kinase inhibitors have been shown to provide sustained disease management in patients with metastasis[10-16], surgical R0 resection of small GISTs without metastasis is the only promising treatment for a permanent cure[8,17]. The best treatment strategy for GISTs is early diagnosis and early resection. However, GISTs are frequently detected as SELs during endoscopy[8,18-20]. The differential diagnoses of SELs are quite broad and can include extra-gastrointestinal tract compression, varices, an ectopic pancreas, and various tumors including GIST, SEL-like cancer, leiomyoma, schwannoma, and lipoma[8,20,21]. GISTs should be diagnosed by immunohistochemical analysis including assessment of KIT, CD34, and/or discovered on gastrointestinal stromal tumor 1 (DOG1)[8,22,23]. However, it is more difficult to obtain a conclusive histologic diagnosis of a GIST than gastrointestinal cancer by standard endoscopic forceps biopsy because a GIST is covered by normal mucosa. Although imaging tests including endoscopic ultrasonography (EUS) and computed tomography (CT) are useful for narrowing down the differential diagnoses of SELs, these techniques are unable to provide a conclusive diagnosis. At present, EUS-guided fine needle aspiration (EUS-FNA) is the most accurate, safe, and reliable preoperative immunohistological test to secure a definitive diagnosis of SELs[8,18,19,23]. Aggressive use of EUS and EUS-FNA for SELs is the key to facilitating early intervention of GISTs[21,23].

This paper provides an overview of the diagnosis and treatment of GISTs, with an emphasis on early diagnosis and management of GISTs using EUS-FNA.

EPIDEMIOLOGY

In epidemiological surveys of GISTs, the estimated mean age at diagnosis is in the sixth decade of life, and the frequency of occurrence is 6.8 to 14.5 cases per million individuals per year[24-26]. GISTs most commonly occur in the stomach (51%), followed by the small intestine (36%), colon (7%), rectum (5%), and esophagus (1%)[24].

HISTOLOGICAL FINDINGS

The main morphologic types of GISTs are the spindle-shaped cell type (70%), epithelial cell type (20%), and mixed type (10%)[27]. It is difficult to differentiate between leiomyomas and neurinomas, two other mesenchymal tumors, using only hematoxylin and eosin staining; differentiation using immunostaining is indispensable[8,22,23]. A GIST is diagnosed in the presence of KIT or CD34 positivity. If the tumor is negative for KIT, CD34, desmin, and S-100, additional tests including DOG1 staining or a mutation search of the KIT or PDGFRA gene are useful for diagnosis of GISTs[28] (Figure 2).

CLINICAL PRESENTATION AND INCIDENTAL GIST

The most common symptoms of GISTs are gastrointestinal bleeding, including acute melena and hematemesis with subsequent anemia; weakness; and abdominal pain, distension, and discomfort due to a tumor-induced mass effect[29]. Previous studies have shown that 15% to 30% of patients with GISTs are asymptomatic, and their GISTs are found incidentally during postmortem autopsy or surgery for treatment of other diseases[6,25,30]. Many pathological studies have highlighted the existence of subclinical microscopic or so-called mini (< 1 cm) GISTs[31-36]. Kawanowa *et al*[31] reported that microscopic GISTs were present in 35% of patients who underwent gastrectomy for treatment of gastric adenocarcinoma. Agaimy *et al*[32] reported that microscopic gastric GISTs were found in 22.5% of consecutive autopsies of patients aged ≥ 50 years. The reported incidence of mini-GISTs according to the affected organ is 3% to 10% in the stomach, 0.2% in the colon, and 0.01% in the

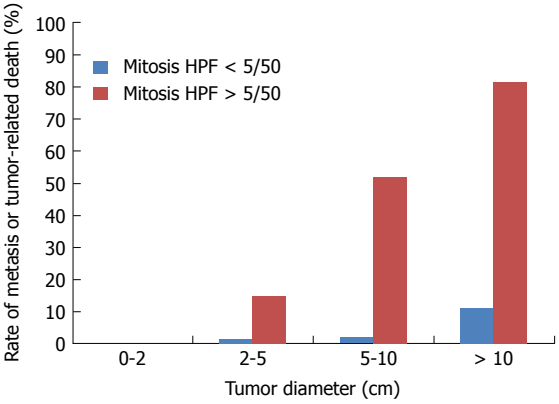

Figure 1 Rate of metastasis or tumor-related death according to tumor diameter and mitotic index. Created using reference[6].

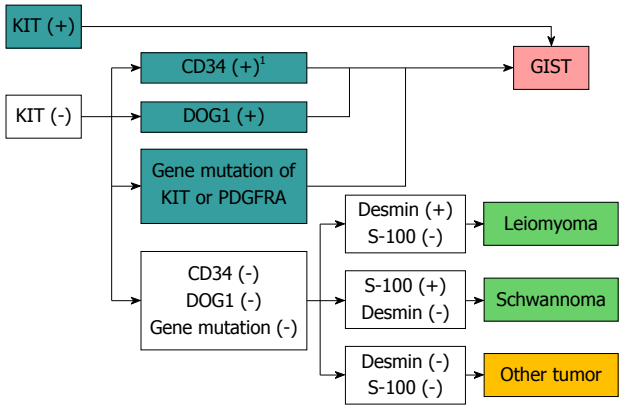

Figure 2 Flow chart of diagnosis of gastrointestinal mesenchymal tumors using immunohistochemical or genetic analysis. [1]Solitary fibrous tumors should be ruled out. Quoted and modified from reference[8]. GIST: Gastrointestinal stromal tumor.

rectum[31-36]. The detection of incidental SELs during gastrointestinal endoscopy has recently increased with the more widespread performance of endoscopic examinations. Gastric SELs are found in 0.36% of middle-aged adults during health examinations, and half of these tumors are considered to be neoplastic[37]. Most gastric SELs found during physical check-ups are small and asymptomatic. GISTs are considered to account for half of these incidentally found SELs in the stomach[31,34]. Based on these studies, GISTs are presumed to be much more common than previously recognized[18].

ENDOSCOPY

SELs are frequently found during ordinary optical endoscopy. The main endoscopic finding of GISTs is common to all SELs: A nonspecific smooth bulge covered with normal mucosa[11,21,38] (Figure 3). Therefore, endoscopic examination provides insufficient information for differential diagnosis of SELs. Irregular borders, ulceration, and/or growth during endoscopic follow-up are considered clinically malignant features on endoscopy[36]. GISTs are usually hard and the cushion sign is negative. When GISTs increase in size, ulceration may be seen on the top of the tumor[19].

EUS

EUS is a key test for differential diagnosis of SELs because it provides high-resolution tomographic imaging using high-frequency ultrasound. EUS provides the following information regarding SELs[39] (Figure 4): The gastrointestinal wall layer from which it originates (within the submucosal layer, in continuity with the muscularis propria, or outside the wall), the nature of the lesion (liquid, fat, solid tumor, or blood vessel), and the true size of the SEL from a cross-sectional image[39]. Thus, EUS is the safest and most useful modality for differential diagnosis and follow-up of SELs[21,40,41]. EUS allows for the conclusive diagnosis of many lesions using echo findings only, such as lipomas (highly echoic masses) (Figure 3A and B), cysts (anechoic masses) (Figure 3C and D), extraluminal compression by surrounding normal organs or lesions[42] (Figure 3E and F), and varices (Figure 3G and H)[21,38,43]. The typical EUS imaging feature of a GIST is a hypoechoic solid mass. EUS can accurately discriminate a SEL suspected to be a GIST (hypoechoic solid mass) from other SELs, including lipomas, cysts, varices, and extra-gastrointestinal compression. According to previous reports, possible high-risk EUS features for GISTs are a size of > 2 cm, irregular borders, heterogeneous echo patterns, anechoic spaces, echogenic foci, and growth during follow-up[44,45]. However, Kim *et al*[46] reported that tumor size and EUS features cannot be used to preoperatively predict the risk of malignancy of medium-sized (2-5 cm) gastric GISTs. At present, estimation of the risk of malignancy of GISTs of < 5 cm by EUS imaging alone seems to be difficult. The finding of a hypoechoic solid mass by EUS is also seen in malignant tumors such as malignant lymphoma, metastatic cancer, neuroendocrine tumor, and SEL-like cancer and in benign conditions such as leiomyoma, neurinoma, and an aberrant pancreas[21]. It is difficult to distinguish among these lesions using EUS findings only. The accuracy of differential diagnosis of SELs by EUS is extremely poor and ranges from 45.5% to 48.0%[47,48]. Because current EUS imaging characteristics alone provide insufficient accuracy in the diagnosis of GISTs, tissue sampling for immunohistochemical analysis using EUS-FNA or biopsy is required for a definite diagnosis before surgery or chemotherapy[18-21].

TUMOR TISSUE SAMPLING METHODS

Endoscopic forceps biopsy

Conventional endoscopic forceps biopsy is limited because these forceps usually cannot reach the tumor beyond the overlying normal mucosa and submucosa[49]. When ulceration is present, a biopsy within the ulcer is effective for a conclusive diagnosis[18,49,50]. Although special methods such as "jumbo" or "bite-on-bite" biopsy

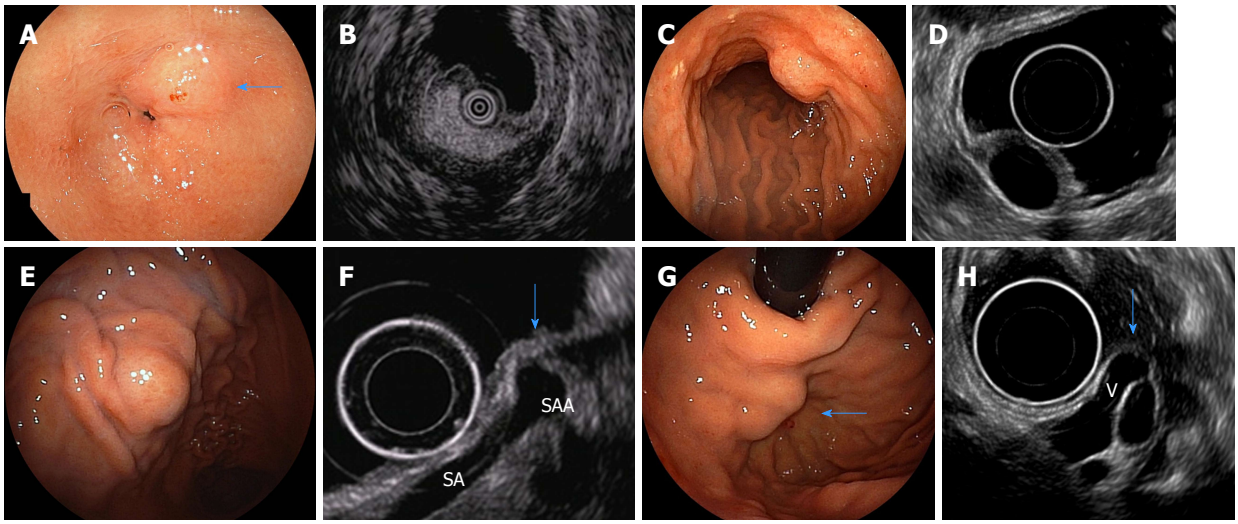

Figure 3 Endoscopic images of subepithelial lesions that can be diagnosed only with endoscopic ultrasound findings and their specific endoscopic ultrasonography images. A: Endoscopic image of a gastric lipoma (arrow); B: Endoscopic ultrasound (EUS) image of A (high-echo mass); C: Endoscopic image of a gastric cyst; D: EUS image of C (anechoic mass); E: Endoscopic image of extra-gastric compression due to splenic artery aneurysm; F: EUS image of E [normal gastric wall is compressed by a splenic artery aneurysm(SAA) (arrow). SA: splenic artery]; G: Endoscopic image of gastric varices (arrow); H: EUS image of G [varices are present in the submucosa from the outside of the wall (V) (arrow)]. Quoted and modified from reference[38] with permission.

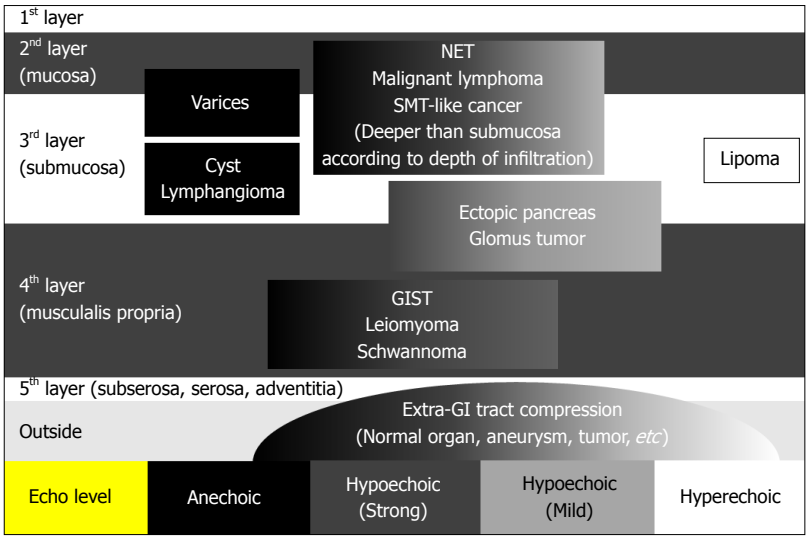

Figure 4 Differential diagnosis of subepithelial lesions by endoscopic ultrasound. Quoted and modified from reference[39] with permission. GIST: Gastrointestinal stromal tumor.

are available, the diagnostic yield of these approaches is poor, ranging from 17% to 59%[51-53]. Additionally, one study showed that significant bleeding occurred in 35.7% of patients after jumbo biopsy, and 34.9% of patients needed subsequent endoscopic hemostasis[53].

EUS-FNA

EUS-FNA is the most established tissue sampling method for SELs and can provide a conclusive immunohistochemical diagnosis safely and accurately (Figure 5) (Video 1). Typical EUS-FNA findings of GISTs are KIT- or CD34-positive spindle-shaped cells or epithelial cells. The diagnostic rate of SELs using EUS-FNA ranges from 62.0% to 93.4%[23,54-56]. The diagnostic rate according to tumor diameter is 71% for 1-cm to 2-cm tumors, 86% for 2-cm to 4-cm tumors, and 100% for > 4-cm tumors[23]. The diagnostic rate tends to be higher as the tumor diameter increases. Unfortunately, EUS-FNA for a subepithelial hypoechoic solid mass of < 1 cm is technically difficult using a standard EUS-FNA scope; thus, EUS-FNA is recommended for masses of > 1 cm[56,57]. However, forward-viewing and curved linear-array echoendoscopes [58] and drill needles[59] have recently been developed and are expected to improve the diagnostic rate of small SELs. The rate of adverse events associated with EUS-FNA using a 22-gauge needle is reportedly close to 0[54-56].

Evaluation of mitosis is important to determine the metastatic risk of GISTs. Unfortunately, the tissue sample volume obtained by EUS-FNA is usually small. Therefore,

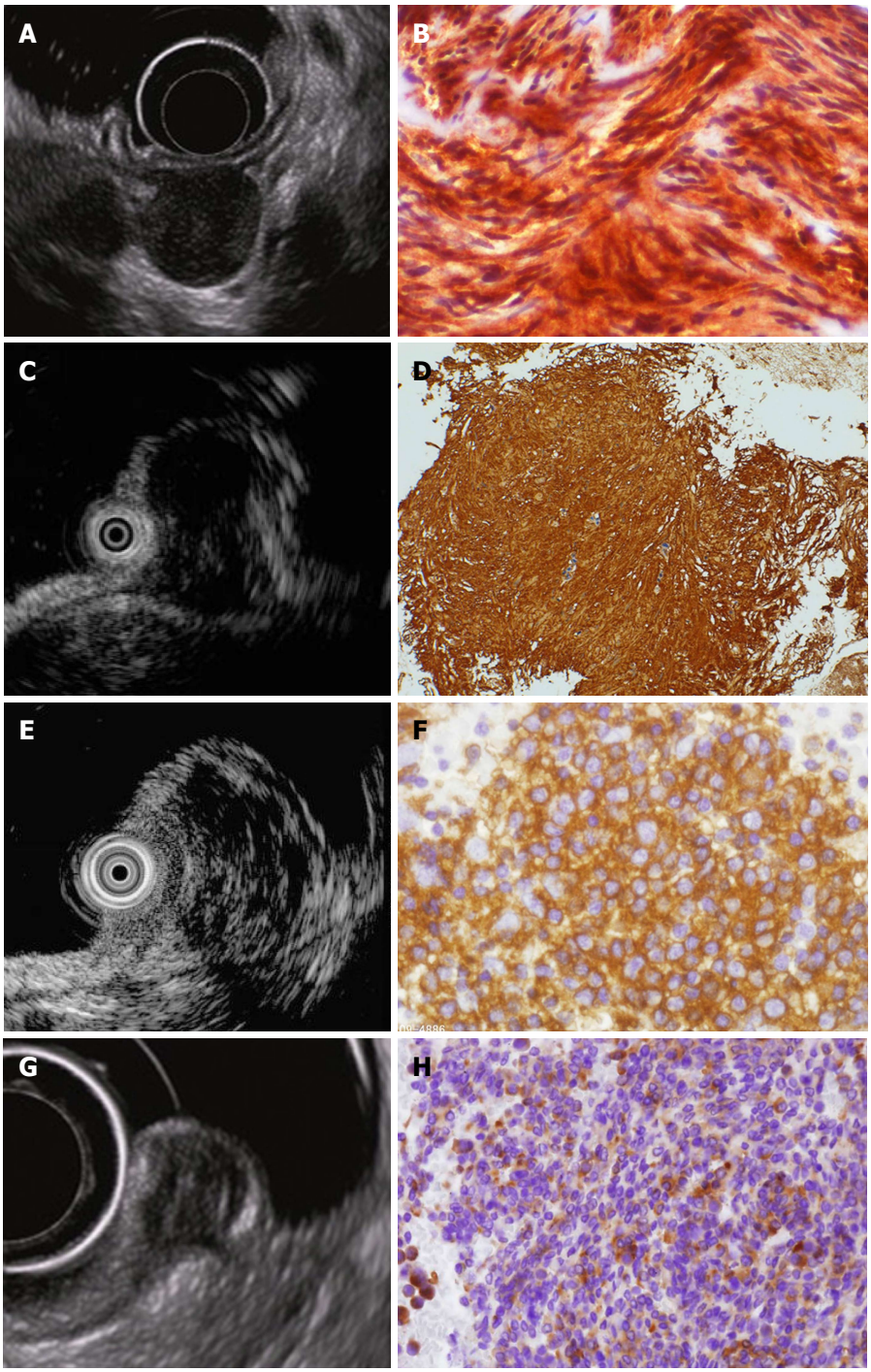

Figure 5 Endoscopic ultrasound images and corresponding endoscopic ultrasonography-guided fine needle aspiration specimens of hypoechoic solid tumors. A: Endoscopic ultrasound (EUS) image of a gastric gastrointestinal stromal tumor; B: EUS-guided fine needle aspiration (EUS-FNA) specimen tissue image of A (KIT-positive spindle-shaped tumor cells are observed); C: EUS image of gastric leiomyoma; D: EUS-FNA specimen tissue image of C [α-SMA-positive spindle-shaped tumor cells are observed; diagnosis of leiomyoma was made by immunohistochemical analysis, which revealed α-SMA (+), KIT (-), CD34 (-), and S-100 (-)]; E: EUS image of gastric malignant lymphoma; F: EUS-FNA specimen image of E (diagnosis of diffuse large B-cell lymphoma was made by CD20-positive lymphoid tumor cells); G: EUS image of rectal neuroendocrine tumor (NET); H: EUS-FNA specimen image of G (diagnosis of NET was made by typical findings of irregular nest of synaptophysin-positive epithelial-like cells). Quoted and modified from reference[38] with permission.

assessment of mitosis by EUS-FNA is difficult. Ando *et al*[60] reported that the MIB-1 labeling index is accurate (100%) for diagnosis of malignant GISTs because Ki-67-positive cells can be easily recognized in the small specimens obtained by EUS-FNA.

Endoscopic biopsy using endoscopic submucosal dissection or endoscopic snare resection techniques

Invasive endoscopic tissue acquisition to obtain a higher tissue volume was recently developed and clinically applied[56-59]. Various endoscopic tissue-obtaining me-

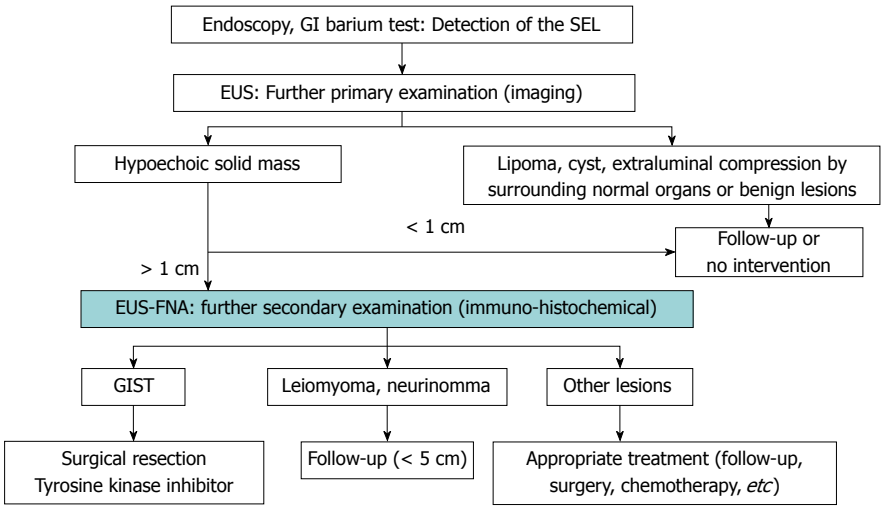

Figure 6 Proposed algorithm for management of subepithelial lesions. Quoted and modified from reference[21]. GIST: Gastrointestinal stromal tumor; GI: Gastrointestinal; SEL: Subepithelial lesion; EUS: Endoscopic ultrasound; EUS-FNA: Endoscopic ultrasound-guided fine needle aspiration.

thods using endoscopic submucosal dissection (ESD) techniques or endoscopic snare resection techniques significantly increase the diagnostic yield when compared with standard forceps biopsy; the reported diagnostic rates range from 85% to 94%[61-64]. An additional advantage of these methods is the ability to evaluate the risk classification of GISTs using the mitotic count per 50 high-power fields[65,66]. However, ESD and endoscopic snare resection are invasive procedures; therefore, endoscopists should pay special attention to intraoperative bleeding and perforation while performing these techniques because such complications may cause severe hypotension or tumor cell seeding. Lee *et al*[63] reported that minor hemorrhage occurred in 56% of patients who underwent endoscopic partial removal with the unroofing technique, but hemostasis was successfully achieved in all patients with argon plasma coagulation, and no perforations occurred. Furthermore, tissue sampling of SELs with an extraluminal growth pattern is difficult[64]. A potential disadvantage of these aggressive endoscopic tissue acquisition techniques using ESD or endoscopic snare resection is the development of perilesional fibrosis, which may render subsequent attempts at submucosal tunneling endoscopic resection[67,68] difficult or even impossible[69].

DIAGNOSTIC PROCESS

GISTs have no specific endoscopic or EUS findings, and diagnosis is difficult to achieve by histopathological examination using hematoxylin and eosin staining alone. Immunohistochemical analysis such as that involving KIT, CD34, or DOG1 measurement is essential for a definitive diagnosis[8,21]. However, because not all SELs are GISTs, it is necessary to identify those SELs that are suspicious for GISTs and perform immunohistochemical analysis of these SELs in clinical practice. Figure 6 shows our institutional algorithm for the detection and management of SELs as discussed herein[21]. First, all SELs are examined by EUS, and the SELs mentioned in the EUS section (Figure 3) that are conclusively diagnosed by EUS findings only are excluded. Second, EUS-FNA using immunohistochemical analysis is performed for the remaining hypoechoic solid masses to differentiate GISTs from other tumors. Narrowing down of SELs by EUS is important for efficient performance of EUS-FNA in the diagnosis of GISTs. In the Japanese clinical practice guidelines for GISTs, biopsy is recommended for exclusion of SEL-like cancer when an SEL is endoscopically diagnosed[70]. However, because SELs also include vascular diseases for which biopsy is contraindicated, such as varices (Figure 3G and H), it is desirable to perform EUS before biopsy.

TREATMENT

The principle treatment strategy for immunohistologically confirmed GISTs is as follows: (1) Surgical resection is the first choice for resectable GISTs without metastasis; and (2) Administration of tyrosine kinase inhibitors such as imatinib is the primary approach for unresectable, metastatic, or recurrent GISTs[70-73]. The objective of surgery is to achieve R0 resection to the greatest extent possible. Lymph node dissection is not recommended except when lymph node metastasis is clinically suspected; most metastasis of GIST is liver metastasis or peritoneal seeding, and lymph node metastasis is extremely rare[74,75]. Therefore, wedge or segmental resection with preservation of organs and organ functions and maintenance of a good quality of life after surgery is recommended[76]. Previous studies have shown that laparoscopic resection is feasible and safe for gastric GISTs and is less invasive than traditional open surgery, with similar oncological outcomes (Figure 7)[77-79]. Other minimally invasive techniques such as submucosal tunneling endoscopic resection[67,68], endoscopic fullthickness resection[80], and laparoscopic endoscopic cooperative surgery[81] have recently shown good clinical outcomes;

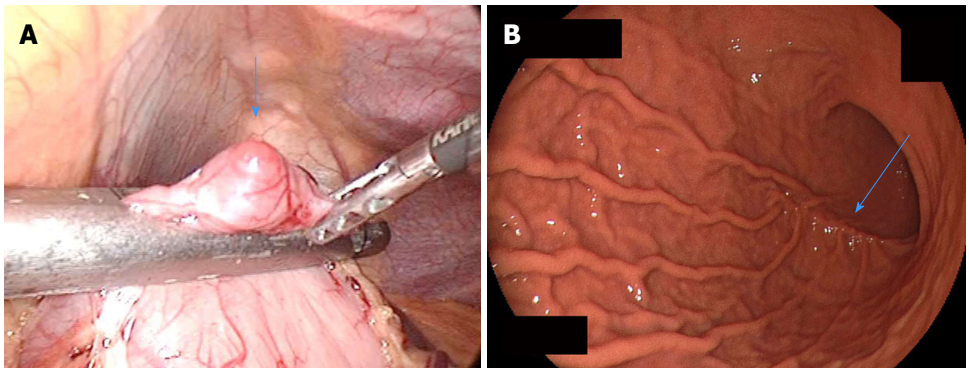

Figure 7 Laparoscopic resection of a small gastric gastrointestinal stromal tumor. A: Laparoscopic view of a small gastric gastrointestinal stromal tumor (arrow) during resection; B: Postoperative endoscopy shows mild postoperative deformity (arrow). Quoted and modified from reference[77] with permission.

Table 1 Modified Fletcher's risk classification

Risk category	Tumor size (cm)	Mitotic index (per 50 HPFs)	Primary tumor site
Very low risk	< 2.0	≤ 5	Any
Low risk	2.1-5.0	≤ 5	Any
Intermediate risk	2.1-5.0	> 5	Gastric
	< 5	6-10	Any
	5.1-10.0	≤ 5	Gastric
High risk	Any	Any	Tumor rupture
	> 10 cm	Any	Any
	Any	> 10	Any
	> 5.0	> 5	Any
	2.1-5.0	> 5	Non-gastric
	5.1-10	≤ 5	Non-gastric

Quoted and modified from reference[7] with permission. HPF: High-power fields.

however, there are still insufficient studies concerning their long-term safety, and they are still at clinical research levels.

In contrast, the introduction of imatinib (first-line tyrosine kinase inhibitor) has dramatically improved the management of GISTs, prolonging recurrence-free survival after surgery[82] and extending overall survival in metastatic or unresectable cases[14]. Three years of adjuvant therapy with imatinib for patients with high-risk GISTs who have undergone macroscopic complete tumor resection (R0 and R1) is recommended because it improves overall survival and recurrence-free survival[82]. Sunitinib (second-line tyrosine kinase inhibitor)[83] and regorafenib (third-line multikinase inhibitor)[84] can be used in advanced GISTs after treatment failure with imatinib. However, it is difficult to obtain a permanent cure by tyrosine kinase inhibitors. Therefore, early diagnosis (early GISTs without metastasis) with early surgical resection is the only promising way to obtain complete cure of this disease[20,21,56].

PROGNOSIS AND RISK CLASSIFICATION

Differentiation between a benign and malignant GIST is difficult even using postoperative histopathological findings. Thus, even if the tumor diameter is small and/or the mitotic rate is low, postoperative metastasis is possible. GISTs are currently regarded as potentially malignant tumors. Discrimination of a benign GIST from a malignant GIST by postoperative histological analysis (tumor diameter, mitotic index, and Ki67 expression level) is difficult; therefore, risk classifications to predict postoperative metastasis have been introduced[7,85,86]. Currently, the modified Fletcher classification (Joensuu classification) is widely used (Table 1)[7]. In addition, contour maps (Figure 8) can be created based on investigation of the prognosis of many cases worldwide. In these maps, the risk of recurrence at the 10th year after surgical treatment of a GIST is calculated using the maximum diameter of the tumor, the number of mitoses, the tumor site, and the presence or absence of tumor capsule rupture; continuous risk assessment is also possible[87]. Using such maps, physicians and patients can predict the probability of recurrence in the 10th postoperative year. This is useful for individual decision-making with respect to adjuvant therapy.

POSTOPERATIVE FOLLOW-UP BY CT

The goal of postoperative follow-up is early detection and management of recurrence. Because the targets of postoperative follow-up observation are local recurrence, liver metastasis, and peritoneal dissemination, abdominal contrast CT, which can be sufficiently evaluated from the diaphragm to the inguinal region, is recommended as a follow-up examination method according to the Japanese clinical practice guideline for GISTs[70]. Based on the above-mentioned risk classification, the following observation intervals are recommended[70]: GISTs with very low, low, and moderate risks are followed up by CT every 6 mo to 1 year, and high-risk and clinically malignant GISTs (those with metastasis, injury to the pseudocapsule, peritoneal dissemination, or infiltration of other organs) are followed up by CT every 4 to 6 mo. The natural history of GISTs is unknown. Previous studies have shown that the estimated 5-year recurrence-free survival rate after surgery is 59.9%; few recurrences occurred after the first 10 years of follow-up[7,88]. Follow-up observation after surgery is considered necessary for

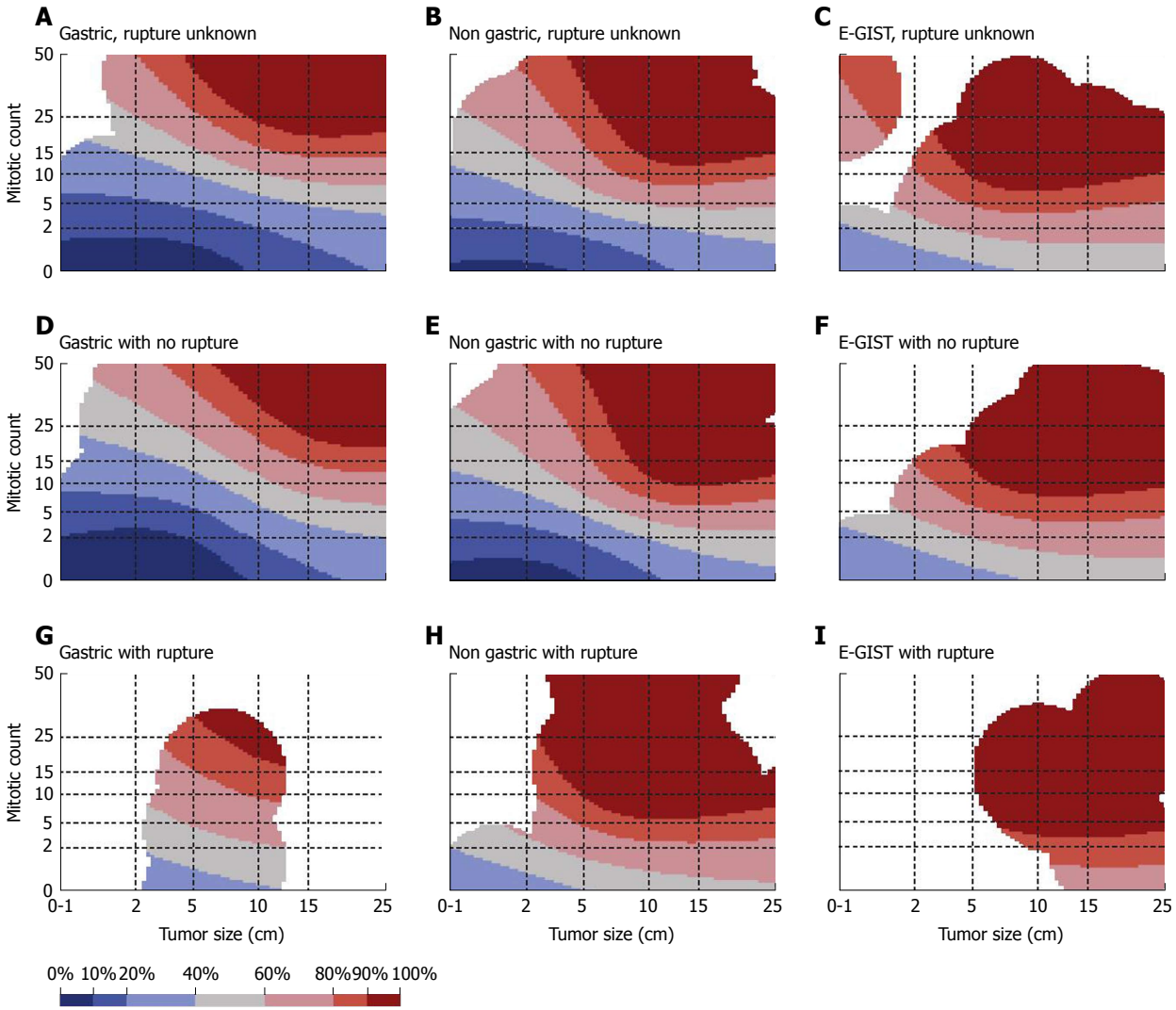

Figure 8 Contour maps for estimating the risk of gastrointestinal stromal tumor recurrence after surgery. Areas of colors according to the recurrence rate at the 10th year after surgical treatment of GIST: Blue-black: 0%-10%, Blue: 10%-20%, Light blue: 20%-40%, Gray: 40%-60%, Pink: 60%-80%, Red: 80%-90%, Dark red: 90%-100%. Reprinted from reference[87] with permission. E-GIST: Extra-gastrointestinal stromal tumor (arising outside the gastrointestinal tract).

more than 10 years.

MANAGEMENT OF SMALL SELS SUSPECTED TO BE GISTS

The detection rate of small GISTs has continuously increased with advancements in endoscopy[89,90]. However, the surveillance and management of GISTs smaller than 2 cm is controversial or lacks evidence-based approaches[41,56,89-92]. Most small GISTs are discovered incidentally and usually show a benign or indolent clinical course. Conversely, strict discrimination between benign and malignant GISTs is considered to be very difficult using both clinical and pathological examinations. Thus, the European Society for Medical Oncology[72], Japanese[70], and Chinese Society of Clinical Oncology[73] GIST guidelines recommend surgical resection when an SEL is immunohistologically diagnosed as a GIST, even when smaller than 2 cm. In contrast, the National Comprehensive Cancer Network[71] guidelines recommend that small GISTs of < 2 cm may be periodically followed up by EUS when they lack high-risk features including an irregular border, cystic spaces, ulceration, echogenic foci, and heterogeneity. However, in the examination of 378 histologically diagnosed GISTs of < 2 cm registered in the National Cancer Institute's Surveillance Epidemiology and End Results database, which is a cancer database in the United States, 11.4% of the patients had regional/distant metastatic disease and the 5-year GIST-specific mortality rate was 12.9%[93]. In a study of 43 surgically resected small GISTs of < 2 cm with immunohistochemical analysis, 23% of lesions were classified as having intermediate risk according to the Joensuu risk stratification[56]. Although GISTs of ⩽ 2 cm are reportedly metastatic at a low frequency (but not 0%)[89,90,93], early tissue diagnosis and early resection with postoperative follow-up are desired. Importantly, therefore, gastroenterologists should consider early interventions such as EUS for incidentally detected small SELs. Active performance of EUS is effective even

for small SELs of ⩽ 2 cm to ensure early detection of hypoechoic solid masses suspected to be GISTs[56]. If EUS imaging of a SEL with an endoscopically negative biopsy shows a hypoechoic solid mass of > 1 cm, subsequent EUS-FNA is needed to obtain a conclusive tissue diagnosis of a GIST[21,56]. Small SELs of < 1 cm are currently recommended to undergo periodic EUS follow-up (every 6 mo or 1 year)[56,91] because EUS-FNA for small SELs of < 1 cm is technically difficult. These aggressive approaches for early diagnosis and early treatment of small SELs, similar to the approaches for gastrointestinal tract cancer, seem to be the only promising way to improve patients' quality of life and prognosis.

CONCLUSION

GISTs are the most common malignant SELs of the digestive tract. According to previous studies, early histologic diagnosis and early surgical resection of small localized disease is currently the most reliable and curative treatment technique for GISTs. However, sufficient prospective studies including small GISTs have not been performed to improve the current clinical GIST management. Further studies are needed to focus on early tissue diagnosis and therapeutic approaches, especially for small SELs.

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REVIEW

Biomarkers of gastric cancer: Current topics and future perspective

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Abstract

Gastric cancer (GC) is one of the most prevalent malignant types in the world and an aggressive disease with a poor 5-year survival. This cancer is biologically and genetically heterogeneous with a poorly understood carcinogenesis at the molecular level. Although the incidence is declining, the outcome of patients with GC remains dismal. Thus, the detection at an early stage utilizing useful screening approaches, selection of an appropriate treatment plan, and effective monitoring is pivotal to reduce GC mortalities. Identification of biomarkers in a basis of clinical information and comprehensive genome analysis could improve diagnosis, prognosis, prediction of recurrence and treatment response. This review summarized the current status and approaches in GC biomarker, which could be potentially used for early diagnosis, accurate prediction of therapeutic approaches and discussed the future perspective based on the molecular classification and profiling.

Key words: Biomarkers; Ccancer diagnosis; Prognostic marker; Predictive marker; Gastric cancer

Core tip: Gastric cancer (GC) is one of the most common leading causes of cancer death in the world. Hence, any effort in early diagnosis, choice of appropriate therapeutic strategies and efficient monitoring can have a pivotal role in reducing the disease related mortalities. Our review purpose the current trends in GC biomarker which are classified as pathologic signaling, genetic or epigenetic changes within the tumor tissue as well as non-invasive biomarkers such as blood or gastric juice based markers. These biomarkers could facilitate more individualized

treatment approaches.

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INTRODUCTION

Gastric cancer (GC) is the fourth most common malignant disease and the second leading cause of cancer-related death worldwide[1]. Despite significant improvements in the survival of patients with GC over the past several decades, GC is often diagnosed at an advanced stage and prognoses is still unsatisfactory due to the high incidence of recurrence[2]. Since GC is mostly asymptomatic until it progresses to advanced stages, the early detection using effective screening approaches is important to impair GC mortalities[2]. Biomarkers are characteristics that are objectively measured and evaluated as an indicator of normal biologic process, pathogenic processes, or pharmacological response to a therapeutic intervention. Various biomarkers related to DNA, RNA, exosome, etc. have been found by recent advances in genome analysis. Development of these biomarkers in the field of cancer treatment is expected to greatly contribute to the progress of cancer, selection of appropriate therapeutic strategies and efficient follow-up programs.

GC is a heterogeneous disease in which each cancer patient exhibits a distinct genetic and molecular profile. Unfortunately, although a numerous studies has been conducted on molecular biomarkers, most of the identified biomarkers failed in the validation studies. Almost patients with advanced GC still cannot be treated with a targeted therapy and currently no diagnostic markers can be seen for secondary prevention. For being able to use GC associated biomarkers in clinical care of patients, comprehensive review to determine the direction for identifying the precise biomarker pinpoint that can be explored for the personalized therapy.

This review aims to classify developing topics for biomarkers in GC, while providing insights on potent candidates based on novel molecular classification that ultimately highlight molecular studies and clinical implementation. These findings should be useful for translating molecular classification and profiling of tumors into therapeutic targets and predictive biomarkers to achieve personalized treatment in the future.

LITERATURE SEARCH

PubMed was searched for English articles using the medical subject heading terms 'gastric cancer', and 'biomarker'. Relevant articles from clinical trials and experimental studies since 1989 were included as well as background articles relevant to the disease processes of interest. Articles which did not include biomarker analysis of GC were excluded from this review.

BIOMARKERS OF GC APLLIED IN CLINICAL PRACTICE

Gastric tumor markers have been used for the diagnosis, the determination of the clinical stage, the evaluation of treatment responses, and the screening for recurrence after successful therapy[3]. Although many biomarkers for GC including carbohydrate antigen (CA) 72-4, alpha-fetoprotein, carbohydrate antigen (CA)12-5, SLE, BCA-225, hCG and pepsinogen Ⅰ/Ⅱ have been reported, carcinoembryonic antigen (CEA) and CA19-9 are still the most frequently used biomarkers in clinical practice for GC.

CEA

CEA is the most widely and frequently used markers in clinical practice in the digestive tract cancer. CEA is known as an independent risk factor for predictive liver metastasis relapse[3]. Increased CEA levels are found in advanced stages of GC in a proportion of all GC patients; therefore, CEA levels are not an effective method of screening. CEA levels in peritoneal lavage fluid are said to accurately predict peritoneal recurrence after a curative resection of GC[4]. The addition of immunohistochemical CEA measurement to conventional cytology resulted in increased sensitivity. Measurement of CEA mRNA using RT-PCR is useful for detecting micrometastasis in the peritoneal cavity[5].

CA19-9

CA19-9 is a glycolipid antigen that has been identified in colorectal cancer, and it is a ligand for E-selectin, which is expressed on the surface of endothelial cells[3]. CA19-9 has previously been a commonly used marker in gastrointestinal cancer; however, it is present in a number of types of cancer, in particular pancreatic and GC. CA19-9-positive GCs demonstrated distinct clinicopathological characteristics such as antral location, differentiated histology, prominent lymphatic and venous invasion, higher proportion of lymph node metastasis, and advanced stage[6]. Previous studies reported that the sensitivity for recurrence of CA19-9 was 56%, with a specificity of 74%[7]. Moreover, the combination of CA19-9 and other tumor markers provided more useful information for prediction of recurrence[8]. The sensitivity was reported to increase to 87% when CA19-9 was combined with CEA.

Other conventional biomarkers

Tumor markers, such as CA72-4, alpha-fetoprotein and CA125 have been widely used for the diagnosis of GC.

Although CA72-4 often represents the superior sensitivity and accuracy compared with CEA, there are few

studies on predictive screening or early detection for CA72-4 under the circumstances. AFP positive GC has the characteristics of high stage and easy occurrence to liver metastasis[9]. AFP producing GC in AFP-positive group also shows the aggressive proliferation and enhanced neovascularization compared with in AFP-negative group[10]. CA12-5 level has been said to be significantly associated with the occurrence of peritoneal dissemination in GC[3]. In patients who have carried out curative surgery, CA125 positivity may serve as the predictor of peritoneal dissemination[11].

HER2

HER2 is the first molecular biomarker available for GC patients in clinical practice. HER2, (a proto-oncogene encoded by *ERBB2* on chromosome 17) is a cell membrane surface-bound receptor tyrosine kinase and is one of the four members of the human EGFR family, including EGFR/HER1, HER2/neu, HER3, and HER4[12]. Although the significance of prognostic and predictive value of HER2 is not established in GC, the importance of HER2 as biomarker is known to be emerged. The studied HER2 amplification in patients with GC ranges from 6% to 23%[13-15]. Histological evaluation revealed the HER2 overexpression/amplification rate was predominantly seen in the intestinal-type than in diffuse-type cancers (32% *vs* 6%)[15-18].

Trastuzumab, a HER2-targeted agent, inhibits HER2-mediated signaling and prevents cleavage of the extracellular domain of HER2[13]. Trastuzumab is the first molecular targeted agent approved as standard treatment in GC. Trastuzumab for Gastric Cancer (ToGA) study, an open-label phase Ⅲ, randomized controlled trial, showed that an addition of trastuzumab to capecitabine or 5-FU and cisplatin demonstrated a clinical benefit compared to chemotherapy alone in terms of tumor response and is now considered to be the standard of care for HER2-positive GC[13]. Moreover, assessment of HER2 expression in the primary gastric tumor is a reliable foundation for examining treatment with anti-HER2 agents in patients with secondary foci[17,18]. There are several other HER2-targeted agents such as pertuzumab, lapatinib and trastuzumab emtansine being investigated in randomized clinical trials in patients with HER2-positive GC[19-21]. However, no significant evidence was found yet. Several obstacles, such as determining the suitable dose of trastuzumab, identifying a predictive biomarker, exist for the advancement of HER2-targeted therapy in GC[22]. Some researches proved the usefulness of several factors for monitoring the efficacy of trastuzumab alone or combined chemotherapy, such as $p27^{Kip1}$ and HER2-extracellular domain[23,24]. Resistance to trastuzumab is also nowadays topic in HER2 positive GCs. One of the most important mechanisms underlying trastuzumab resistance is dysregulation of phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR pathway. It is well known that *PIK3CA* mutations and phosphate and tensin homolog (PTEN) inactivation may affect the effectiveness of HER2-targeted therapy[25]. Thus, combination therapy of trastuzumab with PI3K inhibitors may provide substantial benefit in patients with HER2-positive GC. *CCNE1* amplification, one of the most popular co-occurring copy number alteration, are negatively related with the response to HER2-directed therapy, suggesting its potential role as a biomarker of resistance in patients with *ERBB2* amplified GC[26].

CURRENT TOPICS OF BIOMARKERS IN GC

The measurement of conventional serum tumor biomarkers has been widely accepted in the diagnosis and prediction of recurrence in GC. However, due to their insufficient specificity and sensitivity, these molecular markers cannot be applicated for early GC detection. Therefore, novel and dependable tumor biomarkers are urgently needed.

Metastasis related genes

FGFR2: With the progression of molecular biological techniques over the last several years, investigators have increased pivotal insights into the oncogenesis mechanisms. Besides the well-known pathogenic factor, a variety of experimental procedures have ascertained numerous oncogenes and tumor suppressor genes, including cell cycle genes in the cell growth and signaling pathways[27-29]. A well-organized clarification of these complexity of molecular and genetic profiles will lead to the precise strategies of personalized treatment. The fibroblast growth factor receptors (FGFR) family consists of four members, FGFR1, FGFR2, FGFR3 and FGFR4. These receptors bind to their high-affinity ligands, the fibroblast growth factors (FGFs)[30]. Gene amplification of FGFR induces receptor overexpression, chromosomal translocation, and point mutation or enhanced kinase activity[31]. Various basic diverse cellular behaviors and cellular processes, such as mitogenesis, differentiation, cell proliferation, angiogenesis and invasion are intermediated though FGFRs signaling pathway[30]. The frequency of overexpression of FGFR2 was 31.1% and was more common than EGFR (23.5%), HER2 (11.8%), MET (24.9%)[32]. Thus, FGFRs should attract substantial attention as a useful therapeutic candidate for targeted anticancer agents. FGFR2 amplification was found to be associated with a higher pT stage, higher pN stage, lymph node metastasis and related to poor overall survival[33]. A recent study described that FGFR expression was positively associated with the recurrent rate more than 5 years in patients with stage Ⅱ/Ⅲ GC who undergo curative surgery and adjuvant chemotherapy with S-1[34]. This result indicates that FGFR2 could be the biomarker for predicting long-term failure of adjuvant treatment of S-1 in patients with curative resection for advanced GC.

E-cadherin: E-cadherin is a transmembrane molecule

that is involved in the cellular calcium-mediated adhesion. It is encoded by *CDH1* located on the chromosome 16 (q22.1). E-cadherin closely associates to epithelial gastric cells adhesion and differentiation, which is an important prevention against the malignant formation[35]. *CDH1* is one of the most pivotal tumor suppressor genes in GC, and its disruption of activity has been proven to be closely related with the invasive and metastatic capacity[36]. The E-cadherin gene can be inactivated by several mechanisms, including *CDH1* mutations, hypermethylation, loss of heterozygosity (LOH), *H pylori* infection, transcriptional repression binding to the CDH1-E box element, and tyrosine phospholyration (*e.g.*, EGFR, MET and FGFR)[36]. Hereditary diffuse GC (HDGC) is an autosomal dominate cancer syndrome representing approximately 2% of all GCs[37]. Germline mutations in the *CDH1* gene are identified in HGDC, leading to the histological characteristics similar to diffuse-type GC. The cumulative risk of GC by 80 years of age in male *CDH1* mutation carriers is 83% for advanced GC[38]. Unfortunately, metastatic HGDC patients show lower survival compared with other sporadic GC. A recent study described that E-cadherin/catenin-EGFR crosstalk is closely associated with HDGC. Enhanced sensitivity to EGFR and PI3K kinase inhibition was induced by loss of E-cadherin/catenin-EGFR interaction in HDGC families with *CDH1* germline mutations, suggesting that these inhibitors would be an attractive tool for the targeted therapy in HGC patients in the near future.

Patients with GC showing somatic *CDH1* epigenetic and structural alterations have a worse overall survival than patients with tumors negative for *CDHI* alterations. This finding indicates that the presence of *CDH1* epigenetic and structural alterations in a diagnostic/preoperative biopsy may serve as clinically useful biomarker[39]. A recent study examined the diagnostic role of promoter methylation status of CDH1 in blood samples of patients with GC[40]. Interestingly, the significant facilitation of promoter methylation of CDH1 was shown in blood samples, suggesting that promoter methylation of CDH1 may be a good candidate of biomarkers in patients with GC.

PI3K/Akt/mTOR: PI3K/Akt/mechanistic target of rapamycin (mTOR) signaling is a crucial mediator of many essential cellular processes; genomic instability, cell cycle, growth, metabolism, survival, metastasis and resistance to chemotherapy[41]. The *PIK3CA* gene encoding the PI3K catalytic isoform p110α is the second most frequently mutated oncogene, and *PTEN* encoding the major phosphatidylisositol phosphatase is one of the most mutated tumor suppressor genes Deregulation of the PI3K/Akt/mTOR pathway can occur secondary to oncogenic mutations of *PIK3CA*[42,43]. Genetic deregulations in the PI3K/Akt/mTOR pathway have been identified frequently in GC. PI3K/Akt/mTOR expression has been associated with the lymph node status and poor survival[44]. The *PI3KCA* has been reported to be identified in 4%-25% of patients with GC[25]. Although PIK3CA mutations have a critical role in resistance to antitumor drugs and acquisition of metastatic potential, its mutations did not likely to have an established efficient on prognosis. It has been reported that no ethnic differences in PIK3CA mutation frequencies exist, whereas the PIK3CA mutations are predominantly found in 80% of Epstein Barr virus (EBV) positive subgroups[45]. A recent study pointed that p-AKT negative tumors are more malignant than p-AKT positive but are rescued by the adjuvant chemotherapy for GC patients undergoing gastrectomy regardless of the PIK3CA mutation status[46].

MET: MET is a transmembrane tyrosine kinase receptor identified as the receptor for hepatocyte growth factor/scatter factor (HGF/SF). Activation of MET phosphorylates several signal transduction cascades, leading to cancer cell growth, angiogenesis, migration, and metastases[47]. MET amplification and/or overexpression of its secreted protein has been reported to be involved in the carcinogenesis, therapy efficacy, and outcome of GC[48,49]. The measurement and assessment of HGF activity have been crucial role in understanding the tumor microenvironment that prompt tumor metastasis and drug resistance[47]. The recent immunostaining experiment has presented that MET expression was significantly associated with lymphatic vessel invasion and poor overall survival (OS), implying that the expression of HGF/c-Met pathway might serve as a prospective predictive factor in patients with GC[50,51]. Interestingly, patients with a lower pretreatment HGF level showed a positive response to the treatment of trastuzumab. Serum level of HGF was increased in the patients who had no effect on tastuzumab compared with the pretreated level[52]. In the meanwhile, MET may be a useful predictive marker for chemotherapy, because MET signaling positively related with chemoresistance of GC therapy *via* increasing UGT1A1 level[53].

Vascular endothelial growth factor: Several signal transduction pathways are proved to be associated with tumor-associated angiogenesis, including vascular endothelial growth factor (VEGF)[54]. VEGF is a pivotal growth factor and signaling molecule to promote formation of new blood vessels. Binding to its receptor, VEGFR, activates a complex cascade of downstream signaling pathways, which leads to neovascularization, vasodilation[54]. Inhibition of VEGF and/or VEGFR activity impaired these pathways, which results in reduction of tumor proliferation, survival, and invasion. VEGF and its receptors are upregulated in 40% to 36% of cases, respectively in GC[55].

Antibodies against VEGF and VEGFR have been shown to yield anti-tumor effect, and to date, combined therapy with cytotoxic chemotherapy are adapted as standard first- or second-line treatment of GC. Ramucirumab is a recombinant humanized monoclonal antibody (mAb) specific for VEGF-R2 and impairs its activity by VEGF. Ramucirumab has provided anti-tumor effect in clinical practice as a single agent (REGARD trial) and

in combination with paclitaxel (RAINBOW trial)[56,57]. In a recent, VEGFR-2 as predictive/prognostic biomarkers has been shown in two independent phase-Ⅲ studies evaluating the role of ramucirumab in GC. In the RAISE study, second-line treatment with remucirumab combined with FOLFORI presented that the group of high expression of VEGF-D had a longer survival compared with that of low expression of VEGF-D in colorectal cancer[58]. Therefore, it could be plausible that VEGF-D would be a promising predictive biomarker for ramucirumab efficacy in GC.

TP53: TP53 gene is an extremely crucial tumor suppressor which plays a role as an important regulator of different cellular processes including growth arrest and apoptosis, DNA damage, and aberrant proliferative signals[59]. The mutational site of p53 in GC is wide and the reported incidence of p53 mutations ranges from 3.2% to 65%[60]. The incidence of p53 mutation was significantly lower in EBV-GC (n = 1) when compared with non-EBV-GCs (n = 10)[61]. TP53 mutation is identified most often in the intestinal type of GC[62]. TP53 codon 72 single nucleotide polymorphism (SNP) Arg72Pro was correlated with a shorter outcome in patients with GC. TP53 codon 72 SNP was shown to predict the response to chemotherapy, and related with the time to progression in advanced GC patients treated with paclitaxel and cisplatin chemotherapy[63].

Immune checkpoint

The programmed cell death 1 (PD-1) and 2 (PD-2) are key immune checkpoint receptors expressed on activated T and B lymphocytes, natural killer T cells, and monocytes[64]. Binding of its two ligand, programmed death-1 ligands (PD-Ls) 1 and 2 to PD-1 on activated T cells leads to downregulation of cytotoxic T-cell activity and also induce immune tolerance to tumor. The expression of PD-L1 in patients with GC is ranged in 15% to 70% of cases, and they are correlated with poor outcome[65]. Targeting the PD-1 pathway and immune checkpoint blockade has proved to be a novel tool for GC treatment. Pembrolizumab and nivolumab are an anti-PD1 monoclonal antibody, and they facilitated the capacity of the immune system. A phase Ⅱ study (KEYNOTE-059) demonstrated that application of pembrolizumab alone showed clinical efficacy in previously treated advanced GC[66]. Treatment of pembrolizumab showed a higher overall response rate (ORR) for patients with PD-L1 positive tumors, than in patients with PD-L1 negative tumors. Interestingly, patients with microsatellite-high (MSI-High) revealed higher response compared with in those with non-MSI-High tumors, suggesting the level of PD-L1 and MSI-High may serve as predictive biomarkers for efficacy of pembrolizumab. Besides, up-regulated expression of PD-L1/2 has been shown in the EBV-positive sub-type of tumors[67]. The results of these studies have facilitated the adaptation of immune checkpoint inhibitors generally in patients with GC.

Comprehensive gene analysis

Whole genome sequencing to targeted sequencing has played a crucial role in the identification of the genetic variations and anomalies, which leads to the development of GC. Initiation of GC is closely associated with epigenetic modifications and genome alterations. Recently, human genome project was completed and examination of gene expression profiling has been developed. Several critical genes as biomarker have been identified through genome-wide expression profile for GC[68-70]. For genome analysis, cDNA microarrays and serial analysis of gene expression (SAGE) have been mainly utilized[71]. Similar the microarray technique, SAGE is a powerful technique for worldwide analysis of gene expression in a quantitative manner without previous understanding of the gene sequences[72]. A recent cDNA microarray analysis assumed that seven genes exclusively expressed in patients with positive lymph node metastasis and five genes entirely expressed in lymph node negative patients. Genes (including *Egr-1*) which involved in cell growth, transcription and vascularization were up-regulated, whereas those in apoptosis and cell differentiation was downregulated[73]. Up-regulation of *CEACEM6, APOC1*, and *YF13H12* have been shown to be frequently up-regulated in GC[74]. In the meanwhile, significant correlation of *FUS*, *CDH17*, *COLIA1*, *COLIA2*, and *APOE* with invasion and metastasis was proved. A recent comprehensive analysis using SAGE and *Escherichia coli* ampicillin secretion trap (CAST) detected several gene alterations in GC. Among them, *CDH17*, *REG4*, *OLFM4*, *HOXA10*, *DSC2*, *TSPAN8* and *TM9SF3* were upregulated and *CLDN18* was downregulated in GC[75]. These molecules may not serve as just biomarkers but therapeutic target.

MSI

Microsatellites are repeating 1-6 nucleotide long units of DNA sequences that can be detected in both non-coding and protein coding sequences of DNA[76]. MSI is stated as somatic alterations in microsatellite sequences due to the insertion or deletion of those repeat units, which lead to genomic instability and increasing the susceptibility for the tumor development. Tumors showing 10%-29% of unstable microsatellite are considered MSI-low while tumors with ⩾ 30% of unstable microsatellite are classified as MSI-high. In GC, 15%-30% of tumor display MSI, mainly due to epigenetic silencing thorough promoter hypermethylation of the MLH1[77]. A recent comprehensive analysis from Korea have found that more than 63% of the MSI-high GC identified the mutations within mononucleotide tracts in *TGFBR2*, *CEP164*, *MIS18BP1*, *RNPC3*, *KIAA2018*, *CNOT1* and *CCDC150* genes[78]. The high status of *PIK3CA* mutations in MSI positive GCs has shown the efficiency of *PIK3CA* inhibitors in the personalized treatment of MSI positive patients[79]. Studies have shown a strong association of MSI loci

in GC with intestinal type, which undergoes more genomic instability in comparison to the diffuse type[80]. Interestingly, MSI-high tumors had a better prognosis than MSI-low tumors because MSI-high tumors showed an inferior capacity of invasion and lymph node metastases[81]. A recent randomized clinical trial (MAGIC trial) reported that the prognosis of patients with MSI-high gastroesophageal cancer showed significantly longer compared with those with MSS/MSI-low when treated with surgery alone. In contrast, when patients had a treatment with surgery and perioperative chemotherapy, the prognosis was shorter in patients with MSI-high, suggesting that perioperative chemotherapy may not provide a benefit in patients with MSI-high[82]. These showing results suggest that MSI frequency may be a beneficial predictive and prognostic biomarker in patients with GC.

Epigenetic alterations

Abnormality in the epigenetic system has been caused to pathogenic mechanism, which lead to the carcinogenesis of several cancers. Numerous of research has been performed linking aberrant DNA methylation profiles and histone modifications to progressive diseases, including cancers. The most widely studied epigenetic alteration in cancer is aberrant DNA methylation[83]. In humans, DNA methylation occurs at cytosine residues that precede guanines, called CpG dinucleotides (C-phosphodiester-G). Abnormal DNA methylation in the promoter region of genes, resulted in the inactivation of tumor suppressor and other cancer-relevant genes is the most well-defined epigenetic band in GC. Various risk factors such age, chronic inflammation, and infection with *H. Pylori* and EBV can cause the aberrant gene methylation in GC[84]. Defective DNA methylation in *CDH1*, *CHFR*, *DAPK*, *GSTP1*, *p15*, *p16*, *RARβ*, *RASSF1A*, *RUNX3* and *TFPI2* has been considered as a serum biomarker for the diagnosis of GC[84,85]. Among them, the mitotic checkpoint gene, *CHFR* methylation has been found significantly elevated in mucosa from patients with GC in comparison to mucosa from normal gastric tissue. *CHFR* promoter methylation is related with tumor differentiation and lymph node involving[86]. Aberrant DNA methylation in noncancerous gastric mucosa has been implicated in gastric carcinogenesis and could be a useful biomarker for the assessing risk of GC. A recent study revealed that defect of expression of *FAT4* gene was found in highly methylated GC cell lines and impairment of methylation reduced its expression. *H. Pylori* infection has also related to methylation frequency of *FAT4* gene[87]. The understandings gained from genetic studies on molecular pathogenesis of GC may serve as the inciting cause of various experiments to identify different genetic biomarkers for early diagnosis and prognosis of this type of malignancy.

Genetic polymorphism

Genetic polymorphisms have a pivotal role in human malignancies, and the close association between cancer and genetic polymorphism for tumor initiation has been demonstrated in a variety of experimental studies[88]. One of the important genetic polymorphisms in GC is Interleukin-1β (IL1-β). IL1-β and IL-1RN have a lot of functionally related polymorphism which is associated with the secretion of IL1-β. Existence of IL-1β and IL-1RN polymorphisms with *H. pylori* infection has been shown to provide the progression of chronic atrophic gastritis and GC in an Algerian population[89]. To date, advancements of research have proved the importance of SNP in showing individual specific variations of gene aberrations. A recent study presented that the *CD44* SNP genotype, rs187116 was a meaningful prognostic factor for early recurrent GC and CD44 isoform switching from CD44v to CD44s was closely related with this effect of *CD44* rs187116 on tumor recurrence[90]. Furthermore, this *CD44* SNP was an independent risk factor for disease free survival, suggesting that *CD44* rs187116 may serve as a useful biomarker in GC patient in a Japanese population. A study to detect copy number variations and mutations found that the top mutated genes revealing high frequency were *TP53*, *SYNE1*, *CSMD3*, *LRP1B*, *CDH1*, *PIK3CA*, *ARID1A* and *PKHD*[91]. Copy number variation has been identified for *KRAS*, *JAK2*, *CD274* and *PDCD1LG2* genes using single cell resequencing amplified by different three whole genome amplification[92].

NON-INVASIVE BIOMARKERS; LIQUID BIOPSIES

The main problem to the diagnosis, treatment and surveillance of solid cancers is the necessity for getting appropriate tumor volume frequently and derived tumors does not fully represent the character of total tumor. A 'liquid biopsy' is in principle a sample of any body fluid that may contain genetic material from a tumor, for instance blood, urine, saliva or cerebrospinal fluid[93]. Progress in the field of liquid biopsies may solve the challenges with tissue biopsies by using body fluids to investigate disease biomarkers. Among the liquid biopsy options, blood samples are the most widely studied[93]. Peripheral blood samples from patients with cancer contain circulating tumor cells (CTCs), cell-free DNA, micro RNA, cell - free RNA and cell - derived vesicles, such as exosomes.

CTCs

CTCs are disseminated tumor cells as single cells or, less commonly, as cell clusters, derived from either primary tumors or metastases which are circulating in the bloodstream[94]. The existence of CTCs has been said to be clinically related with progressive or metastatic disease. Hence, CTCs can be used to monitor advanced stage disease without other surveillance markers. In particular, CTCs can be detected at an early stage before the metastasis occurs[94,95]. CTCs can thus identify patients who would have more advantage from adjuvant

treatment after surgery of primary cancer[94].

In GC, a recent meta-analysis of CTCs in patients with GC suggested associations of CTCs with prognosis, tumor staging, histologic type, and lymphovascular invasion[96]. A subset of detected CTCs with stem cell-like characteristics or epithelial-mesenchymal transition (EMT) properties, which should have the capacity for surviving and migrating to secondary foci, may play a pivotal role in tumor stage evaluation and prediction of recurrence. CD44 has been identified as a marker of GC stem cells and increased resistance for chemotherapy- or radiation-induced cell death was found in the CD44-positive GC cells[97]. The expression of epithelial markers pan-CK, E-cadherin were decreased, and mesenchymal markers N-cadherin, vimentin were overexpressed in gastric CTCs, which may provide more useful information for prediction of recurrence[98]. To date, unfortunately, utilizing CTCs in GC is not still established in clinical practice. The novel innovative approaches for detecting EMT CTCs or circulating stem cells are needed to be developed and evaluation in clinical trials should be necessary. Interestingly, a recent phase Ⅱ study presented that preselected patients whose primary tumors were HER2- but who had *HER2*+ CTCs had response rates equivalent to those reported in the trastuzumab-plus-chemotherapy arm of the ToGA study[99].

Circulating cell-free DNA

Circulating cell-free DNA (cfDNA) is cell-free extracellular DNA originating from normal and cancerous cells identicalable in the blood (the plasma or the serum)[100]. The fraction of cell - free DNA that derived from primary tumors, metastases or from CTCs is called ctDNA. Currently, the utility of ctDNA in cancer treatment is the most extensively studied issue in cfDNA research. Compared to the restrictions of conventional biopsy which leads to significant trauma and produces small sample size, ctDNA detection displays several benefits including convenient sampling, minimal invasiveness and high repeatability. Moreover, ctDNA has been shown to be more sensitive than CTC[100]. The potential diagnostic and /or prognostic values of quantifying cf-DNA in GC patients compared to the healthy controls, have been evaluates in a variety of researches.

In GC, methylated promoter regions have been used extensively to identify ctDNA in both serum and plasma by methylation - specific PCR. A recent meta-analysis study showed that detection of ctDNA had an obvious advantage in GC diagnosis specificity, although no superiority of ctDNA over conventional protein biomarkers was detected in sensitivity, such as CEA, CA125 and CA72-4[101]. With regard to prognostic value, significantly poorer DFS and OS in patients were identified. A recent study described that serum APC promotor 1A and RASSF1A promoter hypermethylation in cfDNA was a frequent epigenetic event in patients with early operable GC[102]. Interestingly, cfDNA showing Epstein-Barr virus (EBV) DNA has been proved to be useful for identifying the EBV-associated GC subtype, monitoring tumor development, and managing response in patients with this subtype[103]. Tumor responses to lapatinib plus Capecitabine were closely related with changes in plasma-detected *ERBB2* copy number through serial cfDNA sequencing[26].

MicroRNA

Dysregulations in non-coding regulatory RNAs can contribute to cancer initiation and development[104]. A class of small cellular RNAs, termed microRNAs (miRNAs) are 18 to 24 nucleotides noncoding RNA fragments whose function is to bind the 3'UTR region of their target gene and regulate its expression by impairing the translation[105-107]. MicroRNAs are key players in regulating several biological processes of the cell proliferation, differentiation, migration, and invasion[105].

Expression profiling of microRNAs have shown the distinctive signatures of these small regulatory RNAs in different cancers including GC[108]. Numerous microRNAs have been identified and recognized to be implicated in GC[108,109]. MiRNAs can have a critical role in cancer cell progression through EMT into metastases. The miR-200 family promotes EMT, resulting in cancer cell migration by suppressing E-cadherin and ZEB2 expressions[110]. It is known that miRNAs can increase the expression of oncogenes or reduce the expression of tumor suppressor genes[111] Abundant differentially expressed miRNAs have been associated with different stages of GC. miRNAs such as miR-21, miR-23a, miR-27a, miR-106b-25, miR-130b, miR-199a, miR-215, miR-222-221 and miR-370 were associated with oncogenic activity of GC. Whereas, miR-29a, miR-101, miR-125a, miR-129, miR-148b, miR-181c, miR-212, miR-218, miR-335, miR-375, miR-449, miR-486 and miR-512 reveal tumor suppressive activity[108].

Recently, the research for miRNA as biomarker in human malignancies has facilitated because of the unique feature of miRNAs. Cell-free miRNAs (cfmiRNAs) can be derived from cancer cells to body fluids *via* secreting exosomes particles, which lead to protected from RNase-mediated degradation in circulation, and thus are easily extractable from a variety of body fluids including blood, saliva, urine, feces *etc*. Thus, cf-miRNA could be a useful noninvasive biomarker for diagnosis and relapse of GC. Recent experimental analyses have validated expression levels of cfmiRNAs in serum are consistent with gastric tumor tissue[112]. A study based on analysis of comprehensive expression profiling of miRNAs presented that high expression of two potential biomarkers (miR-331 and miR-21) was observed in peripheral blood than in the vein draining the primary tumor and suggested as a potential diagnostic biomarker[113]. A significantly poorer OS was shown in highly miR-21 expressed group compared with low miR-21 expressed group in meta-analysis study. Several other miRNAs showed significant prognostic value in this study. Among them, miR-20b, 125a, 137, 141, 146a, 196a, 206, 218, 486-5p and 506

showed convincing as prognostic biomarkers in patients with GC[114]. Overexpression of six serum-based miRNAs (miR10b-5p, miR132-3p, miR185-5p, miR195-5p, miR-20a3p, and miR296-5p) was shown in GC compared with normal controls by using qRT-PCR-based Exiqon panel[115]. In the arm not receiving chemotherapy, high expression of miR10b-5p or miR296-5p in tissues correlated with shorter OS. Consequently, cfmiRNAs would play an increasingly important role in the diagnosis, prognosis and/or prediction of recurrence of GC. In contrast, it has been said to be difficult to utilize a miRNA as a cancer biomarker in clinical practice[116]. However, to date, clinical study are ongoing to analyze the expression level of miRNA using next generation sequencing (NGS) in GC tissue and blood by chemotherapy response (NCT03253107). Similarly, a phase Ⅱ study to elucidate whether response to pralatrexate can be predicted by miR-215-5p is currently underway (NCT02050178). When these trials will complete with convincing evidence, miRNAs would be promising markers or new therapeutic targets for drug response prediction and control as well as modification of conventional adjuvant therapy.

Long noncoding RNAs

Long noncoding RNAs (lncRNAs) are sequences of nucleotides longer than 200, that can function as oncogenic or tumor-suppressor[117]. The lncRNAs act as transcriptional mediator, splicing regulator, posttranscriptional processor, enhancer, molecular sponge for miRNAs, chromatin remodeler. The lncRNAs are frequently expressed in a disease - or developmental - specific manner and thus submit potential as a biomarker[111]. Nowadays over 56000 human lncRNAs populating the human genome have been identified and about 135 lncRNAs have been recognized as dysregulated in GC, so they are closely related to tumorigenesis, metastases, and prognosis[117,118]. Impaired expression of ncRuPAR significantly associated with lymph node metastasis, distant metastasis, tumor size and TNM stage in patients with GC[119]. A downregulation in the expression of AI364715, GACAT1, and GACAT2 in GC tissues could also serve as a prognostic marker[120]. LncRNA PVT1 was markedly overexpressed in GC tissues compared with that in the normal control and could be an independent prognostic marker[121,122]. However, further studies about lncRNAs are needed in order to identify their possible clinical utilization.

Exosomes

Exosomes, small cell-derived vesicles, can protect RNAs and miRNAs, from being degraded[123-127]. When exosomes were exposed to RNase the contained RNAs were protected from degradation while cellular RNA was degraded by the same RNase[126]. Exosomes hold great potential for both diagnosis and prognosis of diseases and are exceptionally useful as cancer biomarkers[128]. miR-19b and miR-106a, identified in serum-circulating exosomes, remarkably overexpressed in individuals with GC compared to healthy controls. Furthermore, the validated miRNAs were correlated to lymphatic metastasis and expressed at higher levels in stages Ⅲ and Ⅳ compared to Ⅰ and Ⅱ stages in GC[129]. Similarly, Increased expressions of exosomal miR-21 and miR-1225-5p, isolated peritoneal lavage fluid, were exhibited in patients with T4-stage cancer compared with that in T1- to T3-stage patients[130]. These findings suggest that exosomes may serve as novel diagnostic and therapeutic biomarkers for GC.

STOMACH SPECIFIC BIOMARKER

Gastric washes/gastric juice

Because many mucosal cells can be found in stomach juice, the detection of molecular markers in stomach juice is a possible noninvasive approach to screening for GC. Gastric juice could serve as an excellent source of GC biomarkers, because these are directly released by the tumor without being excluded by the liver. Thus, gastric washes represent an alternative source for detecting aberrant DNA methylation. The analysis for the methylation levels of six genes (*ADAM23*, *GDNF*, *MINT25*, *MLF1*, *PRDM5*, *RORA*) demonstrated that a combination of the markers *MINT25*, *PRDM5* and *GDNF* achieved a high sensitivity (95%) and specificity (92%)[131]. As well, BARHL2 methylation in gastric wash DNA or gastric juice exosomal DNA significantly attenuated after endoscopic resection, suggesting that BARHL2 methylation could be useful for predicting tumor relapse[132]. The levels of PVT1 in gastric juice from gastric patients were significantly higher than those from normal subjects. PVT1 might serve as a promising biomarker for early detection and prognosis prediction of GC[121]. *Gastric juice miR-421, miR-21, miR-106a and miR-129 represent a potential biomarker for screening GC*[133].

Other specific biomarker

Micro-aerophilic, spiral-shaped Gram-negative bacterium *Helicobacter pylori (H.pylori) infection has been said to be associated with the initiation of* GC in clinico-epidemiological studies[134]. *H. pylori* Cytotoxin-associated gene A (CagA) is the first identified bacterial protein playing a positive role in the progression of GC[135]. The molecular mechanism underlying CagA-positive *H. pylori*-induced GC has been widely studied. CagA induces dysregulation of a variety of signaling pathways, including Wnt/β-catenin, PI3K/Akt, JNK, NF-κB, Hedgehog, JAK/ATAT has been identified, which results in the carcinogenesis of GC[136]. Interestingly, the development of EBV-positive GC has been shown to be prompted by *H. pylori* CagA activity, *via* SHP1 inhibition through exhibition of *PTPN6* hypermethylation[137]. In similar, *H. pylori* producing another bacterial toxin vacuolating toxin A (vacA) infection were meaningfully associated with increased risk of GC[138].

Gastrokine 1 (GKN1) is a tissue-specific 18 kDa protein that significantly expressed in gastric tissue and

Table 1 Current topics of molecular markers associated with diagnosis, prognosis, prediction of therapeutic response of gastric cancer

Marker	Alteration	Clinical purpose	Detection method	Ref.
Metastasis related genes				
Growth factors				
HER2, FGFR, PI3K/Akt/mTOR (*PIK3CA*), MET, VEGF (VEGFR-2, VEGF-D)	Overexpression	Diagnostic/prognostic/therapeutic	Tissue	[16-18,25,32,33,44-46,55,58]
Cell cycle regulation				
TP53	Mutation	Diagnostic	Tissue	[60,61,63]
Adhesion molecule				
E-cadherin (*CDH1*)	Mutation/epigenetic alteration	Diagnostic/prognostic	Tissue/blood	[39,40]
Immune checkpoint				
PD-L1	Mutation	Prognostic/therapeutic	Tissue	[66,67]
Comprehensive gene analysis				
CEACEM6, APOC1, YF13H12, CDH17, REG4, OLFM4, HOXA10, DSC2, TSPAN8, TM9SF3, FUS, COLIA1, COLIA2, APOE	Up-regulated	Diagnostic/prognostic/therapeutic	Tissue	[74,75]
ATP4B, S100A9, CYP20A1, ARPC3, DDX5 CLDN18	Down-regulated	Diagnostic/prognostic/therapeutic	Tissue	[74,75]
Microsatellite instability	High level	Prognostic/therapeutic	Tissue	[79,81,82]
Epigenetic alterations				
CDH1, CHFR, DAPK, GSTP1, p15, p16, RARβ, RASSF1A, RUNX3, TFPI2	Hypermethylation	Diagnostic	Tissue	[84-86]
Genetic polymorphism				
IL1-β, IL-1RN, CD44	SNP	Prognostic	Tissue	[89,90]
TP53, SYNE1, CSMD3, LRP1B, CDH1, PIK3CA, ARID1A, PKHD, KRAS, JAK2, CD274, PDCD1LG2	Copy number variations/mutations	Diagnostic/prognostic/therapeutic	Tissue	[91,92]
Circulating tumor cells				
CD44, N-cadherin, vimentin	Overexpression	Diagnostic/therapeutic	Blood	[96]
pan-CK, E-cadherin	Decreased expression	EMT process	Blood	[97]
HER2	Overexpression	Therapeutic	Blood	[99]
Circulating cell-free DNA				
APC promotor 1, RASSF1A	Hypermethylation	Diagnostic	Blood/plasma	[102]
ERBB2	Copy number variations	Therapeutic	Plasma	[26]
MicroRNA				
miR-21, miR-23a, miR-27a, miR-106b-25, miR-130b, miR-199a, miR-215, miR-222-221, miR-370	Up-regulated	Diagnostic/prognostic/therapeutic	Blood/plasma	[108,111]
miR-29a, miR-101, miR-125a, miR-129, miR-148b, miR-181c, miR-212, miR-218, miR-335, miR-375, miR-449, miR-486, miR-512	Up-regulated	Diagnostic/prognostic/therapeutic	Blood/plasma	[108,111]
Cell-free miRNAs				
miR-331 and miR-21	Up-regulated	Diagnostic/Prognostic	Blood	[113]
miR-20b, 125a, 137, 141, 146a, 196a, 206, 218, 486-5p	Up-regulated	Prognostic	Blood/plasma	[114]
miR10b-5p, miR132-3p, miR185-5p, miR195-5p, miR-20a3p, miR296-5p	Up-regulated	Prognostic	Plasma	[115]
Long noncoding RNAs				
ncRuPAR	Down-regulated	Diagnostic/prognostic	Tissue	[119]
AI364715, GACAT1, GACAT2	Down-regulated	Prognostic	Tissue	[120]
PVT1	Up-regulated	Prognostic	Tissue	[121]
Exosomes				
MiR-19b, miR-106a	Up-regulated	Diagnostic/prognostic	Plasma	[129]
miR-21, miR-1225-5p	Up-regulated	Diagnostic/therapeutic	PLF	[130]
Stomach specific biomarker				
ADAM23, GDNF, MINT25, MLF1, PRDM5, RORA	Hypermethylation	Diagnostic	Gastric wash	[131]
BARHL2	Hypermethylation	Diagnostic/therapeutic	Gastric wash/juice	[132]
PVT1	Up-regulated	Diagnostic/prognostic	Gastric juice	[121]
miR-421, miR-21, miR-106a, miR-129	Up-regulated	Diagnostic	Gastric juice	[133]
CagA	Up-regulated	Diagnostic	Tissue	[137]
VacA	Up-regulated	Diagnostic	Tissue	[138]
Gastrokine 1	Inactivation	Prognostic	Tissue	[139]

HER2: Human epidermal growth factor receptor 2; PLF: Peritoneal lavage fluid; FGFR: Fibroblast growth hormone receptor; PI3K: Phosphatidylinositol-3-kinase; mTOR: Mechanistic target of rapamycin; VEGF: Vascular endothelial growth factor; PD-1L: Programmed death-1 ligands; MSI: Microsatellite instability; CagA: Cytotoxin-associated gene A; VacA: Vacuolating toxin A.

is secreted into the stomach but is absent in GC. Its biological function is still unclear, but it is considered to serve as the replenishment of the surface lumen epithelial cell layer, in maintaining mucosal integrity[139]. GKN1 acts as a tumor suppressor and a modulator of apoptotic signals in GC. Due to a facilitated risk of gastric carcinogenesis in patients who have a lower expression of the protein, GKN1 could also be considered a biomarker for cancer specific to stomach. Epigenetic mechanisms leading to the inactivation of *GKN1* play a key role in the multi-step process of gastric carcinogenesis.

CONCLUSION

Through recent rapid advanced understanding of cancer biology, particularly in the field of molecular cell signaling and genetic and/or epigenetic dysregulation, the pattern of gastric carcinogenesis, and the pathways involved have become clearer. These findings may provide precious objectives for the early diagnosis of GC. Reliable prognostic and predictive markers as mentioned above may contribute to improved outcome of advanced GC. Current topics of GC biomarker based on a variety of molecular and genetic feature in this review article were summarized in Table 1. We also classified these biomarkers for early diagnosis, recurrence forecast and chemotherapy benefits assessment (Supplementary Table 1). The use of these new biomarkers such as evaluation of expression levels of various proteins and genes (*i.e.*, FGFR, CDH1, PI3K, MET, VEGFR, TP53, and PD-1) and various body fluid samples (CTC, cfDNA, miRNAs and exosomes) have opened new opportunity for diagnosis and monitoring patients with GC. And these markers will continue to be tested, developed from knowledge of novel approach, such as NGS[140]. This would facilitate more individualized treatment approaches.

FUTURE PERSPECTIVES

Although biological researchers have shown a lot of new findings in regard to biomarkers of GC to numerous publications, only conventional biomarkers (CEA, CA19-9, *etc*.) and HER2 are still in clinical use. It is urgently expected to develop biomarkers that are conventional, noninvasive, highly specific, capable of early detection and leading to treatment choice. Ideal biomarkers for early detection of cancer should be up-regulated in majority of patients with high level in cancerous tissues.

GC is a highly heterogeneous disease where even similar clinical and pathologic features lead to different outcomes, suggesting that previous staging systems may have extended to their limit of benefit for predicting patients' outcome and therapy. Thus, the novel classification of patients with GC to provide preventive and therapeutic approaches based on the genome analysis and clinical evidences are needed. In a recent, the genomic characterization of GC has led to the development of new classification by The Cancer Genome Atlas (TCGA) Research Network. The division of GC into four molecular types: (1) Tumors positive for EBV, (2) MSI-high tumors, (3) genomically stable tumors, and (4) tumors with chromosomal instability, allows identifying patients on the basis of the molecular features[67]. Future strategies aiming to translate molecular classification and profiling of tumors into therapeutic targets and predictive biomarkers in GC will be useful. The subtype of EBV-positive cancer is characterized by recurrent *PIK3CA* and *ARID1A* mutations, and high expression of PD-L1 and PD-L2, extreme DNA hypermethylation, which should be the good candidate as the diagnostic and therapeutic biomarkers. Inhibition of DNA methylation, and the suppression of immune checkpoints are promising target of this subtype. The MSI-high subtype reveals often mutation of multiple genes such as HER2 and HER3. Thus, besides the MSI, ErB family may be considerable as biomarker of this subtype. As mentioned previously, gastric MSI-high tumors represent a high frequency of PD-L1 expression. Hence, this subtype may be a pivotal candidate to anti-PD-1 therapy. The genomically stable subtype has a few somatic copy-number alterations but involves *ARID1A* and *RHOA* mutations or *CLDN18-ARHGAP* gene fusions. RhoA and its related genes could acts as the therapeutic biomarker of this subtype. The subtype with chromosomal instability is rich in TP53 mutations, and has relatively abundant amplifications of RTK genes. Therefore, this subtype can be the target therapy for RTKs, including EGFR and VEGF. The molecular classification of GC will further highlight the need for the identification and use of molecular biomarkers.

Genome wide investigation of cancer transcriptomes identified many new candidate genes. On the contrast, the candidate gene lists generated from comprehensive gene analysis vary considerably among individual studies. Therefore, it is essential to pinpoint the key players that can be explored for the development of biomarkers and leads for better cancer management. On the other hand, with regard to molecular targeting agents, their target molecules and related genes would be suitable for predicting treatment response more accurately.

The discovery of precise biomarker closely related with GC development can also be applied to treatment. We hope that this article will help design to identify the robust biomarkers in clinical care of patients and they can be relevant for the ultimate prevention and treatment of GC.

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MINIREVIEWS

Bowel preparation quality scales for colonoscopy

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Abstract

Colorectal cancer (CRC) is the third most common cancer and second leading cause of cancer-related death in the United States. Colonoscopy is widely preferred for CRC screening and is the most commonly used method in the United States. Adequate bowel preparation is essential for successful colonoscopy CRC screening. However, up to one-quarter of colonoscopies are associated with inadequate bowel preparation, which may result in reduced polyp and adenoma detection rates, unsuccessful screens, and an increased likelihood of repeat procedure. In addition, standardized criteria and assessment scales for bowel preparation quality are lacking. While several bowel preparation quality scales are referred to in the literature, these differ greatly in grading methodology and categorization criteria. Published reliability and validity data are available for five bowel preparation quality assessment scales, which vary in several key attributes. However, clinicians and researchers continue to use a variety of bowel preparation quality measures, including nonvalidated scales, leading to potential confusion and difficulty when comparing quality results among clinicians and across clinical trials. Optimal clinical criteria for bowel preparation quality remain controversial. The use of validated bowel preparation quality scales with stringent but simple scoring criteria would help clarify clinical trial data as well as the performance of colonoscopy in clinical practice related to quality measurements.

Key words: Colonoscopy; Bowel preparation; Aronchick scale; Ottawa Bowel Preparation Scale; Boston Bowel Preparation Scale

Core tip: Adequate bowel preparation is essential for proper visualization of the colonic mucosa to optimize lesion detection for a successful colonoscopy. Clinicians and researchers continue to use a variety of bowel preparation quality measures, including *de novo*, nonvalidated scales in clinical studies, leading to potential confusion, and creating difficulty when comparing bowel preparation quality results across clinical trials. Based on data evaluating different bowel preparation quality scales in the literature, and published criteria that define the most desirable measures to be used in such grading scales, the Boston Bowel Preparation Scale is currently recommended as standard.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer, with an estimated risk of occurring in 1 of 18 persons during their lifetime, and is the second most common cause of cancer-related adult deaths in the United States[1,2]. Approximately 135000 new CRC cases and 50000 CRC deaths were projected to occur in 2017 in the United States[1]. For average risk individuals, the United States Preventive Services Task Force and other public health and professional medical bodies recommend CRC screening using colonoscopy, computerized tomography colonography, sigmoidoscopy, double-contrast barium enema, high-sensitivity guaiac or immunochemical fecal occult blood testing, or stool DNA testing (which is combined with immunochemical blood testing) beginning at the age of 50 years[2-4]. Colonoscopy is a preferred and the most widely used method for CRC screening in the United States[4-6], based on data showing this procedure is correlated with decreased CRC incidence and deaths, most likely through the detection and removal of premalignant polyps[7-10].

Adequate bowel preparation is essential to ensure sufficient visualization of the colonic mucosa and to optimize lesion detection for successful colonoscopy utilized for CRC screening[4,11]. However, study data indicate that up to one-quarter of colonoscopies may be conducted with inadequate bowel preparation[12,13], which is correlated with lower detection of polyps and adenomas *vs* adequate preparation (typically good/excellent quality)[12,14-16]. A meta-analysis of 27 studies found that inadequate bowel preparation for colonoscopy CRC screening reduced detection of small adenomas by 47% (OR = 0.53, CI: 0.46-0.62; $P < 0.001$) *vs* adequate preparation (excellent/good/fair); this relationship was weaker but still significant for advanced adenomas (OR = 0.74, CI: 0.62-0.87; $P < 0.001$)[17]. Other studies have reported overall adenoma miss rates of 42%-48% for initial colonoscopies with inadequate or low-quality bowel preparation, based on findings at repeat colonoscopies[13,18]. Inadequate bowel preparation for colonoscopy may also result in prolonged procedures, more frequent repeat colonoscopies (at shorter than recommended intervals) and related increased costs, lower cecal intubation rates, and higher risk of electrocautery[6,11,19-21]. Studies in various international populations have found that inadequate cleansing is a factor in approximately 20%-70% of incomplete colonoscopies[22-25]. Professional gastroenterology societies recommend that clinical practices aim for minimum adequate bowel preparation rates of 85%-90%, and that bowel preparation quality be documented at the time of the screening[6,26].

Currently, no standard criteria or definition exists for qualitative terms such as "adequate", "inadequate", "excellent", "good", "fair", or "poor"; in some scales, adequate cleansing is defined as a composite of "good" and "excellent"[11,26]. Physician reporting on quality of bowel preparation, as well as overall colonoscopy quality, is highly inconsistent and often missing important elements, which may be attributable to lack of clear and consistent quality assessment standards[27]. Therefore, this review was conducted to summarize and discuss currently available bowel preparation quality scales and highlight the benefits of using a reliable and validated scale in both clinical practice and clinical trials of bowel preparation agents.

COMPONENTS OF A BOWEL PREPARATION QUALITY SCALE

Essential attributes of a dependable bowel preparation quality scale include reliability and validity[11]. Scale reliability involves the degree to which an instrument yields reproducible, or consistent, results for the same investigator (intrarater reliability) or among different investigators (interrater reliability), upon repeated testing[11,28]. Validity indicates how well the scale measures what it is designed to assess, which can be determined *via* several methods[29]. Validity may be assessed by comparison with results of other established and accepted scales used for the same purpose (*i.e.*, bowel preparation quality) in the same test population, referred to as construct validity. Scale validity may also be assessed by correlation with other specific criteria measuring relevant clinical outcomes, in this case, overall colonoscopy quality; this is referred to as criterion-related validity or predictive validity[29,30].

A commonly used criterion for overall quality of CRC screening colonoscopy is the adenoma detection rate (ADR), defined as the proportion of all CRC screening colonoscopies performed by a physician that reveal

at least one adenoma[6,31]. Studies have shown that colonoscopy ADR is strongly, inversely associated with reduced interval CRC rates (CRC diagnosed between the time of screening colonoscopy and the scheduled time of surveillance colonoscopy, which was up to 10 years)[32,33], and that increasing ADRs are correlated with reduced CRC incidence and mortality[34]. Some data also indicate that the polyp detection rate (PDR), the number of patients with at least one polyp removed during screening CRC, may also be a useful parameter of colonoscopy quality, particularly since it appears to correlate well with ADR[6]. However, use of the PDR raises additional questions related to the precise definition of "polyp". Other questions include whether the detection rates of sessile serrated polyps (SSPs), advanced adenomas, and multiple adenomas (as opposed to a "one and done" approach) should be used as key indicators of colonoscopy quality in addition to the ADR and PDR[6]. However, clinical data are insufficient for resolution of these issues, and no guidelines for correlation of bowel preparation quality with detection rates for SSPs, advanced adenomas, and multiple adenomas have yet been established[6]. Thus, ADR appears to be the best criterion currently available, as it is relatively easy to measure and has been shown to correlate with interval cancer rate.

The cecal intubation rate, an indicator of colonoscopy completion (reaching the cecum or anastomosis, if present), is another acknowledged quality measure[6,21,26]. Cecal intubation is essential for visualization of the proximal colon, including the caecum, where many colorectal neoplasms are located, in particular SSPs[6]. However, data on the independent association of cecal intubation rate with CRC risk have been mixed[32,35]. Longer withdrawal time is associated with higher ADR and higher SSP detection and is also considered a key criterion of colonoscopy quality secondary to ADR[6,36-38].

Another recommended criterion of colonoscopy quality is the level of adherence to recommended post-polypectomy and post-cancer surveillance intervals, which are based on study data[2,6,39,40]. The United States Multi-Society Task Force on Colorectal Cancer (USMSTFCC) has recommended that this criterion may serve as the overall indication of clinical adequacy of a bowel preparation[11]. Intra-procedure flushing and suctioning to remove fluid and semisolid debris is often performed during colonoscopy[11]. Therefore, the USMSTFCC recommends that bowel preparation quality should be assessed on withdrawal after washing and suctioning[11]. This criterion relates primarily to clinical adequacy, where washing and suctioning is taken into account, and is less relevant for the comparison of different bowel preparation agents, where pre-wash grading of bowel cleanse quality may better reflect preparation agent efficacy.

VALIDATED BOWEL PREPARATION SCALES

The most well established and commonly used validated bowel preparation quality scales in clinical trials include the Aronchick Scale[41,42], the Boston Bowel Preparation Scale (BBPS)[43-49], and the Ottawa Bowel Preparation Scale (OBPS)[50] (Table 1). Other instruments that have been validated, but are less commonly used, include the Harefield Cleansing Scale (HCS)[51] and the Chicago Bowel Preparation Scale (CBPS)[52] (Table 1). A summary of validation studies is found in Table 2.

Aronchick scale

The Aronchick Scale was the first bowel preparation quality scale to be evaluated for reliability[41,42]. This scale characterizes the percentage of the total colonic mucosal surface covered by fluid or stool, without scoring for separate colon segments, and is performed before washing or suctioning (Table 1). A validity study found that interobserver reliability kappa intraclass correlation coefficients (ICCs) were high for the cecum (0.76) and the total colon (0.77), but were reduced for the distal colon (0.31) and ascending colon segments[42]. The Aronchick Scale is one of the most commonly used validated bowel preparation quality scales in clinical trials and clinical practice.

Ottawa Bowel Preparation Scale

The OBPS measures mucosal cleanliness by colon segment, including the right colon, mid-colon, and rectosigmoid colon, on a scale of 0 (excellent) to 4 (inadequate) for each (Table 1 and Figure 1), and is also scored before washing or suctioning[50]. However, in contrast to the Aronchick scale, the OBPS measures fluid quantity separately, with scores ranging from 0 (small volume) to 2 (large volume) for the total colon. Additionally, the OBPS does not tie scoring to subjective estimates of the percentage of the mucosa that is visible, which the investigators suggested might improve interobserver reliability (Table 1)[50]. In a study of reliability and validity compared with the Aronchick scale, the Pearson correlation coefficients for interobserver ratings were superior for the OBPS *vs* the Aronchick (0.89 *vs* 0.62, respectively; $P < 0.001$)[50]. Similarly, the kappa ICCs also significantly favored the OBPS *vs* the Aronchick scale [0.94 (95%CI: 0.91-0.96) *vs* 0.77 (95%CI: 0.65-0.84), respectively; $P < 0.001$]. Interrater consistency was found to be stronger with the OBPS *vs* the Aronchick scale, and reliability and agreement of the OBPS for the three different colon segments measured were very high, and not significantly different between segments (0.92 kappa, right colon; 0.88 kappa, mid-colon; 0.89 kappa, rectosigmoid; 0.94 kappa, total colon).

A prospective study of the OBPS aimed to identify an optimal cut-off score for bowel preparation adequacy/inadequacy in 211 patients undergoing colonoscopy at a single center[53]. The receiver operating characteristic (ROC) analysis used in this study found that an OBPS score cutoff of $\geqslant 8$ identified inadequate bowel preparation with a sensitivity of 100% and a specificity of 91%. Another study in 150 consecutive patients undergoing colonoscopy reported strong concordance

Table 1 Validated bowel preparation scales

Scale name	Score	Rating/description	Other scale properties/characteristics
Aronchick Scale	1	Excellent: Small volume of liquid; > 95% of mucosa seen	Total score range: Minimum 1 (excellent) to maximum 5 (inadequate)
	2	Good: Clear liquid covering 5%-25% of mucosa, but > 90% of mucosa seen	Scoring performed before washing or suctioning
	3	Fair: Semisolid stool could not be suctioned or washed away, but > 90% of mucosa seen	No separate ratings for segments; global colon rating only
	4	Poor: Semisolid stool could not be suctioned or washed away and < 90% of mucosa seen	No threshold for adequate/inadequate provided
	5	Inadequate: Repeat preparation/screening needed	
Ottawa Bowel Preparation Scale (by colon segment)	0	Excellent: Mucosal detail clearly visible, almost no stool residue; if fluid present, it is clear, almost no stool residue	Total score (obtained by adding scores for each segment + total colon fluid score) range: Minimum 0 (excellent) to maximum 14 (inadequate)
	1	Good: Some turbid fluid or stool residue, but mucosal detail still visible without need for washing/suctioning	Scoring performed before washing or suctioning
	2	Fair: Some turbid fluid of stool residue obscuring mucosal detail; however, mucosal detail becomes visible with suctioning, washing not needed	Rates cleansing by colon segment: Right colon, mid-colon, and rectosigmoid colon (Figure 1)
	3	Poor: Stool present obscuring mucosal detail and contour; a reasonable view is obtained with suctioning and washing	No threshold for adequate/inadequate provided
	4	Inadequate: Solid stool obscuring mucosal detail and not cleared with washing and suctioning	
Ottawa Bowel Preparation Scale (total colon fluid)	0	Small amount of fluid	Total colon fluid score range: Minimum 0 (small amount of fluid) to maximum 2 (large amount of fluid)
	1	Moderate amount of fluid	Scoring performed before washing or suctioning
	2	Large amount of fluid	Single score for the total colon
			No threshold for adequate/inadequate provided
Boston Bowel Preparation Scale (by colon segment)	0	Unprepared colon segment with mucosa not seen because of solid stool that cannot be cleared	Total score (obtained by adding scores for each segment) range: Minimum 0 (very poor) to maximum 9 (excellent)
	1	Portion of mucosa of the colon segment seen, but other areas of segment not well seen because of staining, residual stool, and/or opaque liquid	Scoring performed after washing or suctioning
	2	Minor amount of residual staining, small fragments of stool, and/or opaque liquid, but mucosa of colon segment is well seen	Segments separately rated: Right colon (including cecum and ascending colon); transverse (includes hepatic and splenic flexures); and left colon (descending and sigmoid colon, and rectum)
	3	Entire mucosa of colon segment well seen, with no residual staining, small fragments of stool, or opaque liquid	Threshold optimally is total score of ⩾ 6 AND ⩾ 2 per segment
Harefield Cleansing Scale (by colon segment)	0	Irremovable, heavy, hard stools	Total score (obtained by adding scores for each segment) range: Minimum 0 (very bad) to maximum 20 (very good)
	1	Semisolid, only partially removable stools	Scoring performed after washing or suctioning
	2	Brown liquid/fully removable semi-solid stools	Segments separately rated: Rectum, sigmoid, left, transverse, right colon
	3	Clear liquid	Threshold for successful cleansing = Grade A: no segment scored < 3 or 4, or Grade B: ⩾ 1 segment scored 2 but no segment < 2;
	4	Empty and clean	Unsuccessful cleansing = Grade C: ⩾ 1 segment scored 1 but no segment < 1, or Grade D: ⩾ 1 segment scored 0
Chicago Bowel Preparation Scale (by colon segment)	0	Unprepared colon segment with stool that cannot be cleared (> 15% of mucosa not seen)	Total score (obtained by adding scores for each segment) range: Minimum 0 (unprepared) to maximum 36 (excellent)
	5	Portion of mucosa in segment seen after cleaning, but up to 15% of the mucosa not seen because of retained material	Scoring performed before (fluid) and after (mucosal cleaning) washing or suctioning
	10	Minor residual material after cleaning, but mucosa of segment generally well seen	Segments separately rated: Right (cecum to mid-hepatic flexure), transverse (mid-hepatic flexure to mid-splenic flexure), and left colon (mid-splenic flexure to distal rectum)
	11	Entire mucosa of segment well seen after washing	No threshold for adequate/inadequate provided
	12	Entire mucosa of segment well seen before washing or suctioning	
Chicago Bowel Preparation Scale (total colon)	0	Little fluid (⩽ 50 cc)	Total score range: Minimum 0 (little fluid) to maximum 3 (large amount of fluid)
	1	Minimal amount of fluid (51-150 cc)	Scoring performed before washing or suctioning
	2	Moderate amount of fluid (151-300 cc)	No threshold for adequate/inadequate provided
	3	Large amount of fluid (> 300 cc)	Not incorporated into total score for segments

Table 2 Reliability and validation data for bowel preparation scales

Scale	Study	Colons (*n*)	Raters (*n*)	Reliability	Validity
Aronchick	Aronchick[41], 2004	80	5	ICC values for: Total colon: 0.77 Cecum: 0.76 Distal colon: 0.31	NR
OBPS	Rostom *et al*[50], 2004	97	2	ICC values for: Right colon: 0.92 Mid colon: 0.88 Rectosigmoid colon: 0.89	Comparisons with Aronchick scale PCC: 0.89 OBPS *vs* 0.62 Aronchick ICC: 0.94 OBPS *vs* 0.77 Aronchick
	Chan *et al*[53], 2011	211	NR	NR	Cutoff scores for adequacy/inadequacy Optimal cutoff for inadequate ⩾ 8: Sensitivity, 100%, specificity, 91%
	Martinato *et al*[54], 2013	150	NR	Ratings of physicians *vs* nurses: PCC: $r = 0.60$	Correlations with VAS PCC (physicians *vs* nurses): $r = 0.60$
	Lee *et al*[58], 2016	655	NA	NR	Comparison with BBPS for PDR and ADR PCC: $r = -0.62$ ($P < 0.001$); AUC of ROC analysis similar for PDR, ADR, right-sided adenomas, and SSAs
BBPS	Lai *et al*[47], 2009	633	22	ICC values: 0.74/0.77 wtd κ	PDR by score 40% for scores ⩾ 5 *vs* 24% for scores < 5 ($P < 0.02$) Need for repeat CSP due to inadequate bowel prep 2% for scores ⩾ 5 *vs* 73% for scores < 5 ($P < 0.001$) Correlation with colonoscope insertion time PCC: $r = -0.16$ ($P < 0.003$) Correlation with colonoscope withdrawal time PCC: $r = -0.23$ ($P < 0.001$)
	Calderwood *et al*[43], 2010	119	12	ICC values for: Total colon: 0.91 Right colon: 0.88 Transverse colon: 0.83 Left colon: 0.79	Correlations with ability to exclude polyps > 5 mm 100%, 88%, 82%, 33%, and 0% of physicians deemed bowel preparation adequate to exclude polyps > 5 mm at scores of ⩾ 8, 7, 6, 5, and ⩽ 4 respectively Correlations with surveillance recommendations after normal CSP Score < 5: 100% recommended ⩽ 1 yr Scores 5-6: mean recommended interval 4.3 (± 3.9) yr Scores ⩾ 7: 100% recommended 10 yr
	Calderwood *et al*[44], 2014	2516	74	NR	Physician-recommended CSP interval after negative CSP Scores ⩾ 6 (⩾ 2 each segment): 90% recommended 10 yr Scores 0-2: 96% recommended ⩽ 1 yr
	Schindler *et al*[49], 2016	3	40[1]	ICC values, all raters (all segment and total scores): 0.93	NR
	Gao *et al*[45], 2013	1012	13	ICC values: 0.987/0.671 wtd κ	PDR Scores ⩾ 5 superior *vs* < 5 (35% *vs* 18%; $P < 0.05$)
	Kim *et al*[46], 2014	482	6	ICC values: Total colon: 0.90/0.63 wtd κ Right colon: 0.93/0.91 wtd κ Transverse colon: 0.88/0.86 wtd κ Left colon: 0.50/0.38 wtd κ	PDR Scores ⩾ 8 superior *vs* scores < 8 (44.9% *vs* 33.0%; $P = 0.04$) Colonoscope withdrawal time PCC: $r = -0.167$ ($P < 0.001$) Colonoscope insertion time PCC: $r = 0.018$ ($P = 0.695$)
	Clark *et al*[57], 2016	438	4	ICC values by BBPS scores: 0 and 3: 1.0 2: 0.81 1: 0.80	ADR (> 5 mm) miss rates by BBPS score: 3: 5.6% 2: 5.2% 1: 15.9% Score of 2 noninferior to 3 for missed adenoma > 5 mm
HCS	Halphen *et al*[51], 2013	337	4	ICC value: 0.457 Test-retest κ values: Range, 0.33 to 0.85 Intrarater[2]: 0.28 to 0.64 Internal consistency[3]: 0.81, 0.86	Best score cutoff for satisfactory bowel preparation ⩾ 2 for each segment: Sensitivity, 99% and specificity, 83% Correlation with Aronchick scale PCC: $r = 0.833$ AUC of ROC analysis (*vs* Aronchick scale scores) 0.945 for total colon
CBPS	Gerard *et al*[52], 2013	150	4[4]	ICC values for: Range, 0.624 to 0.702 for all segments	Correlations of scores with adequate cleansing Adequate: Scores of 25-36 (⩾ 95% of mucosa visualized) Inadequate: Scores of 0-24 (< 95% of mucosa visualized)

[1]Raters included endoscopy nurses ($n = 17$), gastroenterology faculty ($n = 14$), and gastroenterology fellows ($n = 9$); [2]Generalized κ for global agreement; [3]Cronbach's alpha; [4]Raters included three gastroenterologists and one physician's assistant. ADR: Adenoma detection rate; AUC: Area under the curve; BBPS: Boston Bowel Preparation Scale; CBPS: Chicago Bowel Preparation Scale; CSP: Colonoscopy; HCS: Harefield Cleansing Scale; ICC: Interobserver reliability kappa intraclass correlation coefficient; NA: Not applicable; NR: Not reported; OBPS: Ottawa Bowel Preparation Scale; PCC: Pearson correlation coefficient; PDR: Polyp detection rate; ROC: Receiver operating characteristic; SSA: Sessile serrated adenoma; VAS: Visual analogue scale; wtd: Weighted.

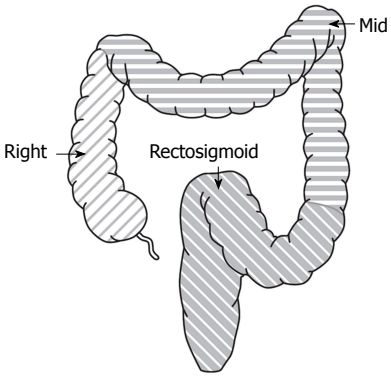

Figure 1 Bowel preparation quality scale segments. Depiction of bowel segments from validation study of Ottawa Bowel Preparation Scale[50]. Before washing or suctioning, each segment is scored on a scale of 0-4 for cleansing, and the total colon is scored for fluid quantity on a scale of 0-2. The total score ranges from 0 (excellent) to 14 (inadequate).

between the OBPS and a visual analogue scale measuring bowel cleansing among both nurses (r = 0.8268) and physicians (r = 0.8095), P < 0.0001 for both[54]. The concordance in scoring between nurses and physicians was r = 0.6010; P < 0.0001.

Boston Bowel Preparation Scale

The BBPS has been validated in multiple clinical studies[11,47,55]. Developed in 2009, this scale was designed to address specific issues affecting bowel preparation quality and scoring: (1) The scale stipulates that scoring is to be conducted upon withdrawal and after all flushing and suctioning of fluid have been completed; (2) scoring is applied by colon segments, as in the OBPS, based on potential for variance in bowel preparation between segments; and (3) subjective, qualitative terms, such as excellent, good, fair, or poor, are replaced by numbered scores that are correlated to more clearly described colonic conditions, including features such as staining, liquid, and stool fragments (Table 1)[47]. Each segment of the colon is scored from 0 to 3, with higher scores indicating superior cleansing, and summed for a total score that can range from 0 to 9 (Table 1).

The initial validation study for the BBPS involved 633 CRC screening colonoscopies in a single center, and was applied by endoscopists who had undergone training on how to use the scale before participating in the study[47]. The median BBPS total score was 6. The ICC for interobserver agreement of total BBPS scores was 0.74 (95% predictive interval: 0.67-0.80), and the weighted kappa value for intraobserver agreement was 0.77 (95%CI: 0.66-0.87)[47]. Validity assessment was based on the correlations of BBPS scores with relevant clinical outcomes and more traditional scale categories, including "excellent", "good", "fair", "poor", or "unsatisfactory". Of the 633 patients who received a CRC screening colonoscopy, 243 (38%) had at least one polyp detected, and the PDR was significantly higher for patients with BBPS scores $\geqslant$ 5 *vs* those for patients with BBPS score < 5 (40% *vs* 24%, respectively; P < 0.02). The frequency of repeat colonoscopy attributable to inadequate bowel preparation was significantly higher in patients with scores < 5 *vs* those with scores $\geqslant$ 5 (73% *vs* 2% of cases, respectively; P < 0.001). Total BBPS scores were inversely associated with colonoscopic insertion (r = -0.16; P < 0.003) and withdrawal times (r = -0.23; P < 0.001). In addition, a significant trend in mean BBPS score correlating with excellent, good, fair, poor, or unsatisfactory, as separately scored by the raters, was observed (P < 0.001 for trend).

A follow-up study investigated interobserver reliability and clinical outcome correlations of BBPS scores for individual segments, and relationship of scores to polyp detection in 119 screening colonoscopies rated by nine full-time faculty and three fellows at a single center[43]. All (100%) raters judged the bowel preparation adequate to exclude polyps > 5 mm with a $\geqslant$ 8 BBPS score, *vs* 88% of physicians when the score was 7, 82% when the score was 6, 33% when the score was 5, and 0% with a score of $\leqslant$ 4. Thus, a score of $\geqslant$ 6 was a particularly important threshold, since approximately 80% of physicians found the bowel preparation adequate at that score *vs* only one-third or less at BBPS scores of $\leqslant$ 5. In patients who had undergone a normal screening colonoscopy, a score of < 5 prompted all physicians to recommend repeat colonoscopy within one year, while a score of $\geqslant$ 7 was correlated with a recommendation for the next colonoscopy to occur in 10 years (among all physicians). BBPS segment scores were positively correlated with improved PDRs for the left and right colon, but no association was found for the transverse colon.

A further validation study was aimed at identifying a cut-off score for adequacy/inadequacy of bowel preparation[44]. This retrospective study of 2516 normal CRC screening colonoscopies performed by 74 endoscopists found that follow-up was recommended in 10 years for 90% of cases with a total BBPS score $\geqslant$ 6 in which all three segments had scores $\geqslant$ 2 (n = 2295), while 96% of examinations with total BBPS scores of 0-2 (n = 26) recommended follow-up within one year (Figure 2). Screenings with total scores of 3-5 (n = 167) had variable recommendations. Based on these findings, the investigators suggested that a total BBPS score of $\geqslant$ 6 and/or all segment scores $\geqslant$ 2 may serve as a standard definition of "adequate for 10-year follow-up"[44]. However, a prospective, observational study in a large, national endoscopic consortium found that inadequate single BBPS segment scores at the initial, average-risk screening colonoscopy were correlated with significantly greater risk of polyps at a second colonoscopy, suggesting that both a total score of $\geqslant$ 6 *and* all segment scores $\geqslant$ 2 should be required as an adequacy standard for 10-year follow-up[56]. This assessment was affirmed by a study in 438 colonoscopies in men, which found that BBPS segment scores of 2 or 3 (with 2 being noninferior to 3) was indicative of adequate bowel preparation for detection of adenomas > 5 mm, and for repeat colonoscopy at standard, guideline-recommended intervals (both parameters are USMSTFCC-recommended criteria for bowel preparation adequacy)[11,57].

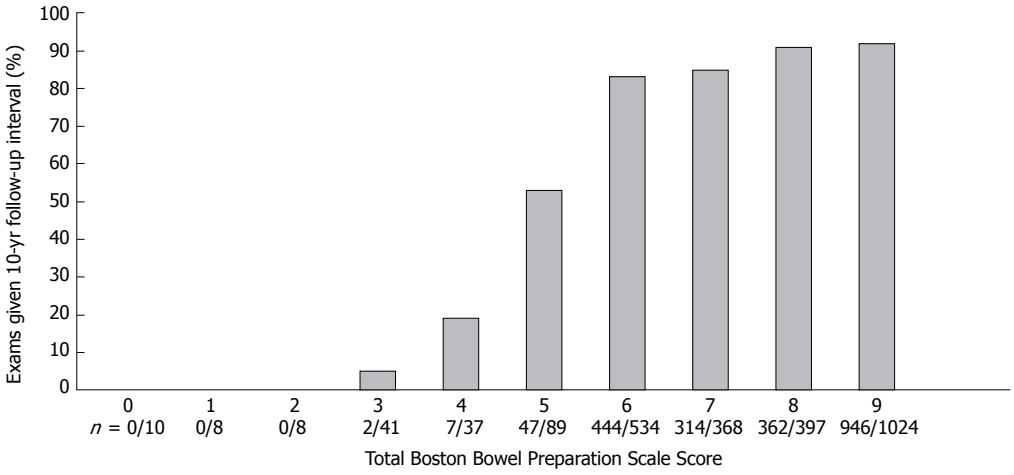

Figure 2 Percentage of screening colonoscopy examinations in which 10-year follow-up was recommended after a negative colonoscopy, stratified by total Boston Bowel Preparation Scale Score[44].

Harefield Cleansing Scale

The HCS, developed in the 1990s, is scored by colon segment, as are the OBPS and BBPS[51]. Like the BBPS, the HCS is also scored after washing and suctioning are completed, and replaces qualitative terms (*e.g.*, "excellent" or "good") with direct descriptions of cleansing quality correlated with score numbers (Table 1)[51]. Grading is performed in five colon segments and ranges from 0-4 (higher numbers indicating better quality of cleanse) for each. Although total scores are derived by adding the separate segment scores, an "acceptable" score is possible only when the mucosa is 100% visible in all five colon segments. A validation study of the HCS compared with the Aronchick scale in 337 colonoscopies reviewed by four gastroenterologists found that there was a high degree of Pearson correlation between the two scales (r = 0.833), and the Spearman correlation coefficient was -0.778 (correlation is negative because improved cleanse quality is represented by different directions in the HCS and Aronchick scale)[51]. The ROC curve analysis *vs* the Aronchick scale showed an area under the curve of 0.945, and a sensitivity of 99% and specificity of 83% at the optimum score cut-off point. Interrater reliability analysis yielded an ICC of 0.457 (95%CI: 0.366-0.539). Cohen kappa scores for individual segments between investigators showed slight-to-fair agreement ranging from 0.15-0.27. Internal consistency was acceptable, based on a Cronbach alpha coefficient of 0.81, and the test-retest reliability assessment showed an overall kappa of 0.639. No analyses of correlations with relevant clinical outcomes such as the ADR or adherence to recall guidelines were performed, due to insufficient patient population.

Chicago Bowel Preparation Scale

Like the HCS, the CBPS was developed to address perceived limitations in other commonly used bowel preparation scales[52]. The main features of the scale are shown in Table 1. Scoring is performed both before and after washing or suctioning, and a separate fluid score is included as a secondary measure (not incorporated into the total score as in the OBPS). The total and fluid scoring categories were designed to measure both the quality of visualization and the intraprocedural effort required to clean the mucosa to attain adequate visualization. These parameters were intended to help clinicians assess the cleansing efficacy of different bowel preparations[52]. A CBPS validation study prospectively compared the results of the CBPS with the OBPS, the BBPS, and a theoretical, dichotomous scale that simply defined "adequate cleansing" as ability to see $\geqslant$ 95% of the mucosa (after it was cleansed), with "inadequacy" being defined as visibility in < 95% in 150 colonoscopies at a single center[52]. In this study, kappa coefficients for interrater agreement were higher for the CBPS (0.624-0.702) than the OBPS (0.493-0.655) and the BBPS (0.545-0.661), but these differences were not significant. Kappa coefficients for the total colon fluid scores for the CBPS and OBPS, and Pearson correlations coefficients for interrater agreement, were also similar. For the OBPS, scores from 8-10 were graded inadequate; for the BBPS, a score of $\leqslant$ 4 was graded inadequate; and for the CBPS, total scores $\leqslant$ 24 were graded inadequate. No clinically relevant parameters were assessed for validation in this study.

ADDITIONAL VALIDATED SCALE COMPARISON DATA

The OBPS and the BBPS were compared in a study that reviewed prospectively collected data from patients who underwent CRC screening or surveillance colonoscopies over a two-year period between August 2013 and July 2015[58]. Of the 655 colonoscopies, overall detection rates for polyp, adenoma, right-side adenoma, and sessile serrated adenoma (SSA) were 42.8%, 32.8%, 20.8%, and 1.2%, respectively. A significant Pearson correlation was observed between the two scales (P < 0.001).

However, the ROC curves for the OBPS *vs* the BBPS were not significantly different for the detection rates, respectively, for polyps (0.550 *vs* 0.513), adenoma (0.544 *vs* 0.519), right-side adenoma (0.469 *vs* 0.516), and SSA (0.712 *vs* 0.790). The investigators concluded that the choice of either the OBPS or the BBPS may not strongly affect the measurement of bowel preparation quality.

DISCUSSION

Quality scales

All currently available bowel preparation quality scales are imperfect, have limitations, and are dependent upon subjective descriptions of luminal contents expressed as categories ("excellent", "good", *etc*.) or numbers, depending on the scale utilized. A standard, fully validated, and universally accepted scale for use in clinical practice and trials has not yet been established. Among the scales, the Aronchick scale is the most well-known and widely used clinically and in clinical trials to date; however, this scale rates cleanse quality of the colon as a whole and provides no details regarding differences between individual segments.

Colon segments cleansing

Guidance is somewhat vague for clinicians regarding grading of the entire colon when individual segments are suboptimally cleansed. This issue may arise more often in the proximal colon, which is harder to clean than other segments and more likely to contain flat lesions such as sessile serrated polyps/adenomas[50,51]. Segment-specific bowel preparation quality scales, such as the OBPS or BBPS, may provide a clearer distinction between cleanse quality of the proximal colon compared with other segments. Furthermore, establishing a minimum acceptable score for adequacy within each colon segment, as has been done for the BBPS, is helpful in determining overall colon cleansing adequacy. A BBPS validation study provided information used to create an "adequate cleansing" threshold score of at least 2 in each of three colon segments.

Need for washing and suctioning

Grading before or after washing and suctioning is another important factor which differs between scales. Many clinicians are using the Aronchick scale incorrectly, as they grade the bowel preparation as good or fair after washing and suctioning. While scales that grade cleanse quality after washing may correlate better with quality measures such as ADR, or the likelihood of an alteration in CRC screening follow-up recommendations, scales that grade before washing can provide a better reflection of a bowel preparation product's efficacy independent of the endoscopist. Similarly, the OBPS gives points based on the total fluid in the colon, which leads to inaccurate grading if using water immersion/exchange.

The OBPS entails scoring by colon segments, thus accounting for variation by segment in bowel preparation quality/visibility; however, it also incorporates the presence of luminal fluid before suctioning[11,50]. The OBPS validation data are largely dependent on correlations with the Aronchick scale, which itself has limited validation and may not correlate with ADR[50]. The BBPS differs in several key aspects from the Aronchick and OBPS scales[47]. To begin, it requires washing and suctioning to be completed before the bowel preparation is graded[47]. The HCS requires rating only after completion of flushing and suctioning, providing a score for the entire colon as well as for individual segments[51,52].

Grading scales validity and reliability

The reliability and validation data for BBPS is more extensive compared with the Aronchick and OBPS scales and include good supporting data correlating scores with key clinical outcomes. These validation studies have provided information to create a threshold for adequate cleansing of a score of at least 2 in each of three colon segments[44,57]. It should also be noted, however, that one study found no significant difference between the BBPS and OBPS regarding key indicators of colonoscopy quality, such as the PDR and ADR, in screening or surveillance colonoscopy[58]. Concerning the HCS and CBPS, each has reported acceptable reliability data, although the CBPS validation study was based on findings from only two raters[51,52]. While the HCS validation assessment was the only one to provide test-retest and internal consistency data for reliability, its validity evaluation was based only on correlations with the Aronchick scale[51]. Although the CBPS was compared with the OBPS and BBPS, no correlations of this scale with key clinical outcomes, such as ADR and adherence to screening and surveillance colonoscopy intervals, have been reported[52]. The CBPS has more specific definitions and requires measurement of fluid suctioned (Table 1), but the complexity may be challenging for the clinician to assess correctly; thus, it may not easily translate to clinical practice. Hence, the usefulness of these scales for clinical practice or trials remains unclear.

Several unique, nonvalidated bowel preparation scales have been developed for use in trials of agents including oral sulfate solution (OSS) (Suprep®, Braintree Laboratories, Braintree, MA, United States)[59], OSS plus sulfate-free electrolyte lavage solution (Suclear®, Braintree Laboratories, Braintree, MA, United States)[60], and polyethylene glycol electrolyte solution plus ascorbic acid (MoviPrep®, Salix Pharmaceuticals, Bridgewater, NJ, United States)[59,61,62]. The grading criteria used in these study- and product-specific scales often differ greatly from validated scales.

The substantial ramification of using nonvalidated scales is illustrated by a *post hoc* analysis of data from two sodium picosulfate and magnesium citrate (P/MC) clinical trials. Investigators analyzed the data from the studies after altering the definition of "adequate" in the Aronchick scale, which had been used in the original trials, to more closely resemble what has been used

in some studies utilizing nonvalidated scales[55]. With this revised definition, > 98% of all P/MC patients were considered responders, compared with 79%-87% using the original OBPS and Aronchick scale categorization criteria. Multiple studies have used more than one validated scale from among the Aronchick, OBPS, and BBPS scales for assessment of bowel preparation quality, providing additional comparative data[63-70]. Generally, the results of these trials have been concordant in assessment of bowel preparation quality, with similar mean total scores being reported for overall quality, and similar comparative assessments of different bowel preparations.

While scales for assessment of bowel preparation quality for CRC screening colonoscopy have improved, establishing a standard, validated scale is essential to optimize CRC colonoscopy screening. The Boston bowel preparation scale has several limitations, but appears nonetheless to be the best available option, and is therefore recommended as the current standard for use in clinical practice. Given the importance preparation plays in multiple colonoscopy quality measures, including the need to repeat the procedure when cleansing is inadequate, it may be advantageous for clinicians to adopt one language to describe cleansing quality. The continued use of multiple scales with varying criteria may undermine the validity of study findings and the accuracy of colonoscopy for CRC screening and surveillance.

For colonoscopy clinical trials, the use of different, and sometimes nonvalidated, scales across studies is one of many reasons comparisons between studies is fraught with difficulties. By incorporating a standard, validated grading scale, we may ensure that the findings are generalizable and comparable with other studies and facilitate progress in the development of future bowel preparations. Future developments in bowel preparation quality assessment are likely to involve establishment of an improved "gold standard" and further refinement of the accuracy of quality assessment. Continued improvement of quality standards for CRC prevention, further studies of ADR and withdrawal time, and recommended years of follow-up are also warranted.

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MINIREVIEWS

Current practices and future prospects for the management of gallbladder polyps: A topical review

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Abstract

A gallbladder polyp is an elevation of the gallbladder mucosa that protrudes into the gallbladder lumen. Gallbladder polyps have an estimated prevalence in adults of between 0.3%-12.3%. However, only 5% of polyps are considered to be "true" gallbladder polyps, meaning that they are malignant or have malignant potential. The main radiological modality used for diagnosing and surveilling gallbladder polyps is transabdominal ultrasonography. However, evidence shows that other modalities such as endoscopic ultrasound may improve diagnostic accuracy. These are discussed in turn during the course of this review. Current guidelines recommend cholecystectomy for gallbladder polyps sized 10 mm and greater, although this threshold is lowered when other risk factors are identified. The evidence behind this practice is relatively low quality. This review identifies current gaps in the available evidence and highlights the necessity for further research to enable better decision making regarding which patients should undergo cholecystectomy, and/or radiological follow-up.

Key words: Gallbladder polyps; Gallbladder cancer; True polyps; Pseudo polyps

Core tip: Evidence for the optimum management of gallbladder polyps is lacking. The main imaging modality used for diagnosis and follow-up is transabdominal ultrasound, but some studies suggest improved accuracy with

endoscopic ultrasound. Other imaging modalities lack evidence. Surgical management involves cholecystectomy and the general consensus is that polyps 10 mm and greater should undergo surgery. However, this is an arbitrary cut-off and high-quality evidence to support this is lacking. Lowering the threshold for cholecystectomy when patients have additional risk factors for gallbladder malignancy may improve the cancer detection rate in polyps smaller than 10 mm, but again, the evidence behind this is lacking.

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INTRODUCTION

A gallbladder polyp is an elevation of the gallbladder mucosa that protrudes into the gallbladder lumen[1,2]. Gallbladder polyps have an estimated prevalence of approximately 5% in the global population, but only 5% of these are considered to be "true" gallbladder polyps[3,4]. The majority of gallbladder polyps are detected incidentally on radiological imaging or histological examination after cholecystectomy. However, a small number of patients with gallbladder polyps may be symptomatic and present with acute cholecystitis due to the polyp obstructing the cystic duct, or cholangitis due to fragments of the polyp breaking off and travelling down into in the bile duct[2,5]. The majority of gallbladder polyps are classified as "pseudo"-polyps, as displayed in Figure 1. "Pseudo"-polyps have no malignant potential and do not require any follow-up or intervention, whereas "true" gallbladder polyps, which include adenocarcinomas or adenomas require surgical removal[2]. Although adenomas are benign, they have malignant potential and there is some evidence to suggest they may follow the adenoma-carcinoma sequence as seen in colorectal cancer[6,7].

Gallbladder cancer is the 20th most common cancer in the world and there are an estimated 178100 new cases diagnosed each year[8]. The highest incidences of gallbladder cancer are seen in South America and Asia, whilst lower incidences are seen in developed regions such as North America and the United Kingdom[9]. For example, the incidence of gallbladder cancer in Chile and Bolivia is 12.8 and 10.9 per 100000 population respectively, whereas in the United Kingdom and North America the incidence is 1.6 and 1.5 per 100000 people[9,10]. The staging of gallbladder cancer as per the American Joint Committee on Cancer 8th edition, ranges from stage 0 to stage 4b. Stage 0 describes carcinoma in-situ when the cancer involves the mucosa only as seen in early polyp cancers, while stage 4b indicates lymph node involvement of 4 or more lymph nodes (N2 disease) or the presence of metastatic disease[11]. Survival in gallbladder cancer patients varies significantly from an 80% 5-year survival in those with in-situ disease, declining to only 8% when lymph nodes are involved, and 2% for patients with stage 4b disease[11]. These figures demonstrate the importance of identifying malignant and pre-malignant polyps to enable early treatment to prevent cancer spread or development of malignancy.

It should be noted that once detected, surgical removal of all gallbladder polyps is not appropriate, given that the majority of polyps are "pseudo"-polyps with no malignant potential and there is a significant risk associated with surgery. In patients with "true" gallbladder polyps, laparoscopic cholecystectomy is the surgical option preferred, although in patients with larger polyps, open cholecystectomy is recommended[12,13]. The risks associated with surgery include damage to intra-abdominal structures during port insertion, bile duct injury (between 0.3% and 1%) and bile leak[14,15]. Furthermore, surgical intervention to repair a bile duct injury and endoscopic retrograde cholangio-pancreatography (ERCP) to manage a bile leak are associated with significant mortality, cholangitis, biliary cirrhosis, pancreatitis, perforation and haemorrhage[16,17].

This review discusses the current evidence that exists regarding the management of gallbladder polyps. Given the low incidence of true polyps within all gallbladder polyps identified, coupled with the high mortality associated with gallbladder cancer and the risk of complications associated with cholecystectomy, it is essential to differentiate between "pseudo"- polyps and true polyps to enable appropriate management. The use of imaging modalities assists with the decision-making process and this review discusses the benefits and shortcomings of the imaging modalities used for identifying and following up gallbladder polyps.

THE ROLE OF DIFFERENT IMAGING MODALITIES IN GALLBLADDER POLYP DIAGNOSIS

Radiological imaging plays the main role in the diagnosis and decision making for the management of gallbladder polyps. The ideal imaging modalities should have three key features. Firstly, they should be able to accurately diagnose polyps and differentiate them from gallstones, sludge, or folds of the gallbladder mucosa. Secondly, "true" polyps need to be differentiated from "pseudo"-polyps, as the latter are benign with no malignant potential and therefore do not require any intervention or follow-up. Thirdly, the size of polyps need to be measured accurately as this is currently the most important factor which determines if patients should undergo cholecystectomy, radiological follow up or cease to be followed up. Given that some patients with gallbladder polyps will require follow-up for many years, it is also important that

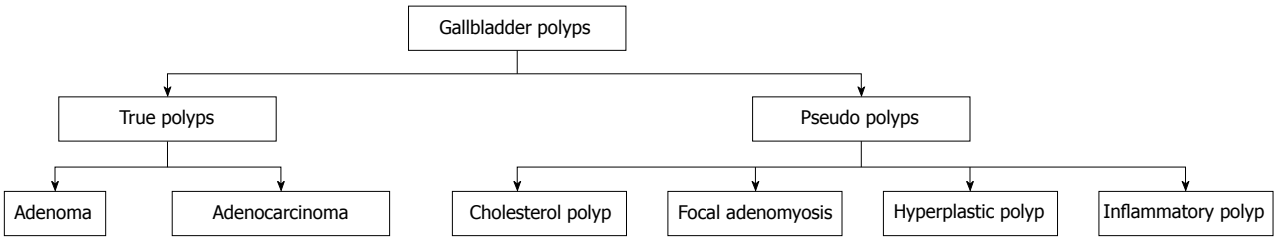

Figure 1 Spider diagram showing the classification of gallbladder polyps.

the imaging modality is acceptable to patients and incurs minimal radiation exposure.

Accurate imaging will prevent unnecessary surgery and ensure true polyps which do not fall into the size criteria for surgical removal category are identified during follow-up. The benefits and shortcomings of different imaging modalities are discussed below. The main modalities discussed include ultrasonography, computed tomography and magnetic resonance imaging.

Transabdominal ultrasonography

Trans abdominal ultrasound (TAUS), encompasses conventional ultrasound (CUS), high-resolution ultrasound (HRUS), three-dimensional ultrasound and contrast enhanced ultrasound (CEUS). CUS and HRUS are easily accessible, cheap, non-invasive tests[18] and are the most widely used modalities for diagnosing and following up gallbladder polyps. However, other studies have been performed to assess the effectiveness of the other forms of ultrasonography mentioned above[18,19].

Ultrasonography is operator dependent and results can be limited by increased body mass index, in particular truncal obesity[20]. Polyp echogenicity is examined to distinguish between "true" polyps and "pseudo"- polyps and the presence of a fixed lesion helps to distinguish between polyps and gallstones. However, in some cases gallstones may be impacted in the gallbladder wall and be incorrectly labelled as a polyp[2]. Features that suggest the presence of a "pseudo"- polyp include a "comet tail" which arises posterior to the lesion but this is not identifiable in all "pseudo"- polyps[21].

CUS uses a low-frequency transducer between 2 and 5MHz but despite this has demonstrated good specificity (71%-98%) and sensitivity (50%-90%) for diagnosing all types of gallbladder polyps[22]. In the same systematic review, CUS had a sensitivity of 47%-67% and specificity of 36-100% for diagnosing malignancy[22] and in polyps 10mm or greater in size, the sensitivity and specificity for identifying malignancy was 78%-100% and 52%-87%, respectively[22].

However, shortcomings in CUS have been reported, for example in a single study by French *et al*[23] which compared histopathology reports from cholecystectomy specimens with findings from the CUS report found that imaging only identified 50% of polyps. This group concluded that CUS should not be used for following up gallbladder polyps[23] .

HRUS operates at a higher frequency than CUS (5-7 MHz) but a lower frequency than endoscopic ultrasound (EUS) (5-12 MHz) and therefore theoretically has a better diagnostic accuracy than CUS but is less accurate than EUS[24]. It does however have the benefit over EUS, in that it is a non-invasive procedure. Kim *et al*[24] demonstrated that HRUS is more accurate than CUS at staging the T-stage of gallbladder cancer and was more accurate for identifying hypoechoic foci in neoplastic polyps which has previously been shown to be a strong predictive factor for neoplastic gallbladder polyps[24,25]. More studies however are required which compare the sensitivity and specificity of CUS and HRUS.

One study has compared HRUS, endoscopic ultrasound (EUS), and computed tomography (CT) in diagnosing and staging gallbladder polyps in 144 patients who all had a polyp greater than 10 mm in size[26]. Diagnostic sensitivities for malignancy were highest in HRUS, compared to the other two modalities and specificity was the same when using EUS and HRUS[26]. The drawback from this study however is that the applicability of this technique to smaller gallbladder polyps remains unknown and polyps of less than 10 mm are diagnostically most difficult group to assess. Furthermore, HRUS was not compared to CUS, which is currently the most commonly used imaging modality.

3D-US is an emerging modality which eliminates the operator dependency seen in 2-dimensional CUS. Research for this imaging modality is minimal but a study of 80 patients with gallbladder polyps found that there was agreement in the diagnosis in 89% of cases when both techniques were applied[27]. This study however found that 3D-US did have difficulty detecting polyps less than 4mm, but it is predicted that as technology continues to evolve this issue will decline in future[27]. Current research therefore does not support the routine use of 3D-US for evaluating gallbladder polyps.

Several small studies have looked at the use of contrast media to improve the diagnostic accuracy of CUS. Contrast aids radiologists to differentiate normal from abnormal conditions. Numata *et al*[28] used galactose palmitic acid contrast injection to assess 35 polyps which were larger than 10 mm in size. Using the criteria of tumour enhancement and tortuous type tumour vessels, this technique had 91% accuracy at identifying malignancy. The downside to this study however, is that it did not compare contrast-enhanced ultrasonography with CUS[28]. Zheng *et al*[29] did compare the two modalities in a study of 116 patients with gallbladder polyps, and

found that CEUS was useful for improving diagnostic accuracy in polyps greater than 10 mm, but not less than 10 mm in size.

Endoscopic ultrasound

EUS works at a higher frequency as described above and enables the transducer to be in closer proximity to the target tissue therefore, hypothetically improving diagnostic accuracy[24]. It is however, an invasive examination associated with a small risk of bleeding and upper gastrointestinal perforation and presents a higher risk of complications than all forms of TAUS[30].

A systematic review has found EUS to have a greater sensitivity (67%-86%) and specificity (84%-91%) for diagnosing malignancy in polyps than CUS[22]. A single study by Sugiyama *et al*[31] compared EUS and CUS in 58 patients who had undergone cholecystectomy. All polyps were 20 mm or less in size, and EUS was more accurate at differentiating between true and "pseudo"- polyps than CUS (97% *vs* 76%). Cheon *et al*[32] however, found that although EUS was more successful at identifying true polyps in those with diameters of 11 mm and greater (83% *vs* 64%), there was not the same success in polyps of diameter 10 mm and less (80% *vs* 72%). Therefore, this imaging technique may play a role in decreasing the number of unnecessary cholecystectomies in larger gallbladder polyps, but more research needs to be done investigating its role in smaller polyps, for which the management is most controversial.

Two studies have been performed looking at the role of contrast- enhanced EUS (CE-EUS) in diagnosing gallbladder polyps. Park studied 34 patients who had a cholecystectomy for gallbladder polyps and found that CE-EUS when attempting to distinguish adenomatous polyps from cholesterol polyps had a sensitivity of 75% and specificity of 66.6%. Unfortunately, in this study CE-EUS was not compared to any other imaging modality. Choi *et al*[33] however compared EUS with CE-EUS and found that diagnostic accuracy was slightly improved with the latter.

Other methods including the use of real time colour Doppler flow EUS has been used to try and improve the diagnostic accuracy of EUS. Kim *et al*[34] found that the presence of a strong colour Doppler flow in a study 115 patients who underwent cholecystectomy for gallbladder polyps may help predict the presence of neoplastic polyps and therefore further research is warranted.

Computed tomography

CT imaging is widely used in the staging of gallbladder adenocarcinoma[2]. However, some research has been performed to assess if it may also play a role in differentiating between true and "pseudo"- polyps and for long-term surveillance[35]. The accuracy of CT imaging was assessed in 31 patients with polypoid lesions of the gallbladder of 3cm or less. The CT diagnosis was accurate in 87% of cases however, only 5 polyps were less than 11 mm and therefore this study provides us with limited evidence regarding the role of CT in this group of patients[35]. Lou *et al*[36] assessed the accuracy of CT biliary cystoscopy in 32 patients and found that CUS accurately detected polyps in 96.9% of cases compared to 93.8% for CT.

This evidence would suggest that CT imaging is best used in staging larger, suspicious malignant polyps, rather than for diagnostic purposes and follow-up, due to lack of superiority to CUS demonstrated in studies to date.

Magnetic resonance imaging

Minimal research has been performed looking at the role of MRI in differentiating between benign and malignant gallbladder polyps. In a small study, Irie *at al*[37] demonstrated in 10 benign polyps and 13 malignant polyps that the ADC values of the malignant lesions were significantly lower than that seen in the benign lesions. They concluded that diffusion-weighted MR imaging may play a role in diagnosing benign and malignant polyps[37]. However, further research is warranted to establish if MRI can improve the accuracy of diagnosing gallbladder polyps.

Other imaging modalities

Other imaging modalities have been considered in small single studies. One study has shown that positive emission tomography can differentiate between benign and malignant disease but more research is needed[2]. Results from a study examining the role of percutaneous transhepatic cholecystoscopy were promising but this is an invasive procedure with significant risk and is difficult for patients to tolerate[2]. Finally, intravenous cholecystography has shown to be of no benefit to date, compared with current imaging modalities[18].

After studying the evidence, TAUS and in particular CUS and HRUS would appear to be the most appropriate imaging modality for detecting gallbladder polyps. Although some studies looking at the role of other forms of ultrasonography in managing gallbladder polyps appear promising, there is still not enough evidence to introduce these modalities into routine practice for the management of gallbladder polyps. Evidence for smaller gallbladder polyps is of particularly low quality. In cases of clear uncertainty however, additional imaging modalities may be deployed to help the clinician in their decision-making process. The role of CT is evident in staging gallbladder cancer but due to a lack of high-quality studies examining a role in gallbladder polyps and the high radiation exposure associated with this imaging, it is not appropriate for either the diagnosis or follow-up of gallbladder polyps.

FACTORS INFLUENCING THE MANAGEMENT OF GALLBLADDER POLYPS

Polyp size

Studies have shown that malignant polyps in general

tend to be larger than benign polyps[5,22]. Kwon *et al*[5] reported in their study of 291 patients that malignant polyps had a mean size of 27.97+/-2.46 mm compared to 8.56+/-0.36 mm in the benign group. Currently, the polyp size on radiological imaging is the biggest contributing factor to the management plan for gallbladder polyps. Multiple retrospective studies have found the risk of malignancy rises sharply from 10 mm and upwards, and the general consensus is that patients with polyps of 10 mm or greater should be treated with cholecystectomy[19,22,38]. Although this is the accepted practice, evidence for this recommendation lacks quality. The most up-to-date guidelines published by the European Society of Gastrointestinal and Abdominal Radiology (ESGAR) support this approach but two recent systematic reviews demonstrate that although the majority of malignant polyps are over 10 mm in diameter, there are a significant number of both malignant polyps or polyps with malignant potential under this sizing threshold[19,22,38].

Babu *et al*[22] performed a systematic review which included 43 studies, of which 20 provided information on the size and histology of 2347 polyps. Of these, 356 were classified as true polyps, of which 228 were malignant - and 29 of these were between 5-10 mm but none below the 5 mm size. Bhatt *et al*[38] in their systematic review also demonstrated that there were a significant number of malignant polyps under 10 mm in size but the probability of malignancy when a polyp was 4.15 mm or smaller was approximately zero. These two large studies demonstrate that although the majority of true polyps are over 10 mm there are a significant number of true polyps under this cut off which will be missed if cholecystectomy is only performed for polyps greater than 10 mm.

Several authors have suggested a change in this cut off with some suggesting polyps of 6 mm and larger should undergo cholecystectomy whilst others have felt that the cut off should be increased to 12mm[39,40]. The argument for lowering the threshold carries more weight, as demonstrated by the findings in the systematic reviews discussed above. The counter-argument of lowering the threshold is that by offering cholecystectomy to those patients with polyps below 10 mm, a greater number of patients may be put through an unnecessary operation associated with significant risk of complications. It has therefore been proposed that polyps under 10 mm should undergo surveillance, based on their size unless significant risk factors are present in which case cholecystectomy should be offered[19].

Surveillance

Polyp surveillance aims to provide a safety net for those patients with true polyps that cannot be differentiated from "pseudo"- polyps on radiological investigations and are under 10 mm in diameter. It is hypothesised that "true" polyps will undergo faster growth, and by careful follow-up these can be identified early and removed[22]. Guidelines state that polyps which reach 10 mm in size or increase in size by 2 mm at follow up transabdominal ultrasonography are recommended to be removed surgically[19]. However, evidence to support this practice is lacking.

There is no consensus on the size of polyps that require follow up, or the frequency or duration of follow up. The most recent set of guidelines published by ESGAR states that patients with polyps of 6-9 mm should be followed up more extensively than patients with polyps of less than 6 mm[19]. Several studies support 6 mm as a lower limit cut-off for less extensive follow up, but go a step further by suggesting the cessation of follow up in polyps less than 6 mm[41,42]. However, this has been contradicted by multiple studies which have found true polyps to be less than 6 mm in size and a single case report that has shown that a 5 mm polyp transformed into a 20 mm carcinoma over a period of two years[22,38,43]. The evidence would suggest that all polyps between 4-10 mm should be followed up equally as although the risk reduces with size, there is still a significant number of true polyps between 4 mm and 6mm. Although no malignant polyps have been shown to be below 4 mm there is still a risk of adenomas and these polyps therefore would still require follow up but on a less frequent basis[22].

The recommended follow up for patients with gallbladder polyps depends on the size of the polyps and the presence of risk factors for malignancy, but opinions differ and the evidence base informing these guidelines is relatively limited. For example, Babu *et al*[22] recommend that the follow up of polyps 5-10 mm should be two scans at six month intervals and following this the surveillance plan should be tailored for individual patients. The ESGAR group recommend that in polyps of 6-9 mm, after two initial six monthly scans there should be yearly scans up to 5 years. However, in polyps under 6 mm there should be imaging at 1, 3 and 5 years but if he patient has risk factors for malignancy there should be more extensive follow-up as those seen for polyps of 6-9 mm with no risk factors[22].

Follow up imaging may have a limited benefit as only a small number of polyps actually change in size during follow up. Babu *et al*[22] identified 10 studies which looked at the follow up of gallbladder polyps between six months and seven years. They found that only 7.6% of polyps increased in size and Bhatt *et al*[38] also found that that 93% of polyps did not change in size during follow up. Neither study stated if growth was more likely to be seen in in pseudo or true polyps and this was supported in a third systematic review[44]. Although there is a lack of evidence comparing growth patterns between pseudopolyps and true polyps, small individual studies have shown that both can undergo sudden growth[2].

RISK FACTORS FOR GALLBLADDER POLYP MALIGNANCY

As discussed above the main determining factor for

Table 1 Summary of evidence for association between potential risk factors and malignant gallbladder polyps

Risk factor	Direction of association	Strength of association	Related notable findings	Key references
Age	Positive	Probability of malignancy was 20.7% in those patients older than 50	This systematic review studied polyps less than 10 mm only	[38]
Sessile morphology	Positive	Probability of malignancy was 13.9% in sessile compared to pedunculated polyps	This systematic review studied polyps less than 10 mm only	[38]
Presence of gallstones	Inconclusive	Aldouri *et al*[47] found increased risk of malignancy with gallstones (HR = 3.2, 95%CI: 1.42-7.22) but Park *et al*[39] found no difference (P = 0.27)	There is no strong evidence to suggest there is a definite association	[39,47]
Indian Ethnicity	Positive	HR = 12.92 (95%CI: 3.77-44.29) This shows a significant HR but the width of the CI's are noted.	This is the only study to compare risk between Indian ethnicity and Caucasian race	[47]
Primary sclerosing cholangitis	Positive	40%-60% of polyps in patients with PSC were malignant	33% of those with benign polyps had associated dysplasia	[56]

gallbladder malignancy is the presence of a polyp greater than 10 mm in size. However, not all polyps under 10 mm are benign and therefore it is important to identify risk factors to enable the clinician to have a higher suspicion for malignancy and therefore perform cholecystectomy below the 10 mm threshold. These potential risk factors are discussed below and summarised in Table 1.

Number of polyps

Evidence is mixed on whether solitary polyps are more likely to be malignant compared to the presence of multiple polyps. In a systematic review by Bhatt *et al*[38], the probability of malignancy in a polyp under 10 mm if it was solitary was 4.3% higher compared to when multiple polyps were present. The authors did not deem this to incur a high enough risk to suggest cholecystectomy in all patients with a solitary polyp under 10mm. Perhaps this is the most useful study as the authors look at the risk exclusively in the 5-9 mm group and it is this cohort in which the evidence is weakest[38]. A study by Kwon *et al*[5] also found that malignant polyps were more likely to be solitary (P = 0.02), but this study only patients who had gallbladder polyps greater than 10 mm. Several other studies however have demonstrated no association between a solitary polyp and malignancy. For example, Park *et al*[39] in a study of 689 patients found that 60% of benign polyps were solitary and 76% of malignant polyps were benign and this was not significantly different (P = 0.11).

Although the probability of malignancy is not high enough to recommend cholecystectomy in all solitary polyps, the presence of a solitary polyp should be considered in combination with other risk factors for malignancy as discussed below.

Sessile morphology

Single studies such as that performed by Kwon *et al*[5] have demonstrated that patients with gallbladder polyps of sessile morphology have a higher risk of malignancy compared to those with pedunculated polyps (OR: 7.70; 95%CI: 2.48-23.95). In the systematic review by Bhatt *et al*[38], malignant polyps under 10 mm were also more likely to be sessile in nature and the probability of malignancy was 13.9% in these patients but cholecystectomy was not recommended. However, if there was a solitary sessile polyp, the probability of malignancy was 24.8% and cholecystectomy was recommended[38]. Although Bhatt *et al*[38] do not recommend cholecystectomy based on sessile morphology alone, the most recent guidelines by the ESGAR group use the strength of this evidence to recommend cholecystectomy for all sessile polyps under between 6 mm and 9 mm.

Age

The risk of most cancers increases with age and a similar pattern is seen for gallbladder cancer. Multiple case series support this but the cut off for an increased risk of malignancy varies significantly between 50 and 65 years old[13,38,39,45]. For example, Park *et al*[39] identified age 57 years and older as a risk factor for malignancy, but in this study one patient who was only 37 years old had a malignant polyp of 10 mm and the one patient who had a malignant polyp under 10 mm in size was only 50 years old. Furthermore, Sarkut *et al*[46] found that there was an increased likelihood of malignancy in patients aged 50 and over, but again this was not exclusive as one patient under 50 had a malignant polyp. The only study to date that looks at the contribution of age to risk of malignancy in polyps solely under 10 mm was performed by Bhatt *et al*[38]. They found that when the polyp was less than 10 mm and the patient was over 50 that the probability of malignancy was 20.7%, and therefore cholecystectomy was recommended[38]. The ESGE group used this evidence to conclude that if patients are aged 50 and have polyps of 6-9 mm they should undergo cholecystectomy[19].

Presence of gallstones

The evidence considering the impact of concurrent gallstones and the risk of malignancy in gallbladder polyps varies significantly and is of relatively low quality. Aldouri *et al*[47] found that if gallstones were present there was an increased risk of malignancy (HR: 3.2;

95%CI: 1.42-7.22) but Park *et al*[39] found that there was no association between the presence of gallstones and malignancy (P = 0.27). In those patients with symptoms due to gallstones, cholecystectomy is already recommended and therefore the decision-making process is simple. However, the evidence is not strong enough to suggest cholecystectomy should be performed in all cases with dual pathology.

Ethnicity

As discussed earlier, gallbladder cancer incidence varies significantly between countries. A study by Aldouri *et al*[47] carried out in the United Kingdom demonstrated that in 5391 patients who underwent cholecystectomy, the risk of malignancy was almost 13 times higher in the Indian population compared to the Caucasian population (HR: 12.92; 95%CI: 3.77-44.29). This is the only study to date which compares risk between different ethnic groups, however the ESGAR felt the evidence was so compelling that their guidelines state that in patients of Indian ethnicity and a polyp between 6-9 mm they should undergo cholecystectomy[19]. Further research needs to be performed comparing other ethnic groups to determine if there should be a lower threshold for cholecystectomy in different ethnicities.

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a recognised risk factor for a gallbladder polyp malignancy, and cholecystectomy is currently recommended in these patients who have a gallbladder polyp irrespective of the polyp size[48]. The largest study to date including 286 PSC patients, found that in 18 patients with a gallbladder polyp, 10 had a malignancy in polyps as small as 5 mm whilst in 9 patients who had no mass lesion they still had dysplasia of the gallbladder[49]. Furthermore, in a case series of 4 patients with PSC and gallbladder polyps, all were shown to have malignant disease including in two polyps under 10 mm in size[50]. Other evidence is less compelling, including a study by Eaton *et al*[51] who found that in 14 patients with PSC and polyps only two were malignant. This group concluded that polyps under 8 mm were less likely to be malignant and in this group and follow up should be applied. Given the presence of research such as this further research would be justified. The difficulty will be recruiting enough patients with both pathologies.

Tumour markers

Limited research has been performed to assess if there is a role for tumour markers in the pre-operative evaluation of gallbladder polyps. The two markers focused on to date has been CEA and CA19-9 but no correlation between malignancy and elevated markers has been found. In a case series of 291 patients, Kwon *et al*[5] found no difference in pre-operative CEA or CA19-9 levels in the benign or malignant groups. Indeed, the CEA level was elevated in more benign cases (5.7%) than malignant cases (2.9%). When comparing the CA19-9 levels, there were 4.9% of benign group who had a raised level and 8.6% of malignant group had a raised level[5]. There is no sufficient evidence to show that tumour markers will assist in the decision-making process for gallbladder polyps.

Genetic risk factors

To our knowledge, no research has studied genetic risk factors for gallbladder polyps, despite multiple studies having investigated genetic contributions to gallbladder cancer. For example, studies from Shanghai and Sweden have noted significantly increased risks of gallbladder cancer in patients with a family history of gallbladder cancer[52,53]. It has also been shown in a recent review that approximately one quarter of cases diagnosed in a Utah cohort study were familial[54]. However, the difficulty with evaluating family history as a proxy for genetic factors is that it may also reflect exposure to similar environmental exposures. A recent review has highlighted the paucity of research on specific genetic polymorphisms with respect to gallbladder cancer risk, and extrapolated some biologically plausible hypotheses from gallstone aetiology[54-56]. Overall, there is only low quality evidence for genetic predisposition to gallbladder cancer, and no studies have been conducted for gallbladder polyps. Robust, genome-wide association studies are required to confirm or deny any potential associations.

CONCLUSION

The gaps in the available evidence to support the current guidelines on the management of gallbladder polyps are outlined above. TAUS is the current mainstay for radiological investigation of gallbladder polyps. EUS and HRUS have shown some promise as an adjunct to TAUS but more work is required to assess the exact role and the category of polyps that they may provide diagnostic accuracy. Although polyps of 10 mm and greater are more likely to be true polyps, this cut-off will miss a significant number of true polyps below this threshold and cholecystectomy will also be performed unnecessarily for pseudopolyps when they are greater than 10 mm. The factoring in of the risk factors discussed above to lower the threshold for cholecystectomy will no doubt decrease the number of missed true polyps in the under 10 mm category but cholecystectomy will also be performed when it is not required. No research has been performed to assess the impact of following these guidelines and therefore larger retrospective and prospective case series need to be performed to assess the success of managing gallbladder polyps as per the current guidelines.

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MINIREVIEWS

New horizons in the endoscopic ultrasonography-based diagnosis of pancreatic cystic lesions

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Abstract

Pancreatic cystic lesions (PCLs) are increasingly being identified because of the widespread use of high-resolution abdominal imaging. These cysts encompass a spectrum from malignant disease to benign lesions, and therefore, accurate diagnosis is crucial to determine the best management strategy, either surgical resection or surveillance. However, the current standard of diagnosis is not accurate enough due to limitations of imaging and tissue sampling techniques, which entail the risk of unnecessary burdensome surgery for benign lesions or missed opportunities of prophylactic surgery for potentially malignant PCLs. In the last decade, endoscopic innovations based on endoscopic ultrasonography (EUS) imaging have emerged, aiming to overcome the present limitations. These new EUS-based technologies are contrast harmonic EUS, needle-based confocal endomicroscopy, through-the-needle cystoscopy and through-the needle intracystic biopsy. Here, we present a comprehensive and critical review of these emerging endoscopic tools for the diagnosis of PCLs, with a special emphasis on feasibility, safety and diagnostic performance.

Key words: Intraductal papillary mucinous neoplasm; Pancreatic cystic lesions; Endoscopic ultrasonography; Confocal endomicroscopy; Mucinous cystadenoma; Through-the-needle cystoscopy; Serous cystadenoma; Through-the-needle forceps biopsy; Contrast harmonic endoscopic ultrasonography

Core tip: This paper provides a focused update on emerging endoscopic technologies for improving the diagnosis and prediction of the malignant potential of pancreatic cystic lesions. Basic principles, diagnostic

performance, safety and limitations are critically reviewed.

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INTRODUCTION

Pancreatic cystic lesions (PCLs), initially thought to be rare, have become an incidental finding increasingly identified because of technological advances and the widespread use of high-resolution abdominal imaging. It is estimated that approximately 3% and 20% of patients undergoing abdominal computed tomography (CT) and magnetic resonance imaging (MRI), respectively, present a PCL[1-2]. Among neoplastic cysts, certain subtypes, basically mucinous cysts, entail a risk of present or future malignancy. Other neoplastic cysts, such as serous cystadenomas (SCAs), are considered benign cysts without potential of malignancy. These two types of neoplastic cysts are sometimes indistinguishable, and therefore, the awareness of possible malignant potential may lead physicians to refer these patients to surgical resection. Despite improvements in pancreatic surgery, considerable morbidity and mortality still occur in 18%-38% and 0.2%-2% of patients who undergo surgery because of PCLs[3-6]. Accurate assessment of malignant potential is therefore of the utmost importance to avoid unnecessary surgery for benign cysts while considering appropriate surveillance for low-risk lesions and surgical treatment for malignant and high-risk cysts.

The initial step of the diagnostic approach to PCLs usually relies on radiological imaging focusing on size and morphological features. Once a PCL has been identified, most commonly by CT, an MRI is recommended due to its higher ability to evaluate nodules and to depict a communication between the cyst and the main pancreatic duct. Owing to the frequent lack of specific radiological features of many PCLs, the overall accuracy of CT and MRI remains low, ranging from 23% to 93%[7-16]. Endoscopic ultrasonography (EUS) further characterizes PCLs and performs better than CT or MRI in assessing the morphology of small cysts and in depicting nodules in mucinous cysts, but EUS imaging alone also shows inadequate accuracy in the range of 40%-94%[17-23]. A major advantage of EUS is the ability to safely obtain cyst fluid for biochemical and cytological analysis. However, the scant cellularity of the cyst fluid accounts for the low diagnostic yield of cytology. Although the cyst fluid carcinoembryonic antigen (CEA) level has been proven to be more accurate than cytology or EUS morphology alone, the reported sensitivity of CEA and cytology for mucinous lesions in a recent meta-analysis do not exceed 63% and 54%, respectively[24].

All the above limitations have led to the recent updated International, AGA and European guidelines based on predictors of malignancy, which have been shown to be far from providing reliable differentiation between the various PCLs, and recommendations provided in these guidelines are based on low-grade evidence[25-27]. Moreover, the Fukuoka International Consensus Guideline, restricted their recommendations to branch-duct IPMNs that are diagnosed easily in a vast majority of cases (multiple cysts, communication with the main pancreatic duct)[28]. The Fukuoka guidelines were evaluated in a prospective study yielding a high negative predictive value (NPV) of 94% for malignancy but a low positive predictive value (PPV) of approximately 38%, frequently prompting unnecessary surgery[29]. Efforts to overcome current limitations have boosted the research in this area. Molecular analysis of DNA-based biomarkers in cyst fluid has been described with promising results[30]. Genomics, miRNA, proteomics and metabolomics in cyst fluid seem to be promising, but validation studies are pending. In addition to molecular testing, endoscopic innovations based on EUS imaging have emerged in the last decade and they are more easily available than the omics technologies though with a longer learning curve. Although not all of these EUS-based innovations have been validated, they warrant a thorough analysis to evaluate their diagnostic performance and potential impact as a part of the diagnostic workflow of PCLs.

In this article, we aim to review the evolving role of emergent EUS-based technologies-contrast harmonic enhanced imaging, needle-based confocal endomicroscopy, through-the-needle cystoscopy, and through-the-needle intracystic biopsy-in the clinical diagnosis of PCLs, with a critical focus on feasibility, safety and diagnostic performance. Novel approaches to cystic fluid analysis, such as omics technologies and other biomarkers, are beyond of the scope of this review.

A literature search was performed for all available studies concerning the EUS diagnosis of PCLs in PubMed and Embase databases. The following search domains (including closely related words) were used: "pancreatic cysts" in combination with "contrast harmonic EUS" or "contrast enhanced EUS" or "needle confocal endomicroscopy" or "cystoscopy" or "intracystic biopsy". The search was limited to papers published in English until December 2017. Titles were then screened for suitability, and the full-text papers were retrieved. A hand-search of the references listed in the articles accessed was also performed to identify other relevant original studies. Both retrospective and prospective studies reporting data on the feasibility, diagnostic performance and safety of the above referred procedures in patients with PCLs were considered for inclusion. Indications, technical details, performance outcomes, impact on final diagnosis and management, complications and mortality were extracted and further discussed.

CONTRAST-HARMONIC ENHANCED ENDOSCOPIC ULTRASOUND

Because perfusion patterns on CT or MRI explorations allow the characterization of focal lesions, EUS has recently incorporated the use of ultrasound contrast agents (UCAs) to depict blood flow in small vessels. Available UCAs consist of microbubbles composed of an inert gas encapsulated by a shell[31]. Gases are compressible, and when exposed to an ultrasound wave, microbubbles alternatively compress under positive pressure and expand under negative pressure, producing a backscattered acoustic signal with harmonic components[31-32]. These harmonic components are higher than those obtained from tissue and may be selectively detected and reproduced on the ultrasound image for displaying the microvascularity pattern[31]. Not until recently, when new UCAs, broadband EUS transducers and contrast-specific software programs became available, was contrast harmonic EUS (CH-EUS) feasible for the first time, allowing the discrimination between tissue and contrast signals. Second-generation UCAs, which contain a soluble gas, unlike air-filled first-generation agents, are commonly used for CH-EUS. They have a resistant but more flexible and longer lasting shell, making possible the use of low acoustic power (a low mechanical index) and continuous real-time assessment[31-35].

Several steps must be followed to perform CH-EUS[31]. After fundamental B-mode exploration of the target area, a dual screen is displayed, simultaneously showing the CH-EUS image and the conventional B-mode image. Next, optimal parameters, notably, a low MI, should be selected on the ultrasound platform. The UCA is then injected intravenously slowly through a large-gauge intravenous catheter of 16G-18G in order to avoid breaking microbubbles. Finally, the venous catheter must be flushed with saline to clear out persistent microbubbles in the vein. After intravenous injection of microbubbles, it takes 10-20 s to observe the arrival of the contrast agent. The arterial phase lasts 30-45 s, during which the enhancement increases progressively. After the arterial phase, there is a progressive washout of the contrast, and the venous phase persists from 30 s to 120 s.

Clinical outcomes: Review of the literature

Among the 71 articles retrieved from PubMed and Embase databases using the terms "pancreatic cysts" and "contrast harmonic EUS", only seven were suitable for further review[36-42]. All of the studies but one were retrospective, and the majority was devoted to the diagnosis of mural nodules and/or malignancy in intraductal pancreatic mucinous neoplasms (IPMNs). Only three articles addressed the issue of the differential diagnosis of PCLs. Selected articles are summarized in Table 1.

Hocke *et al*[36] performed CH-EUS in 125 patients with PCLs. Contrast enhancement of cyst walls, septa and nodules was observed in all PCNs, including mucinous cysts, cystic adenocarcinomas, SCAs and cystic neuroendocrine neoplasms (NENs), but in only 6% of nonneoplastic cystic lesions (PCs and dysontogenic cysts). Further supporting these results, in another study by Fusaroli *et al*[37], of 76 patients with PCLs, most SCAs were hyper-enhanced during CH-EUS, without a significant difference (86% *vs* 89%, P = NS), when in fact, 90% of pseudocysts showed hypoenhancement (P = 0.000004 *vs* serous cysts and P = 0.000005 *vs* mucinous cysts). In addition, Kamata *et al*[38] reported that CH-EUS did not add any advantage to EUS for differentiating mucinous and nonmucinous cysts when the presence of mural nodules was considered a sign of mucinous cysts (sensitivity, 79% *vs* 85%; specificity, 96% *vs* 46%; accuracy, 73% *vs* 84%, respectively, P = 0.057).

Yamashita *et al*[39] used CT, color Doppler EUS and CH-EUS to prospectively study 17 patients with mural nodules in branch duct type IPMN (BD-IPMN) detected by EUS before being referred for surgery. After pathological analysis, 75% of mural nodules corresponded to adenocarcinomas, and 25% corresponded to adenomas. Compared with surgical specimens, CH-EUS depicted vascularity in all pathologically confirmed nodules and in one case with mucous clots (sensitivity, 100%; specificity, 80%; PPV, 92%; NPV, 100%; and accuracy, 94%). Moreover, the sensitivity of CT and color Doppler-EUS was only 41% and 0%, respectively. Comparable results were found in later studies. Fujita *et al*[40] observed that CT, MRI, and EUS detected mural nodules histologically confirmed in 86%, 71% and 100% of cases. Although EUS was highly sensitive, it was not able to distinguish mucous clots from mural nodules that were correctly classified in all cases after assessing the vascular pattern by CH-EUS (Figure 1). CH-EUS was also shown to be more accurate than CT or EUS in diagnosing mural nodules in the study by Harima *et al*[41] (accuracy of 98%, 72% and 92%, respectively).

Fusaroli *et al*[37] observed that all hyper-enhanced solid components during CH-EUS turned out to be malignant, whereas nonenhanced ones were either mucous clots or internal debris. Nevertheless, other authors found it difficult to discriminate between adenomas or adenocarcinomas in BD-IPMN based on the vascular pattern. Only one study evaluated the accuracy of quantitative CH-EUS for differentiating between low-grade dysplasia (LGD) or intermediate-grade dysplasia and high-grade dysplasia (HGD) or invasive carcinoma. In this study, Yamamoto *et al*[42] retrospectively analyzed the time-intensity curve in 30 patients with resected IPMNs who underwent CH-EUS. The analyzed parameters were the echo intensity change and the echo intensity reduction rate of the mural nodule and the nodule/parenchyma contrast ratio. All of the parameters were significantly higher in the HGD/invasive group (P < 0.05), with the nodule/parenchyma contrast ratio being the most accurate parameter (accuracy, 93%). Moreover, a positive linear correlation was observed between the echo intensity change in the mural nodule and the micro-

Table 1 Contrast harmonic enhanced endoscopic ultrasound in pancreatic cystic lesions

Authors	Study	*n*	Main outcomes	Complications
Yamashita *et al*[39], 2013	P	17	Differential diagnosis between mural nodules and mucus clots: CH-EUS: Sen 100%, Spe 80%, PPV 92%, NPV 100%, A 94% CT and Doppler-EUS: Sen 41% and 0% respectively	0
Hocke *et al*[36], 2014	R	125	Differential diagnosis between non-neoplastic cysts and PCNs. Hyperenhancement: 100% PCNs Hypoenhancement: 94% non-neoplastic cysts (PCs and dysontogenic cysts) CH-EUS superior to EUS in differential diagnosis between PCNs and non-neoplastic cysts ($^{a}P < 0.001$)	0
Harima *et al*[41], 2015	R	30	Performance for diagnosing mural nodules CT: Sen 71%, Spe 100%, PPV 100%, NPV 90%, A 92% EUS: Sen 72%, Spe 61%, PPV 50%, NPV 100%, A 72% CH-EUS: Sen 100%, Spe 97%, PPV 93%, NPV 100%, A 98%	0
Fujita *et al*[40], 2016	R	50	Sensitivity for diagnosing mural nodules: CT: 86% *vs* MRI 71% *vs* EUS 100% EUS was not able to distinguish mural nodules from mucus clots CH-EUS correctly differentiated mural nodules from mucus clots in all cases	0
Fusaroli *et al*[37], 2016	R	76	Differential diagnosis between non-neoplastic cysts and PCNs and between benign and malignant cysts. Hyperenhancement: 86% SCAs and 89% mucinous cysts (P = ns) Hypoenhancement: 90% PCs ($^{b}P < 0.000004$ *vs* SCAs and $^{c}P < 0.000005$ *vs* mucinous cysts) Hyperenhanced solid components : 100% malignant cysts Non- hype-enhanced solid components: 100% benign cysts	0
Kamata *et al*[38], 2016	R	70	Mural nodule as a sign of mucinous cyst EUS *vs* CH-EUS: Sen 85% *vs* 79%, Spe 46% *vs* 96%, A 73% *vs* 84% ($P = 0.057$) Mural nodule as a sign of malignancy EUS *vs* CH-EUS: Sen 97% *vs* 97%, Spe 40% *vs* 75%, A 64% *vs* 84% ($^{d}P = 0.0001$)	0
Yamamoto *et al*[42], 2016	R	30	Quantitative CH-EUS in IPMNs Echo intensity change and echo intensity reduction rate, and nodule/parenchyma contrast ratio significantly higher in HGD/invasive carcinoma ($^{e}P < 0.05$) Microvessel density in mural nodule Significantly higher in HGD/invasive carcinoma ($^{f}P < 0.002$) Significant correlation between echo intensity change and microvessel density ($^{g}P < 0.001$)	0

P: Prospective; R: Retrospective; CT: Computed tomography; MRI: Magnetic resonance imaging; EUS: Endoscopic ultrasonography; CH-EUS: Contrast harmonic endoscopic ultrasonography; Sen: Sensitivity; Spe: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; A: Accuracy; PCNs: Pancreatic cystic neoplasms; IPMN: Intraductal papillary mucinous neoplasm; HGD: High grade dysplasia.

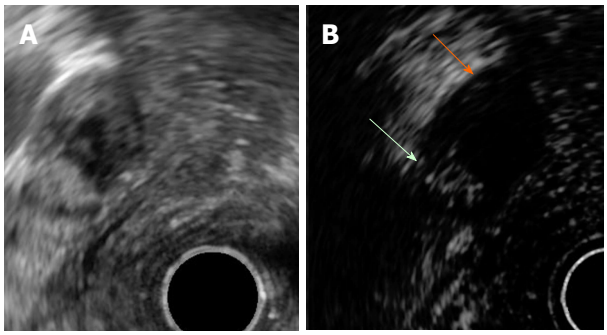

Figure 1 Mural nodule and mucus in branch duct type intraductal pancreatic mucinous neoplasms. A: EUS B mode image. B: Contrast harmonic EUS image. Microbubbles in a mural nodule (green arrow). No bubbles in a mucus clot (orange arrow). EUS: Endoscopic ultrasonography.

vessel density in pathologic specimens ($r = 0.803$, $P < 0.001$).

No mortality or CH-EUS-related adverse events were reported in any study.

NEEDLE-BASED CONFOCAL LASER ENDOMICROSCOPY

Needle-based confocal laser endomicroscopy (nCLE) is an emergent endoscopic modality that enables imaging of a target tissue at a subcellular level of resolution, providing real-time *in vivo* optical biopsy. For CLE imaging, a low-power laser is used to illuminate the tissue. The laser beam is focused on a plane of interest, and the reflected light from the tissue is filtered and transformed into an electrical signal by a detection system and finally translated into grayscale images by a computer system[43,44]. The final result consists of images with very high spatial resolution and magnification of the focal plane examined within the tissue. Because confocal imaging relies on reflected fluorescent light, intravenous injection of a fluorescent dye, most commonly sodium fluorescein, is required. Fluorescein acts as a contrast agent highlighting blood vessels and tissue architecture[43-46].

Recent advances have allowed the incorporation of CLE technology into a miniprobe of 0.85 mm (AQ-Flex) that can be passed through a 19-gauge (19G) EUS needle. This probe is provided with 10.000 optical fibers and has a field of view of 325 μm, 3.5 μm of lateral resolution and 40-70 μm of confocal depth[46].

Before starting the procedure, a 19G EUS needle is preloaded with the AQ-Flex probe that should be inserted until 2 mm of the probe is positioned beyond the needle tip (Figure 2). At this moment, the probe is fixed to the inlet of the needle channel by a locking system. After identifying the cyst, a single pass with the preloaded 19G

Table 2 Needle-based confocal laser endomicroscopy in pancreatic cystic lesions

Authors	Study	*n*	Main outcomes	Complications
Konda *et al*[48], 2011	P	16	94% Technical success (feasibility study)	12% post-procedure pancreatitis
Konda *et al*[49], 2013	P	66	Stage 1: Description of visualized structures (n = 26) with histological correlation Stage 2: Performance assessment of defined criteria (n = 31): Villous pattern: Sen 59%, Spe 100%, PPV 100%, NPV 50%, A 71% for PCNs Significant association with PCNs (^aP = 0.004)	3% post-procedure pancreatitis 4.5% intracystic self-limited bleeding
Nakai *et al*[50], 2015	P	30	Villous pattern in 18 patients with highly certain diagnosis: Sen 80%, Spe 100%, PPV 100%, NPV 80%, A 89% for mucinous cysts Significant association with mucinous cysts (^bP = 0.001)	7% post-procedure (cystoscopy followed by nCLE) pancreatitis
Napoléon *et al*[51], 2015	P	31	Superficial vascular network: Sen 69%, Spe 100%, PPV 100%, NPV 82%, A 87% for SCAs	3% post-procedure pancreatitis
Napoléon *et al*[52], 2016	R	31	Step 1: Description of nCLE patterns for mucinous cysts, PCs and cystic NENs with histological correlation Step 2: Retrospective external validation of nCLE criteria Accuracy 94% for mucinous cysts (90% IPMN - 90% MCA) - 87% SCA - 87% PCs Substantial global IOA: Perfect PC, almost perfect SCA, moderate IPMN, fair MCA	
Kadayifci *et al*[53], 2017	P	20	nCLE performance for mucinous cysts: Sen 66% -Spe 100% -A 80%	0 complications
Krishna *et al*[54], 2017	P	10	Reproducibility of the *in vivo* nCLE criteria in *ex vivo* specimens	
Napoléon *et al*[55], in press	P	209	Diagnostic yield 91% in 78 patients with non-communicating cysts and pathological diagnosis: Sen 95%, Spe 100% - PPV 100% - NPV 98% - A 99% for SCAs Sen 95%, Spe 100% - PPV 100% - NPV 94% - A 97% for mucinous cysts Sen 100%, Spe 95% - PPV 70% - NPV 100% - A 96% for NENs Sen 96%, Spe 95% - PPV 98% - NPV 91% - A R96% for premalignant cysts	1.3% post-procedure pancreatitis
Karia *et al*[56], 2016	R	15	IOA poor to fair for all nCLE variables	
Krishna *et al*[57], 2016	R	49	nCLE performance on 26 patients with definitive diagnosis (23 with pathological diagnosis): Sen 94%, Spe 82% - PPV 88% - NPV 92% - A 89% for mucinous cysts IOA and IOR: Substantial for all nCLE criteria	6.1% post-procedure pancreatitis
Krishna *et al*[58], 2017	R	29	nCLE performance on 29 patients with definitive diagnosis (23 with pathological diagnosis): Sen 95%, Spe 94% - A 95% for mucinous cysts Sen 99%, Spe 98% - A 98% for SCAs Sen 99%, Spe 98% - A 98% for NENs IOA and IOR: Almost perfect for mucinous cysts and SCAs	

P: Prospective; R: Retrospective; Sen: Sensitivity; Spe: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; A: Accuracy; PCNs: Pancreatic cystic neoplasms; PC: Pseudocyst; MCA: Mucinous cystadenoma; SCA: Serous cystadenoma; IPMN: Intraductal papillary mucinous neoplasm; NENs: Neuroendocrine neoplasm; IOA: Interobserver agreement; IOR: Intraobserver reliability.

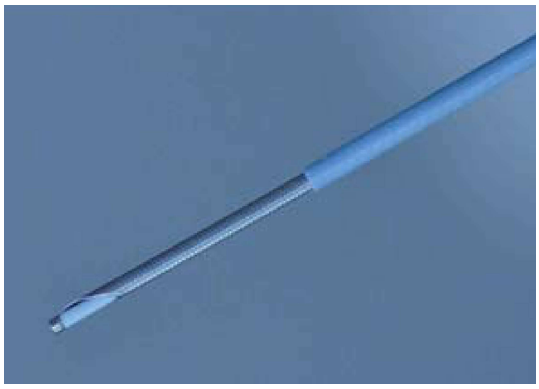

Figure 2 Needle-based confocal laser endomicroscopy probe in a 19G needle.

EUS needle is performed, and the needle is advanced under EUS guidance until the needle tip contacts the cyst wall. Immediately after, 2.5-5 mL of 10% fluorescein is injected; nCLE imaging begins, during which gentle apposition of the probe to the cyst wall is pursued. The elevator, endoscope dials and torquing are useful for imaging in a fanning technique[43]. Because images are obtained at a rate of 12 frames/s, video-recording is always performed for 2-5 min and further reviewed with a dedicated software program. At the end of the procedure, the probe is withdrawn, and the cyst fluid is aspirated as per standard practice. Antibiotic prophylaxis is systematically administered[47].

Clinical outcomes: Review of the literature

A literature search in PubMed and Embase databases identified 24 articles reporting on nCLE in PCLs. Only 11 of these articles were deemed eligible and consisted of 7 prospective and 4 retrospective studies (Table 2)[48-58]. Most of them were focused on feasibility, safety and performance in differentiating mucinous from nonmucinous cysts or in the differential diagnosis among the

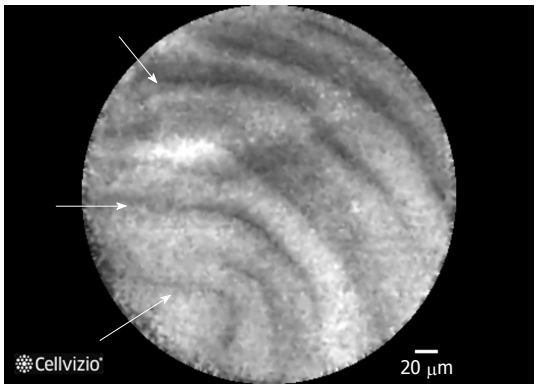

Figure 3 Needle-based confocal laser endomicroscopy image of an intraductal pancreatic mucinous neoplasms displaying multiple papillary projections.

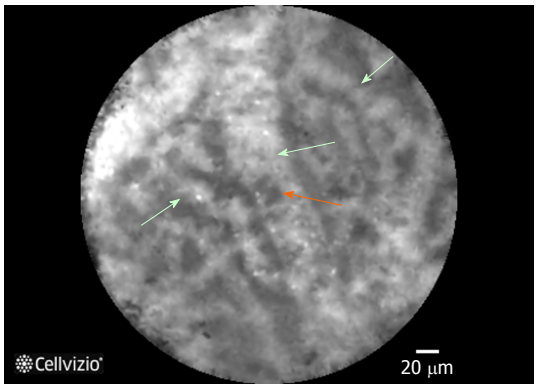

Figure 4 Needle-based confocal laser endomicroscopy image of the superficial vascular network pattern of a serous cystadenoma. Multiple interconnected vessels (green arrows). Red cells inside displayed as black structures (orange arrow).

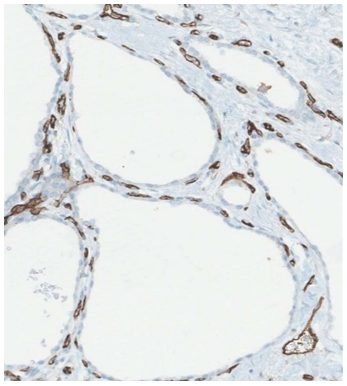

Figure 5 Staining with a vascular marker of serous cystadenomas histological specimen. Capillary necklace with subepithelial vessels showed in brown.

several types of PCLs. Three studies aimed to externally validate the nCLE criteria, and one study performed an *ex vivo* validation of the *in vivo* nCLE criteria.

The first series was reported in 2011 by Konda *et al*[48] and was a feasibility study of 16 cysts and 2 solid masses of the pancreas. The nCLE procedure was feasible in 15 of 16 cysts, although the technical challenges described in 6 of them were related to the transduodenal approach, the post loading technique (the insertion of the CLE probe after positioning the EUS needle inside the lesion) and the longer length of the metallic tip at the distal end of the probe. Complications occurred in two patients, both of whom developed pancreatitis requiring hospitalization. This study was followed by a larger multicenter study (*in vivo* nCLE Study in the Pancreas with Endosonography of Cystic Tumors, INSPECT) that was reported by the same group and that aimed to evaluate the diagnostic potential and safety of nCLE in the differential diagnosis of PCLs in 66 patients[49]. A consensus description of visualized structures on 26 patients was achieved, and the correlation between histology and nCLE was investigated during the first stage of the study. Then, the performance of nCLE criteria to identify PCNs, including mucinous cystadenoma (MCA), IPMN or adenocarcinoma, was assessed in 31 additional patients. The presence of villous structures was highly specific (100%) but provided low sensitivity, at 59%, and it was the only specific finding having a significant association with PCNs (P = 0.004). Post procedure pancreatitis occurred in 3% of cases: one patient experienced transient abdominal pain, and three cases of intracystic bleeding were observed and spontaneously solved.

The DETECT trial (Diagnosis of Pancreatic Cysts: Endoscopic Ultrasound, Through-the-Needle Confocal Laser Endomicroscopy and Cystoscopy Trial) was designed to evaluate the diagnostic yield of cystoscopy followed by nCLE in 30 patients[50]. A highly certain diagnosis was possible in 18 of these patients based on the clinical presentation, other image findings, fluid analysis and cytology. In these patients, a papillary projection (Figure 3) and/or a dark ring on nCLE, corresponding to the villous pattern previously reported by Konda, was associated with mucinous cysts (P = 0.001) with 80% sensitivity, 100% specificity, 100% PPV, 80% NPV and 89% accuracy. Two patients (7%) developed post procedure pancreatitis.

After the above initial experiences, Napoléon and colleagues described new nCLE criteria based on histological correlation in two consecutive studies[51-52]. In the first one, a criterion for *in vivo* diagnosis of SCA was defined and consensually identified as a superficial vascular network (Figure 4). The presence of small and regular structures circulating inside the opacified channels during nCLE suggested the vascular nature of these channels, which was confirmed by histological assessment of surgical specimens (Figure 5). This vascular network was demonstrated to be at a superficial depth of 50-70 μm and, therefore, at the reach of the nCLE probe. Moreover, SCA was the only PCL that featured this pattern among 31 patients with PCLs of unknown diagnosis. The sensitivity, specificity, PPV, NPV and accuracy of this criterion for diagnosing SCA were 69%, 100%, 100%, 82% and 87%, respectively. Only one patient suffered mild acute pancreatitis (3%). In a second study (CONTACT 1), the same authors identified three other nCLE criteria that included a thick gray line

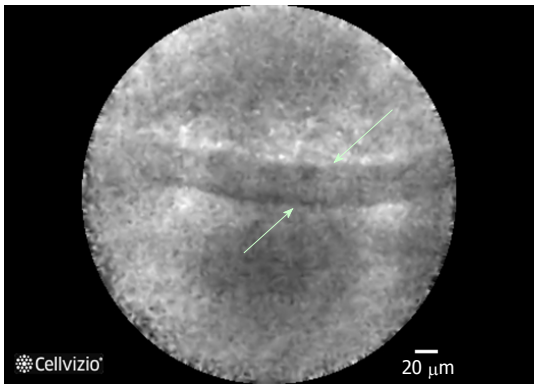

Figure 6 Epithelial border image in a mucinous cystadenoma at needle-based confocal laser endomicroscopy.

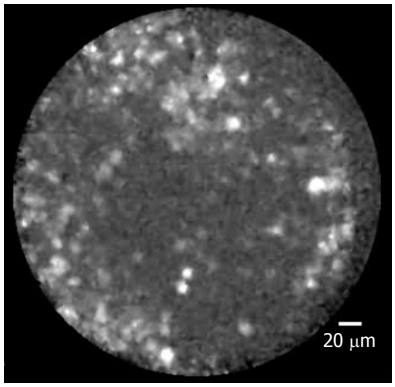

Figure 7 Heterogeneous sized grey and white particles in a pseudocyst at needle-based confocal laser endomicroscopy.

for mucinous neoplasms (Figure 6), a field of bright particles for PCs (Figure 7) and black neoplastic clusters with white fibrous areas for NENs. In this case, the histological correlation was the epithelial border, a mix of inflammatory cells and neoplastic cell proliferation with several forms of architectural organization respectively. In the retrospective validation, four external and blinded reviewers evaluated the diagnostic performance of these criteria. A conclusive diagnosis was achieved in 23 of 31 patients (74%). Overall, the accuracy of nCLE criteria was 94% for mucinous cysts, 87% for SCA and 87% for PC. Trends toward high specificity were also shown (> 90% for mucinous cysts and 100% for nonmucinous cysts). NENs were excluded from the external validation due to the small number in this series (n = 2). More recently, Krishna *et al*[54] have further confirmed the reproducibility of the *in vivo* nCLE criteria in *ex vivo* specimens of 10 patients with surgically resected PCLs.

To overcome the limitations of the reduced number of patients and the lack of pathological confirmation in previous series, Napoléon *et al*[55] designed a larger multicenter and prospective study (CONTACT 2) whose results are now available (submitted). Among 209 enrolled patients with a noncommunicating solitary cyst, 78 patients with a final diagnosis proven by surgical histopathology or cytopathological analysis of cyst fluid by EUS fine needle aspiration (EUS-FNA) were included in the final results. The overall diagnostic yield of nCLE was 91%, and the sensitivity and specificity for the main types of PCLs were higher than 95%. Perfect specificity (100%) was observed for diagnosing SCA and premalignant mucinous cysts. Furthermore, the nCLE area under the curve was significantly higher than that of CEA dosage for differentiating mucinous from nonmucinous cysts (P < 0.01) and higher than that of EUS morphology in differentiating between premalignant and benign PCLs (P < 0.05). However, nCLE criteria were not highly specific for NENs and PCs. NEN criteria were also observed in one cystic solid pseudopapillary neoplasia (CSPPN), one cystic lymphoma and 1 PC. Additionally, one mucinous lesion and one SCA exhibited PC criteria. Acute pancreatitis occurred only in 1.3% of patients. Although the impact of nCLE on patient management was not evaluated, it is noteworthy that in 29% of patients with a previous inconclusive EUS-FNA, nCLE was conclusive in 91% of cases and 100% accurate.

The first reports on interobserver agreement (IOA) yielded diverging results[51,56]. In the first one (CONTACT 1), four external reviewers assessed the IOA of the nCLE criteria in 31 cases[51]. The diagnostic accuracy for mucinous cysts was 94%, and the global IOA was rated as substantial (k = 0.72, 95%CI: 0.52-0.87). A later validation study reported low diagnostic accuracy for the type of PCL (46%), with an IOA ranging from poor to fair for all nCLE variables[56]. However, careful interpretation of these results is advised due to limitations such as poor image quality and short duration of video capture, which may have accounted for the poor results, and other methodological issues such as the lack of intraobserver reliability and few patients with a histological gold-standard diagnosis. More recently, two other studies aimed to validate nCLE criteria. One investigation consisted of a retrospective analysis at a single center[57,58]. nCLE videos from 26 patients (23 with pathological diagnosis) were reviewed by 6 blinded nCLE-naïve observers[57]. Substantial IOA and IOR were achieved for differentiating mucinous from nonmucinous cysts (k = 0.67, 95%CI: 0.57-0.77 and k = 0.78 ± 0.13, respectively) and for detecting all nCLE criteria. These results were further corroborated by an international external interobserver and intraobserver study[58]. In 29 patients, the overall accuracy of nCLE for the diagnosis of mucinous cysts was 95%, with an almost perfect IOA and IOR among six expert endosonographers with nCLE experience (k = 0.81, 95%CI: 0.71-0.90 and k = 0.86 ± 0.11, respectively). Furthermore, nCLE was 98% accurate in diagnosing SCA and the IOA and IOR for recognizing the fern pattern (previously defined as superficial vascular network) were also almost perfect (k = 0.83, 95%CI: 0.73-0.92 and k = 0.85 ± 0.11, respectively).

THROUGH-THE-NEEDLE CYSTOSCOPY

Through-the-needle cystoscopy is a procedure that allows

Figure 8 Moray forceps.

direct assessment of the cyst content as well as the inner cyst wall by means of single-operator cholangioscopy fiberoptic probe (Spyglass®, Boston Scientific, Natick, Mass, United States). The probe has 6000-pixel optic bundles, a 300 cm working length and a diameter of 0.77 mm. It provides a 70-degree field of view and has a 2-7 mm focal length[43,50]. The cystic cavity is accessed under EUS guidance with a 19G EUS needle. Before starting the procedure, the needle stylet is removed, and the fiberoptic probe is preloaded through the 19G needle and prefitted with the advancement of the probe 2 mm beyond the needle tip. Then, the probe is withdrawn 2-3 mm inside the needle, and once inside the cyst, the probe is advanced again to the prefitted position. Prophylactic antibiotics are always given, and cystoscopy images may be recorded for further review[50].

Clinical outcomes: Review of the literature

Of the 8 references retrieved, only 3 suitable articles were identified[50,59-60]. The first one reported on two patients who underwent through-the-needle cystoscopy followed by biliary forceps biopsy[59]. In both cases, it was possible to rule out a pseudocyst because a flat normal mucosa was visualized lining the inner cyst wall. In addition, cystoscopy enabled a better delineation of mural nodules and targeted biopsies of the selected suspicious areas. Severe acute pancreatitis occurred in one patient one month after the procedure. It is not possible to rule out a delayed procedure-related complication, although very unlikely.

In the prospective DETECT study, Nakai *et al*[50] performed through-the needle-cystoscopy followed by nCLE in 30 patients. The cyst content was evaluated for clarity, the presence of mucin or debris, and the smoothness, nodularity and vascularity of the cyst walls were assessed. The median image time of cystoscopy was 4 min, and 33% of the images were rated as fair or poor. The mucinous content was described as viscous and cloudy fluid. Typical findings and their clinical correlations were finger-like projections and a mucin cloud in IPMNs, smooth cyst walls with cloudy fluid in MCNs, and smooth cyst walls with prominent regular vessels in SCAs. However, the only significant association in 18 high-certainty diagnoses was observed between mucin on cystoscopy and mucinous cysts (P = 0.0004), with 90% sensitivity, 100% specificity, 100% PPV, 100% NPV and 94% accuracy. However, finger-like projections were identified in only two of the ten high-certainty mucinous lesions. When cystoscopy and nCLE were combined, the sensitivity increased from 90% to 100%. Post procedure pancreatitis was reported twice.

In a retrospective study published last year, Chai *et al*[60] performed through-the-needle cystoscopy in 43 patients. Based on the blood vessel distribution, the presence of partitions or ridge-like structures and the presence of papilla-like structures, the characteristic findings of different PCLs were defined and then validated by surgical pathology, FNA or fluid cytology. The authors concluded that a tree-like branching pattern of blood vessels may suggest the diagnosis of SCA (specificity, 91%; sensitivity, 69%) and that intracystic papilla-like structures may be characteristic of mucinous cysts (specificity, 92%; sensitivity, 22%). No pancreatitis was observed, and only two patients presented mild abdominal post procedure pain.

THROUGH-THE-NEEDLE FORCEPS BIOPSY

The low sensitivity of EUS-FNA cytology because of relatively acellular samples makes appealing the possibility to obtain biopsies. The design of minibiopsy forceps has led to the development of a new EUS-FNA tissue acquisition technique. Moray® micro forceps (US Endoscopy, Ohio, United States) were designed for use in EUS procedures to enhance sampling from lesions that can occur within and outside the gastrointestinal tract, leading to a more definitive diagnosis (Figure 8). These forceps are 230 cm in length and have serrated jaws (jaw opening of 4.3 mm) and a spring sheath 0.8 mm in diameter, allowing use through a 19G EUS needle[43,61].

Clinical outcomes: Review of the literature

No formal study was retrieved after searching PubMed and Embase for "pancreatic cyst" and "intracystic biopsy". Only two pilot studies reporting on 2 cases each and four additional case reports were identified[59,61-65].

Through-the-needle intracystic biopsy was first described by Aparicio *et al*[59] in two patients. They used 0.8 mm endoscopic retrograde cholangiopancreatography biopsy forces followed by a 3-4 min observation with a fiberoptic probe to rule out immediate bleeding. In both cases, a mucinous-like cylindric epithelium without cellular atypia was observed. As stated above, one patient presented severe acute pancreatitis one month later. After this preliminary experience, six more cases were reported to undergo intracystic biopsy, enabling the correct diagnosis of 5 mucinous cysts (one of them with mild dysplasia) and one benign lymphoepithelial cyst without any complication[61-65].

DISCUSSION

PCLs remain a diagnostic and therapeutic challenge to clinicians. Several issues remain to be solved, notably, how to improve diagnosis and better predict malignant behavior. It has been reported that 36% of SCAs are treated with unnecessary surgery because of an uncertain diagnosis[66]. In the attempt to cover this need,

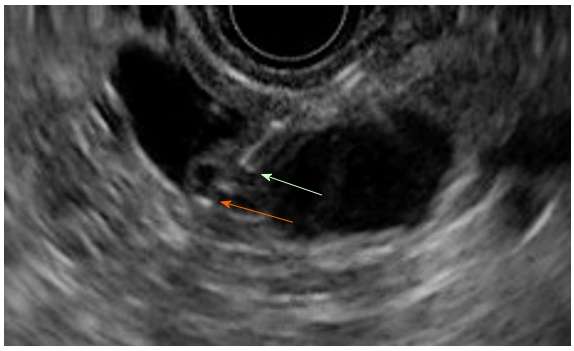

Figure 9 Moray forceps inside a cyst. Green arrow: The tip of the 19G needle. Orange arrow: The tip of the Moray forceps grasping the cyst wall.

several EUS-based tools have emerged recently and some experience is now available using these technologies in the diagnosis of PCLs.

On the grounds that CH-EUS has been proved to be accurate for the differential diagnosis of solid pancreatic masses, it was hypothesized that CH-EUS might also be helpful in PCLs, and some experience has been reported in the last five years[67-70]. Due to its high spatial resolution, CH-EUS may define the inner structure of cysts by depicting small septa or mural nodules that become echogenic during CH-EUS, whereas the intracystic content remains invisible (Figure 9). This modality may assist not only in the differential diagnosis but also in identifying malignancy risk features.

Current evidence suggests that CH-EUS is highly accurate for distinguishing nonneoplastic cysts (PCs and dysontogenic cysts) from neoplastic cysts because the former do not exhibit cystic wall vascularization. This feature prevents pointless and onerous surgery in this benign setting. However, a different scenario is observed among different neoplastic cysts whose biological behavior may be significantly different and where misinterpretations are common in CH-EUS. Benign SCAs, the most common nonmucinous cystic neoplasms, and potentially malignant mucinous cysts show undistinctive features on CH-EUS. Consequently CH-EUS cannot be used for the differential diagnosis of neoplastic cysts.

The presence of mural nodules is considered in the international consensus guidelines on IPMN management as a high-risk stigma and strongly supports surgical resection. Mural nodules may sometimes be too small for detection by CT or MRI. The high spatial resolution of EUS enables better identification in these cases. However, the performance of EUS is not enough to discriminate between mural nodules or mucous clots. Hyperenhancement of solid components during CH-EUS may differentiate mural nodules from mucous clots or debris. This step is essential to avoid unnecessary surgery and is included in the last guidelines[25]. Nodules in BD-IPMNs include not only malignant nodules but also benign adenomas that might be followed without surgery, but the preoperative differentiation between them does not seem possible with qualitative CH-EUS. Angiogenesis plays a key role in tumor growth and progression; in fact, neovascularization was reported to be crucial in the tumorigenesis of invasive IPMNs with a progressive increase in microvessel density from benign to malignant tissue[71]. According to this observation, preliminary experience with quantitative CH-EUS suggests that the analysis of echo intensity changes during CH-EUS may be an accurate method to discriminate between low/intermediate-grade dysplasia and high-grade dysplasia/invasive carcinoma. Further trials are necessary to confirm this interest.

When it comes to safety, CH-EUS exhibits an excellent safety profile with no adverse events in series reporting on PCLs. UCAs are the sole factor adding risk to conventional EUS. Concerns were raised after a study reporting on UCAs used for stress echocardiography, where 4 deaths and 190 serious adverse events were observed and associated with the use of UCAs[72]. Consequently, the Food and Drug Administration issued a black box warning regarding the use of UCAs in several pathologic cardiorespiratory states. Later experience in large cohorts of patients has shown that the rate of UCA-related serious adverse events is lower than 0.01%, with anaphylactoid reactions occurring in 1/10.000 cases[73-74].

More recent evidence confirms that nCLE is feasible during EUS-FNA and allows *in vivo* diagnosis of PCLs with high accuracy; nCLE criteria have been defined for IPMN, MCA, SCA, PC and cystic NEN with a proven histopathological correlation. Unlike CH-EUS, a perfect specificity of 100% has been confirmed with nCLE for benign SCA and for mucinous lesions. The superficial vascular network is exclusive to SCAs, allowing SCA diagnosis with high confidence, therefore preventing unnecessary surgical resection. Sensitivity of this pattern, although high, is not perfect, and epithelium denudation may account for the lack of a vascular pattern in oligocystic SCA. Specificity is also perfect for the overall group of mucinous lesions, but when they are further classified as either IPMN or MCA, the specific findings of papillae or epithelial borders, respectively, may both be present. Other times, papillae or epithelial borders cannot be visualized, and the explanation is the nonuniform distribution of papillae throughout the epithelium of IPMNs or modifications induced by inflammation in some MCAs. These inflammatory changes are also the reason why a field of bright, gray and black particles is not specific for PCs and may be found in other cystic tumors following infection or bleeding. Finally, the presence of dark spots of cell aggregates surrounded by gray areas of fibrosis and vessels, initially attributed to NENs, is not specific and may be present in other premalignant lesions, such as CSPPNs. However, due to the similar premalignant nature, the impact on clinical management of misdiagnosis between NENs and CSPPNs or between IPMNs and MCAs is of little importance.

The major concern related to nCLE is the risk of acute pancreatitis, which has ranged from 1.3% in the largest and most recent study to 12% in the first feasibility study. The use of Spyglass® (Boston Scientific, Natick, Mass, United States) for cystoscopy may also explain

the second highest rate of pancreatitis in the DETECT study[50]. The average rate from the rest of the studies is 2.7%, which is comparable with that of the conventional EUS-FNA procedure[75]. Only one series reported intracystic bleeding in 3 patients (4.5%); however, no intervention was required, and they were solved spontaneously. Several factors that add to the risk of complications have been suggested, such as needle size, duration of the procedure and abrasion of the cyst lining with the needle tip. Some tips have been suggested during nCLE to maximize the procedure safety. The interposition of the main pancreatic duct should be avoided when selecting the puncture site to lower the risk of acute pancreatitis. Once inside the cyst, limited brushing of the cyst wall is recommended to avoid cystic bleeding and pancreatitis; instead, it is preferred to perform consecutive apposition between the probe and the cyst wall. Finally, the exploration should be stopped as soon as nCLE criteria are met, or after 6 min if no specific criteria are found, to minimize the risk of adverse events[43]. No study reported adverse events related to the injection of fluorescein, but severe allergic reactions are possible, although very uncommon (1/222000)[76]. Minor side effects, such as nausea, vomiting, mild epigastric pain, transient hypotension, injection site erythema or diffuse rash, have been reported in 1.4% of cases[77]. The learning curve for nCLE seems to have an important effect on the pancreatitis rate since one study reported three acute-pancreatitis cases among the first 25 patients and none in the successive 34 patients[58]. Technical challenges reported in initial feasibility studies are now overcome, and several tips have been suggested to avoid them (preloading of the nCLE probe, use of the most flexible needle). However, other limitations remain to be solved. The most important one is the limited surface of the cystic wall that is accessible to be scanned. Exploration is feasible over the area in front of the needle tip but less than 50% of the cystic surface is likely to be visualized. The second argued limitation is the high cost of the probe, which is considered a limiting step to implementing nCLE technology in routine practice. Nevertheless, a health economic evaluation carried out in France demonstrated that nCLE resulted in a reduction of 23% of surgical interventions[78]. This finding translated into a reduction in clinical costs of 13% in the public sector and 14% in the private sector. The improved diagnostic accuracy of nCLE reduces the number of false positives and false negatives, avoiding unnecessary surgical interventions and lifelong surveillance for benign cysts.

The heterogeneous distribution of neoplastic tissue in PCLs makes it reasonable to attempt to directly explore the inner cyst walls. Limited experience with through-the-needle cystoscopy has demonstrated the feasibility for direct visualization of cyst walls and has suggested that it may help to target biopsies to suspicious areas. Patterns at cystoscopy have been proposed for SCAs, MCNs and IPMNs but have not been validated yet. Like for nCLE, the main complication is acute pancreatitis, and therefore, limiting the time inside the cyst is strongly recommended. The greatest interest seems to be the ability to detect mucin clots that have a typical appearance on cystoscopy. Nevertheless, the performance of cystoscopy was not higher than the string test that is more simple and free of cost[50]. Beside the cost of the fiberoptic system, other limitations of cystoscopy in reported studies are mainly related to the suboptimal quality of images. Therefore, the real place of cystoscopy need to be established.

Through-the-needle intracystic biopsy has been suggested to be a feasible technique for tissue acquisition in one series and several case reports. It allows histological diagnosis including the grade of dysplasia, although the retrieved samples are small. However, the processing of the samples is not always easy. Because of the small-sized specimens, they sometimes disintegrate during fixation in formalin. In addition, lesions such as mural nodules may not be targetable by the stiff 19G EUS-FNA, especially those located in the uncinate process. Finally, concerns remain about the risk of bleeding following through-the-needle forceps biopsy, similarly to brush cytology, even if no cases of bleeding have been reported. Formal studies in larger series are required to validate the results of this new technique and to confirm its safety.

In summary, CH-EUS seems to be a safe and complementary tool to EUS-FNA for the assessment of PCLs. CH-EUS is especially accurate in differentiating nonneoplastic cysts from PCNs and mural nodules from mucus clots. Moreover, the recent International Association of Pancreatology and the European guidelines have recommended CH-EUS for further evaluation of mural nodules in IPMN and PCN respectively. Larger and prospective studies are required to confirm the role of quantitative CH-EUS in the differential diagnosis between malignant and benign mucinous neoplasms. EUS nCLE is a minimally invasive tool with remarkable potential for diagnosing PCLS. Future research should also address new nCLE criteria associated with the grade of dysplasia or cancer, the additional clinical value of combining nCLE with the current standard of PCLs diagnosis and the cost-effectiveness of nCLE during the initial EUS-FNA or after inconclusive results of EUS-FNA. Meanwhile, and based on available evidence at present, excluding cost-effectiveness, we propose an algorithm of diagnosis for PCLs (Figure 10). Through-the-needle cystoscopy and biopsy must be evaluated in formal studies in larger series to validate their results and to confirm their safety before integrating them in the diagnostic flowchart of PCLs.

CONCLUSION

PCLs are increasingly identified on imaging, but their characterization remains challenging due to limitations of the current endoscopic and imaging techniques. In this article, we presented a comprehensive review about emerging endoscopic tools for the diagnosis of PCLs.

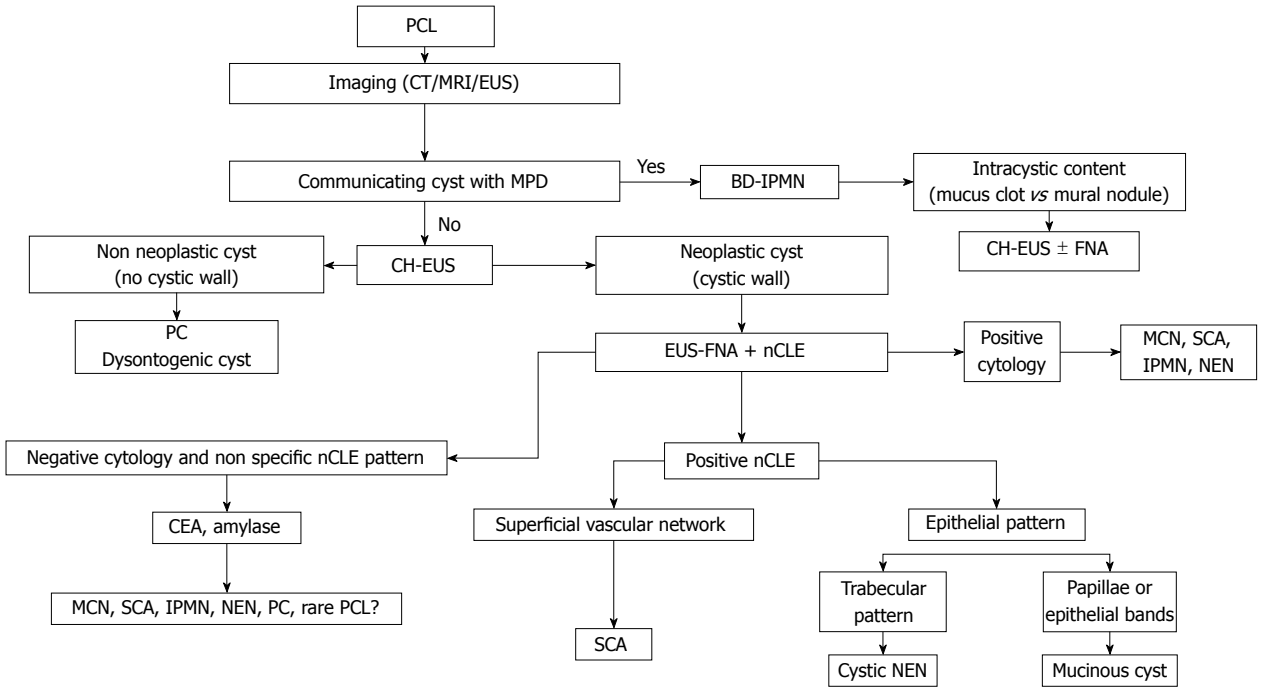

Figure 10 Diagnostic algorithm integrating new endoscopic ultrasonography-based technologies. PCL: Pancreatic cystic lesión; PC: Pseudocyst; MPD: Main pancreatic cyst; BD-IPMN: Branch duct intraductal pancreatic mucinous neoplasm; MCN: Mucinous cystic neoplasm; SCA: Serous cystadenoma; NEN: Neuroendocrine tumour; CT: Computed tomography, MRI: Magnetic resonance imaging; EUS: Endoscopic ultrasonography; CH-EUS: Contrast harmonic enhanced EUS; EUS-FNA: EUS guided fine needle aspiration; nCLE: Needle-based confocal endomicroscopy.

Among them, through-the-needle cystoscopy and biopsy still have the lowest amounts of available evidence, with the most extensive experience reported for CH-EUS and nCLE. Both modalities have been demonstrated to provide valuable information for the decision-making process and to be supplementary techniques to EUS-FNA. Limitations for their widespread implementation are their elevated cost and learning curve. Future studies should address their clinical impact on patient management, the optimal timing for their application in the diagnostic work flow of PCLs and their cost-effectiveness. Through-the-needle cystoscopy and intracystic biopsy must be further evaluated in formal and larger trials. Moreover, because a combination of these new techniques may further improve our ability to diagnose PCLs, multi-arm trials incorporating these new technologies and emergent molecular markers would be of most value to determine the best diagnostic approach.

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ORIGINAL ARTICLE

Basic Study

Total polysaccharides of the Sijunzi decoction attenuate tumor necrosis factor-α-induced damage to the barrier function of a Caco-2 cell monolayer *via* the nuclear factor-κB-myosin light chain kinase-myosin light chain pathway

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Author contributions: Han L conceived and designed research; Lu Y, Li L and Zhang JW performed research; Lu Y and Zhong XQ analyzed data; Lu Y wrote the manuscript; Wei JA and Han L approved and reviewed the final manuscript; all authors have read and agreed with the final manuscript.

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Abstract

AIM

To explore the protective effects and underlying mechanisms of total polysaccharides of the Sijunzi decoction (TPSJ) on the epithelial barriers *in vitro*.

METHODS

Caco-2 cell monolayers were treated with or without TPSJ in the presence or absence of TNF-α, and paracellular permeability and transepithelial electrical resistance (TEER) were measured to evaluate the epithelial barrier function. Immunofluorescence and western blotting were respectively used to evaluate the distribution and expression of the tight junction proteins claudin 1, claudin 2, zo3, and occludin in Caco-2 cells. Western blotting was also used to evaluate the cellular expression of myosin light chain (MLC), phosphorylated MLC (pMLC), MLC kinase (MLCK), and nuclear factor (NF)-κB p65.

RESULTS

TPSJ promoted the proliferation of Caco-2 cells and inhibited TNF-α-induced secretion of pro-inflammatory cytokines. Furthermore, TPSJ significantly ameliorated both

the reduction of TEER and the increased paracellular permeability observed in tumor necrosis factor (TNF)-α-damaged Caco-2 monolayers. Furthermore, TPSJ remarkably attenuated TNF-α-induced morphological changes, downregulated the expression of claudin 1, claudin 2, zo3, and occludin, and markedly suppressed TNF-α-mediated upregulation of p-MLC and MLCK expression. Finally, TPSJ inhibited the activation and expression of NF-κB p65.

CONCLUSION

Our results demonstrate that TPSJ alleviates the TNF-α-induced impairment of the intestinal epithelial cell barrier function by suppressing NF-κB p65-mediated phosphorylation of MLCK and MLC.

Key words: Inflammatory bowel disease; Tight junction; Total polysaccharides of the Sijunzi decoction; Nuclear factor-κB pathway

Core tip: Total polysaccharides of the Sijunzi decoction (TPSJ) comprise the active ingredient of Sijunzi decoction, which has long been used to treat gastrointestinal tract disorders. However, the mechanisms by which TPSJ affects the intestinal epithelial barrier remain unclear. Our study results demonstrated that TPSJ attenuated tumor necrosis factor (TNF)-α-induced intestinal barrier dysfunction in a Caco-2 cell monolayer. Furthermore, TPSJ inhibited TNF-α-induced upregulation of myosin light chain (MLC) phosphorylation, which is mediated by MLC kinase and NF-κB, suggesting that this mechanism might underlie the protective effects of TPSJ against intestinal epithelial barrier dysfunction triggered by proinflammatory cytokines.

Lu Y, Li L, Zhang JW, Zhong XQ, Wei JA, Han L. Total polysaccharides of the Sijunzi decoction attenuate tumor necrosis factor-α-induced damage to the barrier function of a Caco-2 cell monolayer *via* the nuclear factor-κB-myosin light chain kinase-myosin light chain pathway. *World J Gastroenterol* 2018; 24(26): 2867-2877 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i26/2867.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i26.2867

INTRODUCTION

The intestinal epithelial barrier plays a crucial role in separating luminal microbes and antigenic molecules from the internal milieu. Accordingly, intestinal barrier dysfunction can destroy immune homeostasis and induce an inflammatory response[1,2]. Tight junctions (TJs), which are mediated by proteins such as claudins, occludin, and zonula occludens, are necessary for epithelial barrier maintenance[3-5]. Disruption of the intestinal epithelial barrier can increase intestinal permeability, a crucial pathogenic contributor to intestinal inflammation[6,7]. Intestinal epithelial barrier disruption is common to many inflammatory enteropathies, including Crohn's disease (CD), ulcerative colitis (UC), and infectious diarrhea[8-11].

Many locally released pro-inflammatory cytokines and mediators, including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1β, Th17 type cytokines (IL-17, IL-23, IL-22, and IL-6), and nitric oxide (NO) have been recognized to contribute to intestinal barrier dysfunction during the course of inflammatory bowel disease *in vitro* and *in vivo*[12-17]. Additionally, myosin light chain kinase (MLCK), which mediates the phosphorylation of myosin light chain (MLC), is thought to play a critical role in proinflammatory cytokine-induced intestinal barrier disruption[18,19].

Sijunzi decoction (SJZD) is a traditional medicinal formula comprising four Chinese herbs: *Ginseng Radix et Rhizoma* or *Codonopsispilosula, Atractylodes Macrocephalae Rhizoma*, *Poria*, and *Glycyrrhizae Radix et Rhizoma Praeparatecum Melle*. This decoction, which is used to strengthen the spleen and tonify the qi, has been used to treat gastrointestinal tract diseases since ancient times[20-22]. In a previous study, SJZD was shown to regulate both digestive system and immune system function[23]. In mice, total polysaccharides of the Sijunzi decoction (TPSJ) were shown to antagonize cyclophosphamide-induced injury to the intestinal mucosal associated lymphoid tissues[24], suggesting that these polysaccharides could improve intestinal mucosal immune function. Another study found that polysaccharides of the SJZD can restore intestinal function and protect against indomethacin-induced damage to IEC-6 rat intestinal epithelial cells[25].

Our previous studies suggested that TPSJ could inhibit the proliferation of IEC-6 cells *in vitro*. However, no reports have discussed the ability of TPSJ to regulate the intestinal epithelial barrier. In the present study, we explored the effects of TPSJ on the intestinal barrier formed by Caco-2 human colon adenocarcinoma cells damaged by the proinflammatory cytokine TNF-α, as well as the underlying mechanism. Our results indicate that TPSJ could relieve the intestinal epithelial barrier dysfunction induced by TNF-α, and that this function was mediated by the downregulation of MLCK-dependent MLC phosphorylation in a manner dependent on nuclear factor (NF)-κB p65.

MATERIALS AND METHODS

Plant materials

The Sijunzi Decoction (SJZD) used in this research comprised *Ginseng Radix et Rhizoma* or *Codonopsispilosula, Atractylodes Macrocephalae Rhizoma*, *Poria* and *Glycyrrhizae Radix et Rhizoma Praeparatecum Melle*. These four drugs were pharmacopoeia-grade. All herbs were obtained from Kangmei Pharmaceutical Company Ltd. (Guangzhou, Guangdong, China).

Reagents

Dulbecco's modified Eagle's medium (DMEM), non-

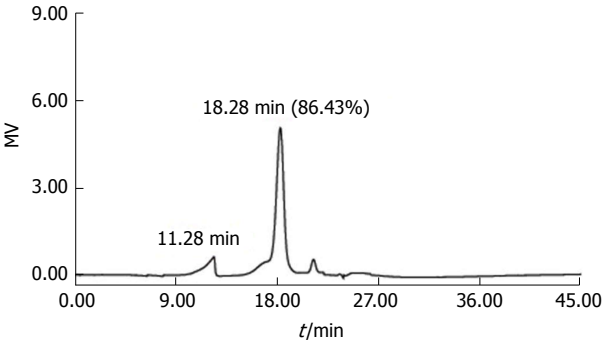

Figure 1 Gel permeation chromatography of total polysaccharides of the Sijunzi decoction.

essential amino acids (NEAA), and fetal bovine serum (FBS) were obtained from GIBCO Laboratories (Grand Island, NY, United States). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and phenol-sulfonphthalein were obtained from Sigma-Aldrich (St. Louis, MO, United States). Fluoroisothiocyanate (FITC)-conjugated Annexin V and propidium iodide (PI) were purchased from Lianke Biotechnology Co (Hangzhou, China). Antibodies specific for claudin 1, claudin 2, zo3, occludin, MLC (phospho S20), MLC (pan), and MLCK and the NF-κB p50/p65 transcription factor assay kit were all purchased from Abcam (Cambridge, MA, United States). Enzyme-linked immunosorbent (ELISA) kits for TNF-α, IL-6 and IL-8 were obtained from eBioscience (San Diego, CA, United States).

Preparation of TPSJ

SJZD comprised *Ginseng Radix et Rhizoma* or *Codonopsispilosula, Atractylodes Macrocephalae Rhizoma*, *Poria*, and *Glycyrrhizae Radix et Rhizoma Praeparatecum Melle* at a ratio of 3:3:3:2 to yield a total weight of 1100 g. All of the herbs were placed in a container to which a volume of cold water approximately 12 times (7.2 L) the solid volume was added. After soaking the herbs for 2 h, we boiled the mixture for 30 min and filtered the herbs, reserving the filtrate. Subsequently, we added another volume of water approximately 5 times the volume of herbs to the container and boiled the mixture for 30 min, followed by filtration. We then mixed the two filtrates and concentrated the liquid to 1.4 L. Subsequently, we added ethanol to the filtrates to yield an alcohol concentration of 75% and stored them at 4 ℃ overnight. The next day, we filtered, precipitated, and dissolved the ethanol mixture in approximately 1.6 L of ultrapure water, followed by centrifugation at 8400 rpm for 15 min. The resulting supernatant was frozen and dried to yield the total polysaccharide. A phenol-sulfuric acid spectrophotometry method was used to measure the polysaccharide content (as glucose), which was 70.61% ± 1.70%, according to at least three independent experiments. Figure 1 depicts the gel permeation chromatography (GPC) analysis of TPSJ[26].

Cell culture

Caco-2 human colon adenocarcinoma cells were obtained from the Cell Culture Unit of Shanghai Science Academy (Shanghai, China). The cells were grown in DMEM supplemented with 10% FBS and 1% NEAA and incubated in a humidified atmosphere with 5% CO_2 atmosphere at 37 ℃.

MTT assay

Cell viability was determined using a MTT reduction assay. Cells were seeded into 96-well plates in DMEM + 10% FBS + 1% NEAA at a density of 5000 per well and treated with 100 ng/mL TNF-α. After a 24-h incubation, TPSJ or DMEM (control) was added to the wells, followed by another 24-h incubation. Subsequently, 10 μL of MTT solution was added to each well, and the plates were incubated for 4 h. Finally, we lysed the cells with 0.04 N HCl in isopropyl alcohol and read the absorbance of each well at 570 nm.

Flow cytometric quantification of apoptosis

To assess apoptosis, we harvested Caco-2 cells. After two washes with phosphate-buffered saline (PBS), we resuspended the cells in 200 μL of Annexin-V binding buffer (10 mmol/L HEPES, 140 mmol/L NaCl, 2 mmol/L $MgCl_2$, 5 mmol/L KCl, 2.5 mmol/L $CaCl_2$, pH 7.4) and added 10 μL of FITC-conjugated Annexin V to each tube according to the manufacturer's protocol. Following a 15 min incubation in the dark at room temperature, we added 10 μL of PI and 200 μL binding buffer to each tube. Finally, we analyzed the samples on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, United States).

Measurements of electrical resistance

We used an EVOM TEER meter (Millipore, Bedford, MA, United States) to monitor the transepithelial electrical resistance (TEER) of Caco-2 cells. Specifically, an increase in TEER to a steady state exceeding 200 Ω cm^2 at day 7 indicated the complete formation of tight junctions and full epithelial barrier integrity. In our experiments, we treated cell monolayers with recombinant human TNF-α (100 ng/mL) for 24 h and subsequently added 150 μg/ml TPSJ or not to the wells. Monolayers treated with cytokine alone or DMEM alone were used as controls.

Permeability study by colorimetric assay

Caco-2 cells were grown on inserts. Firstly, we washed the cell monolayers with PBS. Next, we added phenol-sulfonphthalein to the apical compartment to a final concentration of 20 mg/L in ultrapure water. We added only water to the basolateral compartment. After a 4-h incubation, we removed 150 μL aliquots from the basolateral compartment into tubes containing 1.5 mL NaOH (20 μmol/mL). We then analyzed the absorbance of each tube at 570 nm using a spectrophotometer.

ELISA

We collected culture medium of from Caco-2 cells and used ELISA kits (eBioscience) to measure the amounts of TNF-α, IL-6, and IL-8 according to the manufacturer's instruction.

Immunofluorescence

We seeded Caco-2 cells on glass cover slips placed in the wells of a 6-well plate and treated the cells with TNF-α (100 ng/mL) for 24 h without or with 150 μg/mL TPSJ. The immunofluorescence assay was performed according to the protocol with antibodies specific for claudin 1, claudin 2, zo3, and occludin, followed by incubation with a FITC-conjugated anti-rabbit IgG (1:200 dilution). Images were captured using a fluorescence microscope (Olympus, BX51, Tokyo, Japan).

Measurement of NF-κB p65 activity

We used a NF-κB p50/p65 transcription factor assay kit to measure the NF-κB p65 activity in prepared cellular extracts according to the manufacturer's instructions.

Western blot

We plated Caco-2 cells in 6-well plates at a density of 1 × 10^6 per well. The cells were treated with recombinant human TNF-α (100 ng/mL) for 24 h without or with 150 μg/mL TPSJ for different time intervals.

At different time points, we lysed the cells in lysis buffer [50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 0.1% sodium dodecylsulfate (SDS), 1% sodium deoxycholate, 1 mmol/L phenylmethylsulphonyl fluoride, 1% Triton X-100 and protease inhibitors] and subsequently removed cell debris by centrifugation (15000 rpm, 15 min, 4 ℃). After determining the protein concentrations of the samples, we separated equal amounts of protein by 12.5% SDS-PAGE and transferred the proteins to nitrocellulose membranes. After blocking the membranes, we incubated them overnight at 4 ℃ with different primary antibodies, followed by a 1 h incubation with secondary antibodies. Finally, protein expression was evaluated using a Bio-Rad Imaging System (Bio-Rad Biosciences, Hercules, CA, United States).

Statistical analysis

The results are expressed as means ± standard errors of the means (SEM). Student's t-test was used for the statistical analysis, and a P value < 0.05 was considered significant. At least three independent experiments were performed.

RESULTS

TPSJ boosts the proliferation of TNF-a-damaged Caco-2 cells

We first used a MTT assay to assess the effect of TPSJ on TNF-α-damaged Caco-2 cells. As shown in Figure 2A, TPSJ dramatically induced the growth of TNF-α-treated Caco-2 cells in a dose-dependent manner, particularly at a concentration of 150 μg/mL. However, TPSJ treatment had no significant effect on the frequency of cell apoptosis in comparison to the TNF-α control group (Figure 2B and C).

TPSJ ameliorates the intestinal epithelial barrier dysfunction induced by TNF-α

Many investigators have shown that TNF-α disrupts intestinal barrier function by decreasing the TEER and increasing paracellular permeability[19,27]. Therefore, to investigate the effects of TPSJ on intestinal barrier function, we treated a Caco-2 cell monolayer with TNF-α for 24 h and subsequently analyzed the TEER and permeability of the cells after TPSJ treatment.

As shown in Figure 3A, the TEER of the TNF-α-damaged Caco-2 cell monolayer decreased significantly compared with the control group, indicating that TNF-α upregulated the paracellular permeability of ionic solutes. By contrast, TPSJ treatment significantly increased the TEER.

As shown in Figure 3B, the phenolsulfonphthalein flux was significantly higher in the TNF-α-damaged Caco-2 cell monolayer than in the control monolayer, indicating this inflammatory cytokine increased the paracellular permeability of nonionic macromolecules. However, TPSJ markedly decreased the increased phenolsulfonphthalein flux induced by TNF-α. These results suggest that TPSJ can attenuate the intestinal epithelial barrier dysfunction induced by TNF-α.

TPSJ decreased the secretion of pro-inflammatory cytokines by TNF-α-induced Caco-2 cells

The ELISA results shown in Figure 4 demonstrate significant increases in the levels of TNF-α, IL-6, and IL-8 secreted by Caco-2 cells into the culture medium after TNF-α treatment. However, TPSJ markedly decreased the secretion of these cytokines in response to TNF-α. These results indicate that TPSJ can regulate TNF-α-induced production of pro-inflammatory factors.

TPSJ protected a Caco-2 cell monolayer from TNF-α-induced barrier dysfunction by regulating tight junctions

Increasing evidence suggests that altered tight junction protein expression contributes to the proinflammatory cytokine-induced disruption of barrier function[15,28]. Therefore, we examined the effects of TPSJ on the expression of the tight junction proteins claudin 1, claudin 2, zo3, and occludin in Caco-2 cell monolayers treated with or without TNF-α. As shown in Figure 5, the expression of claudin 1, claudin 2, and zo3 proteins were significantly downregulated by TNF-α. After TPSJ treatment, however, the expressions of all three proteins were upregulated markedly at different time points. By contrast, the expression of occludin was not significantly affected by treatment with or without TNF-α or in the absence or presence of TPSJ.

Reports have demonstrated an association of proinflammatory cytokine-induced intestinal barrier dysfunction with the morphological alterations and relocalization of the tight junction[29-31]. Thus, we next determined whether TPSJ affected the morphological localization of tight junctions in Caco-2 cell monolayers treated with or without TNF-α. As shown in Figure 6, claudin 1, claudin 2, zo3, and occludin were localized along the edges of cells in the control group. However, a 24-h treatment with TNF-α rendered the tight junction distribution irregular and discontinuous, and led to the partial internalization of occludin into cytoplasmic vesicles. After TPSJ treatment,

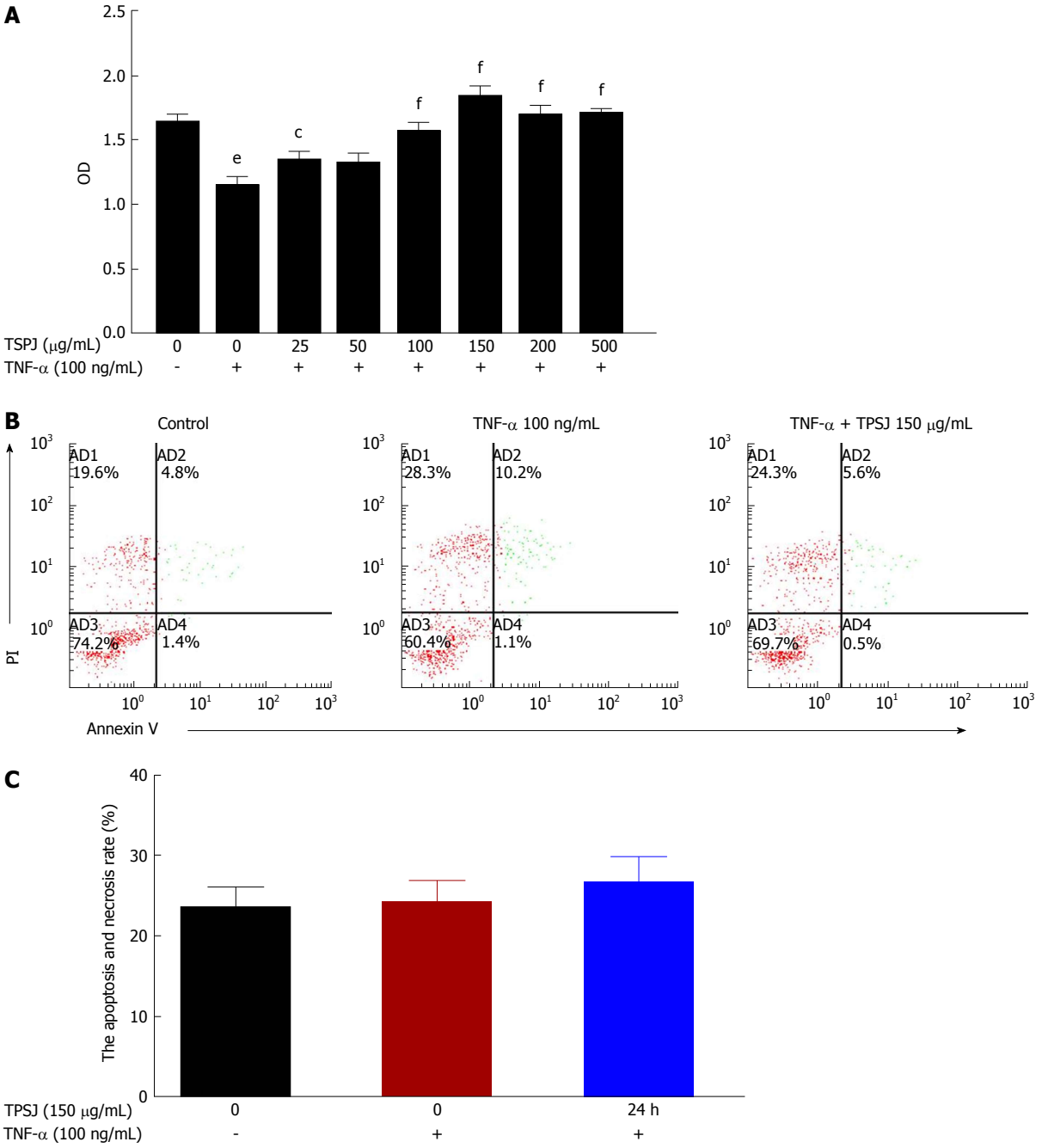

Figure 2 Total polysaccharides of the Sijunzi decoction promoted the proliferation of tumor necrosis factor-α-damaged Caco-2 cells. A: Caco-2 cells were treated with various concentrations of TPSJ (25-500 μg/mL) or control DMEM for 24 h. The effect of TPSJ on cell viability was measured using the MTT assay; B: TNF-α-damaged Caco-2 cells were incubated with 150 μg/mL TPSJ or control DMEM for 24 h. The induction of apoptosis was determined using an Annexin V-FITC/propidium iodide staining assay; C: Quantification of the numbers of apoptotic and necrotic cells. Data are shown as the means ± standard errors of the means of at least three independent experiments ($^eP < 0.001$ *vs* control Caco-2 cells; $^cP < 0.05$, $^fP < 0.001$ *vs* TNF-α-damaged Caco-2 cells). TPSJ: Total polysaccharides of the Sijunzi decoction; TNF: Tumor necrosis factor; DMEM: Dulbecco's modified Eagle's medium; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

however, the reorganization of claudin 1, claudin 2, zo3, and occludin was significantly attenuated. These results indicate that TPSJ can prevent the proinflammatory cytokine-induced reorganization of tight junctions in a Caco-2 cell monolayer.

TPSJ suppresses TNF-α-induced upregulation of MLC phosphorylation and MLCK expression

The MLCK-mediated phosphorylation of MLC has been reported to play a crucial role in the regulation of intestinal epithelial tight junctions and paracellular leakage pathways[3,4]. Given the protective effect of TPSJ on intestinal barrier function, we wished to explore whether TPSJ could alleviate TNF-α-induced barrier dysfunction and tight junction disruption by inhibiting MLC phosphorylation. As shown in Figure 7A and B, treatment of a Caco-2 monolayer with TNF-α induced a significant increase in the ratio of phosphorylated to total MLC. However, TPSJ treatment markedly downregulated this ratio at 6 h and 9 h (compared with the TNF-α control

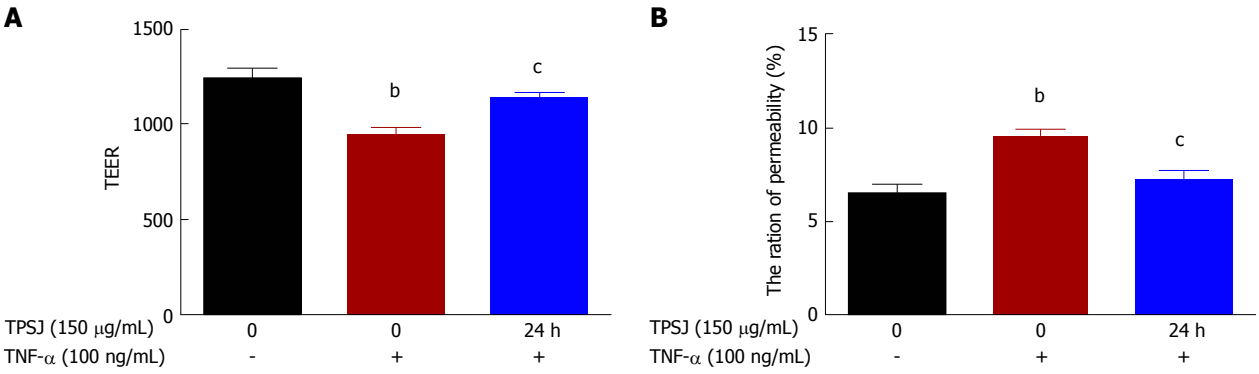

Figure 3 Total polysaccharides of the Sijunzi decoction attenuated intestinal epithelial barrier dysfunction induced by tumor necrosis factor-α. A: Caco-2 monolayers were incubated with or without 100 ng/mL TNF-α in the absence or presence of TPSJ for 24 h. TPSJ significantly increased the transepithelial electrical resistance of Caco-2 cell monolayers damaged by TNF-α; B: TPSJ markedly attenuated phenolsulfonphthalein flux across the epithelial monolayers. Data are shown as the means ± standard errors of the means of at least three independent experiments ($^{b}P < 0.01$ *vs* control Caco-2 cells; $^{c}P < 0.05$ *vs* TNF-α-damaged Caco-2 cells). TPSJ: Total polysaccharides of the Sijunzi decoction; TNF: Tumor necrosis factor.

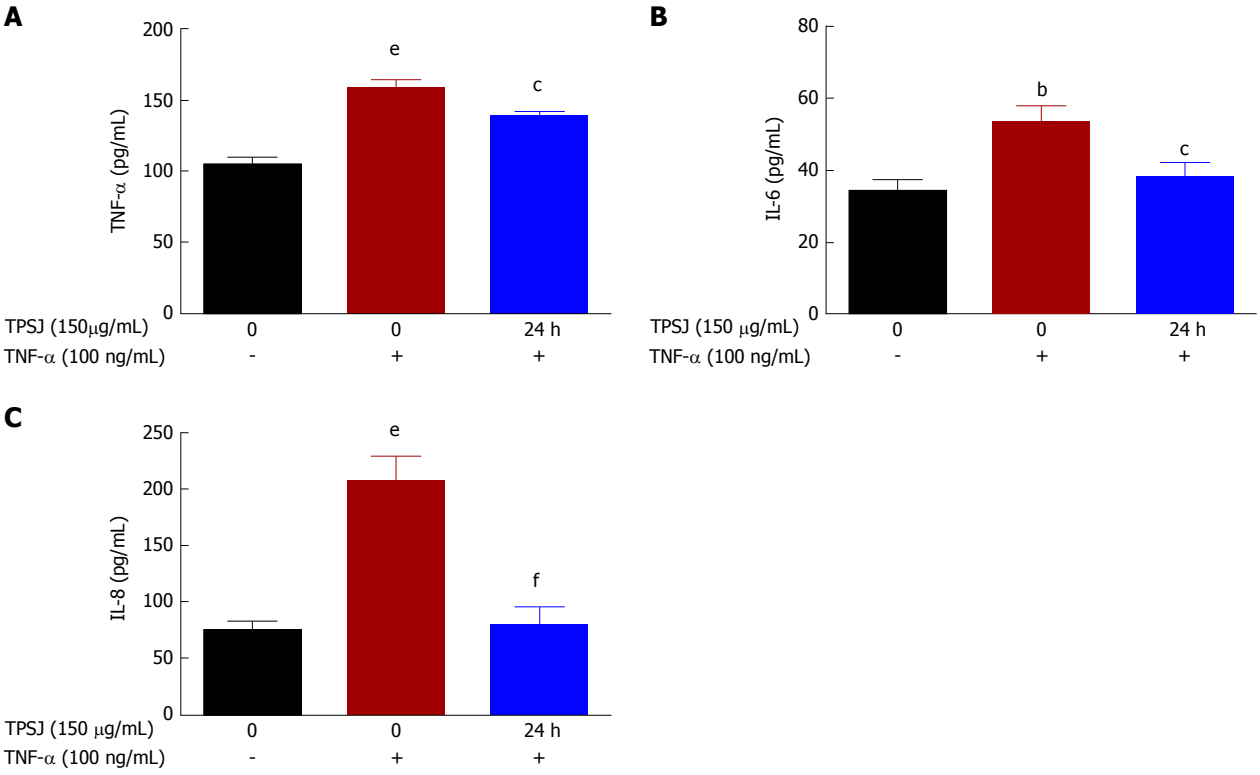

Figure 4 Total polysaccharides of the Sijunzi decoction regulated the secretion of inflammatory cytokines by Caco-2 cells. Caco-2 cells were incubated with or without 100 ng/mL TNF-α in the absence or presence of TPSJ for 24 h. Cell culture media were collected, and the concentrations of TNF-α (A), IL-6 (B), and IL-8 (C) were detected by ELISA ($^{b}P < 0.01$, $^{e}P < 0.001$ *vs* control Caco-2 cells; $^{c}P < 0.05$, $^{f}P < 0.001$ *vs* TNF-α-damaged Caco-2 cells). TPSJ: Total polysaccharides of the Sijunzi decoction; TNF: Tumor necrosis factor.

group).

MLCK is a well-known and predominant regulator of MLC phosphorylation, and many studies have indicated an association of MLCK upregulation with tight junction dysfunction and paracellular hyperpermeability[30,31]. Therefore, we next investigated the effect of TPSJ on MLCK expression in Caco-2 cell monolayers treated with or without TNF-α. As shown in Figure 7C and D, MLCK expression increased significantly in the TNF-α-treated monolayer. However, TPSJ treatment significantly reduced MLCK expression at 9 h, compared with the TNF-α control group. These results suggest that TPSJ attenuates TNF-α-induced intestinal barrier disruption by suppressing the MLCK-mediated phosphorylation of MLC.

TPSJ attenuated TNF-α-induced intestinal epithelial barrier dysfunction by inhibiting NF-κB p65

Studies have shown that NF-κB activation plays a role in intestinal barrier dysfunction as well as in the upregulation of MLCK in TNF-α treated intestinal epithelial cells[32,33]. Based on the above results, we aimed to investigate further the potential involvement of the NF-κB signa-

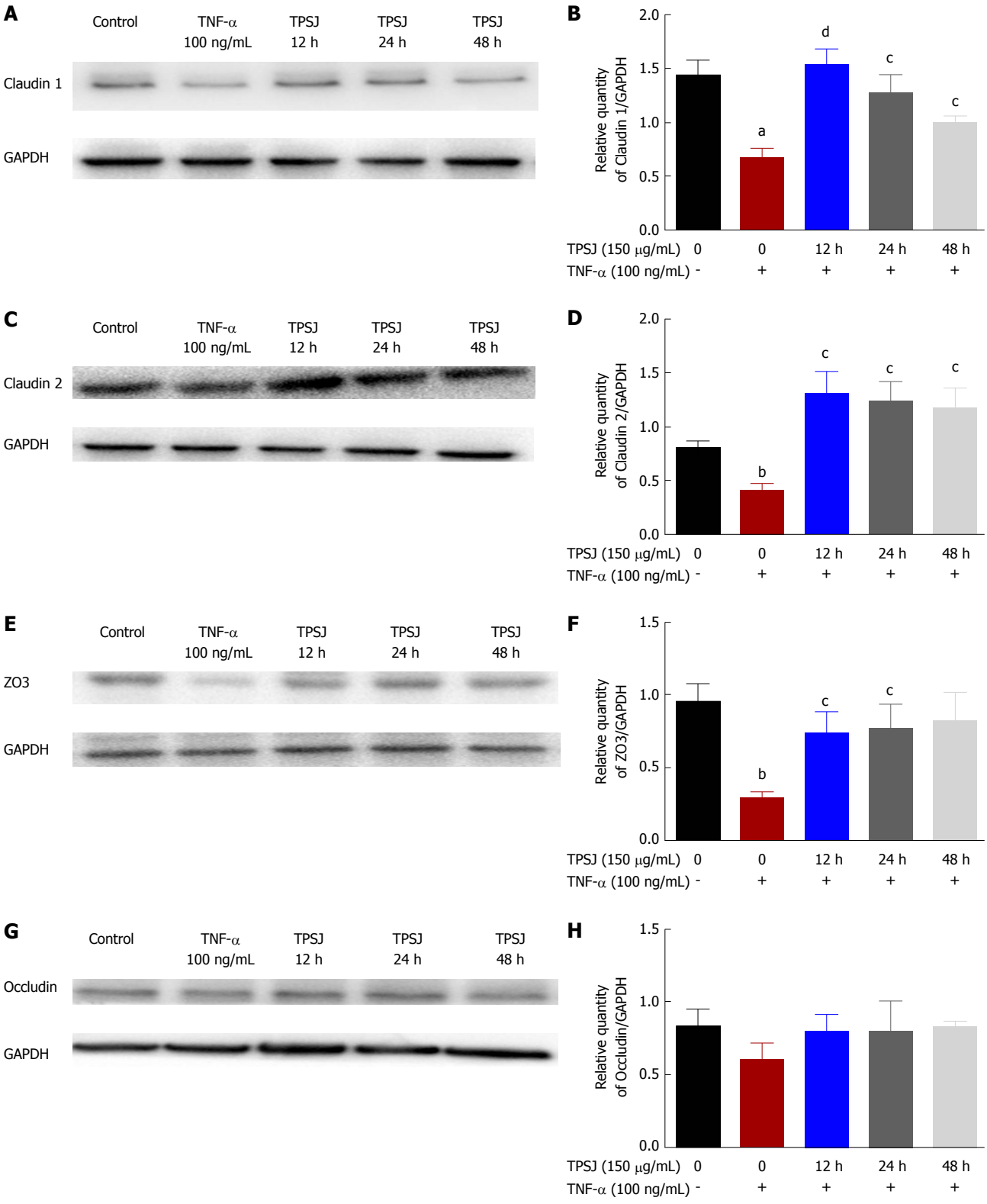

Figure 5 Total polysaccharides of the Sijunzi decoction modified the expression of claudin 1, claudin 2, zo3, and occludin. A: A representative western blot of claudin 1 expression in TNF-α-damaged Caco2 cells treated with TPSJ for 12 h, 24 h, and 48 h; B: Quantification of the amounts of claudin 2 relative to GAPDH at different time points; C: A representative western blot of claudin 2 in TNF-α-damaged Caco-2 cells treated with TPSJ for 12 h, 24 h, and 48 h; D: Quantification of the amounts of claudin 2 relative to GAPDH at different time points; E: A representative western blot of zo3 in TNF-α-damaged Caco-2 cells treated with TPSJ for 12 h, 24 h, and 48 h; F: Quantification of the amounts of zo3 relative to GAPDH at different time points; G: A representative western blot of occludin in TNF-α-damaged Caco-2 cells treated with TPSJ for 12 h, 24 h, and 48 h. H: Quantification of the amounts of occludin relative to GAPDH at different time points. Data are shown as the means ± standard errors of the means of at least three independent experiments ($^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control Caco-2 cells; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* TNF-α-damaged Caco-2 cells). TPSJ: Total polysaccharides of the Sijunzi decoction; TNF: Tumor necrosis factor.

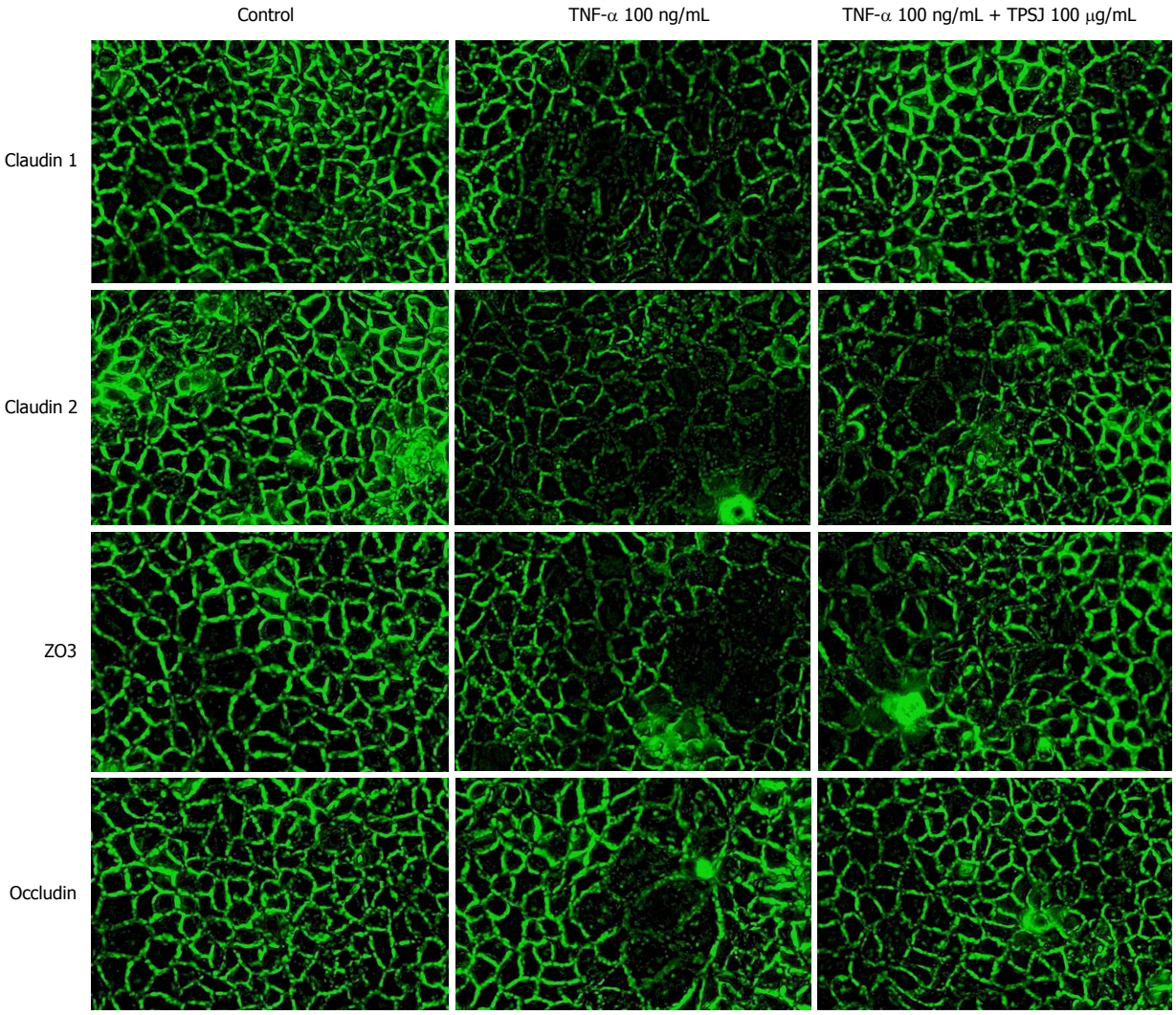

Figure 6 Immunofluorescence analysis of the effects of total polysaccharides of the Sijunzi decoction on the tight junction proteins claudin 1, claudin 2, zo3, and occludin in tumor necrosis factor-α-damaged Caco-2 cells. Results are representative of three independent experiments. Original magnification = 400 ×.

ling pathway in the protective effect of TPSJ against TNF-α-induced epithelial barrier dysfunction. As demonstrated in Figure 8A, NF-κB p65 activity was upregu-lated in the TNF-α-treated Caco-2 cell monolayer. TPSJ treatment, however, significantly inhibited NF-κB p65 activity at 6 h and 9 h compared with the TNF-α-treated Caco-2 monolayer. Similarly, as shown in Figure 8B and C, the nuclear expression of NF-κB p65 increased following TNF-α treatment, whereas TPSJ markedly downregulated the nuclear expression at 6 h and 9 h.

DISCUSSION

Inflammatory bowel diseases, including UC and CD, are well-known chronic and recurring inflammatory diseases of the intestinal tract. Although the pathogenesis of inflammatory bowel diseases is not fully elucidated, all are characterized by the overproduction of proinflammatory cytokines within the mucosa and the disruption of epithelial barrier function. However, many research groups have demonstrated that proinflammatory cytokines may disrupt intestinal barrier function both *in vivo* and *in vitro*[28,29,34]. Therefore, restoration of the intestinal barrier function is a worthwhile strategy for the treatment of inflammatory bowel diseases.

As noted above, TPSJ was previously reported to antagonize cyclophosphamide-induced mucosal-associated lymphoid tissue injury in an animal model, suggesting a potential beneficial role in intestinal mucosal immune function[24]. In this study, we showed that TPSJ could attenuate intestinal epithelial barrier dysfunction caused by proinflammatory cytokines in a Caco-2 cell monolayer. Specifically, TPSJ alleviated the TNF-α-induced decrease in TEER and increase in paracellular permeability, enhanced the expression of claudin 1, claudin 2, and zo3, and preserved the morphological distributions of these three tight junction proteins and occludin.

The molecular mechanism by which TPSJ ameliorates the proinflammatory cytokine-induced intestinal barrier dysfunction is currently unknown. Many research groups have demonstrated that the upregulation of MLCK and subsequent increase in MLC phosphorylation are es-

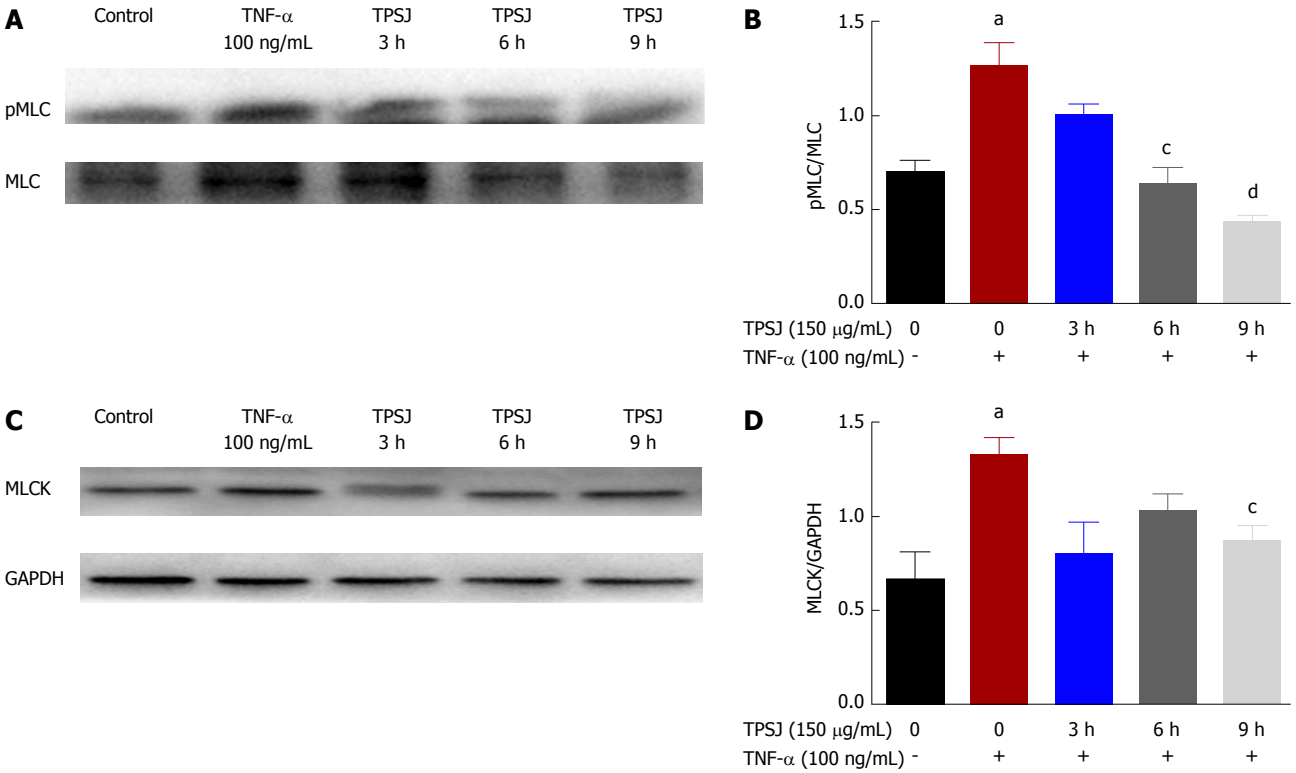

Figure 7 Total polysaccharides of the Sijunzi decoction inhibited tumor necrosis factor-α-induced increases in myosin light chain phosphorylation and myosin light chain kinase protein expression. A: A representative western blot of pMLC and MLC in TNF-α-damaged Caco-2 cells treated with TPSJ; B: Quantification of the amounts of pMLC relative to MLC; C: A representative western blot of MLCK in TNF-α-damaged Caco-2 cells treated with TPSJ; D: Quantification of the amounts of MLCK relative to GAPDH. Data are shown as the means ± standard errors of the means of at least three independent experiments ($^{a}P < 0.05$ *vs* control Caco-2 cells; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* TNF-α-damaged Caco-2 cells). TPSJ: Total polysaccharides of the Sijunzi decoction; MLC: Myosin light chain; MLCK: MLC kinase; TNF: Tumor necrosis factor.

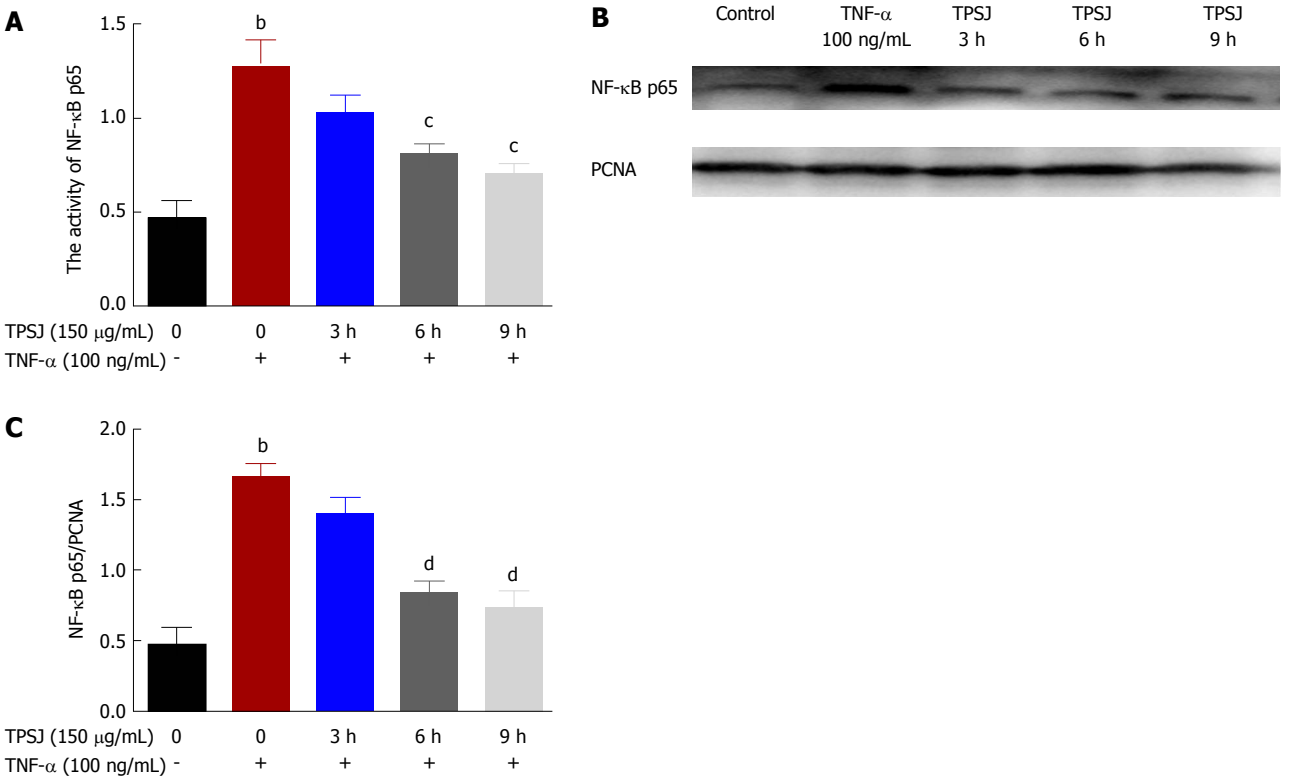

Figure 8 Effects of total polysaccharides of the Sijunzi decoction on the nuclear factor-κB signaling pathway. A: TPSJ upregulated the activity of the NF-κB transcription factor p65; B: A representative western blot of NF-κB p65 in TNF-α-damaged Caco-2 cells treated with TPSJ; C: Quantification of the amounts of p65 relative to PCNA. Data are shown as the means ± standard errors of at least three independent experiments $^{b}P < 0.01$ *vs* control Caco-2 cells; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* TNF-α-damaged Caco-2 cells). TPSJ: Total polysaccharides of the Sijunzi decoction; NF: Nuclear factor; TNF: Tumor necrosis factor.

sential to the induction of intestinal barrier defects by proinflammatory cytokines[9,12]. In our study, we found that TPSJ inhibited increases in MLC phosphorylation and MLCK expression in Caco-2 cell monolayers exposed to TNF-α, suggesting that TPSJ may attenuate proinflammatory cytokine-induced intestinal barrier dysfunction by inhibiting MLCK activation and subsequent MLC phosphorylation. However, other potential molecular mechanisms remain to be investigated further.

According to previously published reports, activated NF-κB mediates the increased intestinal epithelial tight junction permeability induced by TNF-α[27] and contributes to MLCK upregulation in a Caco-2 cell monolayer exposed to proinflammatory cytokines[12,32]. In this study, we showed that TPSJ could suppress the activation and expression of NF-κB p65 in a Caco-2 cell monolayer treated with TNF-α. Our results suggest that the mechanism by which TPSJ attenuates TNF-α-induced barrier dysfunction in the Caco-2 cell monolayer is mediated by the NF-κB signaling pathway.

In conclusion, our results demonstrate that TPSJ attenuates the intestinal barrier dysfunction elicited by TNF-α treatment in a Caco-2 cell monolayer. We further demonstrate that TPSJ inhibits the TNF-α-induced upregulation of MLC phosphorylation, which is mediated by MLCK and NF-κB. These factors may comprise the mechanism by which TPSJ protects the intestinal epithelial barrier from destruction triggered by proinflammatory cytokines.

ARTICLE HIGHLIGHTS

Research background

Sijunzi decoction (SJZD) is a traditional Chinese medicinal prescription that has been used to treat gastrointestinal tract diseases since ancient times. Our previous studies suggested that total polysaccharides of the Sijunzi decoction (TPSJ) could inhibit the proliferation of IEC-6 rat intestinal epithelial cells *in vitro*. However, no report has discussed the regulatory effects of TPSJ on the intestinal epithelial barrier.

Research motivation

Although TPSJ may inhibit intestinal epithelial cell proliferation, the mechanism by which it mediates barrier protection remains unclear.

Research objectives

To explore the protective effects of TPSJ on the epithelial barrier and the mechanism by which it mitigates tumor necrosis factor α (TNF-α)-induced damage in a Caco-2 cell monolayer.

Research methods

We first used a MTT assay to assess the effect of TPSJ on TNF-α-damaged Caco-2 cells. Secondly, we treated a Caco-2 cell monolayer with TNF-α for 24 h and subsequently analyzed the TEER, permeability, and cytokines of the cells after TPSJ treatment. Third, we examined the effects of TPSJ on the expression of the tight junction proteins claudin 1, claudin 2, zo3, and occludin by immunofluorescence and western blotting. Finally, we investigated the NF-κB-MLCK-MLC pathway in TNF-α treated intestinal epithelial cells.

Research results

TPSJ promoted the growth of TNF-α-treated Caco-2 cells in a dose-dependent manner and decreased the secretion of pro-inflammatory cytokines in response to TNF-α. Secondly, TPSJ treatment significantly increased the TEER and decreased the increased phenolsulfonphthalein flux induced by TNF-α. Third, TPSJ markedly upregulated the expression of claudin 1, claudin 2, and zo3 proteins and attenuated the reorganization of claudin 1, claudin 2, zo3, and occludin. Finally, TPSJ suppressed the TNF-a-induced upregulation of myosin light chain (MLC) phosphorylation, MLC kinase (MLCK), and NF-κB p65.

Research conclusions

TPSJ promoted proliferation of TNF-α-treated Caco2 cells. In Caco2 cell monolayers, TPSJ alleviated the TNF-α-induced decrease in TEER and increase in paracellular permeability, enhanced the expression of claudin 1, claudin 2, and zo3, and preserved the morphological distributions of these three tight junction proteins and occludin. Further, we found that the barrier protective effect of TPSJ was mediated through suppressing the NF-κB p65-mediated phosphorylation of MLCK and MLC.

Research perspectives

Our findings provide evidence that TPSJ is a potential protective agent of intestinal barrier function. Further investigation into the mechanism of TPSJ on intestinal barrier as well as *in vivo* research is required.

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ORIGINAL ARTICLE

Retrospective Study

Efficacy and safety of endoscopic submucosal tunnel dissection for superficial esophageal squamous cell carcinoma and precancerous lesions

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Author contributions: Wang J, Zhu LL, Gan T and Yang JL designed the research; Wang J, Zhu XN, Zhu LL, Chen W and Ma YH performed the research; Wang J, Zhu XN, Chen W and Ma YH collected the data; Wang J, Zhu LL and Gan T analyzed the data; Wang J, Zhu LL and Yang JL wrote the paper.

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Data sharing statement: No additional data are available.

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Abstract

AIM

To evaluate the clinical outcomes of patients who underwent endoscopic submucosal tunnel dissection (ESTD) for esophageal squamous cell carcinoma (ESCC) and precancerous lesions.

METHODS

ESTD was performed in 289 patients. The clinical outcomes of the patients and pathological features of the lesions were retrospectively reviewed.

RESULTS
A total of 311 lesions were included in the analysis. The en bloc rate, complete resection rate, and curative resection rate were 99.04%, 81.28%, and 78.46%, respectively. The ESTD procedure time was 102.4 ± 35.1 min, the mean hospitalization time was 10.3 ± 2.8 d, and the average expenditure was 3766.5 ± 846.5 dollars. The intraoperative bleeding rate was 6.43%, the postoperative bleeding rate was 1.61%, the perforation rate was 1.93%, and the postoperative infection rate was 9.65%. Esophageal stricture and positive margin were severe adverse events, with an incidence rate of 14.79% and 15.76%, respectively. No tumor recurrence occurred during the follow-up period.

CONCLUSION
ESTD for ESCC and precancerous lesions is feasible and relatively safe, but for large mucosal lesions, the rate of esophageal stricture and positive margin is high.

Key words: Superficial esophageal squamous cell carcinoma; Endoscopic submucosal tunnel dissection; Efficiency; Safety; Esophageal stricture

Core tip: Endoscopic submucosal tunnel dissection (ESTD) is a modified technique based on endoscopic submucosal dissection. In this paper, we found ESTD is feasible and relatively safe for treating esophageal squamous cell carcinoma (ESCC) and precancerous lesions. The en bloc rate was high, while the adverse event rate was relatively low. When treating large mucosal lesions, ESTD has a high rate of esophageal stricture and positive margin, which requires further treatment. Furthermore, we found that the pathology of preoperative biopsies had to be upgraded after ESTD, which suggests that the accuracy of biopsy to diagnose ESCC should be reconsidered.

Wang J, Zhu XN, Zhu LL, Chen W, Ma YH, Gan T, Yang JL. Efficacy and safety of endoscopic submucosal tunnel dissection for superficial esophageal squamous cell carcinoma and precancerous lesions. *World J Gastroenterol* 2018; 24(26): 2878-2885 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i26/2878.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i26.2878

INTRODUCTION

Endoscopic submucosal dissection (ESD) is becoming the standard treatment for early gastrointestinal cancers, as it has a higher en bloc resection rate and a lower recurrence rate than endoscopic mucosal resection (EMR)[1,2] and it can be used to resect lesions with a diameter greater than 2 cm[3]. However, esophageal ESD faces many difficulties because of the narrow esophageal lumen and thin walls[4-6]. When using conventional ESD treatment for large mucosal lesions, and especially for lesions with a circumference that exceeds three fourths of the esophageal lumen, multiple submucosal injections are required, which could prolong the procedure time and thereby increase the risk of complications[5]. Even worse, with the resected mucosa blocked in the lumen, the endoscopic view becomes unclear and may increase the difficulty of complete resection[6]. To overcome these difficulties, some modified ESD techniques have been introduced, such as the line traction method[6], the clip traction method[7], and the thread-traction method[8]; however, none of these methods was suitable for extensive application.

In 2009, Linghu *et al*[9] used a "submucosal tunnel" to resect successfully a circumferential esophageal mucosal lesion, which was subsequently termed an endoscopic submucosal tunnel dissection (ESTD)[5]. Compared with conventional ESD, ESTD has many technical advantages, as it resects the mucosal lesions by creating a submucosal tunnel between the mucosal layer and muscular layer, after which therapeutic endoscopy can enter the tunnel and acquire a clear operative view. Moreover, the CO_2 injected in the operation can help the blunt dissection of the mucosal layer, thereby reducing the number of submucosal injections, shortening the procedure time, increasing the resection speed, and reducing the injury of the muscular layer[10-12]. This approach can also incise the submucosa more completely, thereby reducing the risk of tumor metastasis and recurrence, as shown in our previous study[13]. However, there are no studies that have verified the feasibility of ESTD in superficial esophageal squamous cell carcinoma (ESCC) and precancerous lesions in a large sample. The aim of this study was to assess the efficacy and safety of ESTD in treating superficial ESCC and precancerous lesions in a relatively large sample.

MATERIALS AND METHODS

Patients and endoscopic characteristics

A prospectively collected endoscopic therapy database was analyzed retrospectively. All of the patients with superficial ESCC and precancerous lesions who underwent ESTD in the Digestive Endoscopy Center of West China Hospital from March 1, 2013 to May 1, 2017 were enrolled. A total of 355 patients with superficial esophageal cancer underwent endoscopic treatment. We excluded patients with esophageal adenocarcinoma, EMR/ESD procedure, and incomplete clinical data. Finally, 289 patients with 311 lesions were analyzed (Figure 1). All of the lesions were confirmed by pathological evaluation from biopsy specimens according to the Japanese classification of ESCC[14] before ESTD procedure. The clinical records and ESTD records were collected, and demographic and endoscopic characteristics were retrospectively reviewed. The endoscopic type of lesion was assessed according to the Paris endoscopic classification[15]. This study was approved

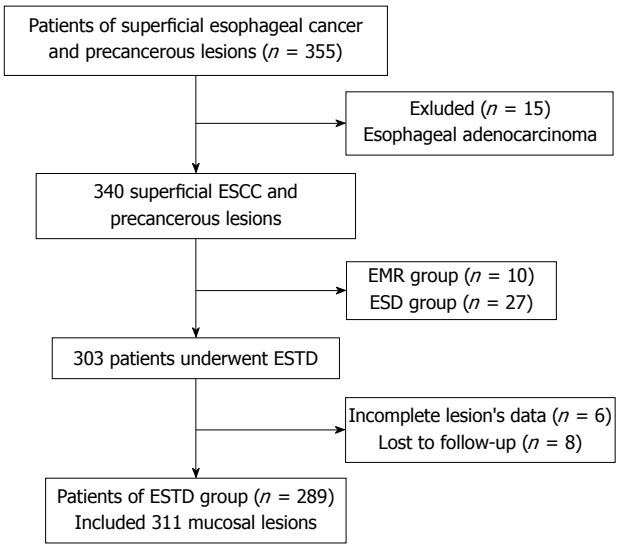

Figure 1 Flowchart of the enrollment process. ESCC: Esophageal squamous cell carcinoma; ESTD: Endoscopic submucosal tunnel dissection; EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection

by the Ethics Committee of the West China Hospital of Sichuan University.

ESTD procedure

Before ESTD, all of the lesions were evaluated by endoscopy, enhanced ultrasound (EUS), and computed tomography (CT) of chest and abdomen. Prophylactic antibiotics were used in patients with large mucosal lesions (circumference ⩾ 3/4) at half an hour before ESTD. ESTDs were performed by one endoscopist with an experience of more than 200 cases of ESD procedure. ESTD procedure included six steps, as shown in Figure 2. When the lesion was detected by white light endoscopy, it was carefully observed under narrow band imaging (NBI) and iodine staining. Next, the margins were marked by a dual knife (KD-650Q, Olympus, Tokyo, Japan). A liquid mixture of 1:10000 adrenaline saline, sodium hyaluronate, glycerin fructose, and indigo carmine was used in submucosal injection. Both anal-side and oral-side incisions were made after submucosal injection, after which the submucosal tunnel was established from the oral side to the anal side and stopped at the anal-side incision. Thereafter, the remaining lateral margin incisions were made; thus, the lesion was completely resected. Finally, wound hemostasis was carefully performed by hemostatic forceps (FD-410LR, Olympus) or argon plasma coagulator (ERBE Corporation).

Postoperative strategies and follow-up

Patients were allowed to feed orally from the third day after ESTD, while treatment with proton pump inhibitors, hemostatics, and nutritional supports was initiated. The vital signs were monitored, and gas-related complications were closely detected, including subcutaneous emphysema, mediastinal emphysema, and pneumoperitoneum. All patients were asked to join in the follow-up plan, and surveillance endoscopy with iodine staining was performed at 1, 3, 6, 12, 24, and 36 mo after ESTD. Biopsies for suspicious lesions were also recommended. The patients with non-curative resection underwent either additional treatment (re-ESD, radiotherapy, surgery) or close surveillance.

Outcome measures

The primary outcomes included en bloc resection rate, complete resection rate, and curative resection rate as well as the data acquired from ESTD procedure, such as procedure time, dissection speed, and the specimen area. The secondary outcomes were the rates of adverse events, including intraoperative and postoperative bleeding, perforation, muscular injury, postoperative infection, esophageal stricture, positive margin, and local tumor reoccurrence. The symptom score of esophageal stricture was assessed according to Stooler's dysphagia score[16].

Definitions

Procedure time was defined as the time from lesion marking to the termination of therapeutic endoscopy. Specimen area was calculated by the formula: S = (a + b)/2 × (c + d)/2, (a and b represent the maximum and minimum values of the length diameter, respectively, while c and d represent the maximum and minimum values of the width diameter, respectively). En bloc resection was defined as resection of the lesion by an entire specimen, while complete resection/R0 resection was defined as an en bloc resection with neoplasia-free margins (both horizontal and vertical margins). Curative resection was pathologically defined as a complete resection with a differentiated carcinoma with < 200 μm submucosal invasion and no lympho-vascular invasion.

Intraoperative bleeding was defined as blood volume > 50 mL and bleeding that could be effectively stopped in ESTD procedure. Postoperative bleeding was defined as the symptoms of hematemesis or/and melena, with hemoglobin levels being decreased by more than 20 g/L within 30 d after ESTD procedure[17]. Perforation was defined as a visible hole in the esophageal wall or the presence of subcutaneous emphysema, pneumothorax, mediastinal emphysema, or pneumoperitoneum.

Esophageal stricture was defined when the standard GIF-Q260J (Olympus) gastroscopy could not pass through the esophageal lumen and if the patient had dysphagia[18]. A positive margin was defined as the presence of a neoplastic cell in the horizontal or vertical margins. Residual tumor was defined as the presence of new tumor lesions in the primary resection site and its surrounding 1 cm area within 6 mo after ESTD. Tumor reoccurrence was defined as the presence of new tumor lesions in the primary resection site and its surrounding 1 cm area over 6 mo after ESTD.

Statistical analysis

Continuous variables are represented by average ± SD and were compared by Student's *t*-test. Categorical variables are represented by the rate and evaluated by Pearson Chi square test or Fisher exact test (SPSS

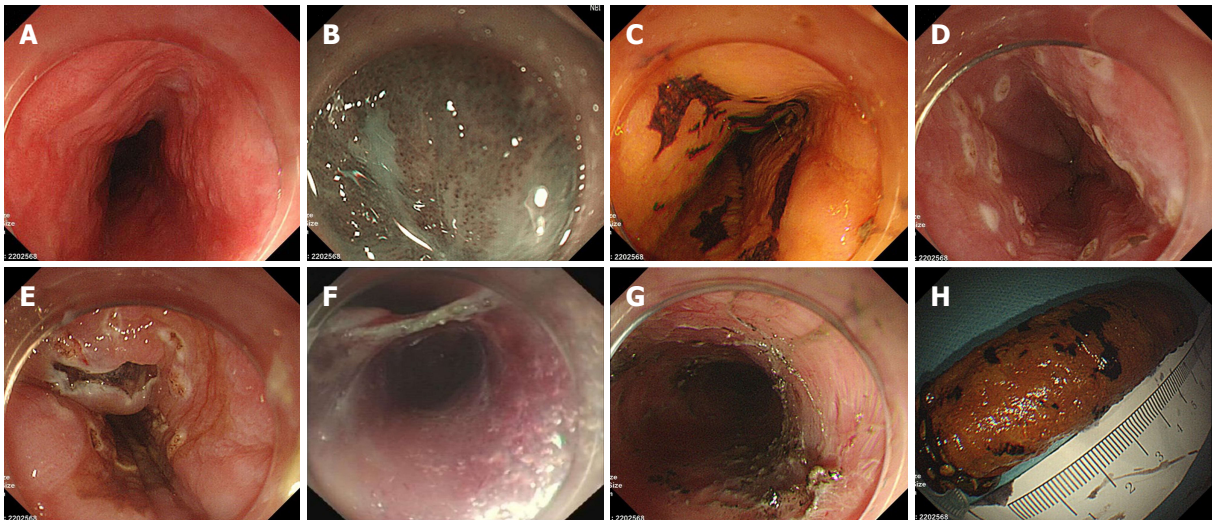

Figure 2 Endoscopic submucosal tunnel dissection procedures. A: Lesion was detected under white light endoscopy; B: Lesion was observed under narrow band imaging (NBI); C: Lesion was observed under iodine staining; D: The margin of the lesion was marked; E: Anal-side and oral-side incisions after submucosal injection; F: Creating the submucosal tunnel and resecting the lesion; G: The artificial wound after endoscopic submucosal tunnel dissection; H: The *in vitro* specimen encircled in the body of a syringe after iodine staining.

Table 1 Baseline characteristics of 311 lesions treated with endoscopic submucosal tunnel dissection *n* (%)

Category	ESTD (*n* = 311)
Sex, male/female	213/98
Age, yr, mean (range)	61.4 ± 8.1 (40-83)
Tumor location	
Upper third	24 (7.72)
Middle third	200 (64.31)
Lower third	87 (27.97)
Paris classification	
0-Ⅰ	18 (5.79)
0-Ⅱa	111 (35.69)
0-Ⅱb	94 (30.23)
0-Ⅱc	35 (11.25)
0-Ⅱa-Ⅱc	50 (16.08)
0-Ⅲ	3 (0.96)
Circumferential level	
≤ 1/4	11 (3.54)
≤ 1/2	163 (52.41)
≤ 3/4	65 (20.90)
≤ 7/8	41 (13.18)
≤ 1	31 (9.97)

ESTD: Endoscopic submucosal tunnel dissection.

version 24.0, SPSS Inc, Armonk, NY, United States). *P*-value < 0.05 indicated statistical significance.

RESULTS

Baseline characteristics of patients

A total of 355 superficial esophageal patients underwent endoscopic treatments from March 1, 2013 to May 1, 2017, of which 66 patients were excluded for the following reasons: (1) Adenocarcinoma (*n* = 15); (2) EMR procedure (*n* = 10) or ESD procedure (*n* = 27); (3) incomplete lesion data (*n* = 6); and (4) lost to follow-up (*n* = 8), as shown in Figure 1. The demographic data are shown in Table 1. The average age of the patients was 61.39 ± 8.07 years with a male/female ratio of 2.17 (213/98). The lesions were mainly located in the middle third of the esophagus (64.31%). Thirty-one circumferential lesions were included in the final analysis (Table 1). The most common preoperative histological type was HGIN, as shown in Table 2.

Table 2 Pre-endoscopic submucosal tunnel dissection and post-endoscopic submucosal tunnel dissection pathology *n* (%)

Pathology	Pre-ESTD	Post-ESTD	Pre-ESTD and Post-ESTD coincidence
Inflammation	0	3 (0.96)	0
LGIN	67 (21.54)	43 (13.83)	36 (11.57)
HGIN	159 (51.13)	52 (16.72)	37 (11.90)
M1	74 (23.79)	74 (23.79)	18 (5.79)
M2	11 (3.54)	47 (15.11)	3 (0.96)
M3	0	51 (16.40)	0
SM1	0	23 (7.40)	0
> SM1	0	18 (5.79)	0

ESTD: Endoscopic submucosal tunnel dissection; LGIN: Low-grade intraepithelial neoplasia; HGIN: High-grade intraepithelial neoplasia; M1: Carcinoma in situ; M2: Carcinoma infiltrated to laminae propria; M3: Carcinoma infiltrated to muscularis mucosae; SM1: Submucosal invasion < 200 μm; SM2: Submucosal invasion > 200 μm.

Treatment outcomes and complications

Three hundred eleven lesions were successfully resected from 289 patients. The average specimen area was 14.1 ± 3.6 cm^2, the mean procedure time was 102.4 ± 35.3 min, and the mean dissection speed was 18.6 ± 2.1 mm^2/min. A total of 308 (308/311) lesions were resected by en bloc (99.04%), of which 49 were diagnosed with horizontal or vertical margin involvement by pathological evaluation; thus, the R0 resection rate was 81.28%

Table 3 Endoscopic submucosal tunnel dissection procedure characteristics *n* (%)

Category	ESTD (*n* = 311)
Specimen area, cm^2, mean ± SD	14.1 ± 3.6
Tumor width diameter, cm, mean ± SD	3.1 ± 0.6
Tumor length diameter, cm, mean ± SD	4.2 ± 0.9
Procedure time, min, mean ± SD	102.4 ± 35.3
Dissection speed, mm^2/ min, mean ± SD	18.6 ± 2.1
En bloc resection	308 (99.04)
R0 resection	259 (81.28)
Curative resection	244 (78.46)
Hospitalization day, d, mean ± SD	10.3 ± 2.8
Hospitalization expense, dollars, mean ± SD	3766.5 ± 846.5

ESTD: Endoscopic submucosal tunnel dissection.

Table 4 Endoscopic submucosal tunnel dissection-related complications *n* (%)

Category	ESTD (*n* = 311)
Post-operative infection	30 (9.65)
Bleeding	
Intraoperative bleeding	20 (6.43)
Postoperative bleeding	5 (1.61)
Muscular injury	98 (31.51)
Perforation	6 (1.93)
Esophageal stricture	46 (14.79)
Positive margin	
Horizontal margin	35 (11.25)
Vertical margin	10 (3.22)
Horizontal and vertical margin	4 (1.29)
Lymphovascular invasion	12 (3.86)

ESTD: Endoscopic submucosal tunnel dissection.

(259/311). Twelve patients were diagnosed with lymphovascular invasion (3.86%), of which five were combined with positive margin. We evaluated the invasion depth under microscopy and observed that seven lesions had a submucosal invasion deeper than 200 μm. As a result, the curative resection rate was 78.46% (244/311). After post-ESTD pathological evaluation, three patients were diagnosed with residual cancer in horizontal margin and 12 in vertical margin, of which five patients had vascular invasion. Another seven patients simply showed vascular invasion. All of the 22 patients were recommended an additional surgery. Finally, 17 patients underwent surgery, while the other five refused and were closely observed. The mean hospitalization stay was 10.3 ± 2.8 d, while the average hospitalization expense was 3766.5 ± 846.5 dollars (Table 3).

After ESTD procedure, 30 patients had postoperative infection, of which 29 were pulmonary infection, and one was urinary-tract infection. All of the infections were cured by intravenous infusion of antibiotics. Moreover, 20 (6.43%) patients had intra-operative bleeding, and five patients had postoperative bleeding. All of these patients underwent endoscopic hemostasis, and no severe complications with regard to bleeding were observed. Six patients had esophageal perforation and were cured by conservative treatment. Forty-six patients had postoperative esophageal stricture, of which 36 (78.26%) underwent an average of 4.1 (2-19 times) endoscopic balloon dilations in a mean follow-up time of 20.2 mo. In addition, the dysphagia was almost relieved (Table 4), while the other 10 patients with obstinate stenosis were further managed by receiving endoscopic balloon dilatation every 2 wk till dysphagia was relieved.

Pathology analysis

We analyzed the pathological change between pre-ESTD biopsies and post-ESTD specimens and observed that HGIN accounted for 51.13% (159/311) of pre-ESTD biopsies, while in post-ESTD pathology, superficial invasive carcinoma accounted for 44.70% (139/311). The pre-ESTD and post-ESTD coincidence rate was 30.23% (94/311). Also, 50.21% (117/233) of HGIN and M1 lesions had a pathological upgrade after ESTD to superficial invasive carcinoma.

DISCUSSION

This study evaluated the efficacy and complications of ESTD in 289 patients with 311 esophageal mucosal lesions. ESTD is a new technique developed from ESD and tunnel endoscopy. There are currently few studies that have reported the efficacy and complications of ESTD in large samples. Gan *et al*[13] reported endoscopic submucosal multi-tunnel dissection (ESMTD) for seven circumferential lesions, in which all patients achieved R0 resection but suffered from esophageal stricture. Huang *et al*[10] compared the efficacy and complication rate between ESD and ESTD using a propensity score matching analysis and observed that ESTD can improve procedure efficacy and reduce injury to muscular layer due to a better view, more efficient vessel coagulation, and longer lasting submucosal liquid cushion. In our previous ESD procedure, we observed that the dissected mucosa shrank and blocked the lumen, making it difficult to obtain a clear view. While ESTD can avoid this obstacle by creating a submucosal tunnel, when therapeutic endoscopy enters the submucosal tunnel, it will acquire a clear operative view to facilitate observation of the submucosal vessels and muscular layer, thereby reducing the bleeding and perforation rate. For this reason, it is especially appropriate for large mucosal lesions[11]. Zhai *et al*[19] obtained similar findings and noted that ESTD is indicated when (1) lesions do not invade deeper than sm1 and have no evidence of lymph node metastasis and (2) the lesion's circumference level ⩾ 1/3 or the diameter ⩾ 2 cm.

The reported en bloc and R0 resection rates of ESTD were 97.8% (92%-100%) and 85.6%（81.8%-100%）, which are similar to our study outcomes. However, our curative resection rate was 78.46% (244/311), mainly because we included large mucosal lesions. Also, 44.05% (137/311) of our lesions had a circumference level > 1/2, which may increase the risk of incomplete resection.

Patients' mean hospitalization stay was 10.3 ± 2.8 d, which is closely related to less hospitalization expenses (3766.5 ± 846.5 dollars) compared with surgical treatment.

We evaluated the post-ESTD specimens' pathological features and observed that 50.21% (117/233) of HGIN and M1 lesions upgraded to superficial invasive carcinoma after ESTD. Several reasons might contribute to this: First, the heavier the lesion and the wider its range, the poorer the representativeness of the pre-ESTD biopsy. In large or multifocal lesions, even if multiple biopsies are taken, it is difficult to represent the whole picture of the lesion. Moreover, the esophagus wall is thin; thus, too deeply drawn or frequent biopsies will lead to bleeding, perforation, and other biopsy-related complications. Therefore, we think that the reference significance of preoperative biopsy requires further evaluation and should be combined with iodine staining, narrow band imaging with magnifying endoscopy (ME-NBI), and radiological examination.

Postoperative infection, bleeding, and perforation are common in ESD procedure. Previous studies reported the bleeding and perforation rates of ESD to be 0-6% and 1.7%-4.0%[20-22], respectively. In our study, the total bleeding rate and perforation rate associated with ESTD were 8.04% and 1.93%, respectively. The significant bleeding that needs postoperative hemostatic treatment is relatively low (1.61%), indicating that ESTD is a safe treatment method for superficial esophageal squamous cell carcinoma and precancerous lesions. Thirty (9.65%) patients had postoperative infection. There are no available studies that have reported on post-ESD infection, although it is relatively common especially for the elderly. We speculate that the infection is caused by the patient hypoimmunity and the history of previous pulmonary disease or inhalation pneumonia related to anesthesia; however, further studies are needed to confirm this etiology.

Esophageal stricture and positive margin are serious complications of ESTD procedure, and the incidence rates found in our study were 14.79% (46/311) and 15.76% (49/311), respectively. It was reported that the circumference level and the area of the lesion are risk factors for esophageal stricture[23,24]. The incidence rate of esophageal stricture in patients with circumference level > 3/4 is above 70%-90%[25,26]. When the lesion area is large enough, the artificial esophageal ulcer causes excess absence of epithelial cells and results in fibrous repair in the submucosa[27], which is the primary cause of esophageal stricture. To prevent esophageal stricture, the administration of steroids is useful, as previously reported[28,29], while endoscopic balloon dilation and esophageal stent implantation can also be options[30,31]. For positive margin, previous studies reported its incidence after ESD to be 3%-17%[18,32,33]. Wen reported that the lesion area and invasion depth are risk factors of positive margin[33], and hypothesized that a greater lesion area and deeper invasion level corresponded to higher positive rates of the incisal margin. When treating large and multiple lesions, the risk of positive margin is relatively high, and thus accurate preoperative labeling and intraoperative complete resection are important. There are no standard guidelines to address positive margin after endoscopic resection; therefore, we recommended additional surgery for all patients in our study with positive basal margin, horizontal margin carcinoma involvement, and vascular invasion; however, several patients refused and entered the follow-up cohort. No residual or recurrent tumor was observed during the follow-up period.

The present study is the largest sample research of ESTD technique to date, and our observation indicators are complete, the follow-up period is long, the results are credible, and there is strong reference significance in clinical work. Moreover, our study also performed a detailed evaluation of postoperative pathology and emphasized its guiding role in the postoperative management of the patients. However, this study has several limitations. Firstly, this was a retrospective study and thus has inherent case selection bias. Secondly, this was a single center study; therefore, the operation level of ESTD in this study cannot be fully represented in whole.

In conclusion, ESTD for superficial esophageal squamous cell carcinoma and precancerous lesions is effective and safe, exhibiting high en bloc resection rate as well as low bleeding and perforation rates. When using ESTD to resect large mucosal lesions, the incidence of postoperative esophageal stricture and positive margin is high, and thus other effective preventative measures should be considered. We also observed that preoperative biopsies cannot represent the whole specimen, while half of the biopsies' pathology upgraded after ESTD procedure; therefore, the choice of therapy cases should be made cautiously before ESTD.

ARTICLE HIGHLIGHTS

Research background

Endoscopic submucosal dissection (ESD) is becoming the standard treatment for early gastrointestinal cancers, as it has a higher en bloc resection rate and a lower recurrence rate than endoscopic mucosal resection (EMR). However, when treating large mucosal lesions, ESD always faces many difficulties, such as multiple submucosal injections times, long procedure time, and low complete resection rate. To overcome these difficulties, a modified technique named endoscopic submucosal tunnel dissection (ESTD) has been proposed. Compared with ESD, ESTD could reduce the number of submucosal injections, shorten the procedure time, increase the resection speed, and reduce the injury of the muscular layer. However, there are no studies that verify the feasibility of ESTD in superficial esophageal squamous cell carcinoma (ESCC) and precancerous lesions in a large sample.

Research motivation

To our knowledge, the present study is the largest sample research of ESTD technique to date, and our observation indicators are complete, the follow-up period is long, the results are credible, and there is strong reference significance in clinical work.

Research objectives

This study aims to evaluate the clinical outcomes of patients who underwent ESTD for ESCC and precancerous lesions.

Research methods
ESTD was performed in 289 patients with 311 lesions. The clinical outcomes of the patients and pathological features of the lesions were retrospectively reviewed.

Research results
A total of 311 lesions were included. The en bloc rate, complete resection rate, and curative resection rate were 99.04%, 81.28%, and 78.46%, respectively. The ESTD procedure time was 102.4 ± 35.1 min, the mean hospitalization time was 10.3 ± 2.8 d, and the average expenditure was 3766.5 ± 846.5 dollars. The intraoperative bleeding rate, postoperative bleeding rate, the perforation rate, and the postoperative infection rate were 6.43%, 1.61%, 1.93%, and 9.65%, respectively. Esophageal stricture and positive margin were severe adverse events, with an incidence rate of 14.79% and 15.76%, respectively. No tumor recurrence occurred during the follow-up period.

Research conclusions
ESTD for ESCC and precancerous lesions is feasible and relatively safe, but the rates of esophageal stricture and positive margin are high for large mucosal lesions.

Research perspectives
The present study is a retrospective study to describe the general characteristics of ESTD. In the future, case control studies and prospective studies are considered necessary to evaluate further the feasibility and safety of ESTD for treating ESCC and precancerous lesions.

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ORIGINAL ARTICLE

Retrospective Study

Impact of the number of examined lymph nodes on outcomes in patients with lymph node-negative gallbladder carcinoma

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Author contributions: Fan DX and Sun MY designed the research and critically revised the manuscript for important intellectual content; all authors performed the research, analyzed the data, and wrote the paper.

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Abstract

AIM

To determine whether the number of examined lymph nodes (LNs) is correlated with the overall survival of gallbladder carcinoma (GBC) patients.

METHODS

Patients were collected from the Surveillance Epidemiology and End Results database (2004-2013) and categorized by the number of LNs into six groups: 1 LN, 2 LNs, 3 LNs, 4 LNs, 5 LNs, and ≥ 6 LNs. Survival curves for overall survival were plotted with a Kaplan-Meier analysis. The log-rank test was used for univariate comparisons.

RESULTS

In a cohort of 893 patients, the median number of examined LNs was two for the entire cohort. The survival for the 1 LN group was significantly poorer than those of the stage Ⅰ and Ⅱ disease groups and for the entire

cohort. By dichotomizing the number of LNs from 1 to 6, we found that the minimum number of LNs that should be examined was four for stage Ⅰ, four or five for stage Ⅱ, and six for stage ⅢA disease. Therefore, for the entire cohort, the number of examined LNs should be at least six, which is exactly consistent with the American Joint Committee on Cancer criteria.

CONCLUSION

The examination of higher numbers of LNs is associated with improved survival after resection surgery for N0 GBC. The guidelines for GBC surgery, which recommend that six LNs be examined at least, are statistically valid and should be applied in clinical practice widely.

Key words: Gallbladder carcinoma; Lymph node; N0 stage; Prognostic factor

Core tip: Six lymph nodes were recommended as the minimum number of examination in the 8th edition American Joint Committee on Cancer tumor-node-metastasis criteria for gallbladder carcinoma, but the rationality has not been evaluated yet. Thus, we aimed to explore the optimal lymph node number using the Surveillance Epidemiology and End Results database.

Fan DX, Xu RW, Li YC, Zhao BQ, Sun MY. Impact of the number of examined lymph nodes on outcomes in patients with lymph node-negative gallbladder carcinoma. *World J Gastroenterol* 2018; 24(26): 2886-2892 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i26/2886.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i26.2886

INTRODUCTION

Gallbladder carcinoma (GBC) is one of the most lethal carcinomas and has a poor prognosis[1-3]. To date, surgery remains the only radical treatment strategy for patients, translating into 5-year survival rates of approximately 5%[4-7]. Lymph node (LN) status is an important prognostic factor for GBC patients[8]. Unfortunately, LN metastases occur in more than 50% of patients, and LN-positive patients are widely known to have very poor survival[4].

The role of regional and extended lymphadenectomy for GBC has been previously investigated[9-12], but there is not a general consensus about the number of LNs that should be examined. In the 8th edition of the tumor-node-metastasis (TNM) staging system for GBC from the American Joint Committee on Cancer (AJCC), the N category was defined by the number of metastatic LNs instead of the location of the metastatic LNs, as used in the previous edition, and was correlated with prognosis. These guidelines recommend examining a minimum of six LNs to accurately classify patients with GBC[13]. Thus, this study aimed to assess patients with LN-negative (N0) GBC to determine whether the number of examined LNs was correlated with overall survival of GBC patients. We used the Surveillance, Epidemiology, and End Result (SEER) database to determine the influence of the number of examined LNs on prognosis in patients with N0 GBC.

MATERIALS AND METHODS

Patients

The SEER database (2004-2013) was used to identify patients with GBC. Patients who met the following criteria were included: (1) Pathologically confirmed diagnosis; (2) radical surgical treatment; (3) definite cancer stage according to the 8th edition of the AJCC criteria; (4) first primary tumor; (5) number of positive LNs equal to zero; (6) no distant metastases; (7) one or more LNs examined; and (8) active follow-up. The exclusion criteria were as follows: (1) Age < 18 years; (2) unavailable follow-up data or 0 d of follow-up; (3) unknown cause of death; (4) number of LNs examined coded with SEER codes 95 to 99 (the information about the number of LN is not available); and (5) T4 disease.

Statistical analysis

The clinicopathological characteristics were compared among the stage Ⅰ, Ⅱ, and ⅢA disease subgroups by the independent *t* test for continuous variables and the chi-square test for categorical variables. Overall survival (OS) was determined from the SEER record of survival time (total number of months) and vital status. The relationship between the number of examined LNs and OS was assessed separately for the entire cohort and for stage Ⅰ, Ⅱ, and ⅢA patients. Patients were categorized by the number of examined LNs into the following six groups: 1 LN, 2 LNs, 3 LNs, 4 LNs, 5 LNs, and ⩾ 6 LNs. The optimal number of examined LNs was determined with X-tile software (Yale University, Version 3.6.1). Survival curves for OS were plotted with a Kaplan-Meier analysis. The log-rank test was used for univariate comparison. A Cox proportional hazard method was used to identify factors associated with mortality and to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). The variables, including age, sex, race, radiation therapy, number of examined LNs, grade, and stage, that were significant in univariate analysis, were included in the Cox model. All the statistical analyses were performed using SPSS, version 20 (Armonk, NY, United States). A two-tailed *P*-value of < 0.05 was considered statistically significant.

RESULTS

Among the 893 patients who were finally eligible for this analysis, 228 patients (25.5%) had stage Ⅰ disease, 444 patients (49.7%) had stage Ⅱ disease, and 221

Table 1 Demographic and tumor characteristics for patients with lymph node-negative gallbladder carcinoma *n* (%)

Characteristics	Entire cohort *n* = 893 (100%)	Stage Ⅰ[1] *n* = 228 (25.5%)	Stage Ⅱ[1] *n* = 444 (49.7%)	Stage ⅢA[1] *n* = 221 (24.7%)	*P*[2] Value
Age, yr					
Median (range)	67 (21-96)	67 (21-92)	67 (25-96)	67 (35-93)	0.575
Sex					
Male	272 (30.5)	63 (27.6)	139 (31.3)	70 (31.7)	
Female	621 (69.5)	165 (72.4)	305 (68.7)	151 (68.3)	0.559
Race					
White	675 (75.6)	162 (71.1)	34.1 (76.8)	172 (77.8)	
Black	106 (11.9)	33 (14.5)	50 (11.3)	23 (10.4)	
Others	112 (12.5)	33 (14.5)	53 (11.9)	26 (11.8)	0.261
Grade[1]					
Well	187 (20.9)	67 (29.4)	102 (23.0)	18 (8.1)	
Moderate	414 (46.4)	101 (44.3)	215 (48.4)	98 (44.3)	
Poor	210 (23.5)	26 (11.4)	96 (21.6)	88 (39.8)	
Undifferentiated	11 (1.2)	1 (0.4)	5 (1.1)	5 (2.3)	
Unknown	71 (8.0)	33 (14.5)	26 (5.9)	12 (5.4)	< 0.001
Radiation					
Yes	151 (16.9)	8 (3.5)	75 (16.9)	68 (30.8)	
No	742 (83.1)	220 (96.5)	369 (83.1)	153 (69.2)	< 0.001
Vital status					
Alive	547 (61.3)	151 (66.2)	308 (69.4)	88 (39.8)	
Dead	346 (38.7)	77 (33.8)	136 (30.6)	133 (60.2)	< 0.001
Number of LN					
Median (range)	2 (1-80)	1 (1-80)	2 (1-40)	2 (1-24)	0.590

[1]AJCC/TNM 8th edition. [2]Factors were compared by the independent t test and the χ^2 test for continuous and categorical variables, respectively. LN: Lymph node.

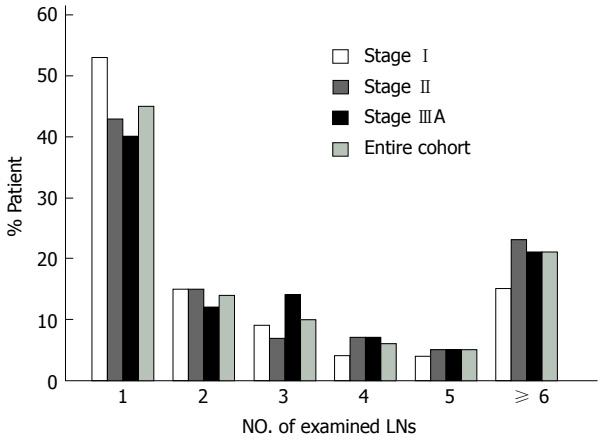

Figure 1 Number of examined lymph nodes was categorized into subgroups. The bars in the graph reflect the percentage of patients who fell into each subgroup. Entire cohort, *n* = 893; stage Ⅰ, *n* = 228; stage Ⅱ, *n* = 444; stage ⅢA, *n* = 221.

patients (24.7%) had stage ⅢA disease. The median age at diagnosis for the entire cohort was 67 years (range 21-96 years), and 272 patients (30.5%) were male.

The clinical characteristics of the entire cohort and patients with stage Ⅰ, Ⅱ, and ⅢA disease are listed in Table 1. There was no difference among patients with stage Ⅰ, Ⅱ, or ⅢA disease in terms of age, sex, race, and number of LNs examined. In addition, compared with patients with stage Ⅰ and Ⅱ disease, a larger proportion of patients with stage ⅢA disease had poor/undifferentiated tumors and received radiation therapy. The median number of examined LNs was 2 for the entire cohort, 1 LN for the stage Ⅰ group, 2 LNs for the stage Ⅱ group, and 2 LNs for the stage ⅢA group. More than 40% of the patients had only 1 LN examined, and a lower proportion of patients had more LNs examined (Figure 1). The number of examined LNs did not differ by stage ($P = 0.59$).

Patients were categorized by the number of examined LNs into the following 6 groups: 1 LN, 2 LNs, 3 LNs, 4 LNs, 5 LNs and ⩾ 6 LNs. Survival in relation to the number of examined LNs was assessed separately for the entire cohort and patients with stage Ⅰ, Ⅱ and ⅢA disease (Table 2). For the entire cohort, a median survival of 18 mo and a 5-year survival rate of 0.393 were noted for patients with one LN examined ($n = 398$). The survival for the 1 LN group was significantly poorer than that of the other groups ($P < 0.001$, Figure 2). However, there was no difference in survival among the other five groups ($P > 0.05$). For patients with stage Ⅰ disease, the median survival for the 1 LN, 2 LNs, 3 LNs, 4 LNs, 5 LNs, and ⩾ 6 LNs groups was 24, 43, 30, 22, 38, and 26 mo, respectively. Similar survival results according to the LN groups were demonstrated for patients with stage Ⅰ and Ⅱ disease but not for patients with stage ⅢA disease (Table 2). However, compared with patients with stage Ⅰ and Ⅱ disease, the median survival and 5-year survival rate of patients with stage ⅢA disease was obviously decreased in all the LN groups. For example, the 5-year survival rate in the 1 LN group was 0.473 for stage Ⅰ, 0.445 for stage Ⅱ, and 0.177 for stage ⅢA disease. As shown in Table 3, there was no difference in

Table 2 Survival by lymph node group and stage

Number of LN	Entire cohort *n* = 893 (100%)	Stage Ⅰ[1] *n* = 228 (25.5%)	Stage Ⅱ[1] *n* = 444 (49.7%)	Stage ⅢA[1] *n* = 221 (24.7%)
1 LN				
Patients (*n*)	398	121	189	88
Median OS, mo	18	24	20	10
3-yr SR (95%CI)	0.503 (0.474-0.532)	0.603 (0.553-0.653)	0.608 (0.566-0.650)	0.271 (0.217-0.325)
5-yr SR (95%CI)	0.393 (0.361-0.425)	0.473 (0.418-0.528)	0.445 (0.394-0.496)	0.177 (0.128-0.226)
2 LNs				
Patients (*n*)	129	35	68	26
Median OS, mo	28	43	27	15
3-yr SR (95%CI)	0.711 (0.665-0.757)	0.808 (0.737-0.879)	0.775 (0.713-0.837)	0.425 (0.317-0.533)
5-yr SR (95%CI)	0.579 (0.524-0.634)	0.725 (0.640-0.810)	0.586 (0.504-0.668)	0.340 (0.225-0.455)
3 LNs				
Patients (*n*)	85	20	33	32
Median OS, mo	21	30	27	11
3-yr SR (95%CI)	0.587 (0.525-0.649)	0.722 (0.603-0.841)	0.697 (0.606-0.788)	0.379 (0.277-0.481)
5-yr SR (95%CI)	0.466 (0.396-0.536)	0.602 (0.454-0.750)	0.639 (0.539-0.739)	0.203 (0.109-0.297)
4 LNs				
Patients (*n*)	55	8	31	16
Median OS, mo	22	22	27	10
3-yr SR (95%CI)	0.638 (0.560-0.716)	0.833 (0.681-0.985)	0.760 (0.671-0.849)	0.295 (0.154-0.446)
5-yr SR (95%CI)	0.533 (0.438-0.628)	0.833 (0.681-0.985)	0.652 (0.526-0.778)	0.148 (0.022-0.274)
5 LNs				
Patients (*n*)	43	10	21	12
Median OS, mo	24	38	24	18
3-yr SR (95%CI)	0.750 (0.671-0.829)	0.857 (0.725-0.989)	NA	0.292 (0.133-0.451)
5-yr SR (95%CI)	0.652 (0.557-0.747)	0.714 (0.543-0.885)	0.857 (0.725-0.989)	0.146 (0.016-0.276)
⩾ 6 LNs				
Patients (*n*)	183	34	102	47
Median OS, mo	26	26	26	21
3-yr SR (95%CI)	0.696 (0.655-0.737)	0.817 (0.731-0.903)	0.753 (0.701-0.805)	0.498 (0.412-0.584)
5-yr SR (95%CI)	0.594 (0.547-0.641)	0.817 (0.731-0.903)	0.671 (0.610-0.732)	0.306 (0.221-0.391)

[1]AJCC/TNM 8th edition. LN: Lymph node; OS: Overall survival; SR: Survival rate; NA: Not available.

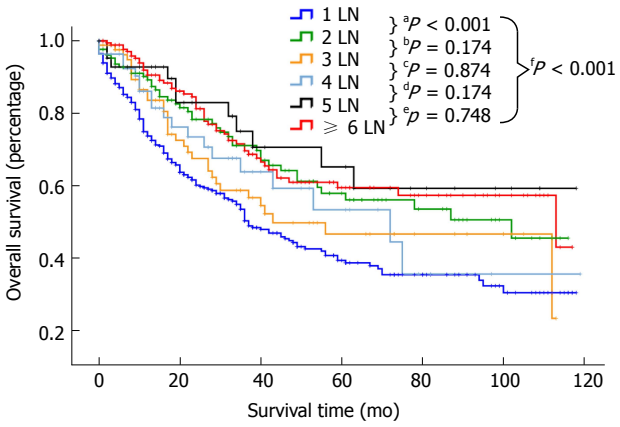

Figure 2 Overall survival curves for the entire cohort comparing patients with 1, 2, 3, 4, 5, and ⩾ 6 examined lymph nodes.

survival between the stage Ⅰ and Ⅱ groups (HR: 1.089, 95%CI: 0.793-1.497, *P* = 0.598 for stage Ⅱ, referred to stage Ⅰ), but the survival of patients with stage ⅢA disease was significantly lower than that of patients with stage Ⅰ disease (HR: 3.730, 95%CI: 2.635-5.280, *P* < 0.0001).

To identify the cutoff point for the optimal number of examined LNs, we compared the survival of the entire cohort with stage Ⅰ, Ⅱ and ⅢA groups with X-tile software. The ranges for the significant dichotomization LN numbers varied among the three stages. The largest survival difference was observed at 4 LNs for stage Ⅰ disease (*P* = 0.004; Figure 3A), at 4 or 5 LNs for stage Ⅱ disease (*P* < 0.001 for both; Figure 3B and C), and at 6 LNs for stage ⅢA disease (*P* = 0.019; Figure 3D). For the entire cohort, the optimal number of examined LNs was 4, 5, or 6 (*P* < 0.001 for all; Figure 3E-G).

A stepwise Cox regression identified race and sex as significant prognostic factors for the entire cohort (Table 3); however, race was not a significant factor for patients with stage Ⅱ and ⅢA disease. Grade was a significant prognostic factor for patients with stage Ⅰ, Ⅱ, and ⅢA disease but not for the entire cohort; and radiation therapy was a significant prognostic factor only for patients with stage ⅢA disease.

DISCUSSION

GBC is associated with a high incidence of invasion through the layers of the gallbladder wall into adjacent structures and LNs. The influence of LN metastases on primary GBC is supported by one series of reports, which showed that the 5-year survival rate of T1N0 patients was 33% compared with a 3% survival rate in T1N1 patients[14]. As a consequence, several large-scale studies were conducted to examine the role of extended LN dissection to determine whether the removal of

Table 3 Multivariate analyses for overall survival in patients with lymph node-negative gallbladder carcinoma

Variable	Entire cohort *n* = 893 (100%)		Stage Ⅰ[1] *n* = 228 (25.5%)		Stage Ⅱ[1] *n* = 444 (49.7%)		Stage ⅢA[1] *n* = 221 (24.7%)	
	HR (95%CI)	[a]*P*	HR (95%CI)	[b]*P*	HR (95%CI)	[c]*P*	HR (95%CI)	[d]*P*
Age, yr	1.001 (1.000-1.002)	0.504	1.001 (1.000-1.002)	0.934	1.001 (1.000-1.002)	0.449	1.001 (1.000-1.002)	0.057
Sex								
Male	1		1		1		1	
Female	0.684 (0.532-0.879)	0.003	0.317 (0.156-0.645)	0.002	0.553 (0.346-0.881)	0.013	0.494 (0.299-0.818)	0.006
Race								
White	1		1		1		1	
Black	1.475 (1.008-2.160)	0.046	4.593 (1.625-12.981)	0.004	1.645 (0.775-3.490)	0.195	2.245 (0.904-5.575)	0.082
Others	0.821 (0.551-1.224)	0.334	0.262 (1.975)	0.719	0.509 (0.245-1.056)	0.070	1.362 (0.609-3.047)	0.452
Grade1								
Well	1		1		1		1	
Moderate	0.840 (0.609-1.157)	0.286	0.418 (0.194-0.902)	0.026	1.568 (0.905-2.715)	0.109	0.297 (0.125-0.704)	0.006
Poor	1.291 (0.908-1.835)	0.155	1.339 (0.449-3.994)	0.601	2.412 (1.306-4.453)	0.005	0.629 (0.259-1.531)	0.307
Undifferentiated	2.101 (0.891-4.954)	0.090	/	/	4.059 (1.033-15.951)	0.045	1.434 (0.323-6.373)	0.636
Unknown	0.856 (0.503-1.455)	0.565	0.384 (0.134-1.106)	0.076	2.376 (0.857-6.583)	0.096	0.260 (0.068-1.003)	0.050
Radiation								
No	1		1		1		1	
Yes	0.727 (0.515-1.027)	0.071	1.055 (0.302-3.688)	0.933	0.781 (0.430-1.419)	0.417	0.390 (0.215-0.708)	0.002
No. of LNs examined	1.001 (1.000-1.002)	0.162	1.001 (1.000-1.002)	0.910	1.001 (1.000-1.002)	0.382	1.001 (1.000-1.002)	0.167
Stage[1]								
Ⅰ	1		/	/	/	/	/	/
Ⅱ	1.089 (0.793-1.497)	0.598	/	/	/	/	/	/
ⅢA	3.730 (2.635-5.280)	0.000	/	/	/	/	/	/

[1]AJCC/TNM 8th edition. OS: Overall survival.

additional LN basins would influence the survival of GBC patients[9,15].

Early retrospective reports suggested improved survival for late-stage GBC patients treated with extended regional lymphadenectomy compared with standard regional lymphadenectomy[9]. However, the minimum clearance and/or number of LNs that should be examined have yet to be established. In the study, we evaluated the impact of the number of examined LNs on survival in N0 GBC. Using the SEER database, we discovered that the median number of examined LNs was two for the entire cohort, one for stage Ⅰ, two for stage Ⅱ, and two for stage ⅢA disease. We used the smallest median value, 1 LN, as the basis for our categorization of the SEER patient cohort into six groups that reflected the extent of lymphadenectomy: 1 LN, 2 LNs, 3 LNs, 4 LNs, 5 LNs, and ⩾ 6 LNs. There was a significant difference in survival among the six groups for the entire cohort and for the stage Ⅰ and Ⅱ groups but not for the stage ⅢA group. With X-tile software, we found that the minimum number of LNs that should be examined was four for stage Ⅰ, four or five for stage Ⅱ, and six for stage ⅢA disease. Therefore, for the entire cohort, the number of examined LNs should be at least six, which is exactly consistent with the AJCC guidelines.

The general phenomenon that the more LNs are examined, the better the survival for N0 disease, has some potential explanations. For example, the final LN count may be a proxy for surgeon experience and surgical technique and may be reflective of more thorough pathological assessment and identification of nodes from the surgical specimen[16]. It is also related to the concept of stage migration, where inadequate removal of LNs may result in the misclassification of LN-positive patients as N0[17] . However, the removal of too many LNs may result in side effects such as lymphatic leakage. Our results were consistent with those of previous studies that investigated the relationship between LN count and survival in GBC patients and demonstrated that at least six LNs should be examined to improve survival after resection surgery[18]. Notably, except for LN dissection, nerve dissection may be required, especially for T3 or T4 disease[19].

In conclusion, the analysis suggests that examining higher numbers of LNs is associated with improved survival after resection surgery in N0 GBC. As recommended in the AJCC guidelines, at least six LNs should be examined for patients with N0 GBC.

ARTICLE HIGHLIGHTS

Research background

The American Joint Committee on Cancer (AJCC) tumor-node-metastasis staging system for gallbladder carcinoma (GBC) has been updated recently to the 8th edition. The N category is re-defined by the number of metastatic lymph nodes (LNs) instead of the location of the metastatic LNs, as defined in the 7th edition.

Research motivation

The new staging system for GBC has not been validated yet. Thus, we used Surveillance, Epidemiology, and End Result (SEER) database to evaluate its impact on clinical practice.

Research objectives

The primary purpose of this study was to evaluate the impact of the number

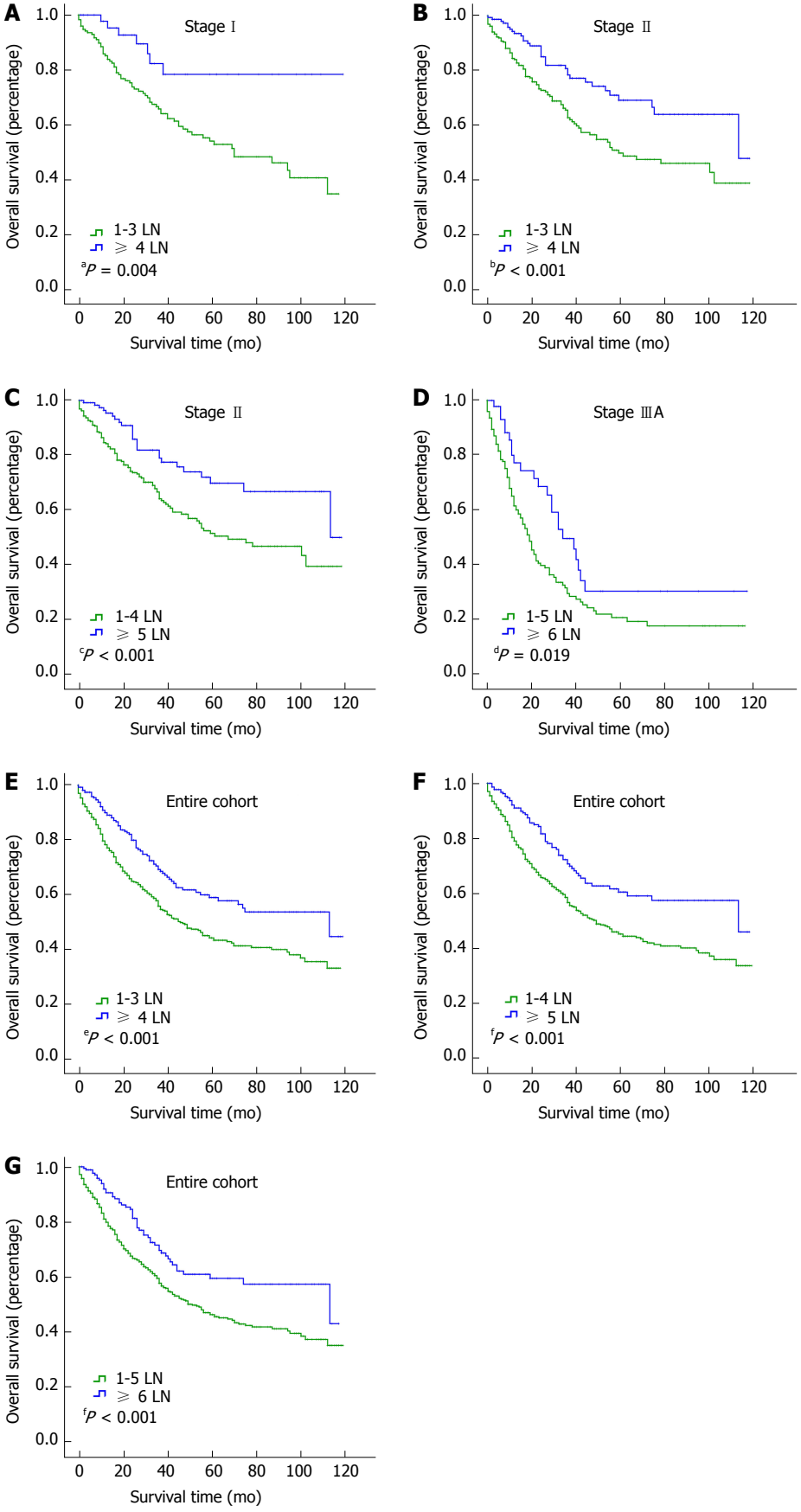

Figure 3 Overall survival curves for the entire cohort and the stage Ⅰ, Ⅱ, and ⅢA groups and optimal dichotomization by the number of examined lymph nodes. A: Stage Ⅰ, LN = 4, $P = 0.004$; B: Stage Ⅱ, LN = 4, $P \leqslant 0.001$; C: Stage Ⅱ, LN = 5, $P \leqslant 0.001$; D: Stage ⅢA, LN = 6, $P = 0.019$; E: Entire cohort, LN = 4, $P \leqslant 0.001$; F: Entire cohort, LN = 5, $P \leqslant 0.001$; G: Entire cohort, LN = 6, $P < 0.001$. LN: Lymph node.

of examined LNs on the prognosis of N0 GBC. The secondary purpose was to verify the rationality of the guideline recommendation that at least six LNs should be harvested and evaluated.

Research methods

Patients were collected from the SEER database (2004-2013) and categorized by the number of LNs into six groups: 1 LN, 2 LNs, 3 LNs, 4 LNs, 5 LNs, and ≥ 6 LNs. Survival curves for overall survival were plotted with a Kaplan-Meier analysis. The log-rank test was used for univariate comparisons.

Research results

The survival for the 1 LN group was significantly lower than that of the stage Ⅰ and Ⅱ disease groups and for the entire cohort. By dichotomizing the number of LNs from one to six, we found that the minimum number of LNs that should be examined was four for stage Ⅰ, four or five for stage Ⅱ, and six for stage ⅢA disease. Thus, at least six LNs should be examined for the entire cohort, which was exactly consistent with the AJCC criteria.

Research conclusions

The examination of higher numbers of LNs is associated with improved survival after resection surgery for N0 GBC. As recommended in the guidelines, at least six LNs should be examined for patients with N0 GBC.

Research perspectives

The results validated the new recommendation in the AJCC guidelines, which can be applied widely in clinical practice.

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ORIGINAL ARTICLE

Observational Study

Upper gastrointestinal tract capsule endoscopy using a nurse-led protocol: First reported experience

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Author contributions: McAlindon ME designed the study, assisted with interpretation of results and critically appraised the paper; Healy A and Thurston V led the data collection; Ching HL assisted with data collection, performed the data analysis, drafted the initial manuscript and is guarantor; Sidhu R assisted with interpretation of results and critically appraised the paper; Hale MF critically appraised the paper; all authors approved the final manuscript.

Institutional review board statement: This study was registered as service evaluation with the clinical effectiveness unit (CEU number 7073), Sheffield Teaching Hospitals NHS Foundation Trust, United Kingdom.

Informed consent statement: Capsule endoscopy was performed on patients who declined to undergo gastroscopy and all provided written informed consent for the capsule examination which was performed in all cases as part of routine clinical practice. The capsule examinations were not performed as part of a clinical research trial. In these patients who refused to have gastroscopy, the capsule endoscopy protocol was registered as a service evaluation with the department of clinical effectiveness unit (CEU number 7073, Sheffield Teaching Hospitals NHS Foundation Trust) and the evaluation is presented in this paper.

Conflict-of-interest statement: Professor McAlindon ME has acted as a consultant for Medtronic Ltd. All remaining authors have no conflict of interest to report.

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Abstract

AIM

To test the feasibility and performance of a novel upper gastrointestinal (GI) capsule endoscope using a nurse-led protocol.

METHODS

We conducted a prospective cohort analysis of patients who declined gastroscopy (oesophagogastroduodenoscopy, OGD) but who consented to upper GI capsule endoscopy. Patients swallowed the upper GI

capsule following ingestion of 1 liter of water (containing simethicone). A series of positional changes were used to exploit the effects of water flow and move the upper GI capsule from one gravity-dependent area to another using a nurse-led protocol. Capsule transit time, video reading time, mucosal visualisation, pathology detection and patient tolerance was evaluated.

RESULTS

Fifty patients were included in the study. The mean capsule transit times in the oesophagus and stomach were 28 s and 68 min respectively. Visualisation of the following major anatomical landmarks was achieved (graded 1-5: Poor to excellent): Oesophagus, 4.8 (± 0.5); gastro-oesophageal junction (GOJ), 4.8 (± 0.8); cardia, 4.8 (± 0.8); fundus, 3.8 (± 1.2); body, 4.5 (± 1); antrum, 4.5 (± 1); pylorus, 4.7 (± 0.8); duodenal bulb, 4.7 (± 0.7); second part of the duodenum (D2), 4.7 (± 1). The upper GI capsule reached D2 in 64% of patients. The mean video reading time was 48 min with standard playback mode and 20 min using Quickview (P = 0.0001). No pathology was missed using Quickview. Procedural tolerance was excellent. No complications were seen with the upper GI capsule.

CONCLUSION

The upper GI capsule achieved excellent views of the upper GI tract. Future studies should compare the diagnostic accuracy between upper GI capsule and OGD.

Key words: Capsule endoscopy; Upper gastrointestinal; Gastroscopy; Oesophagus; Stomach

Core tip: The demand for diagnostic upper gastrointestinal (GI) endoscopy is high. Capsule endoscopy is well tolerated and is a first line small bowel investigative modality. Capsule endoscopy of the upper GI tract has previously been limited by technology and complexity of use. We demonstrate the feasibility of a nurse-led protocol using simple patient positional changes to move the novel upper GI capsule around a water-filled stomach. This technique provides excellent mucosal views in the oesophagus, stomach and (battery life allowing) duodenum and is well tolerated. The upper GI capsule might be a potential non-invasive, patient-friendly, alternative for diagnostic upper GI endoscopy.

Ching HL, Healy A, Thurston V, Hale MF, Sidhu R, McAlindon ME. Upper gastrointestinal tract capsule endoscopy using a nurse-led protocol: First reported experience. *World J Gastroenterol* 2018; 24(26): 2893-2901 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i26/2893.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i26.2893

INTRODUCTION

Capsule endoscopy is the method of choice to image the small bowel mucosa and is an accepted alternative to colonoscopy[1,2]. However, the short length of the oesophagus, the volume of the stomach and the convoluted shape of the gastroduodenum present challenges to its role as a non-invasive upper gastrointestinal examination technique: Transit can be rapid through a straight lumen[3] and visualisation may be limited to the dependent part of the stomach.

The PillCam® ESO2 capsule (Given Imaging Ltd., Yoqneam, Israel) has cameras at both ends and is capable of high image acquisition rates (18 frames per second) to maximise oesophageal imaging and a 30-min battery life. Meta-analyses have shown that it is an effective tool to detect Barrett's oesophagus, oesophageal varices and oesophagitis[4-6]. Three studies have also shown that it can be used to identify patients with suspected upper gastrointestinal bleeding who need gastroscopy[7-9]. In a comparative study in dyspeptic patients, Marelli *et al*[10] identified all major pathology detected by gastroscopy using an ESO2. These examinations were performed following a fast alone: better visualisation may be achieved after ingestion of simethicone and water to distend the stomach[11-13].

The upper gastrointestinal (UGI) capsule (Medtronic Ltd, Dublin, Ireland) represents the most recent technological advance in this field. Preserving dual-camera image capture, each with a 174° field of view, the UGI capsule captures as many as 35 frames per second for 10 min followed by 18 frames per second for a further 80 min. This study describes the first reported experience of UGI capsule endoscopy using a simple, nurse-led protocol comprising a sequence of patient positional changes following the ingestion of water and simethicone.

MATERIALS AND METHODS

Study population

We performed a prospective observational study at our tertiary hospital. Patients were offered UGI capsule endoscopy if they refused gastroscopy. All indications were considered. Those who had Crohn's disease were required to undergo a PillCam Patency capsule (Medtronic Ltd.) examination first.

Simple positional interchange technique

The UGI capsule endoscopy system includes an external portable data recorder. The recorder is connected to the patient by an array of leads on the chest and abdominal skin during the examination. This interface supports data export from the capsule to the memory drive of the data recorder. A small monitor in the recorder allows real-time viewing. When the procedure is complete, the data recorder is docked onto a workstation installed with Rapid 9® software (Medtronic Ltd.) and video images are exported for further analysis by the physician.

The simple positional interchange technique (SPIT) was performed by nursing staff on the Clinical Investigation Unit, Royal Hallamshire Hospital. Patients first drank one litre of water containing 80 mg simethicone. Immediately before swallowing the UGI capsule, 20 mg

Table 1 Upper gastrointestinal mucosal visualisation grading

Grade	Description
1	Poor view. More than 75% obscured by debris/bubbles/poor image clarity/illumination
2	Sub-optimal view. More than or equal to 50% obscured by debris/bubbles/poor image clarity/illumination
3	Reasonable view. Less than 50% obscured by debris/bubbles/poor image clarity/illumination
4	Good view. Less than 25% obscured by debris/bubbles/poor image clarity/illumination
5	Excellent. 100% complete view of the landmark

Views of each major landmark were graded; oesophagus, gastro-oesophageal junction; gastric cardia, fundus, body (anterior, posterior wall, greater and lesser curve), antrum, pylorus, and the first (D1) and second part of the duodenum (D2).

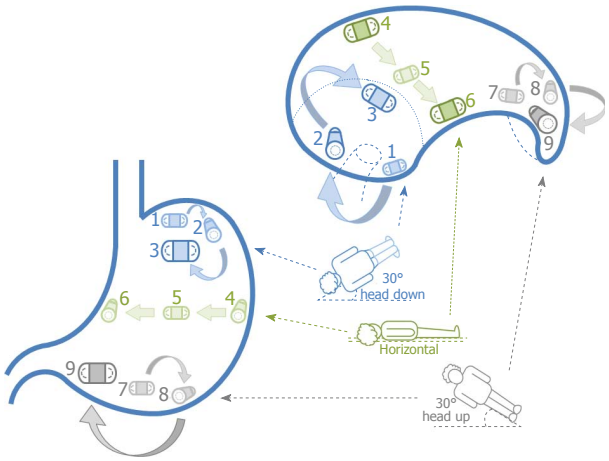

Figure 1 Schematic of the simple positional interchange technique. Coronal views are illustrated on the left and transverse views (with the cranial end closest to the reader) on the right. Capsule movement is achieved by exploiting the effects of water flow from one gravity dependent area to another with patient positional change. Once the UGI capsule enters the stomach, the examination bed is tilted 30° head down (depicted in blue) and patients lie supine (position 1), on their left lateral (position 2) and then prone (position 3). The bed is returned to the horizontal plane (depicted in green) and patients lie on their left lateral (position 4), supine (position 5) and then right lateral (position 6). The bed is finally adjusted to 30° head up (depicted in grey) and patients lie supine (position 7), on their left lateral (position 8) and then prone (position 9). UGI: Upper gastrointestinal.

of hyoscine butylbromide was given intramuscularly to reduce gastric peristalsis[14] and optimise gastric views. Patients were asked to swallow the UGI capsule in the right lateral position using an adaptation of the previously described simplified ingestion procedure (SIP)[15]. In brief, this entailed swallowing small sips of water (approximately 15mL) every 30 s until the UGI capsule entered the stomach. If patients were unable to swallow the capsule while lying in the horizontal plane, the head of the bed was incrementally elevated until swallowing was successful. If this failed, then patients swallowed the capsule sitting upright. The real-time views detected when the UGI capsule entered the stomach. Once the capsule entered the stomach, patients were asked to position themselves to face three planes (left/right lateral decubitus and supine/prone) at three angles (30° head down/up and horizontal) for 2 min per position (Figure 1). Additional positional changes and sips of water were used to improve views of the gastric mucosa as necessary. When complete gastric mucosal assessment was achieved patients were asked to sit upright to assist passive capsule movement towards the pylorus. If the capsule had not reached the first part of the duodenum 60 min after ingestion then 10 mg of intramuscular metoclopramide was administered as per our standard protocol[11]. Patient tolerance in the form of procedural pain, discomfort and distress scores were recorded using previously validated visual analogue scales (VAS. 0: No symptom; 10: Intolerable symptom)[16,17].

Video interpretation and analysis

UGI capsule videos were reported by one of two co-authors (Sidhu R and McAlindon ME), each with experience of reading over 1000 small bowel capsule endoscopy videos. Rapid 9® software (Medtronic Ltd.) was used to review videos and has the capacity to playback recordings up to 100 frames per second in an accelerated reading mode. Analysis of videos included grading of mucosal visualisation (Table 1) using an adapted protocol[18]. Capsule transit time, video reading time, completion of examination to the second part of the duodenum (D2), pathology detection and procedural complications were recorded. The service evaluation was registered with the Clinical Effectiveness Unit (registration number 7073), Sheffield Teaching Hospitals NHS Foundation Trust (STH), United Kingdom.

SPSS V.22.0 (IBM) was used for statistical analysis. Continuous data was represented as mean ± SD: The student's *t*-test or one-way analysis of variance (ANOVA) was used for comparisons. Categorical data was represented as an absolute number and/or percentage: The χ^2 test or Fisher's exact probability test was used for comparisons. $P < 0.05$ (two-sided) was considered statistically significant.

RESULTS

Patient demographics

Fifty patients (40% male) with a mean age of 57 (± 15.7) years were included in the study protocol. Indications for investigation included dyspepsia (32%), iron deficiency anemia (14%), variceal screening (42%), suspected upper GI Crohn's disease (4%) and assessment of oesophageal ulcer healing (8%).

Performance characteristics

SPIT was achieved in 90% of patients: Five had difficulty lying prone. Complete examination to D2 was achieved

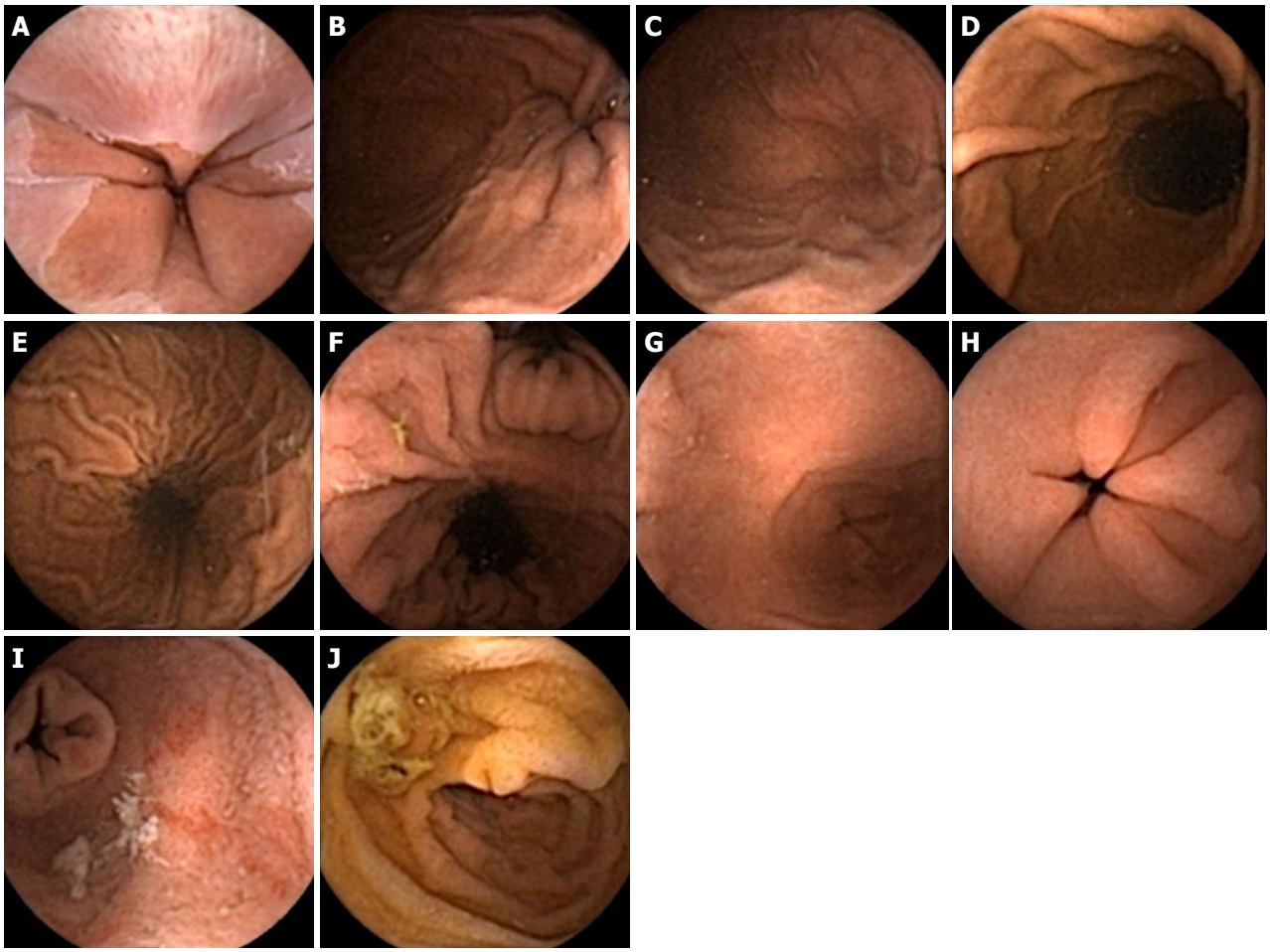

Figure 2 Normal views of the upper gastrointestinal tract seen with the upper gastrointestinal capsule. A: Gastroesophageal junction; B: Cardia; C: Fundus; D: Greater curvature; E: Lesser curvature; F: Incisura angularis; G: Antrum; H: Pylorus; I: First part of duodenum (retrograde view); J: Second part of duodenum (ampulla also seen).

in 64%. The mean (± SD) time of capsule transit in the oesophagus, stomach and duodenum was 28 (± 95) s, 68 (± 25) min and 11 (± 15) min respectively. Routine administration of hyoscine was abandoned after the first 33 patients because of concern that it might be delaying capsule entry into the duodenum. Analysis, however, failed to demonstrate any delaying effect of the drug on gastric transit: The mean gastric transit time with hyoscine butylbromide was 69 (± 25) min and 66 (± 26) min without (P = 0.67).

Mucosal visualisation and pathology detection

The mean reading time for capsule videos was 48 (± 18) min with standard mode. All 50 studies were subsequently de-identified and re-read by one reader (MEM) in a randomised, blinded fashion using the Quick-view (Medtronic Ltd.) option in the pre-set mode (the software selecting 10% of the most relevant lesions for viewing by the reader) to examine the stomach (oesophagus and duodenum being read in standard mode with frame rate selected by the reader according to his usual practice): Reading time was significantly reduced to 20 (± 5) min (P = 0.0001).

Visualisation of the upper GI tract was graded as follows: Oesophagus, 4.8 (± 0.5); gastro-oesophageal junction (GOJ), 4.8 (± 0.8); cardia, 4.8 (± 0.8); fundus, 3.8 (± 1.2); body, 4.5 (± 1); antrum, 4.5 (± 1); pylorus, 4.7 (± 0.8); duodenal bulb (D1), 4.7 (± 0.7); D2, 4.7 (± 1) (Figure 2). Withdrawal of hyoscine administration did not affect any visualisation scores. The visualisation grade at the fundus was significantly lower when compared to all other areas of the upper GI tract (P < 0.05 for comparisons to the oesophagus, GOJ, cardia, body, D1 and D2) (Figure 3). The whole circumference of the Z-line was seen in 92.5% of cases. Inability to achieve prone positions during SPIT did not render lower overall gastric visualisation compared to complete SPIT; combined mean scores of cardia, fundus, body, antrum and pylorus visualisation were 4 (± 1) *vs* 4.2 (± 1.4), respectively (P = 0.38). Detected pathology included: oesophagitis (n = 12), Barrett's oesophagus (n = 1), hiatus hernias (n = 7), Cameron's ulcer (n = 1), gastric inlet patch (n = 1), oesophageal varices (n = 8), gastric varices (n = 2), portal hypertensive gastropathy (n = 5), gastritis (n = 20), benign gastric polyps (n = 10), gastric ulcers (n = 2), duodenitis (n = 4), duodenal polyp (n = 1), villous atrophy (n = 1) and angioectasia (n = 7) (Figure 4). No pathology was missed using

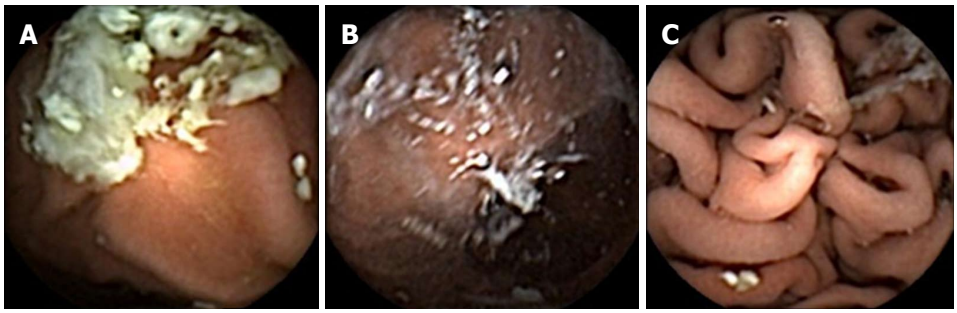

Figure 3 Suboptimal views in the fundus. A: Mucus; B: Bubbles; C: Insufficient distension.

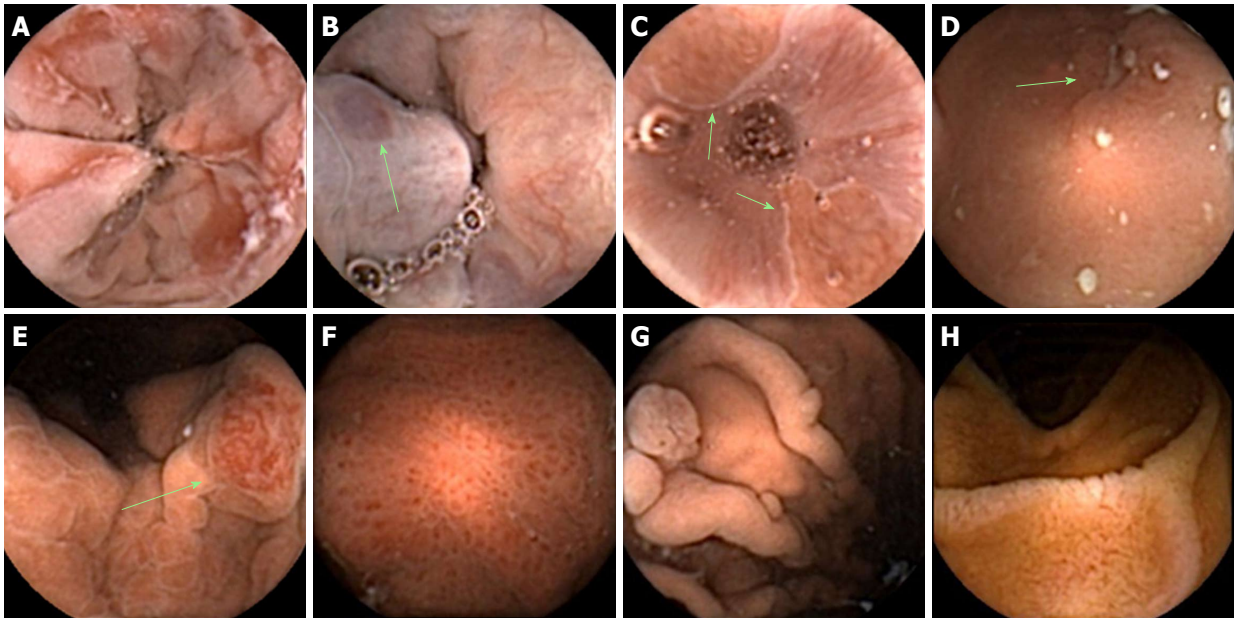

Figure 4 Pathology detected by upper gastrointestinal capsule. A: Erosive esophagitis; B: Oesophageal varices; C: Barrett's oesophagus; D: Gastric ulcer; E: Gastric angioectasia; F: Portal hypertensive gastropathy; G: Benign cystic fundic gland polyps; H: Coeliac disease.

the Quickview reading software in the stomach when compared to standard mode.

Patient tolerance and safety

Mean procedural pain, discomfort and distress scores were: 0.4 (± 1), 0.4 (± 1) and 0.3 (± 0.9) respectively. No complications were seen. All patients were willing to undergo a repeat procedure if it was necessary.

DISCUSSION

UGI capsule endoscopy achieved oesophagogastric examination in all patients, although limited battery life precluded duodenal examination in a third. All studies using swallowed water for gastric distension, simethicone and the SPIT were performed by nursing staff according to protocol. Patients were able to comply with the SPIT in 90% of cases although difficulties with lying prone in the remainder did not affect outcome. SPIT provided excellent views of all areas of the oesophagus and stomach, both D1 and D2 were visualised clearly when the capsule traversed the pylorus within the 90-minute time frame and pathology was identified throughout. The procedure was extremely well tolerated and no complications occurred.

Gastroscopy is performed in 1% of the United Kingdom population per annum[19]. In the United States, an increase in 50% of gastroscopy utilisation was estimated within the space of a decade between 2000 and 2010[20]. However, gastroscopy is an uncomfortable procedure[16,21,22] and the majority of findings do not significantly affect management[23]. This would suggest a role for a well-tolerated, non-invasive alternative that could select the minority of patients who need upper gastrointestinal biopsies or endoscopic therapy. Unlike the small and large bowel, which are long, relatively straight with constant lumina, the upper gastrointestinal tract comprises three quite different structures: the short, tubular, small diameter oesophagus and duodenum and the voluminous stomach, the gastroduodenum being convoluted in shape. Technologies to date have tried to address these challenges by developing capsules with cameras at both ends, maximising image capture rate and battery life and controlling capsule movement.

Although there is no equivalent data for the oesophagus, there is evidence that a double-ended pill camera is better than a single-ended one in terms of diagnostic yield in the small bowel[24,25]. Intuitively it seems likely that a single-ended capsule leading with the blind end is less likely to get complete views of the GOJ than one with cameras at both ends. Similarly, our experience is that a single ended device may miss proximal lesions in the duodenal bulb if transit through the bulb is rapid[26].

The Pillcam® ESO, capturing a total of fourteen frames (seven from each end) per second[27] was superseded by the ESO2[28], capturing a total of 18 frames per second. The 35 frames per second delivered by the UGI capsule would deliver almost 1000 oesophageal images in the average transit time of 28 s shown in our evaluation. This improvement is likely to have resulted in better oesophageal views: The entire GOJ was seen in only 50% of ESO2 studies[3] compared to 92.5% in this series. Whether or not this translates to better diagnostic yield in the oesophagus and the rest of the upper gastrointestinal tract needs to be confirmed.

We, and others, have demonstrated some degree of control with an external handheld magnet[11,29,30], which has shown promise in comparison with conventional gastroscopy[26,31]. Rey *et al*[32] visualised between 85%-93% of gastric landmarks in a controlled trial comparing gastroscopy with capsule endoscopy controlled using a large fixed external magnet developed by Olympus and Siemens. Both modalities identified 58% of pathologies and both missed lesions identified by the other. A similar system was found to have a sensitivity of only 62% in comparison to gastroscopy but only 21 of 189 patients recruited had focal pathology[33]. More recently, Liao *et al*[12] demonstrated that capsule endoscopy controlled by a robot magnet achieved 90% sensitivity (irrespective of size and location) in detecting focal lesions compared to gastroscopy in a large 350 patient multicenter study in Chinese patients with dyspepsia. Such techniques, however, require expertise and cost-effectiveness studies are needed. Therefore, the prospect of a simple, nurse-led, protocol driven UGI examination is attractive: cost and expertise required is mainly limited to the capsule and the interpretation of the videos.

The SPIT protocol is easy to follow in clinical practice. The patient is asked to rotate along their longitudinal axis almost 360° from the right lateral to prone position, a series of manoeuvres which are performed 30° head down, horizontal and 30° head up. This aims to achieve complete gastric imaging as was reported for capsule endoscopy using handheld external[34] and static robot magnets[35]. Qian *et al*[35] demonstrated the benefits of the left lateral, supine and right lateral positions for imaging the fundus, cardia and antropyloric regions respectively. Rahman *et al*[34] found that visualising incisura, antrum and pylorus was best achieved by using the handheld magnet to position the capsule opposite the gravity-dependent positions on the greater curve and antrum in the supine patient. We have used the prone position to achieve the same capsule position and viewpoints. The combination of patient positional changes in Rahman's study achieved good to excellent views of all areas of the upper gastrointestinal tract. These previous studies were performed using single ended camera capsules: it is likely that greater coverage is obtained using a double-ended capsule providing a view of almost 360°. Studies comparing diagnostic yield of the two modalities are warranted. Five patients were unable to achieve the prone position but otherwise completed SPIT without obvious impact on landmark visualisation. Nonetheless, SPIT may not be feasible for all those with mobility restrictions.

Capsule reading was time consuming at 48 min and most of the viewing is repetitive gastric imaging making reading a tedious task. However, image recognition software continues to be developed which can exclude sequentially identical images, or select images which are different or identified as pathological, thereby reducing the size of the video to be viewed. The Quickview system is such a software and in its previous iteration in the Pillcam® SB2 (Given Imaging Ltd.) was shown to have a sensitivity of 92.3% in detecting small bowel pathology[36]. Perhaps such software may prove more useful in the large volume stomach in which the capsule images the same areas repeatedly, compared to the small bowel in which transit distally is more constant and subject to less repetitive imaging of the same region. No pathology was missed when Quickview was used to view the stomach. In this study, videos were re-read with Quickview in a randomised order and anonymised. Even so, they were re-read by MEM, one of the co-authors involved in the initial video interpretation using standard mode. Unbiased Quickview video interpretation by an independent reader, blinded to the findings at standard reading would provide more reliable comparison. Future larger comparative studies are needed to confirm the value Quickview in UGI capsule endoscopy.

The UGI capsule visualised the fundus less well. This is consistent with other studies using capsule endoscopy, even with external actuation techniques such as magnetic steering[18,30]. During gastroscopy, gas insufflation is used to inspect the proximal stomach, which is collapsed in the fasted state. While varying amounts of water have been used to distend the stomach during upper GI capsule endoscopy[10,11], we have previously shown that 1000 mL improves mucosal clarity and distension compared to 200 mL[11]. Some UGI videos were obscured by adherent mucus in the proximal stomach. The use of mucolytics such as N-acetylcysteine or pronase has been shown to be of benefit in improving mucosal visibility during gastroscopy[37-39], although this did not translate to the only capsule endoscopy study to date[40]. Routine use of hyoscine has been advocated to improve visualisation in OGD[14]. This did not appear to make a difference in our experience, although as with water- and gas- distension techniques and mucolytics, the potential benefits of these

agents should be investigated further.

A 64% complete examination to D2 was disappointing. Hyoscine may delay gastric emptying[41], but although this was not a study powered to investigate its effects, hyoscine did not appear to have an obvious effect on gastric transit in this small cohort. Meltzer *et al*[42] found that only one half of their ESO2 (30 min) examinations reached the duodenum. Using a modified version of the ESO2 (with a 90-min battery life) and pre-procedural intravenous erythromycin, Gralnek *et al*[7] achieved duodenal entry of the capsule in 97.8% of cases. Therefore the use of promotility agents might be considered, unless rendered redundant by further improvements in battery life.

The development of transnasal and single-fibre endoscopy as well as Cytosponge acknowledges the need for less-invasive technologies for upper gastrointestinal screening and surveillance[43]. In this feasibility study, anxiety, discomfort and pain scores associated with the UGI capsule and SPIT were excellent, consistent with previous studies of capsule endoscopy of the oesophagus[44,45], small bowel[16] and colon[46]. Furthermore, Gupta *et al*[47] found that adult subjects expressed a preference for capsule endoscopy compared to sedated endoscopy for Barrett's oesophagus screening, raising the possibility that compliance with investigation might be better if less-invasive techniques are offered.

There are limitations to this study and with the technologies. This is an observational cohort study that suggests that UGI capsule endoscopy is feasible, and when technological development allows more reliable duodenal imaging, randomised controlled trials of diagnostic yield compared to gastroscopy are needed. Cost effectiveness studies should consider the costs of the supporting systems and their maintenance (endoscopes, stack systems, monitors, computer software), disinfection, accessories and disposables (which includes the capsule), training requirements and the time taken to perform procedures (including interpreting images). Capsule endoscopy at present remains only diagnostic. The technology to biopsy lesions has been reported but remains in the experimental phase[48]. However, whilst most endoscopists have a low threshold for taking biopsies, the use of non-invasive tests for *Helicobacter pylori* might reduce this and our experience of investigating patients with dyspepsia is that biopsies only increased diagnostic yield by 2.4%[23].

Within the context of the limitations, this study shows that upper GI capsule endoscopy can be performed by nurses in a protocol-driven manner using the novel UGI capsule (Medtronic Ltd.). The SPIT, combined with gastric insufflation using water and simethicone appears to allow excellent visualisation of the whole stomach, albeit with slightly reduced visibility in the fundus. The oesophagus and gastro-oesophageal junction are well seen although further work is needed to allow more reliable visualisation of the duodenum. The procedure is extremely well tolerated by patients.

ARTICLE HIGHLIGHTS

Research background

Upper gastrointestinal (UGI) endoscopy (gastroscopy) is the method of choice to investigate dyspepsia, but is an uncomfortable test which carries the risk of intubation and sedation. Dyspepsia is a common symptom of which potential malignant lesions are an uncommon cause. Therefore a non-invasive alternative which might appropriately select those patients who require gastroscopy in order to obtain biopsy samples for histological analysis or for endotherapy is desirable. Capsule endoscopy is well tolerated and is a first line small bowel imaging tool, but lack of control of capsule movement limits visualisation to the dependent parts of the stomach only. Control can be achieved using external magnets, but this requires operator skill and magnetic devices which may be expensive. A simpler method would be to use swallowed water as a medium in which to move the capsule in the flow of water to different dependent parts of the stomach using patient positional change.

Research motivation

Several techniques using magnets to control capsule movement have been developed, but movement in water flow induced by patient positional change might offer an effective, simpler and less expensive alternative which has not been studied. An assessment of the areas of the upper gastrointestinal tract a capsule endoscope is capable of visualising is necessary in order to determine if such a technique might be feasible. Were this to be so, comparative trials with gastroscopy in identifying pathology would be warranted.

Research objectives

Our aims were to determine the visualisation quality of different upper gastrointestinal landmarks using a capsule endoscope moved around a water-filled stomach using a novel patient positional change technique, to assess procedural completion and patient tolerance of the procedure and time taken to read and report the videos.

Research methods

This was an observational study of a cohort of patients undergoing capsule endoscopy because they declined to undergo gastroscopy. Visualisation quality of different landmarks (oesophagus, gastro-oesophageal junction, cardia, fundus, body, antrum, pylorus, duodenal bulb and second part of duodenum) was scored (1-5: Poor-excellent) as was patient tolerance in terms of pain, discomfort and distress (0-10: No - intolerable). Video reading times in both standard and Quickview mode were compared.

Research results

Complete oesophagogastric examination was achieved with excellent views in all 50 patients. However, the battery-life for the UGI capsule expired before reaching D2 in 36%. Future adaptations are necessary to either promote earlier exiting of the capsule from the stomach into the duodenum (by positional change or prokinetics) or extend battery life. Reading time was lengthy, at 48 min. Using Quickview reduced this to 20 min and no pathology was missed. Further blinded comparative trials are needed to determine the reliability of Quickview in this setting. For patients, the procedure was extremely well tolerated and no complications were seen with the UGI capsule in this study.

Research conclusions

Our study demonstrates the feasibility of achieving excellent views of the oesophagus, stomach and duodenum (when seen) using a novel nurse-led protocol to move the upper gastrointestinal (GI) capsule through a series of patient positional changes. Future randomised control trials assessing diagnostic yield against gastroscopy will be needed to demonstrate reliability. However, the results we report suggest that this protocol may be a well-tolerated and less invasive alternative means to examining the upper GI tract endoscopically.

Research perspectives

These findings suggest that UGI capsule endoscopy is feasible, allows visualisation of all oesophagogastric landmarks and is extremely well tolerated by patients. Technological improvement, for example in battery life,

is likely to ensure more reliable imaging of the duodenum. If so, the simple positional interchange technique using the UGI capsule should be compared to gastroscopy in terms of diagnostic yield. Further studies to improve video reading time are needed.

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SYSTEMATIC REVIEWS

Role of band ligation for secondary prophylaxis of variceal bleeding

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Abstract

AIM

To summarize and critically examine the role of band ligation in secondary prophylaxis of variceal bleeding in patients with cirrhosis.

METHODS

A literature review was performed using the MEDLINE and PubMed databases. The search terms consisted of the words "endoscopic band ligation" OR "variceal band ligation" OR "ligation" AND "secondary prophylaxis" OR "secondary prevention" AND "variceal bleeding" OR "variceal hemorrhage" AND "liver cirrhosis". The data collected from relevant meta-analyses and from the most recent randomized studies that were not included in these meta-analyses were used to evaluate the role of endoscopic band ligation in an effort to demonstrate the most recent advances in the treatment of esophageal varices.

RESULTS

This study included 11 meta-analyses published from 2002 to 2017 and 10 randomized trials published from 2010 to 2017 that evaluated the efficacy of band ligation

in the secondary prophylaxis of variceal bleeding. Overall, the results proved that band ligation was superior to endoscopic sclerotherapy. Moreover, the use of β-blockers in combination with band ligation increased the treatment effectiveness, supporting the current recommendations for secondary prophylaxis of variceal bleeding. The use of transjugular intrahepatic portosystemic shunt was superior to combination therapy regarding rebleeding prophylaxis, with no difference in the survival rates; however, the results concerning the hepatic encephalopathy incidence were conflicting. Recent advances in the management of secondary prophylaxis of variceal bleeding have targeted a decrease in portal pressure based on the pathophysiological mechanisms of portal hypertension.

CONCLUSION

This review suggests that future research should be conducted to enhance current interventions and/or to develop innovative treatment options with improved clinical endpoints.

Key words: Band ligation; Variceal bleeding; Rebleeding; Liver cirrhosis; Endoscopic therapy; Variceal eradication; Secondary prophylaxis; Esophageal varices

Core tip: Variceal bleeding is a life-threatening complication of liver cirrhosis. The current guidelines recommend the use of band ligation together with β-blockers in the setting of secondary prophylaxis for variceal bleeding in patients with cirrhosis. This review summarizes data from meta-analyses and randomized trials to demonstrate the most recent advances in the management of variceal rebleeding. The current evidence suggests that the efficacy of band ligation is increased by adding β-blockers in accordance with the current guidelines. However, combination therapy does not procure a survival advantage. Innovative interventions and more effective novel strategies aiming to improve clinical outcomes should be developed.

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INTRODUCTION

Approximately half of liver cirrhosis patients have developed gastroesophageal varices at diagnosis[1]. In the absence of proper treatment, approximately 30% of patients with varices will suffer a bleeding episode within the first 2 years following the cirrhosis diagnosis[2]. Variceal bleeding is considered one of the most severe complications of portal hypertension and constitutes a life-threatening condition for the cirrhosis patient. Patients surviving this first attack have an increased risk for rebleeding, especially during the first 6 weeks following the initial event. Overall, a second episode of variceal bleeding occurs in approximately 60% of this group of patients within 2 years[3,4]. The most common risk factors for variceal bleeding are the sizes of the varices[2,5,6], the severity of the liver disease[2] and the presence of red color signs on the variceal wall[2,7]. Patients with small varices have a low bleeding risk (approximately 5% per year), whereas patients with large varices have a higher bleeding rate of approximately 15% per year[1,8,9]. The 1-year bleeding probability in Child-Pugh class A cirrhosis patients with large varices and red signs is 24% compared with a 20% probability for Child-Pugh C patients with small varices and no red signs, indicating that variceal size constitutes the most useful predictor for variceal bleeding[2,6]. The aforementioned predictive factors have been combined in the North Italian Endoscopic Club index to classify patients according to the risk of a first variceal bleeding episode[2]. Variceal bleeding is associated with an increased hepatic venous pressure gradient (HVPG) (exceeding the threshold value of 12 mmHg). In contrast, a HVPG beneath 12 mmHg or a decrease in the HVPG gradient of more than 20% from the baseline level is related to a considerable reduction in the risk of variceal hemorrhage.

Patients who survive a first bleeding episode have a high risk of recurrence[8]. Therefore, these patients should receive appropriate treatment[10,11]. The primary aim of secondary prophylaxis is the prevention of further episodes of variceal hemorrhage and a reduction of associated mortality in cirrhosis patients. Available management options for secondary prophylaxis of variceal bleeding include pharmacotherapy, endoscopic treatment, transjugular intrahepatic portosystemic shunt (TIPS) and surgical shunting[8,12]. According to the Baveno Ⅵ guidelines and the practice guidance of the American Association for the Study of Liver Diseases (AASLD), the combination of non-selective β-blockers (propranolol or nadolol) and endoscopic band ligation constitutes the preferred treatment option for secondary prophylaxis in patients with liver cirrhosis[10,11]. Endoscopic band ligation should not be used alone unless the patient cannot tolerate β-blockers or there is a contraindication for non-selective β-blocker administration[10]. Patients who have not responded to the combination therapy should undergo covered TIPS insertion[10].

Systematic reviews and meta-analyses comparing these interventions have highlighted the differences in efficacy between the different modalities. The primary objective of this study is to summarize and critically review the existing data with a focus on the most updated randomized trials of the role of endoscopic band ligation in the secondary prophylaxis of variceal bleeding in liver cirrhosis patients.

MATERIALS AND METHODS

Search strategy

We conducted a review of the literature using the MEDLINE and PubMed databases. Data regarding the role of band ligation in secondary prophylaxis of variceal bleeding in liver cirrhosis patients were extracted from the relevant full articles. The search terms consisted of the words "endoscopic band ligation" OR "variceal band ligation" OR "ligation" AND "secondary prophylaxis" OR "secondary prevention" AND "variceal bleeding" OR "variceal hemorrhage" AND "liver cirrhosis".

Two reviewers (Aggeletopoulou I and Konstantakis C) independently reviewed all the titles and abstracts retrieved from the search after applying the inclusion criteria. A third reviewer (Triantos C) made the final decision in cases of disagreement. All manuscripts that compared endoscopic band ligation intervention *vs* other interventions were evaluated. Data collected from relevant meta-analyses and the most recent randomized studies not included in these meta-analyses were used to evaluate the role of endoscopic band ligation in an effort to demonstrate the most recent advances in the treatment of esophageal varices. All disagreements were resolved after full discussions within the research group.

Inclusion criteria

The inclusion criteria were as follows: (1) Full articles; (2) meta-analyses or systematic reviews comparing endoscopic band ligation *vs* other interventions (monotherapy or combination); (3) most recent randomized studies comparing endoscopic band ligation *vs* other interventions (monotherapy or combination) that were not included in the existing meta-analyses; (4) patients with liver cirrhosis; (5) studies containing the information of interest as subgroup analyses were included; and (6) Criteria 1 and 2 were applied in the setting of secondary prevention.

RESULTS

Meta-analyses

The meta-analyses that compared the effectiveness of endoscopic band ligation to that of other treatment options are presented in Table 1. Overall, 11 meta-analyses evaluated the efficacy of band ligation from 2002 to 2017[13-23]. Cheung *et al*[17] compared the efficacy of band ligation, pharmacotherapy [β-blockers alone or with isosorbide mononitrate (ISMN)] and their combination for the secondary prevention of variceal bleeding. The authors found no difference in the mortality and complication rates between the different treatment options and concluded that all treatment modalities were equally efficient for the prevention of rebleeding[17]. Similar results were found by Ding *et al*[13], who demonstrated no difference in rebleeding, mortality and complication rates between the band ligation and the β-blockers plus ISMN groups. Band ligation was compared with β-blockers plus ISMN in one additional meta-analysis; the results showed no significant difference between band ligation and β-blockers with regard to all-cause mortality, bleeding-related mortality and the occurrence of adverse events[18]. However, a significant decrease in variceal bleeding was noted in patients who underwent band ligation compared to patients administered β-blockers that was attenuated when the analysis included only studies with adequate randomization and allocation concealment[18]. Thiele *et al*[19] assessed the effectiveness of band ligation with medical therapy compared with monotherapy (band ligation or medical therapy) and suggested that the combination treatment decreased the risk of rebleeding but did not influence the mortality rate compared with monotherapy. However, patients treated with combination therapy exhibited an increased trend towards the development of serious adverse events[19]. A subgroup analysis was performed in 2 meta-analyses to examine the efficacy of band ligation compared to band ligation plus pharmacotherapy; both meta-analyses agreed that combination therapy decreased the overall and variceal rebleeding rates[14,15]. Similar results reported by Ko *et al*[21] indicated that the combination therapy (β-blockers plus band ligation) was superior to pharmacotherapy alone for reduction of variceal rebleeding but not for overall rebleeding and mortality, which exhibited no differences between the two groups[21]. Lastly, another meta-analysis compared band ligation plus β-blockers to monotherapy (band ligation or β-blockers) after stratifying the patients according to their cirrhosis severity (Child-Pugh A *vs* B/C classes)[23]. The outcomes showed that the combination therapy was more effective in preventing rebleeding in the compensated patients but had no influence on the mortality rates[23]. In the decompensated patients, band ligation alone demonstrated an increased risk of rebleeding and mortality compared to combination therapy[23].

Nonsurgical therapeutic endoscopic approaches (endoscopic sclerotherapy and band ligation) for the control and prevention of bleeding episodes were compared by Dai *et al*[20], Karsan *et al*[22] and Singh *et al*[16]. Lower rebleeding, adverse event and mortality rates and higher variceal eradication were reported by Dai *et al*[20] in patients treated with band ligation compared to sclerotherapy, suggesting that endoscopic ligation should be the first-choice therapy. Furthermore, comparison of the combination of band ligation plus sclerotherapy with ligation alone failed to demonstrate significant differences in rebleeding prevention and mortality, and the former approach was associated with higher complication rates[16,22]. In contrast, a meta-analysis that evaluated the effectiveness of 12 prophylactic modalities for secondary prevention of variceal bleeding using multiple treatments indicated that band ligation combined with sclerotherapy could be used as a first-choice therapy[24]. Lastly, comparison of the efficacy of endoscopic procedures to that of pharmacotherapy showed that both methods were equally effective in terms of rebleeding prevention and all-cause mortality[25]. However, the combination of these methods was superior compared to endoscopic therapy

Table 1 Results from meta-analyses comparing band ligation with other interventions in terms of all-cause related rebleeding, variceal rebleeding, all-cause related mortality, bleeding related mortality and complication rates

Study (reference)	Publication year	Country	Method	Number of studies	Number of patients	All-cause related rebleeding RR or OR/CI/I^2	Variceal rebleeding RR or OR/CI /I^2	All-cause related mortality RR or OR/CI /I^2	Bleeding related mortality RR or OR/CI/ I^2	Complications RR or OR/CI/ I^2
Singh *et al*[16]	2002	United States	EBL *vs* EST + EBL	7	453	NR	1.12/ 0.69-1.81/ NR	NR	1.1/ 0.70-1.74/ NR	0.37/ 0.21-0.62/ NR
Karsan *et al*[22]	2005	United States	EBL *vs* EST + EBL	8	520	NR	1.05/ 0.67-1.64/ NS	0.99/ 0.68-1.44/ NS	NR	NR
[1]Gonzalez *et al*[15]	2008	Spain	[2]Combination therapy *vs* EBL	4	404	0.62/ 0.44-0.87/ 40%	NR	0.79/ 0.44-1.43/ 54%	NR	NR
Cheung *et al*[17]	2009	Canada	EBL *vs* PT	6	698	0.96/ 0.73-1.30/ 62%	NR/ NR/ 79%	1.20/ 0.92-1.57/ 0	NR	0.90/ 0.70-1.15/ 0
			EBL+PT *vs* EBL	4	404	0.57/ 0.31-1.08/ 60%	0.38/ 0.19-0.76/ 0	0.90/ 0.41-1.98/ 45%		3.4/ 1.4-8.2/ 74%
			EBL+PT *vs* PT	2	279	0.76/ 0.56-1.03/ 0	0.58/ 0.40-0.85/ 0	0.94/ 0.54-1.63/ 31%		NR
Ding *et al*[13]	2009	China	β-blockers + ISMN *vs* EBL	4	476	0.94/ 0.64-1.38 71.50%	NR	0.81/ 0.61-1.08/ 0	0.76/ 0.31-1.42/ 38.90%	1.26/ 0.93-1.70/ 42.70%
[1]Funakoshi *et al*[14]	2010	France	EBL *vs* EBL + β-blockers	3	252	3.16/ 1.76-5.34/ 0	NR	1.78/ 0.92-3.43/ 0	NR	NR
Li *et al*[18]	2011	China	EBL *vs* β-blockers + ISMN	6	687	0.95/ 0.65-1.40/ NR	0.89/ 0.53-1.49/ NR	1.25/ 1.01-1.55/ NR	1.16/ 0.68-1.97/ NR	NR
Thiele *et al*[19]	2012	Denmark	3EBL+PT *vs* monotherapy	9	955	0.68/ 0.54-0.85/ 1%	0.67/ 0.54-0.84/0	0.89/ 0.65-1.21/ 0	0.52/ 0.27-0.99/ NR	1.42/ 0.94-2.13/ 69%
Ko *et al*[21]	2012	South Korea	EBL + β-blockers *vs* β-blockers	4	409	0.78/ 0.58-1.04/ NR	0.60/ 0.41-0.88/ NR	1.21/ 0.88-1.65/ NR		
Dai *et al*[20]	2015	China	EBL *vs* EST	14	1236	0.68/ 0.57-0.81/ 9.00%	NR	0.95/ 0.77-1.17/ 32.80%	NR	0.28/ 0.13-0.58/ 86.50%
Albillos *et al*[23]	2017	Spain	EBL + β-blockers *vs* EBL	4	416	0.36/ 0.21-0.59/ NR	0.52/ 0.25-1.11/ NR	0.50/ 0.28-0.89/ NR	NR	NR
			EBL + β-blockers *vs* β-blockers	3	389	1.0/ 0.68-1.47/ NR	0.81/ 0.53-1.23/ NR	1.19/ 0.76-1.87/ NR		

[1]These results represent a subgroup analysis of the examined meta-analysis; [2]The term combination therapy includes endoscopic therapy combined with injection sclerotherapy or band ligation combined with drug therapy (β-blockers); [3]The term monotherapy includes endoscopic band ligation alone or medical therapy alone (β-blockers alone or combined with ISMN). RR: Risk ratio; OR: Odds ratio; CI: Confidence interval; I^2: Study heterogeneity; EBL: Endoscopic band ligation; EST: Endoscopic sclerotherapy; NR: Not reported; NS: Nonsignificant; PT: Pharmacotherapy; ISMN: Isosorbide mononitrate.

alone[25].

Randomized trials

The most recent randomized studies evaluating the role of band ligation in secondary prophylaxis (*vs* other interventions) that were not included in the existing meta-analyses were reviewed. Ten trials on secondary variceal bleeding prophylaxis in 770 patients with liver cirrhosis from 2010 to 2017 were included in this study (Table 2). The characteristics and clinical profiles of the patients are summarized in Table 3.

Three trials compared the efficacy of band ligation *vs* endoscopic sclerotherapy[26-28], 3 trials compared band ligation *vs* pharmacotherapy[29-31], 2 trials compared band ligation *vs* TIPS[32,33], one trial compared band ligation *vs* cyanoacrylate injection[34] and one trial compared band ligation combined with sclerotherapy *vs* band ligation combined with microwave coagulation[35]. The results of these studies in terms of variceal obliteration, rebleeding and variceal recurrence are summarized in Table 4, and

Table 2 Characteristics of the included randomized trials

Study (reference)	Publication year	Country	Number of subjects
Monici *et al*[35]	2010	Brazil	70
Luz *et al*[26]	2011	Brazil	83
Santos *et al*[34]	2011	Brazil	38
Lo *et al*[31]	2013	Taiwan	118
Stanley *et al*[30]	2014	United Kingdom	64
Chen *et al*[28]	2016	China	96
Holster *et al*[32]	2016	Netherlands	72
Mansour *et al*[27]	2017	Egypt	120
Lv *et al*[33]	2017	China	49
Hanif *et al*[29]	2017	Pakistan	60

the results regarding mortality are summarized in Table 5.

Band ligation *vs* endoscopic sclerotherapy: Three studies evaluated the efficacy of band ligation *vs* endoscopic sclerotherapy alone[26] or in combination[27,28]. The comparison of band ligation *vs* endoscopic sclerotherapy showed no differences in bleeding control or in the early re-bleeding, complication and mortality rates[26]. Conflicting results emerged when band ligation was compared to sclerotherapy and band ligation[27,28]. Mansour *et al*[27] reported that sclerotherapy and band ligation were superior to band ligation for variceal obliteration, whereas Chen *et al*[28] showed that band ligation alone was more effective than the combination of ligation and sclerotherapy in terms of rebleeding. However, both studies demonstrated no differences in the adverse event rate and survival[27,28].

Band ligation *vs* pharmacotherapy: Stanley *et al*[30] assessed the efficacy of band ligation *vs* carvedilol and found no difference in the prevention of rebleeding. However, a trend towards an improved survival rate was observed in the patients who received carvedilol[30]. The effectiveness of band ligation plus propranolol *vs* propranolol alone was evaluated by Hanif *et al*[29], who suggested that the combination therapy was superior for secondary prophylaxis compared to the use of propranolol alone. Band ligation combined with proton pump inhibitors (PPIs) was compared to band ligation combined with vasoconstrictors; the results showed that adjuvant therapy with PPIs was similar to vasoconstrictors in relation to initial hemostasis and the very early rebleeding rate, but the combination treatment with PPIs demonstrated a lower rate of adverse events[31].

Band ligation *vs* TIPS: Band ligation plus β-blocker combination treatment was compared to TIPS in 2 trials. Both trials agreed that TIPS was superior to combination therapy for rebleeding prophylaxis; however, no difference was found in the survival rates[32,33].

Band ligation *vs* cyanoacrylate injection: One study evaluated the efficacy of band ligation compared to cyanoacrylate injection[34]. The results showed no significant difference between the two methods in terms of mortality, variceal obliteration and the adverse event rates but reported that patients treated with cyanoacrylate injection presented with more minor complications, earlier variceal recurrence and more bleeding episodes than the ligation group[34].

Band ligation plus sclerotherapy *vs* band ligation plus microwave coagulation: One study evaluated the rate of variceal recurrence in patients who received band ligation combined with either sequential microwave coagulation or endoscopic sclerotherapy in a cohort of Child-Pugh A and B patients[35]. The results showed that although the application of thermal therapy after ligation was safe and effective, no difference was found between the two methods in terms of variceal eradication, complications and variceal recurrence[35].

DISCUSSION

The aim of this review was to evaluate the effectiveness of endoscopic band ligation for secondary prophylaxis of esophageal variceal bleeding in liver cirrhosis patients. In this study, we incorporated data from meta-analyses that evaluated the efficacy of band ligation in comparison to (or in combination with) other interventions as well as the most recent data from randomized clinical trials that were not included in the aforementioned meta-analyses. We collected these data with the intention of identifying conflicting results from previous studies and obtaining precise estimates of treatment outcomes in terms of secondary prevention of variceal hemorrhage. Overall, current data favor the use of band ligation over endoscopic sclerotherapy. In addition, use of β-blockers combined with band ligation increases the treatment efficacy due to the reduced risk of rebleeding from the upper gastrointestinal system and esophageal varices. These findings are in agreement with the current clinical practice recommendations for secondary prophylaxis of variceal bleeding. Despite its proven benefits, the effect of combination therapy on survival remains uncertain. Therefore, further high-quality (and volume) studies and the development of novel treatment options are required.

Esophageal variceal bleeding constitutes a life-threatening complication of portal hypertension with a mortality rate of 12%-16% (depending on the analyzed cohort) and a high incidence of early rebleeding within the first 6 wk of the initial bleeding episode[36]. Endoscopic band ligation is a proven therapeutic option for achieving both initial hemostasis and preventing further bleeding episodes. The aim of band ligation is to eradicate varices through their "constriction" with rubber rings that are placed using a device attached to the endoscope tip called a "multiband ligator"[37]. The varices are sucked into the cap of the multiband ligator and then ligated through the release of a rubber band, which is responsible for the interruption of blood flow into the ligated varix[37]. Application of the bands initiates at

Table 3 Baseline characteristics of the patients included in the review

Study (reference)	Patients	Gender (M/F)	Age (range or ± SD)	CP class (A/B/C)	Cirrhosis etiology (agent %)
Monici *et al*[35]	EBL + EST: 36	25/11	47.8 (30-68)	28/8/0	Alcohol/virus/alcohol+virus/cryptogenic/autoimmune/PSC/PBC 12/13/3/5/2/1
	EBL + MC: 34	26/8	48.5 (22-71)	29/5/0	Alcohol/virus/alcohol+ virus/cryptogenic/autoimmune/PSC/PBC 8/11/3/9/1/2
Luz *et al*[26]	EBL: 44	NR	NR	2/22/20	Alcohol/virus/secondary biliary cirrhosis/cryptogenic/PBC 43.2/43.2/9.1/2.3/2.3
	EST: 39	NR	NR	3/21/15	Alcohol/virus/secondary biliary cirrhosis/cryptogenic/PBC 43.6/38.5/7.7/5.1/5.1
Santos *et al*[34]	EBL: 20	13/7	52 ± 12.6	0/4/16	Alcohol/HCV/alcohol+HCV/other 30/30/15/25
	CI: 18	14/4	51 ± 8.2	0/3/15	Alcohol/HCV/alcohol+HCV/other 39/33/6/22
Lo *et al*[31]	EBL+ vasoconstrictors: 60	49/11	52.5 ± 14.4	18/32/10	Alcohol/HBV/HCV/HBV+HCV/cryptogenic 40/22/30/3/5
	EBL+PPIs: 58	49/9	54.2 ± 9.7	15/24/19	Alcohol/HBV/HCV/HBV+HCV/cryptogenic 38/29/26/3/2/2
Stanley *et al*[30]	EBL: 31	21/10	49.6 ± 12.87	11/28/25	Alcohol/NAFLD/PBC/DICLD 91/5/3/2
	Carvedilol: 33	22/11	51.4 ± 10.8		
Chen *et al*[28]	EBL: 48	32/16	56 ± 10	19/29/0	HBV/HCV/Alcohol/autoimmune/other 59/4/6/8/23
	EST: 48	31/17	54 ± 11	20/28/0	HBV/HCV/alcohol/autoimmune/other 75/0/2/10/13
Holster *et al*[32]	EBL+β-blockers: 35	23/12	54 (30-71)	13/18/4	Alcohol/HBV+HCV/alcohol + HBV+HCV/autoimmune liver+biliary disease/other 51/3/8/26/11
	TIPS: 37	18/19	56 (37-75)	13/19/5	Alcohol/HBV+HCV/alcohol + HBV+HCV/autoimmune liver+biliary disease/other 35/19/8/24/14
Mansour *et al*[27]	EBL: 60	34/26	NR	8/20/32	HCV/HBV/HCV+HBV 86.67/6.66/6.66
	EBL + EST: 60	44/16	NR	14/22/24	HCV/HBV/HCV+HBV 86.67/6.66/6.66
Lv *et al*[33]	EBL+propranolol: 25	16/8	46 (38-56)	10/14/1	HBV/HCV/alcohol/AH/HBV+AH/cryptogenic 86.67/13.3/0
	TIPS: 24	13/12	49 (46-62)	9/13/2	HBV/HCV/alcohol/AH/HBV+AH/cryptogenic 83/4/4/4/0/4
Hanif *et al*[29]	EBL+ propranolol: 30	25/5	56.30 ± 5.80	NR	NR
	Propranolol: 30	13/17	57.63 ± 5.98	NR	NR

CP: Child Pugh; EBL: Endoscopic band ligation; EST: Endoscopic sclerotherapy; PSC: Primary sclerosing cholangitis; PBC: Primary biliary cirrhosis; MC: Microwave coagulation; NR: Not reported; HCV: Hepatitis C virus; HBV: Hepatitis B virus; CI: Cyanoacrylate injection; AH: Autoimmune hepatitis; NAFLD: Non-alcoholic fatty liver disease; PPIs: Proton pump inhibitors; DICLD: Drug-induced chronic liver disease; TIPS: Transjugular intrahepatic portosystemic shunt; NSBBs: Non-selective β-blockers.

the gastroesophageal junction and moves upwards in a helical manner for approximately 5-8 cm.

After initial control of bleeding, the band ligation sessions should be repeated at 1-wk to 4-wk intervals according to the practice guidelines of the AASLD[11] and at 1-wk to 8-wk intervals according to the American Society for Gastrointestinal Endoscopy guidelines[38] until the varices are eradicated. The complete eradication process typically requires 2 to 4 ligation sessions[39]. Variceal obliteration is achieved in approximately 90% of patients who undergo band ligation[40]. Once variceal obliteration has been achieved, a surveillance endoscopy is performed 3 mo to 6 mo after obliteration and every 6 to 12 mo thereafter to evaluate variceal recurrence[41]. Episodes of variceal recurrence after obliteration are common, with an incidence range of 20%-75% (within 1 year of therapy)[37].

The most recent consensus guidelines recommend the use of a combination of β-blockers and band ligation as the first-line therapy for the prevention of variceal rebleeding[10]. Non-selective adrenergic β-blockers, such as propranolol or nadolol, are preferred. The effect of non-selective adrenergic β-blockers relies on the reduction of portal pressure by decreasing the portal blood flow, because increased portal pressure is the driving force that enhances variceal growth and subsequent rupture, whereas band ligation only has a local effect[42]. The beneficial effect of combination treatment on variceal rebleeding was confirmed in 5 meta-analyses that assessed the efficacy of combined endoscopic and β-blocker therapy *vs* monotherapy in the prevention of variceal hemorrhage in cirrhosis patients[14,15,19,21,23]. However, the effect of combination therapy on survival was uncertain, because no significant

Table 4 Results of individual trials comparing band ligation with other interventions in terms of variceal obliteration, rebleeding and variceal recurrence

Study (reference)	Treatment	Mean sessions to obliterate	Rate of obliteration /time to obliterate (%)	Rebleeding rate (%)	Variceal recurrence rate
			Endoscopic band ligation *vs* endoscopic sclerotherapy		
Luz *et al*[26]	EBL	NR	75 at 5 d	25 at 5 d	NR
	EST		84.6 at 5 d	15.4 at 5 d	
Mansour *et al*[27]	EBL	3.43 ± 0.67	100 at 15.6 wk	16.70	26.7 at 3 mo
					10 at 6 mo
	EBL + EST	2.22 ± 0.92	100 at 8.64 wk	13.30	20 at 3 mo
					10 at 6 mo
Chen *et al*[28]	EBL	3 ± 0.5	25	14.60	NR
	EBL + EST	3 ± 0.6	16.30	35.40	
			Endoscopic band ligation *vs* β-blockers		
Stanley *et al*[30]	EBL	NR	65	35.50	NR
	Carvedilol		68	36.40	
			Endoscopic band ligation + β-blockers *vs* β-blockers		
Hanif *et al*[29]	EBL + propranolol	NR	NR	10	NR
	Propranolol			40	
			Endoscopic band ligation + PPIs *vs* endoscopic band ligation + vasoconstrictors		
Lo *et al*[31]	EBL + vasoconstrictors	NR	NR	1.7 at 6 d	NR
				8.3 at 6-42 d	
	EBL + PPIs			1.7 at 6 d	
				8.6 at 6-42 d	
			Endoscopic band ligation + β-blockers *vs* TIPS		
Holster *et al*[32]	EBL + β-blockers	NR	71 at 2 yr	26 at 2 yr	NR
	TIPS		73 at 2 yr	0 at 2 yr	
Lv *et al*[33]	EBL + propranolol	NR	NR	37 at 6 mo	NR
				45 at 12 mo	
				45 at 24 mo	
				52 at 30.4 mo	
	TIPS			5 at 6 mo	
				15 at 12 mo	
				20 at 24 mo	
				17 at 30.9 mo	
Santos *et al*[34]	EBL	3.17 ± 1.15	90 at 75.4 d	0	33 at 14.6 mo
	CI	3 ± 1.36	78 at 55.4 d	10	57 at 7.9 mo
			Endoscopic band ligation + endoscopic sclerotherapy *vs* endoscopic band ligation + microwave coagulation		
Monici *et al*[35]	EBL + EST	2.75 ± 1.92	97.30	8.30	27.7 at 9.5 mo
					19.5 at 12 mo
	EBL + MC	2.38 ± 1.63	97.10	0	17.6 at 9.16 mo
					17.5 at 12 mo

EBL: Endoscopic band ligation; EST: Endoscopic sclerotherapy; NR: Not reported; PPIs: Proton pump inhibitors; TIPS: Transjugular intrahepatic portosystemic shunt; CI: Cyanoacrylate injection; MC: Microwave coagulation.

difference was observed[14,15,19,21,23]. This result could be explained by a possible link between band ligation and the development of new or the exacerbation of previous complications, such as a ligation-related ulcer, portal hypertensive gastropathy, or the development of fundal varices[43-45]. The meta-analysis by Albillos *et al*[23] reported that the addition of band ligation with β-blockers resulted in a higher but not significant risk of mortality [incidence rate ratio (IRR) = 1.40; 95%CI: 0.87-2.27), all-source rebleeding (IRR = 1.36; 95%CI: 0.87-2.14) and variceal rebleeding (IRR = 1.24; 95%CI: 0.75-2.05) in patients with Child-Pugh class B/C, suggesting a potential deleterious effect of band ligation in this setting and highlighting the use of β-blockers as a key element of combination therapy[23]. The use of β-blockers enhances nonhemodynamic effects, such as a decrease in the drive of the sympathetic nervous system, and hemodynamic effects, such as a reduction in the splanchnic or gastroesophageal collateral blood flow and portal pressure[46,47]. Moreover, β-blockers may have a favorable effect on overall mortality, because they reduce the frequency of complications of cirrhosis, such as ascites, hepatorenal syndrome, portal hypertensive gastropathy[9,48] and spontaneous bacterial peritonitis[49-51]. A recent study described the "window hypothesis", which proposed that β-blockers had a beneficial impact on survival during the early phase of decompensated liver cirrhosis[46]. However, this benefit seems to diminish/disappear in well-compensated and end-stage cirrhosis patients[46,52]. Over the past few decades, variceal bleeding-related mortality has decreased. Conversely, deaths related other causes that are not associated with endoscopic treatment or pharmacotherapy, such as hepatocellular carcinoma, have demonstrated an increasing trend. Other studies, including a meta-analysis and four studies comparing

Table 5 Results of individual trials comparing band ligation with other interventions in terms of mortality

Study (reference)	Treatment	Mean hospitalization days (range or ± SD)	Mortality rate (%)	Follow up (range or ± SD)
		Endoscopic band ligation *vs* endoscopic sclerotherapy		
Luz *et al*[26]	EBL	NR	13.60	5 d
	EST		7.70	5 d
Mansour *et al*[27]	EBL	NR	No difference	6 mo
	EBL + EST			
		Endoscopic band ligation *vs* endoscopic band ligation + endoscopic sclerotherapy		
Chen *et al*[28]	EBL	NR	2.10	6 mo
	EBL + EST		6.30	
		Endoscopic band ligation *vs* β-blockers		
Stanley *et al*[30]	EBL	NR	51.60	26.3 mo
	Carvedilol		27.30	
		Endoscopic band ligation + β-blockers *vs* β-blockers		
Hanif *et al*[29]	EBL + propranolol	NR	NR	6 mo
	Propranolol			
		Endoscopic band ligation + PPIs *vs* endoscopic band ligation + vasoconstrictors		
Lo *et al*[31]	EBL + vasoconstrictors	9.4 ± 2.3	6.7 at 42 d	42 d
	EBL + PPIs	8.8 ± 3.8	5.2 at 42 d	
		Endoscopic band ligation + β-blockers *vs* TIPS		
Holster *et al*[32]	EBL + β-blockers	8.8 ± 5.4	20 at 2 yr	23.4 mo
	TIPS	12.4 ± 11.2	22 at 2 yr	
Lv *et al*[33]	EBL + propranolol	NR	12 at 6 mo	30.4 mo
			12 at 12 mo	
			16 at 24 mo	
			33 at 30.4 mo	
	TIPS		16 at 6 mo	30.9 mo
			17 at 12 mo	
			27 at 24 mo	
			33 at 30.9 mo	
		Endoscopic band ligation *vs* cyanoacrylate injection		
Santos *et al*[34]	EBL	NR	55	338 ± 189 d
	CI		56	
		Endoscopic band ligation + endoscopic sclerotherapy *vs* endoscopic band ligation + microwave coagulation		
Monici *et al*[35]	EBL + EST	NR	5.50	36.1 (15-53) mo
	EBL + MC		5.88	33.6 (14-54) mo

EBL: Endoscopic band ligation; EST: Endoscopic sclerotherapy; NR: Not reported; PPIs: Proton pump inhibitors; TIPS: Transjugular intrahepatic portosystemic shunt; CI: Cyanoacrylate injection; MC: Microwave coagulation.

band ligation *vs* combined ligation and β-blockers, found no significant differences in the rebleeding and mortality rates[17]. Several studies have compared the effectiveness of band ligation *vs* β-blockers with or without nitrates. Their results are compiled in 3 meta-analyses, which demonstrated comparable results for both the rebleeding and mortality rates[13,17,18].

Endoscopic sclerotherapy, which is another therapeutic intervention for variceal obliteration, has proven to be inferior to band ligation due to its higher complication rates and the number of sessions required for variceal obliteration[37,53]. However, endoscopic sclerotherapy achieves better results in cases of deeper paraesophageal varices, possibly because sclerotherapy induces fibrosis and eradication of perforating veins in contrast to band ligation, which does not affect collateral vessels in the deeper layers[54]. A randomized study that compared the early effects of endoscopic sclerotherapy *vs* band ligation on the HVPG values during acute bleeding episodes showed a sustained increase in the portal pressure levels after sclerotherapy that was followed by a higher rebleeding rate; in contrast, the HVPG values in the ligation group returned to the baseline levels within 48 h[55]. A recent meta-analysis evaluated these two endoscopic approaches and concluded that band ligation was superior in terms of the rebleeding and mortality rates[20]. The combination of band ligation plus sclerotherapy was assessed by Singh *et al*[16] and Karsan *et al*[22], who found no advantage over ligation alone in the prevention of rebleeding and reduction of mortality.

Endoscopic band ligation, endoscopic sclerotherapy, drug therapy and TIPS constitute the nonsurgical therapeutic options for control of variceal bleeding and prevention of rebleeding episodes. Band ligation is considered the preferred initial approach, whereas TIPS is recommended in patients who fail endoscopic and pharmacological therapy or coagulation and those who are at high risk of treatment failure[10,11,56]. Portal vein thrombosis (PVT) is a frequent complication in patients with liver cirrhosis, with a prevalence rate ranging from 10% to 23%[57]. Acute variceal bleeding occurs in patients with PVT under certain circumstances. PVT is related to an increased risk of variceal bleeding and higher failure rates of primary and secondary prophylaxis of variceal

bleeding, resulting in higher mortality rates compared to those of cirrhosis patients without PVT. TIPS insertion has been well established as a safe and effective method for the secondary prophylaxis of variceal bleeding and recanalization of the portomesenteric system in patients with liver cirrhosis and PVT[58-62].

Recent randomized studies assessed the efficacy and safety of covered TIPS *vs* band ligation with β-blockers in patients with and without PVT[32,33]. Both studies suggested that TIPS implementation resulted in decreased variceal rebleeding rates but similar survival rates when compared to patients who received combination treatment[32,33]. In patients with PVT, TIPS insertion was also related to a higher rate of portal vein patency[33]. However, conflicting results were found regarding the incidence of hepatic encephalopathy. In patients with PVT, both groups demonstrated similar risks of hepatic encephalopathy[33]. In contrast, TIPS was associated with higher rates of early hepatic encephalopathy development in patients without PVT[32]. A meta-analysis showed a significant reduction in variceal rebleeding episodes and rebleeding-related mortality in patients undergoing TIPS *vs* endoscopic techniques; although TIPS increased the rate of post-treatment encephalopathy, the overall mortality rate remained the same for both groups[63]. Another meta-analysis that evaluated various interventions for secondary prophylaxis of variceal bleeding reported that TIPS, β-blockers combined with sclerotherapy and band ligation combined with sclerotherapy were superior to β-blockers alone in decreasing the rebleeding rates[24]. Moreover, TIPS was superior to β-blockers, band ligation, sclerotherapy, β-blockers combined with ISMN and β-blockers combined with sclerotherapy in terms of bleeding-related mortality[24]. These results were confirmed by a recent meta-analysis that evaluated the efficacy of TIPS compared to endoscopic treatment (band ligation, endoscopic sclerotherapy and cyanoacrylate injection) for the secondary prevention of variceal bleeding, the incidence of post-treatment hepatic encephalopathy and the survival of cirrhosis patients[64]. The results showed that the incidence of bleeding following TIPS was significantly lower than that in the endoscopic treatment group. Moreover, TIPS had a survival benefit in patients with Child-Pugh class C and those who underwent TIPS with a covered stent and did not increase the risk of hepatic encephalopathy. These results suggested that the use of covered TIPS was the preferred choice in patients with severe liver disease[64].

Other approaches that have been proposed to improve the outcome of band ligation, particularly variceal recurrence and rebleeding, include the following. Harras *et al*[65] proposed a combination of band ligation and argon plasma coagulation as an effective method to facilitate the rapid obliteration of varices accompanied by a low recurrence rate without obvious adverse events[65]. Another approach involves the injection of a monomeric liquid compound [cyanoacrylate (n-butyl-2-cyanoacrylate)], which is quickly polymerized when it comes into contact with the tissue surface and results in immediate eradication of the vessel[66]. Several randomized controlled studies have evaluated the efficacy of cyanoacrylate injection compared to other treatment modalities for esophageal varices[34,67,68]. Band ligation was compared with cyanoacrylate injection in two randomized studies, and the results showed no significant differences between the two groups in terms of variceal obliteration, mortality and major complications[34,67]. However, Santos *et al*[34] reported significantly more frequent minor complications, variceal recurrence and a clear trend towards an increase in bleeding episodes in the cyanoacrylate injection group than in the ligation group. Lastly, microwave coagulation, which is another thermal endoscopic treatment method, has been proposed in conjunction with band ligation for the treatment of esophageal varices[35]. Monici *et al*[35] evaluated the efficacy of band ligation plus microwave coagulation compared to band ligation plus endoscopic sclerotherapy and found that application of the microwave coagulation method was safe and gave similar results to the sclerotherapy group.

Recent advances in the management of secondary prophylaxis of variceal bleeding have emerged by targeting a decrease in portal pressure through the pathophysiological mechanisms of portal hypertension. First, the lipid-lowering agent simvastatin, which reduces the portal pressure and improves hepatocellular function, has been added to the standard treatment (β-blocker and band ligation) for variceal bleeding in cirrhosis patients. A recent placebo-controlled randomized trial showed that simvastatin administration was related to a significant amelioration of survival in Child-Pugh A and B patients[69]. However, no improvement was found in the rebleeding rates compared to those of patients who received the placebo[69]. Second, the use of alternative and more powerful β-blockers, which further reduce the HVPG compared to the effects of those used at present. The most recent guidelines recommend the use of propranolol or nadolol with or without ISMN for the prevention of variceal bleeding. However, reduction of HVPG is achieved in approximately 40% of patients, and the variceal bleeding risk is increased in hemodynamic non-responders. Studies have suggested that the use of carvedilol, which is a β-blocker with additional alpha-1 adrenoceptor inhibition properties, promotes a better hemodynamic response than propranolol or nadolol, prevents the progression of small esophageal varices and is more potent in reducing HVPG[70-73]. Lastly, portal pressure-guided therapy has been used to further improve the prevention of variceal rebleeding episodes. Villanueva *et al*[74] showed that the use of HVPG-guided therapy resulted in a significantly lower risk of rebleeding [hazard ratio (HR) = 0.53; 95%CI: 0.29-0.98], a decreased decompensation (HR = 0.68; 95%CI: 0.46-0.99), and mortality rate (HR = 0.59; 95%CI: 0.35-0.99) compared to the control group (combination of nadolol, nitrates and band ligation). Moreover, the hemodynamic responders in the HVPG-guided therapy group received

monotherapy with β-blockers, whereas the non-responders received combination treatment with β-blockers and band ligation. All patients in the control group received the combination treatment[74]. These results conclude that the addition of band ligation will not be beneficial for improving the outcomes if there is no hemodynamic response to β-blockers and set the stage for reevaluation of which patients should receive band ligation[75].

In conclusion, recently, management of variceal bleeding has markedly improved. These gains stem mainly from improvement of the overall strategy for secondary variceal prophylaxis of the cirrhosis population resulting from better understanding of the underlying mechanisms of the pathogenesis of portal hypertension, which guides the rationale behind each therapeutic intervention. In light of current evidence, endoscopic band ligation constitutes an effective treatment option for the prevention of recurrent variceal bleeding. However, the efficacy of band ligation is clearly increased by adding β-blocker therapy, and this combination is suggested as the first-line treatment for the prevention of rebleeding. Although the incidence of rebleeding is reduced by combined therapy in most studies, this option does not result in an overall survival advantage. However, other treatment modalities could also be considered in selected clinical scenarios. In the future, innovative endoscopic techniques and more effective treatment strategies or combinations of novel drugs should be developed with an aim of better clinical management of these patients.

ARTICLE HIGHLIGHTS

Research background

Variceal bleeding is considered one of the most severe complications of portal hypertension and constitutes a life-threatening condition for cirrhosis patients. Recurrent variceal bleeding occurs in approximately 60% of patients within 2 years, with a six-week mortality rate of approximately 12%-16%. Available treatments for the secondary prophylaxis of variceal bleeding include pharmacotherapy, endoscopic treatment, transjugular intrahepatic portosystemic shunt (TIPS) placement and surgical shunting. The most recent guidelines suggest that the combination of non-selective β-blockers (propranolol or nadolol) and endoscopic band ligation constitutes the preferred treatment option for prevention of rebleeding in liver cirrhosis patients. Endoscopic band ligation should not be used alone unless the patient cannot tolerate β-blockers or there is a contraindication for non-selective β-blocker administration. Covered TIPS insertion is recommended for patients who do not respond to combination treatment.

Research motivation

Systematic reviews and meta-analyses have compared these interventions and highlighted differences in the efficacy of the different modalities. However, conflicting data are present in the existing literature.

Research objectives

The authors aimed to summarize and critically examine existing data focusing on the most updated randomized trials of the role of endoscopic band ligation in the secondary prophylaxis of variceal bleeding in liver cirrhosis patients.

Research methods

A systematic search of the MEDLINE and PubMed databases was performed. All manuscripts comparing the endoscopic band ligation intervention *vs* other interventions were studied. Data from the relevant meta-analyses and the most recent randomized studies t not included in these meta-analyses were analyzed.

Research results

The results demonstrated that band ligation was more effective than endoscopic sclerotherapy. The use of β-blockers in combination with band ligation increased the treatment efficacy, supporting the current guidelines regarding secondary prevention of variceal bleeding. TIPS placement was superior to combination therapy in terms of rebleeding prophylaxis, with no difference in the survival rates. However, the data concerning the incidence of hepatic encephalopathy were conflicting.

Research conclusions

This review demonstrated the most recent advances in the role of endoscopic band ligation for the treatment of esophageal variceal rebleeding. Endoscopic band ligation constitutes an effective treatment option for the prevention of recurrent variceal bleeding. However, the efficacy of band ligation is clearly increased by the addition of β-blocker therapy. Other treatment modalities could also be considered in selected clinical scenarios.

Research perspectives

Innovative endoscopic techniques and more effective treatment strategies or combinations of novel drugs should be developed in the future, with an aim of better clinical management of these patients.

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CASE REPORT

Gastric adenocarcinoma of fundic gland type with signet-ring cell carcinoma component: A case report and review of the literature

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Abstract

A depressed lesion was found at a gastric angle of 76-year-old Japanese woman by esophagogastroduodenoscopy. Four years prior, she was diagnosed with a *Helicobacter pylori* infection but no eradication was performed. The pathological diagnosis of biopsy specimens was signet-ring cell carcinoma. Endoscopic submucosal dissection (ESD) was performed. Histopathological examination of the ESD specimen revealed proliferation of well-differentiated tubular adenocarcinoma mimicking fundic gland cells at the deep layer of the lamina propria mucosae. These tumor cells expressed focally pepsinogen-I, diffusely MUC6, and scattered H^+/K^+ ATPase according to immunohistochemistry. Therefore, we diagnosed this tumor as gastric adenocarcinoma of fundic gland type (GA-FG). Adjacent to the GA-FG, proliferation of signet-ring cell carcinoma which diffusely expressed MUC 2 and MUC 5AC was observed. Intestinal metaplasia was focally observed in the surrounding mucosa of the signet-ring cell carcinoma. To the best of our knowledge, this is the first case report of GA-FG with a signet-ring cell carcinoma component. The origin of signet-ring cell carcinoma, *i.e.*, whether it accidentally arose from a non-neoplastic mucosa and coexisted with the GA-FG or dedifferentiated from the GA-FG is unclear at present. We expect the accumulation of similar cases and further analysis to clarify this issue.

Key words: Gastric adenocarcinoma of fundic gland type; Endoscopic submucosal dissection; *Helicobacter pylori*; Intestinal metaplasia; Signet-ring cell carcinoma

Core tip: Gastric adenocarcinoma of fundic gland type is a very rare variant of a well-differentiated gastric adenocarcinoma. To the best of our knowledge, this is the first case report of gastric adenocarcinoma of fundic gland type with a signet-ring cell carcinoma component.

Kai K, Satake M, Tokunaga O. Gastric adenocarcinoma of fundic gland type with signet-ring cell carcinoma component: A case report and review of the literature. *World J Gastroenterol* 2018; 24(26): 2915-2920 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i26/2915.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i26.2915

INTRODUCTION

Gastric adenocarcinoma showing chief cell differentiation was initially reported by Tsukamoto *et al*[1] in 2007. In 2010, Ueyama *et al*[2] reported 10 cases of gastric adenocarcinoma showing chief cell differentiation which expressed pepsinogen-Ⅰ (a marker for chief cells) and proposed the concept of gastric adenocarcinoma of fundic gland type (GA-FG). Since then, the concept of GA-FG has been widely recognized, and reported cases and studies have been gradually accumulated.

Because GA-FG is thought to originate from the gastric mucosa of the fundic gland region without chronic gastritis or intestinal metaplasia, it has been generally considered that GA-FG develops without *Helicobacter pylori* (*H. pylori*) infection[3]. However, cases of GA-FG with current *H. pylori* infection or post-eradiation therapy were recently reported[4,5]. GA-FG generally presents as a well-differentiated adenocarcinoma with mild nuclear atypia and is generally considered to have a low potential for malignancy, although an extremely rare case of advanced GA-FG showing high-grade malignancy was reported[6]. To the best of our knowledge, no GA-FG case with a poorly differentiated adenocarcinoma or signet-ring cell carcinoma component has been reported.

We recently encountered a case of GA-FG with a signet-ring carcinoma component which developed in a patient with current *H. pylori* infection, and we report the case as follows.

CASE REPORT

A 76-year-old Japanese woman visited a nearby clinic complaining of a dull feeling in the stomach. Esophagogastroduodenoscopy (EGD) revealed a depressed lesion at a gastric angle of the greater curvature side. She was referred to our hospital for further examination. She had been found to have an *H. pylori* infection by a urease test four years ago, but no eradication was performed. The depressed lesion was confirmed by an EGD performed at Koga Hospital 21 (Figure 1A) and narrow band imaging (Figure 1B) showed a relatively demarcated lesion with an irregular microsurface pattern. A biopsy of the depressed lesion was performed. Histologically, the biopsy specimens consisted of several fragments of gastric mucosa with intestinal metaplasia. Among the glands with intestinal metaplasia, a small number of atypical cells showing a signet-ring-cell-like appearance were found (Figure 1C). As these atypical cells were positive for immunohistochemistry of pan-cytokeratin (AE1/AE3), a pathological diagnosis of signet-ring cell carcinoma was made (Figure 1D). Endoscopic submucosal dissection (ESD) was performed.

Pathological findings

The ESD specimen showed a slightly depressed lesion measuring 28 mm × 14 mm. In that lesion, a deeper depressed lesion measuring 12 mm × 3 mm was found. Histologically, a well-differentiated tubular adenocarcinoma mimicking the fundic gland cells, mainly the chief cells, proliferated at the deep layer of the lamina propria mucosae (Figure 2A). The tumor cells had slightly enlarged nuclei and showed mild nuclear atypia. The structure and differentiation toward the surfaces of the fundic gland were significantly disturbed compared to normal fundic glands (Figure 2B). The tumor had invaded into the submucosal layer, and the maximum depth of invasion was 400 μm (Figure 2C). No lymphatic or venous invasion was observed. The mucosal surface was covered with non-neoplastic foveolar epithelium.

Adjacent to the well-differentiated tubular adenocarcinoma mimicking fundic gland cells, proliferation of a signet-ring cell carcinoma producing intra- and extracellular mucin was observed (Figure 2A). Proliferation of the signet-ring cell carcinoma was restricted within the lamina propria mucosae, and no lymphatic or venous invasion was observed. Focally, intestinal metaplasia was observed at the mucosa surrounding the signet-ring cell carcinoma (Figure 2A).

In immunohistochemistry, the tumor cells of well-differentiated tubular adenocarcinoma expressed focally (30%) pepsinogen-Ⅰ (Figure 3A), diffusely MUC6 (Figure 3B) and scattered (5%) H^+/K^+ ATPase (Figure 3C). Therefore, we diagnosed this tumor as GA-FG. The tumor cells of GA-FG were negative for MUC 2 but diffusely positive for MUC 5AC (Figure 3D). Meanwhile, the tumor cells of the signet-ring cell carcinoma were diffusely positive for MUC 2 (Figure 3E) and MUC 5AC (Figure 3F) but negative for pepsinogen-Ⅰ, MUC6, and H^+/K^+ ATPase. The immunohistochemistry results are summarized in Table 1.

Based on these HE and immunohistochemical findings, we made the final diagnosis of GA-FG with a signet-ring cell carcinoma component. The mapping based on histology revealed that GA-FG was distributed at a slightly depressed lesion (28 mm × 14 mm) and the signet-ring cell carcinoma was distributed at a deeper depressed lesion (12 mm × 3 mm) in the slightly depressed lesion

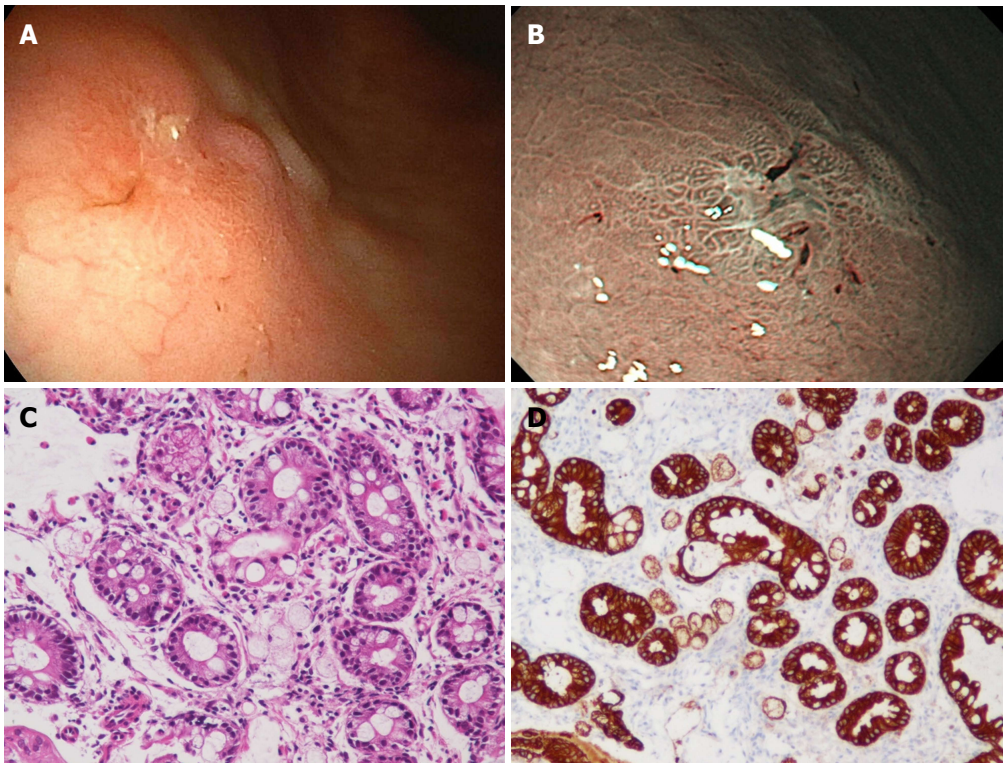

Figure 1 Image from esophagogastroduodenoscopy. A: Depressed lesion was found at gastric angle of the greater curvature side; B: The narrow band imaging of the EGD showed a relatively demarcated lesion with an irregular microsurface pattern; C: The biopsy specimen from the depressed lesion. Among the glands with intestinal metaplasia, a small number of signet-ring cell carcinoma cells were found (HE; × 200). D: Signet-ring cell carcinoma cells were positive for immunohistochemistry of pan-cytokeratin (× 200). EGD: Esophagogastroduodenoscopy.

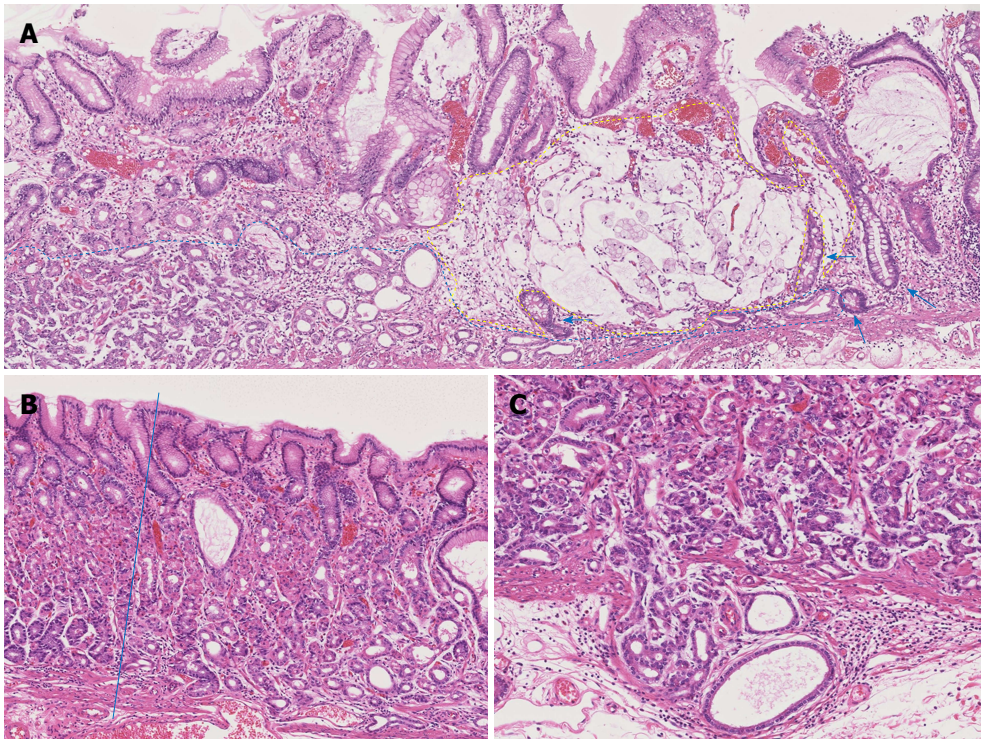

Figure 2 Pathological findings. A: Representative histological photograph of the specimens of endoscopic submucosal dissection (HE; × 50). Proliferation of gastric adenocarcinoma of the fundic gland type (GA-FG) are observed at the deep layer of the lamina propria mucosae in the left half of the photo (blue dot line: the border of GA-FG). Adjacent to the GA-FG, proliferation of the signet-ring cell carcinoma producing intra- and extracellular mucin is observed in the right half of the photo (yellow dotted line: border of the signet-ring cell carcinoma). Intestinal metaplasia was observed at the mucosa surrounding the signet-ring cell carcinoma (arrows); B: Structure and differentiation toward the surfaces of the fundic gland were significantly disturbed at the GA-FG compared to the normal fundic glands (HE; × 50). The blue line is the border of the GA-FG and the normal fundic glands. The mucosal surface was covered with non-neoplastic foveolar epithelium. Intestinal metaplasia cannot be observed in this photo; C: GA-FG invaded into the submucosal layer (HE; × 100).

Table 1 Results of Immunohistochemistry

	MUC 6	H+/K+ ATPase	Pepsinogen-Ⅰ	MUC5AC	MUC2
Gastric adenocarcinoma of fundic gland type	+ (Diffuse)	+ (Scattered, 5%)	+ (Focal, 30%)	+ (Diffuse)	-
Signet-ring cell carcinoma	-	-	-	+ (Diffuse)	+ (Diffuse)

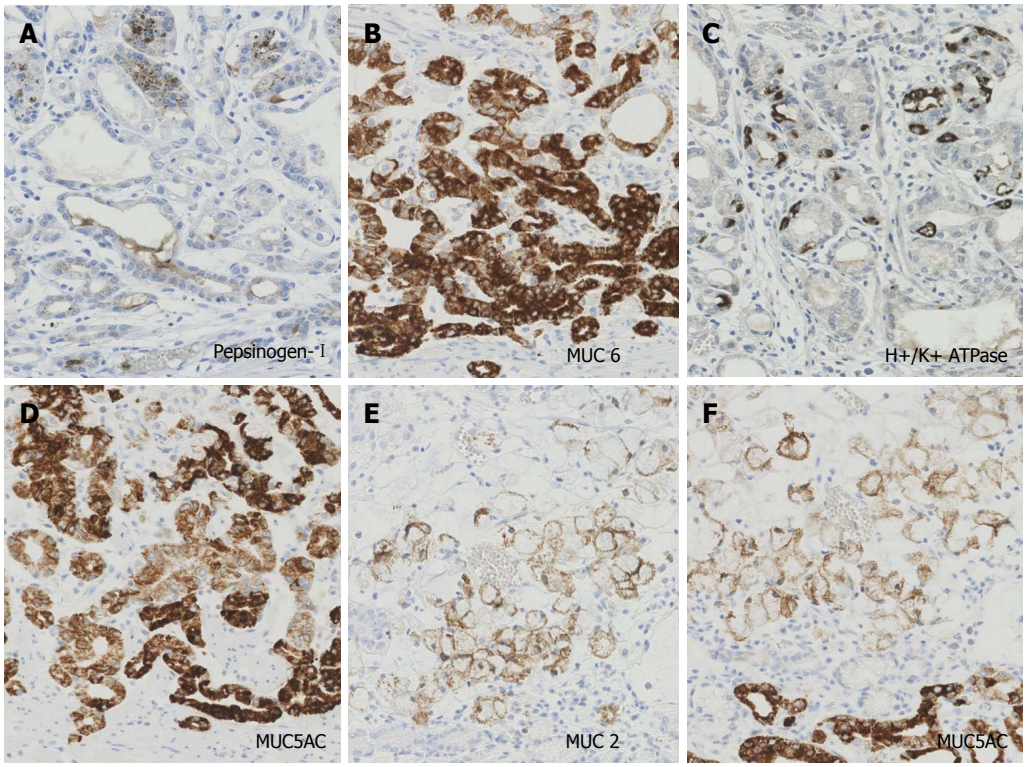

Figure 3 Photographs of immunohistochemistry. The magnifications of all photographs are × 200. The tumor cells of GA-FG expressed focally (30%) pepsinogen-Ⅰ (A), diffusely MUC6 (B), scattered (5%) H+/K+ ATPase (C), and diffusely MUC5AC (D). The tumor cells of the signet-ring cell carcinoma diffusely expressed MUC 2 (E) and MUC 5AC (F). GA-FG: Gastric adenocarcinoma of fundic gland type.

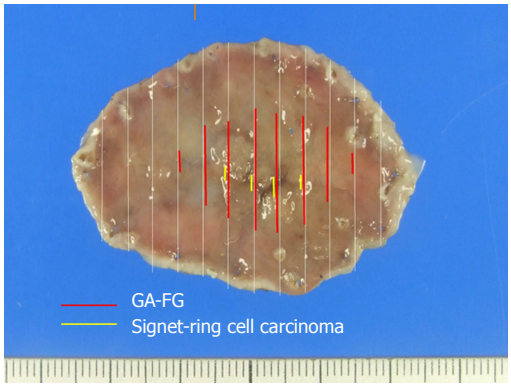

Figure 4 Mapping of the endoscopic submucosal dissection specimen based on histology. GA-FG distributed at a slightly depressed lesion measuring 28 mm × 14 mm (red line) and signet-ring cell carcinoma distributed at a deeper depressed lesion measuring 12 mm × 3 mm in the slightly depressed lesion (yellow line). GA-FG: Gastric adenocarcinoma of fundic gland type.

(Figure 4).

DISCUSSION

GA-FG is a very rare variant of a well-differentiated gastric adenocarcinoma accounting for 1.6% of gastric adenocarcinomas[7]. GA-FGs are characterized by the following: (1) They arise most commonly from the normal gastric mucosa of the fundic gland region without intestinal metaplasia; (2) they are recognized as smooth elevated or depressed lesions; (3) they often invade the submucosal layer, while lymphatic and venous invasion are rare; and (4) the atypia of the tumor cell is usually mild[8]. The Wnt/β-catenin signal signaling pathway and *GNAS* mutations are considered to contribute to the development and progression of GA-FG[7,9,10].

Immunohistochemically, GA-FG variably express the following biomarkers of fundic gland cells: MUC6 for mucous neck cells; H^+/K^+ ATPase for parietal cells; and pepsinogen-Ⅰ for chief cells. Typical cases diffusely express pepsinogen-Ⅰ and MUC6 and show scattered positivity for H^+/K^+ ATPase. These cases are referred to as GA-FG of the chief cell predominant type[2]. GA-FGs do not express the intestinal-type mucin of MUC2.

The distinctive feature of present case was the co-existence of the signet-ring cell carcinoma and GA-FG. To the best of our knowledge, no GA-FG case which contains signet-ring cell carcinoma has been reported. The signet-ring cell carcinoma component in our case expressed the

intestinal type of MUC2, and intestinal metaplasia was focally observed in the background mucosa. In addition, the present case had a current *H. pylori* infection. These are unusual findings for GA-FG.

The origin of the signet-ring cell carcinoma is a very interesting subject. We propose two hypotheses regarding this issue. First, these two lesions (GA-FG and the signet-ring cell carcinoma) may have accidentally coexisted. Usually, GA-FGs develop at the fundic gland in a deep layer of the gastric mucosa, and the normal foveolar epithelium remains at the surface. In the present case, intestinal metaplasia due to chronic inflammation caused by the *H. pylori* infection was focally observed at the surface of the mucosa. Therefore, it seems reasonable that the signet-ring cell carcinoma producing intestinal-type mucin developed at the surface of the mucosa from the intestinal metaplasia and that GA-FG simultaneously developed from the fundic gland of the deep layer of the mucosa. However, the probability for this situation to occur is considered extremely low.

The second hypothesis is that a part of the GA-FG dedifferentiated into signet-ring cell carcinoma. Although dedifferentiation or transformation is often observed in various types of malignant tumors, no GA-FG case showing dedifferentiation or transformation has been reported. Usually, GA-FGs do not express MUC5AC, which is a marker of the foveolar epithelium; however, it is known that GA-FGs rarely express MUC5AC[2,8,11]. In the present case, both the GA-FG and signet-ring cell carcinoma expressed MUC5AC. Ueyama *et al*[2] speculated that MUC5AC is only expressed in advanced GA-FG lesions with a large diameter and massive submucosal invasion, suggesting that cell differentiation changes from the fundic gland type to the foveolar type during disease progression. This speculation regarding MUC5AC seems to support the potential for the transformation of GA-FG. However, we believe it is impossible to conclusively determine the origin of the signet-ring cell carcinoma in the present case because of a lack of reliable evidence.

In conclusion, we have reported the first case of GA-FG with a signet-ring cell carcinoma component which expressed an intestinal type of mucin. Our case had a current *H. pylori* infection and showed focal intestinal metaplasia in the background mucosa. The origin of the signet-ring cell carcinoma is unclear at present. We expect the accumulation of the similar cases and further analysis of whether dedifferentiation or transformation can really occur in GA-FG.

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ARTICLE HIGHLIGHTS

Case characteristics

A 76-year-old Japanese woman visited a nearby clinic complaining of a dull feeling in the stomach.

Clinical diagnosis

Esophagogastroduodenoscopy (EGD) revealed a depressed lesion at a gastric angle of the greater curvature side.

Differential diagnosis

The clinical diagnosis of early gastric cancer was considered by EGD findings.

Laboratory diagnosis

No specific finding was obtained by laboratory testing.

Imaging diagnosis

The narrow band imaging of EGD showed a relatively demarcated lesion with an irregular microsurface pattern.

Pathological diagnosis

Pathological findings of endoscopic submucosal dissection (ESD) specimens indicated the diagnosis of gastric adenocarcinoma of fundic gland type (GA-FG) with a signet-ring cell carcinoma component.

Treatment

Only ESD was performed for treatment.

Related reports

To the best of our knowledge, no GA-FG case with a poorly differentiated adenocarcinoma or signet-ring cell carcinoma component has been reported.

Term explanation

The term GA-FG describes gastric adenocarcinoma of fundic gland type.

Experiences and lessons

This is the first case report of GA-FG with a signet-ring cell carcinoma component.

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