

# World Journal of *Gastroenterology*

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2014-2017

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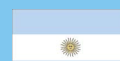
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## Portal vein thrombosis in cirrhosis: Controversies and latest developments

Damian J Harding, M Thamara PR Perera, Frederick Chen, Simon Olliff, Dhiraj Tripathi

Damian J Harding, M Thamara PR Perera, Dhiraj Tripathi,  
 Liver Unit, Queen Elizabeth Hospital, Birmingham B15 2TH,  
 United Kingdom

Frederick Chen, Department of Haematology, Queen Elizabeth  
 Hospital, Birmingham B15 2TH, United Kingdom

Simon Olliff, Department of Imaging and Interventional  
 Radiology, Queen Elizabeth Hospital, Birmingham B15 2TH,  
 United Kingdom

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**Correspondence to:** Dr. Dhiraj Tripathi, Liver Unit, Queen  
 Elizabeth Hospital, Queen Elizabeth Medical Centre, Edgbaston,  
 Birmingham B15 2TH,  
 United Kingdom. [dhiraj.tripathi@uhb.nhs.uk](mailto:dhiraj.tripathi@uhb.nhs.uk)  
 Telephone: +44-121-3714645  
 Fax: +44-121-4141833

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### Abstract

Portal vein thrombosis (PVT) is encountered in liver

cirrhosis, particularly in advanced disease. It has been a feared complication of cirrhosis, attributed to significant worsening of liver disease, poorer clinical outcomes and potential inoperability at liver transplantation; also catastrophic events such as acute intestinal ischaemia. Optimal management of PVT has not yet been addressed in any consensus publication. We review current literature on PVT in cirrhosis; its prevalence, pathophysiology, diagnosis, impact on the natural history of cirrhosis and liver transplantation, and management. Studies were identified by a search strategy using MEDLINE and Google Scholar. The incidence of PVT increases with increasing severity of liver disease: less than 1% in well-compensated cirrhosis, 7.4%-16% in advanced cirrhosis. Prevalence in patients undergoing liver transplantation is 5%-16%. PVT frequently regresses instead of uniform thrombus progression. PVT is not associated with increased risk of mortality. Optimal management has not been addressed in any consensus publication. We propose areas for future research to address unresolved clinical questions.

**Key words:** Portal vein thrombosis; Liver cirrhosis; Anticoagulation; Transjugular intrahepatic portosystemic stent-shunt

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**Core tip:** Portal vein thrombosis is a complication of liver cirrhosis. Optimal management of portal vein thrombosis in cirrhosis has not been addressed in any consensus publication. There has been recent interest in the impact of portal vein thrombosis on the natural history of cirrhosis, and several authors have now described specific treatments for portal vein thrombosis, particularly with transjugular intrahepatic portosystemic stent-shunt and anticoagulation. We review current literature on portal vein thrombosis in cirrhosis and propose areas for future research to



address unresolved clinical questions.

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## INTRODUCTION

Portal vein thrombosis (PVT) is a relatively common finding in advanced cirrhosis, often found in asymptomatic subjects as part of routine ultrasonography. There has been no published consensus on non-malignant PVT in cirrhosis.

We aim to provide an analysis of the current literature and explore options for optimal management of non-malignant PVT in cirrhosis. Literature was identified by a search strategy using MEDLINE and Google Scholar using search terms that included "liver cirrhosis" OR "cirrhosis" AND "portal vein" AND "thrombosis" OR "venous thrombosis" OR "embolism and thrombosis." Eligible studies referred to aspects of the incidence and prevalence, pathophysiology, aetiology, diagnosis and management of PVT in cirrhosis. Because liver transplantation is an important treatment for cirrhosis, studies that referred to PVT and liver transplantation were also eligible. Studies that referred to non-cirrhotic or hepatocellular carcinoma-related PVT were excluded. We identified 2967 search results with MEDLINE and 2860 results with Google Scholar.

## ANATOMY

The portal vein is a valveless, approximately 6-8 cm long conduit that arises from the confluence of the superior mesenteric and splenic veins posterior to the neck of the pancreas. It accounts for 75% of the blood supply to the liver. In the porta hepatis, the portal vein divides into right and left branches that ultimately empty into the hepatic sinusoids of the right and left lobes respectively<sup>[1]</sup>.

PVT is a condition caused by the formation of blood clot within the extra-hepatic portion of the portal vein. In the presence of cirrhosis, PVT is most commonly associated with portal vein stasis, or caused by tumour invasion from hepatocellular carcinoma or portal vein occlusion by cholangiocarcinoma in patients with primary sclerosing cholangitis. PVT may also occur following ablative therapy for hepatocellular carcinoma or fine needle aspiration of pancreatic mass<sup>[2,3]</sup>. PVT can also occur as an unusual condition in non-cirrhotic individuals: in the Western Hemisphere there is commonly an underlying pro-thrombotic aetiology or

local intra-abdominal inflammation, such as pancreatitis or cholangitis. In Southern Asia omphalitis from neonatal umbilical sepsis or cannulation is a cause of childhood PVT<sup>[4]</sup>. Tumour-related and non-cirrhotic PVT is not further discussed in this review.

## INCIDENCE AND PREVALENCE

There have only been limited studies of the incidence and natural history of PVT in cirrhosis. A prospective study of 1243 patients with Child's A and B cirrhosis found the cumulative incidence of new PVT after 1 and 5 years 4.6% and 10.7% respectively<sup>[5]</sup>. Another prospective study of 73 cirrhotics, with a mean baseline MELD score of 15.1, showed an annual incidence of 16%<sup>[6]</sup>. In one cohort of 251 patients with cirrhosis listed for transplantation the incidence of new PVT was 7.4% during a mean follow up of 12 mo<sup>[7]</sup>. The cumulative incidence of PVT after 1 year was 12.8% in a study by Maruyama *et al*<sup>[8]</sup> that followed 150 patients with viral hepatitis-related cirrhosis and no baseline PVT. The risk of developing PVT has been related to the severity of liver disease, with a risk less than 1% in those with well-compensated cirrhosis<sup>[9]</sup>.

The prevalence of PVT in cirrhotic populations is between 0.6% to 26%<sup>[10]</sup>. In studies published since 2000 the prevalence of PVT in patients undergoing transplantation or evaluation for transplantation is between 5% to 16%<sup>[11-16]</sup>.

## PATHOPHYSIOLOGY

### Acute phase

In acute PVT there is new formation of either partially or completely occlusive thrombosis in the portal vein. The episode may be asymptomatic, or may be associated with abdominal pain - particularly if the superior mesenteric vein is involved. Acute obstruction of the superior mesenteric vein and mesenteric arches can lead to intestinal ischaemia, and life-threatening infarction: this seldom occurs in patients with cirrhosis where the onset and progression of PVT is a more gradual and slower process, allowing alternative venous drainage to be established.

Following acute complete occlusion of the portal vein there is intense compensatory hepatic arterial vasodilatation ("arterial rescue") that stabilises liver function.

### Chronic phase

After the period of arterial vasodilatation a phase of "venous rescue" follows with formation of venous collaterals that bypass the occluded segment, forming a "cavernoma" in 3 to 5 wk. For practical purposes an acute PVT can be differentiated from chronic PVT by the absence or presence of a cavernoma of porto-portal collateral vessels on imaging<sup>[17,18]</sup>.

### Complications of PVT

Complications of PVT include variceal haemorrhage, intestinal ischaemia and portal biliopathy (enlarged collateral veins on the surface of the common bile duct causing partial or complete bile duct obstruction)<sup>[19]</sup>. There is conflicting evidence regarding the role of PVT in the natural history of cirrhosis (see below). It has been reported that subjects with cirrhosis and PVT are at an increased risk of variceal haemorrhage compared with cirrhotics without PVT<sup>[20]</sup>. The incidence of intestinal ischaemia following PVT is not widely reported. Harki *et al*<sup>[21]</sup> prospectively assessed for symptoms and clinical evidence of ischaemia (by measuring small intestinal mucosal saturation measurements with visible light spectroscopy). In their small cohort ( $n = 17$ ) of subjects with non-malignant, non-cirrhotic PVT, 67% had both exercise-induced abdominal pain and low visible light spectroscopy findings consistent with ischaemia. No similar studies have been reported. The risk of intestinal infarction has not been well characterised for reasons explained earlier. Prospective studies have not identified cases of intestinal infarction<sup>[8,22]</sup>. A retrospective study of databases from 11 hospitals in Sweden reported on 176 patients with PVT over a median 2.5 years. Abdominal pain was less common in cirrhotic than non-cirrhotic patients. 3% of the cohort required bowel resection for intestinal ischaemia or infarction<sup>[23]</sup>.

## AETIOLOGY OF PVT IN CIRRHOSIS

### Venous stasis

Cirrhosis is associated with increased intra-hepatic vascular resistance and reduced portal blood flow into the liver<sup>[24]</sup>. Low portal blood flow seems to be the most important risk factor for PVT in cirrhosis and has been found to be predictive of future PVT<sup>[6,25,26]</sup>.

Many patients with cirrhosis are treated with non-selective beta-blockers, which reduce portal blood flow and velocity<sup>[27]</sup>. The role of non-selective beta-blockers in influencing survival in patients with decompensated cirrhosis remains controversial; whether they are implicated in the pathogenesis of PVT has not been evaluated with the exception of Nery *et al*<sup>[5]</sup> who did not find any association between the use of non-selective beta-blockers and the development of PVT<sup>[10,28-30]</sup>.

### Thrombophilia

Levels of both pro- and anti-coagulation proteins are reduced in cirrhosis with impaired synthetic function, usually with maintained haemostatic balance and no tendency for bleeding or thrombosis<sup>[31]</sup>. Thrombin generation in cirrhosis is only impaired in the presence of severe thrombocytopenia<sup>[32]</sup>. The international normalized ratio (INR) in liver disease likely overestimates the risk of bleeding because the international sensitivity index used is determined

by means of plasma from patients on vitamin K antagonists<sup>[33]</sup>. Other conventional coagulation tests in patients with cirrhosis do not take into account the reduction in anti-coagulant proteins<sup>[34]</sup>.

Several large population studies have demonstrated that the incidence of venous thromboembolism (deep vein thrombosis, pulmonary embolism) in individuals with cirrhosis is at least similar to that in subjects without liver disease<sup>[35-38]</sup>.

Factor VIII is an important pro-coagulant involved in thrombin generation. Concentrations of factor VIII increase progressively with worsening cirrhosis<sup>[39]</sup>. Protein C is an important anti-coagulant: levels of protein C are often reduced in cirrhosis<sup>[40]</sup>. The ratio of factor VIII to protein C may be predictive of a hypercoagulability<sup>[41]</sup>.

Some pro-thrombotic genotypes, including factor V Leiden G1691A mutation, methylenetetrahydrofolate reductase (MTHFR) C677T mutation and prothrombin G20210A mutation may be more frequent in cirrhotic patients with PVT compared with cirrhotic patients without PVT<sup>[42-44]</sup>.

Anticardiolipin antibodies may be more common in PVT in cirrhosis<sup>[45]</sup>. Bacteraemia from *bacteroides fragilis* has been associated with an increased risk of PVT due to transient appearance of anticardiolipin antibodies<sup>[46]</sup>. However in a prospective longitudinal study of cirrhotic patients in France and Belgium the presence of G20210A prothrombin or factor V mutations was not associated with the development of PVT<sup>[5]</sup>.

### Endotoxaemia

Bacterial translocation and endotoxaemia are common with worsening liver disease, as a result of intestinal mucosal barrier damage<sup>[47-50]</sup>. Inflammation from bacterial infection increases portal pressure<sup>[51-53]</sup>.

Portal endotoxaemia may facilitate activation of the coagulation cascade within the portal venous system<sup>[54]</sup>. Villa *et al*<sup>[55]</sup> demonstrated that the use of enoxaparin in cirrhosis was associated with reduced bacterial translocation, and proposed that this was because of improvements in intestinal microcirculation sufficient to ameliorate portal hypertensive enterocyte damage<sup>[55]</sup>. Reducing portal pressure with non-selective beta blockers is associated with a reduced risk of spontaneous bacterial peritonitis or bacteraemia<sup>[56,57]</sup>.

## HISTORICAL RISK FACTORS FOR PVT IN CIRRHOSIS

Reported historical risk factors for PVT in cirrhosis include complications of, and previous treatments for complications of portal hypertension (previous variceal haemorrhage, endoscopic sclerotherapy, splenectomy, shunt surgery) and the presence of hepatocellular carcinoma<sup>[7,15,26]</sup>. The presence of hepatocellular

carcinoma (in the absence of macro-vascular invasion) appears to be a risk factor for non-neoplastic PVT<sup>[13]</sup>. Severity of underlying cirrhosis, and time spent on a waiting list for liver transplantation are risk factors for PVT<sup>[7,58]</sup>.

Low platelet count, and the development of collateral vessels have been associated with increased risk of developing PVT<sup>[7,8]</sup>. These findings are compatible with the presence of reduced portal blood flow in cirrhosis with portal hypertension, likely the most important causative factor for PVT in cirrhosis<sup>[6]</sup>.

## DIAGNOSIS AND SCREENING

Imaging is appropriate as part of the initial evaluation of subjects with cirrhosis, and periodically during follow up. Because of the risk of hepatocellular carcinoma in cirrhosis computed tomography (CT) or magnetic resonance imaging (MRI) evaluation is advisable following new ultrasound diagnosis of PVT, to look for the presence of liver tumour. Endoscopic screening for varices should also take place because of the increased risk of varices in the presence of cirrhosis with PVT.

### Ultrasound and Doppler ultrasound

Ultrasound and Doppler ultrasound are usually sufficient to diagnose PVT according to published series, however the incidence of PVT is much higher than that is routinely detected by above means<sup>[59,60]</sup>. Ultrasound may demonstrate hyperechoic material in the vessel lumen, but there is variation and operator dependent aspect to this diagnosis. Most of the diagnosis is reliant on Doppler scan that demonstrates absence of flow in part of, or all of the lumen. It can also show flow velocity and direction. "False negatives" have been reported with ultrasound at the time of transplantation<sup>[14]</sup>. Such findings may occur because of *de novo* thrombus formation between imaging and transplantation or false negatives. Different grades of PVT (discussed below) further complicate these findings. Using three-monthly Doppler ultrasound on their cohort of 251 cirrhotic patients awaiting transplantation, Francoz *et al*<sup>[7]</sup> diagnosed PVT in 9 patients at the time of transplantation. Eight of these patients had only partial thrombosis: transplantation was technically feasible in all cases. Data from 1491 patients who underwent liver transplantation at Queen Elizabeth Hospital Birmingham between January 2000 to August 2012 show a PVT prevalence of 119 (8%). Thirty-four (29%) of these cases were diagnosed at the time of surgery. For these subjects the mean interval from last screening imaging to transplantation was 2.25 mo. This suggests that some of the PVTs diagnosed are "interval" thromboses. Overall there were no survival differences between "diagnosed", "incidental" PVT cases and matched controls without PVT<sup>[61]</sup>.

### Cross-sectional imaging

Multiphase CT is alternatively recommended to diagnose PVT during evaluation of cirrhosis. Ultrasound is accurate detecting thrombus in the trunk of the portal vein and intrahepatic branches. CT better assesses the superior mesenteric vein, the presence of porto-systemic shunts, renal veins and inferior vena cava, and the extent of thrombus. CT can help diagnose hepatocellular carcinoma and intestinal ischaemia<sup>[62,63]</sup>.

MRI is an alternative to CT, although has reduced definition in the presence of significant ascites<sup>[64]</sup>.

MRI with contrast is helpful for demonstrating the portal venous system flow and thrombus like CT. MRI is advised for repeated imaging in younger patients to avoid the radiation associated with repeated CTs.

## EFFECTS OF PVT ON THE NATURAL HISTORY OF CIRRHOSIS

The effects of PVT on the natural history of cirrhosis, including its effects on survival may not be deleterious. The risk of PVT appears to increase with severity of cirrhosis<sup>[9]</sup>, but there is little data to demonstrate that PVT is an independent prognostic factor in cirrhosis<sup>[16,65]</sup>.

### Effects on survival

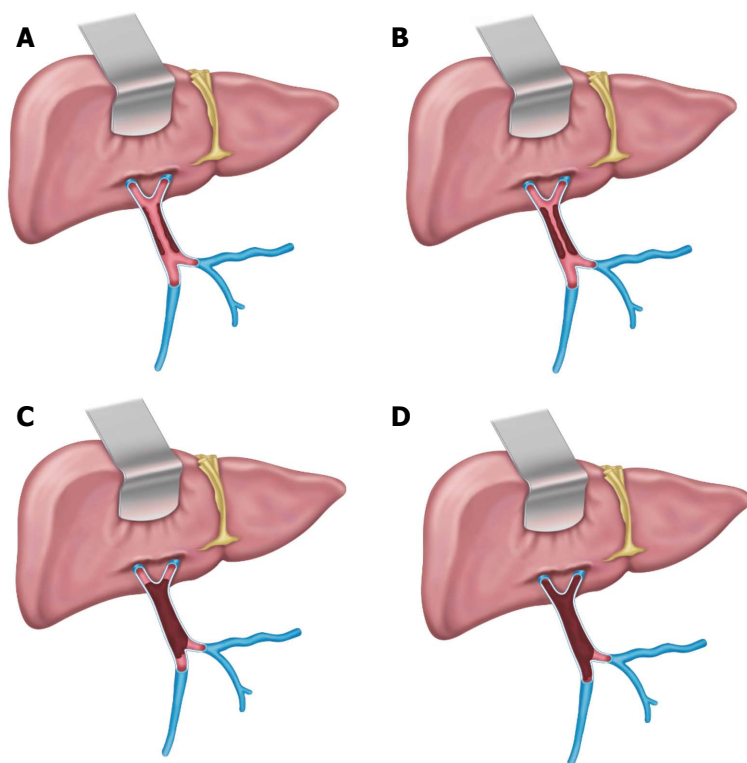
A review, using UNOS registry data from 2002 to 2013 of 66506 patients without hepatocellular carcinoma who were awaiting liver transplantation, found that the presence of PVT was not associated with an increased risk of death or reduced chance of undergoing transplantation<sup>[66]</sup>.

Maruyama *et al*<sup>[8]</sup> followed up 150 patients with viral hepatitis-related cirrhosis, without PVT at baseline. Of the 42 (28%) patients who developed PVT the thrombus progressed in 7.2%, was unchanged in 45.2% and improved in 47.6%. The cumulative survival rates were similar between the thrombosis and non-thrombosis groups<sup>[8]</sup>.

### Effects on disease progression

In a prospective study of 1243 patients with cirrhosis and a mean follow-up of 47 mo, the development of PVT was not associated on multivariate analysis with the risk of disease progression. 118 subjects developed a new PVT, of which 87 were non-occlusive (one year cumulative incidence 4.6%). Non-occlusive thrombus varied over time, disappearing on follow-up in 70% of cases<sup>[5]</sup>.

Natural history studies have identified relatively high rates of PVT regression instead of uniform thrombus progression. One study of 42 consecutive patients with cirrhosis (mean MELD 12.1; range 7-20) and untreated extra-hepatic, non-malignant PVT followed up subjects for a mean 27 mo. PVT worsened



**Figure 1 Yerdel's Classification of portal vein thrombosis**<sup>[19]</sup>. A: Grade I portal vein thrombosis. Partial portal vein thrombosis (< 50% lumen) with or without minimal extension in to the superior mesenteric vein (SMV); B: Grade II portal vein thrombosis; > 50% occlusion with or without minimal extension into the SMV; C: Complete thrombosis of both portal vein and proximal SMV. Distal SMV is open; D: Complete thrombosis of portal vein, proximal and distal SMV.

in 48% of patients and improved in 45%. There was no clear association between progression or regression of PVT and clinical outcome, with baseline Child-Pugh score the only independent predictor of survival or hepatic decompensation<sup>[22]</sup>.

The positive findings of a study that examined the effects of primary prevention of PVT with anticoagulation in subjects with cirrhosis might suggest that PVT does have a role in the progression of cirrhosis: improved survival and less episodes of hepatic decompensation were seen in the study's active arm<sup>[55]</sup>. However the study's authors did not attribute the difference in hepatic decompensation to the prevention of PVT: they postulated that enoxaparin therapy lead to improved intestinal microcirculation and endothelial function, which had a protective effect on the course of the liver disease by reducing bacterial translocation. No other published studies have confirmed their findings.

### Complications

The presence of PVT has been associated with a longer time to achieve endoscopic eradication of varices, but once achieved did not influence their recurrence<sup>[67]</sup>.

The potential for life-threatening intestinal infarction in the presence of complete thrombus occlusion of the portal and superior mesenteric veins is known, although the actual risk of this event is not known.

Sudden clinical deterioration in a cirrhotic patient, such as the development of diuretic resistant ascites or

bacterial peritonitis is suggestive of the development of PVT and should be thoroughly evaluated. The PVT may be the cause of, or the consequence of such events. A stable patient on diuretics may develop a PVT leading to diuretic resistance, leading to SBP. On the other hand bacterial infection in the peritoneum may lead to development of PVT.

## IMPACT OF PVT ON LIVER TRANSPLANTATION

PVT, particularly complete thrombosis affects rates of complications, and possibly survival with liver transplantation. It was historically seen as a contraindication to transplantation.

### Surgical considerations

The Yerdel classification of PVT is widely used to describe PVT because it correlates thrombosis extent with surgical technique and risk of complications (Figure 1)<sup>[15]</sup>.

Pre-existing knowledge of a patient's PVT and use of the Yerdel classification allows appropriate graft selection and planning of the transplant surgical procedure. (Intra-operative diagnosis of incidental PVT may cause problems of added surgical explant time, increased risk of significant bleeding and prolonged cold ischaemia time for the selected graft). For Yerdel grades I to III, operative techniques include thrombectomy, with



**Table 1** Key studies of portal vein thrombosis and liver transplantation

	Number of patients	Prevalence PVT, <i>n</i> (%)	PVT characteristics	Outcomes
Englesbe <i>et al</i> <sup>[65]</sup>	22291 (2001-2007)	897 (4.02)	Not described	PVT was not predictive of waiting list mortality (HR = 0.90, <i>P</i> = 0.23) PVT was predictive of post-transplant mortality (HR = 1.32, <i>P</i> = 0.02)
Sringeri <i>et al</i> <sup>[61]</sup>	1491 (2000-Aug 2012)	119 (8.0)	Not described	Prolonged theatre time, increased blood transfusion rates <sup>1</sup> . No difference mortality up-to 140 mo
Ravaioli <i>et al</i> <sup>[13]</sup>	889 (1998-2008)	91 (10.2)	Partial 51 (56%) Complete 40 (44%)	No difference 1 yr (85% <i>vs</i> 86%) and 5 yr (68% <i>vs</i> 73%) survival between PVT and non-PVT subjects Survival improved significantly for patients with complete PVT in the second era (2003-2008) (57% <i>vs</i> 89% at 1 yr <sup>1</sup> )
Yerdel <i>et al</i> <sup>[15]</sup>	779 (1987-1996)	63 (8.1)	Grade 1: 24, Grade 2: 23, Grade 3: 6, Grade 4: 10	Reduced 5 yr survival between PVT and non-PVT subjects (65.3% <i>vs</i> 76.3% <sup>1</sup> ) But improved 5 yr survival from 1 <sup>st</sup> to 2 <sup>nd</sup> era in all patients (from 72% to 83% <sup>1</sup> )

<sup>1</sup>The *P* value is < 0.05. PVT: Portal vein thrombosis.

or without creation of an interposition graft, followed by direct porto-portal anastomosis. In cases where the lumen of the portal vein has been narrowed by cicatrization of the thrombus, the narrowed segment can be resected and a donor iliac vein graft used as an interposition graft, resulting in a patent, larger diameter vein. Thrombectomy is still possible with Yerdel grade III PVT as long as the portal vein is carefully examined down to the junction of the superior mesenteric and splenic veins with extraction of all thrombus.

Cases of Yerdel grade IV and some grade III cases may be considered as contraindications to transplantation in some centres, while taken on by experienced high volume centres. Complex vascular reconstruction techniques may be necessary with meso-portal "jump grafts" from donor veins or synthetic vascular grafts, creation of porto-caval shunt or portal vein arterialisation. Such complex procedures for extensive or grade IV PVT carry a high (approximately 50%) risk of post-transplant portal hypertension<sup>[16]</sup>. There are a few case series of patients with diffuse PVT who have undergone multivisceral transplantation<sup>[68]</sup>. The procedure is only offered in a few centres, but should be considered in patients with severe bowel dysfunction due to porto-mesenteric venous ischaemia or refractory portal hypertensive gastrointestinal bleeding where diffuse PVT is present.

### Outcomes of liver transplantation with PVT

A large American series described outcomes post-liver transplantation between September 2001 and December 2007 in 22291 subjects where the prevalence of PVT was 4.02% (*n* = 897). PVT was not classified according to grade, or whether occlusive/non-occlusive. The presence of PVT was associated with higher post-transplant mortality only during the first year of follow up in this cohort (HR = 1.32, *P* = 0.02)<sup>[65]</sup>. Our own institution's experience of 1491 transplants between January 2000 and August 2012 found the presence of PVT was associated with significant increases in intra-operative blood product use and

theatre time, but no difference in survival<sup>[61]</sup>.

Several papers describe outcomes based upon the classification of thrombosis. In subjects with non-occlusive PVT, post-transplant mortality outcomes are no different from non-PVT patients<sup>[15,69,70]</sup>.

Mortality rates likely increase in the presence of occlusive PVT, but may be better in larger centres with greater experience of PVT-surgical management. In a review of 25753 transplants performed in different centres between 1984 and 2008 the 30 d and 1 year mortality rates for subjects with PVT were higher than for those without PVT (10.5% and 18.8% *vs* 7.7% and 15.4%): only complete PVT accounted for this difference<sup>[69]</sup>. Mortality rates were higher still in subjects with grade IV PVT. Studies of transplant recipients where end-to-end portal anastomoses were not feasible describe high rates of post-operative morbidity due to persistent portal hypertension, and higher rates of early post-operative mortality (25%)<sup>[71-73]</sup>. More recent data from high volume centres with specific experience in PVT-surgical treatment do not show any effect of PVT on survival. Two studies provide analysis of outcomes for patients with Yerdel grades 3 and 4 PVT. Outcomes for these centres have improved: Ravaioli *et al*<sup>[13]</sup> showed no survival differences for patients with complete PVT when their 10 year data were restricted to the last 5 years (Table 1)<sup>[13]</sup>.

### PVT following liver transplantation

Thrombosis of the portal vein, particularly early following transplantation carries a poor prognosis<sup>[74]</sup>. The rate of PVT occurrence post-transplantation in subjects without a history of preceding PVT is between 0%-2%<sup>[12,14,15,75]</sup>. PVT post-transplantation can occur at the anastomosis site when there is significant mismatch of the donor: recipient vein diameters<sup>[76]</sup>. The rate of post-transplant PVT recurrence in subjects with previous PVT is higher: 2%-3%<sup>[14,61,77,78]</sup>. It is not clear whether thrombosis rates are greater following more complex procedures. There are no standardised approaches to post-liver transplant prevention of re-

thrombosis. It is expected that the risk of recurrent PVT should be reduced by the correction with transplantation of the haemodynamic abnormalities associated with cirrhosis and portal hypertension. In considering approaches to managing the risk of PVT recurrence, they should be weighed against risks of post-operative bleeding.

## LIVING DONOR TRANSPLANTATION AND PVT

Living donor transplantation is performed in many centres because of a shortage of cadaveric donors. For the safety of donors partial grafts obtained from living donors have only a very short length of portal vein. To complete the anastomosis it is vital that there is an adequate length of recipient portal vein, which is not always feasible, particularly in the presence of recipient PVT<sup>[79]</sup>. Procurement of additional vessels to allow complex interposition or jump grafts is also limited, making living donor liver transplantation for patients with complete PVT technically more difficult with high reported mortality. Outcomes in the presence of partial PVT are similar to those in recipients without PVT<sup>[80]</sup>. For cases of complete PVT the use of re-canalised umbilical vein, saphenous vein of the donor or the recipient, or the hepatic veins of the explanted cirrhotic liver have all been used. Another option is to use the cryopreserved vessels from cadavers or cadaveric donors but their use has been associated with worse outcomes due to an increased risk of re-thrombosis<sup>[81]</sup>.

## MANAGEMENT OF PORTAL VENOUS THROMBOSIS IN CIRRHOSIS

The natural history of PVT in cirrhosis remains controversial: this has affected the ability to provide clear management consensus. The presence of PVT does affect liver transplantation surgery and potentially outcome. In candidates for transplantation the main objective of management is to achieve at least partial recanalisation to allow portal flow to the graft with a conventional end to end PV anastomosis. If recanalisation cannot be achieved the objective is to prevent extension of thrombus, particularly to the superior mesenteric vein. Careful screening during evaluation and throughout follow up is important to achieve these aims. In patients with PVT there are different possible approaches to treatment: anticoagulation, transjugular intrahepatic portosystemic stent-shunt (TIPSS), and endovascular procedures with fibrinolysis. The use of primary preventative strategies could also be considered for patients at risk of developing PVT.

PVT in cirrhosis is associated with a higher risk of variceal haemorrhage than in cirrhotic individuals without PVT: assessment with upper gastrointestinal

endoscopy is warranted to assess for oesophageal varices<sup>[82]</sup>.

## ANTICOAGULATION

Six published studies describe anticoagulation in 199 cirrhotic patients using warfarin (with target INR 2-3) or low molecular weight heparin for means of between 6 mo to 302 d<sup>[7,55,83-86]</sup> (Table 2). Two case reports describe the use of rivaroxaban, an oral factor Xa inhibitor, in the management of acute PVT in six subjects with well-compensated Child's A cirrhosis<sup>[87,88]</sup>. With the exception of studies reported by Villa *et al*<sup>[55]</sup> and Senzolo *et al*<sup>[85]</sup>, all of the reported studies are case-control or retrospective series of subjects with cirrhosis and partial or occlusive acute PVT. These published studies do not describe treatment of chronic PVT associated with cavernoma.

### Primary prevention

Villa *et al*<sup>[55]</sup> performed a randomised, controlled study of enoxaparin (4000 IU daily) for 48 wk in 70 patients with Child's B7 to C10 cirrhosis and no PVT (34 active arm, 36 controls). The study's primary outcome, prevention of PVT in subjects with cirrhosis, was successful: there were no PVTs in the active arm at the end of follow up (at 2 years), compared with the 27.7% rate of PVT in the control arm. Of clinical importance, rates of hepatic decompensation (ascites, encephalopathy, bacterial peritonitis, portal hypertensive bleeding) were significantly lower in the treatment arm (38.2%) compared with controls (83%,  $P < 0.0001$ ). Treatment with enoxaparin was associated with a reduction in bacterial translocation, which was thought at least partly responsible for the lower rates of decompensation. No relevant side effects or haemorrhagic events were reported.

### Secondary prevention

Senzolo *et al*<sup>[85]</sup> prospectively evaluated treatment with low molecular heparin (nadraparin) for at least 6 mo compared with standard care in 35 actively treated and 21 control subjects. The patients had cirrhosis (mean MELD 12.6 active arm) and either partial or complete acute PVT. In the active arm the incidence of complete recanalisation was 60%, with stabilisation or partial recanalisation achieved in 20%. Amongst controls recanalisation occurred in only one subject (5%) with partial recanalisation or stabilisation in 5 (24%): the incidence of thrombus progression in controls was 71.4%.

Amongst the reported studies of anticoagulation therapy for secondary prevention, treatment was associated with recanalisation rates of between 39.3% to 75%, and an incidence of thrombus progression between 0% and 14.3%. This compares favourably with rates of recanalisation or thrombus progression reported for control subjects by Senzolo *et al*<sup>[85]</sup>.

Table 2 Summary of studies reporting the use of anticoagulation for portal vein thrombosis in cirrhosis

	Study type	n (controls)	Baseline severity liver disease	Duration and type of anticoagulation	PVT characteristics (none/partial/complete occlusion)	Recanalisation	Partial recanalisation/ stabilisation	Progression	Bleeding complications
Francoz <i>et al</i> <sup>[7]</sup>	Prospective case control	19 (10)	mean MELD 12.8	Warfarin (INR 2-3) Mean 8.1 mo	0/18/1	8/19 (42%) <i>vs</i> 0/10 non-anti-coagulated ( <i>P</i> = 0.002)	0	1	1 bleeding episode following endoscopic variceal band ligation
Amitrano <i>et al</i> <sup>[83]</sup>	Prospective	28	?	Enoxaparin 200 IU/kg per day: 6 mo for responders and non-responders. Partial responders continue until end of follow up.	0/23/5	21 (75%) at median 11 mo	5 (17.9%)	2	Mild anaemia in patient with portal hypertensive gastropathy
Delgado <i>et al</i> <sup>[84]</sup>	Retrospective	55	mean MELD 12.8 +/- 3.8	Warfarin (INR 2-3) or enoxaparin mean 6.8 mo	0/41/14	25 (45.5%)	30 (54.5%)	0	6 variceal bleeds, 1 lower GI bleed, 1 obscure GI bleed, 1 oral bleed post-dental extraction, 1 severe vaginal bleed
Senzolo <i>et al</i> <sup>[85]</sup>	Prospective case control	33 (21)	MELD 12.6 (controls MELD 13.7)	Nadraparin low molecular weight heparin until end of follow up, or until 6 mo following complete recanalisation.	0/24/11	12/33 (36%) active arm <i>vs</i> 1/21 (5%) controls	Partial: 9/33 active arm. Stabilisation: 7/33 active arm. Partial recanalisation or stabilisation in 5/21 controls.	5/33 (14.3%) active arm <i>vs</i> 15/21 (71.4%) control arm ( <i>P</i> < 0.001)	Active arm: 1 cerebral haemorrhage, 1 epistaxis, 1 haematuria, 1 variceal bleed Control arm: 5 variceal bleeds
Werner <i>et al</i> <sup>[86]</sup>	Retrospective	28	MELD 7-29	Warfarin Mean 302 d	not described	11 (39.3%)	17 (60.7%)	0	1 significant vaginal bleed
Villa <i>et al</i> <sup>[85]</sup>	Prospective randomised controlled trial	34 (36)	Child's 7-10	Enoxaparin 4000 IU/d 48 wk treatment. Follow up to 2 yr	Primary prevention study: No PVTs at baseline	N/A	N/A	Treatment arm: No PVT at 2 yr. Control arm: PVT in 10/36 (27.8%) at 2 yr ( <i>P</i> = 0.001)	Active arm: 2 variceal bleeds, 2 epistaxis Control arm: 1 variceal bleed, 1 epistaxis

PVT: Portal vein thrombosis.

Amitrano *et al*<sup>[83]</sup> and Senzolo *et al*<sup>[85]</sup> reported a mean time until venous recanalisation of 6.5 and 5.5 mo respectively. Delgado *et al*<sup>[84]</sup> reported that that up to 39% of subjects who had achieved portal vein recanalisation developed re-thrombosis after stopping anti-coagulation. Where assessed for, the prevalence of thrombophilic abnormalities was between 5 and 16% (Werner and Delgado)<sup>[84,86]</sup>.

Complications

With the exception of the study reported by Delgado *et al*<sup>[84]</sup>, all study patients were screened for large varices, with endoscopic obliteration provided before commencement of anticoagulation. Patients with cavernoma were generally excluded. Patients in the study by Delgado *et al* received standard primary or secondary management of varices following recognised international guidelines<sup>[89]</sup>. In the studies of patients who received pre-emptive endoscopic obliteration of varices (*n* = 144) there were 4 episodes of variceal haemorrhage on treatment. There were 6 episodes of variceal haemorrhage amongst the 57 control subjects.

Senzolo *et al*<sup>[85]</sup> report one cerebral haemorrhage leading to hemiparesis on treatment. Other bleeding complications on treatment were: 2 epistaxis (1 epistaxis in a control subject), 1 haematuria, 2 significant vaginal bleeding, 1 obscure and 1 lower gastro-intestinal haemorrhage. Among the control subjects there were 2 episodes

**Table 3** Summary of new oral anti-coagulants

Name	Dabigatran	Apixaban	Edoxaban	Rivaroxaban
Action	Direct thrombin inhibitor	Activated factor Xa inhibitor	Activated factor Xa inhibitor	Activated factor Xa inhibitor
Clearance	80% renal clearance	73% hepatic 27% renal clearance	50% hepatic 50% renal clearance	65% hepatic 35% renal clearance
CYP3A4 interaction?	No	Yes (minor)	Minimal	Yes
Absorption with food?	No effect	No effect	Up to 20% more	40% more therefore intake with food
Elimination half life	12-17 h	12 h	9-11 h	8-9 h young 11-13 h elderly

of intestinal ischaemia (one fatal), and two subjects went on to have liver transplantation that required caval hemi-transposition. Delgado *et al.*<sup>[84]</sup> identified baseline platelet count of  $< 50 \times 10^9/L$  as a risk factor for bleeding complications.

In the study reported by Senzolo *et al.*<sup>[85]</sup> control patients experienced greater rates of complications than the active arm: 2 of the 21 controls developed intestinal ischaemia (one fatal), and 2 required caval hemitransposition at liver transplantation. Delgado *et al.*<sup>[84]</sup> study showed that complications associated with deteriorating cirrhosis were more common in patients who did not achieve recanalisation.

### New oral anti-coagulants

These agents work by direct inhibition of thrombin or activated factor Xa, and are licenced for the prevention of primary or recurrent venous thromboembolism, or prevention of stroke in non-valvular atrial fibrillation<sup>[90-93]</sup>. Their practical advantages include oral administration, the lack of any requirement for monitoring with blood tests, and no effect on INR, an important component of the MELD score. The new oral anticoagulants have no antidote: of clinical importance when considering individual patients' risks of bleeding complications<sup>[94]</sup>. However specific antidotes, such as Andexanet Alfa (Clinicaltrials.gov: NCT01758432) are under development. The new oral anti-coagulants can be affected by drugs that are P-glycoprotein substrates. Drugs that inhibit or induce CYP3A4 can significantly affect concentrations or effects of rivaroxaban<sup>[95]</sup>. The potential for drug interaction is important to consider for patients intending to commence new directly acting antiviral therapies for hepatitis C (Table 3)<sup>[96]</sup>.

Recent case reports describe the use of rivaroxaban to treat acute PVT in well-compensated cirrhosis. Unfortunately for patients with severe liver disease the drug has not been evaluated in decompensated cirrhosis, where concerns exist that its pharmacological effects will be altered<sup>[97,98]</sup>.

From these small studies and series it is clear that anticoagulation with warfarin or low molecular heparin is feasible in cirrhosis, may prevent the onset of PVT or its extension once present, and may even slow down progression of liver cirrhosis. Further controlled studies with larger numbers are indicated to validate these findings. These published experiences may justify

the current use of anticoagulation in some settings such as cirrhotics with partial or occlusive PVT who are on transplant waiting lists. There is no consensus on which anticoagulant is best: low molecular weight heparin can be given until transplantation, but requires administration of an injection. Warfarin impacts upon patients' MELD scores and requires monitoring of INR. Rivaroxaban cannot be provided to patients with decompensated cirrhosis (Table 2).

### TIPSS

TIPSS (with bare or covered stents) may be a treatment option to manage PVT as an alternative to anticoagulation, particularly in the presence of severe complications of portal hypertension (recurrent or refractory variceal haemorrhage or ascites), or contraindications to anticoagulation. The goal of TIPSS is to re-permeate the portal vein and restore portal flow through the low resistance shunt, thereby preventing recurrent thrombosis. TIPSS may have a role in liver transplant candidates in maintaining portal vein patency, avoiding PVT propagation, and enhancing the feasibility of transplantation. TIPSS may even be feasible in some patients with cavernoma<sup>[99,100]</sup>. TIPSS can prevent total portal vein occlusion in liver transplantation candidates with partial PVT<sup>[101]</sup>. There are no studies that compare anticoagulation, TIPSS, or conservative treatment in the management of PVT in cirrhosis.

### Outcomes

Experience of TIPSS in more than 200 subjects with cirrhosis and PVT has been published<sup>[99-106]</sup>. Rates of feasibility between 70% to 100% are described. Successful TIPSS placement is associated with clinical improvement, low rates of re-thrombosis, and low rates of recurrent portal-hypertensive bleeding. Because of the low rates of re-thrombosis following complete portal vein recanalisation, systemic anticoagulation following TIPSS is probably only indicated in the presence of a documented pro-thrombotic state<sup>[101-103]</sup>.

The use of TIPSS has been described in cirrhotic patients with PVT and complications of portal hypertension, bleeding or ascites. A small number of individuals have undergone TIPSS with the aim of preventing complete occlusive PVT while awaiting liver



transplantation<sup>[102,105]</sup>. D'Avola *et al*<sup>[101]</sup> describe TIPSS in 15 cirrhotic subjects with partial PVT waiting for transplantation. These individuals were compared with 8 matched controls who did not undergo TIPSS. There were no significant complications associated with the TIPSS procedure. There were no differences between the groups' post-transplantation outcomes, transplant operating times or use of blood products. Wang *et al*<sup>[105]</sup> compared a group of 25 patients with cirrhosis and PVT who were treated successfully with TIPSS with a cohort of 25 patients with cirrhosis and PVT who were managed conservatively including endoscopic variceal ligation). Successful TIPSS was associated with portal vein recanalisation and not surprisingly lower rates of variceal bleeding. Interestingly there were no differences in survival between the two groups, which were followed up for a mean of 25.1 mo (Table 4).

While elective TIPSS use in cirrhosis carries a higher risk with high MELD scores<sup>[107]</sup>, between 16% to 33% of cases where baseline severity of liver disease was recorded had baseline Child's C disease severity.

### Complications and technical failure

Technical failure has been associated with extensive main PVT<sup>[100]</sup>, and the absence of a patent intra-hepatic portal vein branch that can be punctured<sup>[101,103,105]</sup>. TIPSS placement may compromise an intended liver transplant procedure if it is sited distally into the portal vein trunk and superior mesenteric vein<sup>[108]</sup>. Lower rates of success are reported in the presence of cavernoma<sup>[100,101]</sup>. Reduced rates of TIPSS dysfunction have been reported with the use of covered stents<sup>[103]</sup>.

Unlike anticoagulation, TIPSS is associated with a risk of developing hepatic encephalopathy<sup>[109]</sup>. Han *et al*<sup>[99]</sup> and Luca *et al*<sup>[102]</sup> reported rates of post-TIPSS encephalopathy between 25% to 32%, although Senzolo *et al*<sup>[104]</sup> reported only one out of 28 subjects developing encephalopathy in their series of both cirrhotic and non-cirrhotic patients.

## ENDOVASCULAR FIBRINOLYSIS

Results from published experiences of thrombolysis in non-cirrhotic patients have been disappointing with high incidence of major bleeding complications and low rates of recanalisation<sup>[110-112]</sup>. Experience of thrombolysis, alone or in conjunction with TIPSS, in cirrhotic patients with PVT is limited<sup>[113,114]</sup>.

## CONCLUSION

PVT is a common problem in patients with advanced cirrhosis, with diagnosis occurring more frequently because of the greater prevalence of ultrasound screening in cirrhosis. While PVT has been associated with some important clinical complications, including worsening portal hypertension (at least in the short term), mesenteric infarction and portal biliopathy;

its overall prognostic significance is still not fully understood. PVT is clinically relevant where liver transplantation is anticipated.

While reduced portal vein velocity is likely the most important risk factor for PVT in cirrhosis, other causes such as thrombophilic disorders and endotoxaemia may play an important role in some individuals. Future studies examining the impact of more targeted use of non-selective beta-blockers in advanced cirrhosis, or strategies aimed at reducing bacterial translocation in cirrhosis may demonstrate a beneficial impact on the incidence of PVT<sup>[28,115-117]</sup>. Larger studies are warranted to repeat the work of Villa *et al*<sup>[55]</sup> to establish whether primary prevention of PVT with anticoagulation has a role in selected subjects with cirrhosis.

When PVT is first diagnosed in a cirrhotic individual, it is important to ensure that the thrombosis is not associated with the presence of hepatocellular carcinoma: this can be assessed with the use of multiphase CT or MRI liver<sup>[118]</sup>. Endoscopic screening for varices is warranted. A thrombophilic disorder may be a contributing causative factor -and should be looked for if diagnosis will have longer term clinical implications, for example in liver transplant candidates.

The increasing experience of using specific therapies to prevent or to treat PVT in cirrhotic patients is very interesting. It has been argued that there is sufficient evidence or experience to warrant the use of anti-coagulation in patients with cirrhosis and PVT who are listed for liver transplantation, following prophylactic management of oesophageal varices<sup>[20,119]</sup>. However in our opinion there remains a lack of adequately powered, randomised studies to demonstrate clearly the role, benefits and risks of anticoagulation or TIPSS to manage PVT in cirrhosis, and whether such interventions are appropriate in all cirrhotics, or appropriate in only certain groups, such as potential liver transplant candidates. There is no clear evidence to support the routine use of anticoagulation or TIPSS in primary prevention.

Prospective cohort studies are warranted to assess the impact of PVT on patients referred for liver transplantation; to evaluate its impact on eligibility for transplantation, on the natural history of patients waiting for transplantation and on outcomes of transplantation.

Randomised controlled studies are warranted to compare current conservative management with the use of anticoagulation or TIPSS to treat acute PVT in cirrhosis. To evaluate outcomes it may be preferable to perform a study in patients referred for or waiting for transplantation. Outcomes of interest should include incidence and maintenance of portal vein recanalisation, survival and effects on MELD or progression of underlying liver disease, effects on portal hypertensive complications, and effects on transplant surgery and outcomes. Future study should compare the use of warfarin, low-molecular weight

Table 4 Summary of retrospective case series reporting the use of transjugular intrahepatic portosystemic stent-shunt for portal vein thrombosis in cirrhosis

Study type and stent characteristics	n	Baseline severity liver disease: Child's A/B/C (%)	TIPS indication (%)	PVT characteristics: Complete/ partial/ cavernoma (%)	Successful cannulation (%)	Outcome	Significant complications/ notes
Luca <i>et al</i> <sup>[102]</sup> Series 2003-2010 13 bare Wallstent, 57 covered Viatorr ePTE covered (WL Gore and Associates)	70	A: 17 (24) B: 42 (60) C: 11 (16)	Bleeding: 48 Ascites/ hydrothorax: 18 Specific treatment of PVT: 4	Complete: 24 Cavernoma: 2	70/70 (100) cannulation. Complete recanalisation or significant reduction in thrombosis: 61 (87)	Complete recanalisation in 40 (57%); 38 maintained patency at mean follow up 20.7 mo.	
Perarnau <i>et al</i> <sup>[100]</sup> Series 1990-2004 Palmaz (Cordis) or Wallstent (Boston Scientific) bare stents	34	A: 3 (14) B: 11 (52) C: 7 (33) (incomplete details)	Bleeding: 27 (79) Ascites: 5 (15) Other: 2	Complete acute: 15 Complete + cavernoma: 19	No cavernoma: 15/15 (100) Cavernoma: 12/19 (63)	Mean F/U 30 mo. 26/34 (72%) long-term patency	Failed cannulation in presence of thrombosed intrahepatic PV branches or peri-hilar cavernoma
Senzolo <i>et al</i> <sup>[104]</sup> Series 1994-2005 26 Memotherms (Angiomed) bare stents, 3 Viatorr covered stents	28 (15 non-cirrhotic)	Not stated	Bleeding: 15 Ascites: 5 Portal biliopathy: 3 Specific treatment PVT: 1	Complete: 8 (3 with, 5 without cavernoma) Partial: 5	19/28 (73%)	Primary patency mean 18 mo in 14/19. Stent thrombosis in 2 non-cirrhotic subjects (Budd-Chiari syndrome)	
Han <i>et al</i> <sup>[99]</sup> Series 2001-2008 Uncovered stents in all patients	57	A: 25 (44) B: 26 (46) C: 6 (30)	Bleeding: 56 Ascites: 1	Complete: 14 Cavernoma: 30 Partial: 35	Overall: 43/57 (75) Complete PVT: 8/14 (57) Partial PVT: 35/35 (100) Cavernoma: 16/30 (53%)	Primary patency maintained in 26/43 (17 required shunt revisions to maintain patency)	Failure related to presence of cavernoma. 1 case of delayed severe intra-abdominal haemorrhage following percutaneous trans-hepatic approach.
Van Ha <i>et al</i> <sup>[106]</sup> Series 1995-2003 12 bare Wallstent (Boston Scientific), 1 bare Zilver stent (Cook) Series 1995-2009 Bare and covered stents	15  15 (+ 8 controls with PVT)	B: 11 (73) C: 4 (27)  Mean Child's 8	Bleeding: 10 Ascites: 5  Prevention of complete PVT pre-liver transplant: 8 Bleeding: 6  Ascites: 1	Complete: 4/partial: 7/complete with cavernoma: 4  All partial PVT	Overall: 13/15 (87) Cavernoma: 3/4 No cavernoma: 10/11 (91)  Series describes only patients who successfully underwent TIPS	Mean F/U 17 mo. 1 stent occlusion  3/15 TIPS thrombosis: all successfully treated. Median time TIPS to transplant: 185 d. 100% portal vein patency at time of transplant vs 50% patency at transplant in controls (P = 0.008) 1/9 re-thrombosed. 2 patients transplanted with no PVT present	
Bauer <i>et al</i> <sup>[103]</sup> Series 1999-2005 3 covered stents; others bare stent Case series All bare stents	9  7	Cirrhosis: disease severity not stated  Cirrhosis: disease severity not stated	Primary indication: maintain PV patency for future liver transplantation Bleeding: 7	Complete: 7 Partial: 2 Cavernoma: 4 PVT severity not stated. No cavernoma.	Series describing only patients who successfully underwent TIPS Series of successful cases		

PVT: Portal vein thrombosis; TIPS: Transjugular intrahepatic portosystemic stent-shunt; F/U: Follow up.

heparin and new oral anti-coagulants.

Some centres routinely provide anti-coagulation to patients with cirrhosis and acute PVT who are waiting for transplantation. It would be useful to combine and publish the available efficacy and safety data from these centres.

Management of PVT in cirrhosis at present remains an individualised decision, according to the risk of thrombus extension, the likelihood of transplantation, and whether there are other clinically significant issues, such as intractable ascites or portal hypertensive bleeding that would warrant use of TIPSS.

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## REFERENCES

- Schmidt S, Demartines N, Soler L, Schnyder P, Denys A. Portal vein normal anatomy and variants: implication for liver surgery and portal vein embolization. *Semin Intervent Radiol* 2008; **25**: 86-91 [PMID: 21326549 DOI: 10.1055/s-2008-1076688]
- Shimada T, Maruyama H, Kondo T, Sekimoto T, Takahashi M, Motoyama T, Ogasawara S, Suzuki E, Ooka Y, Tawada A, Chiba T, Kanai F, Okabe S, Yoshikawa M, Yokosuka O. Clinical features and natural history of portal vein thrombosis after radiofrequency ablation for hepatocellular carcinoma in Japan. *Hepatol Int* 2013; **7**: 1030-1039 [DOI: 10.1007/s12072-013-9470-z]
- Matsumoto K, Yamao K, Ohashi K, Watanabe Y, Sawaki A, Nakamura T, Matsuura A, Suzuki T, Fukutomi A, Baba T, Okubo K, Tanaka K, Moriyama I, Shimizu Y. Acute portal vein thrombosis after EUS-guided FNA of pancreatic cancer: case report. *Gastrointest Endosc* 2003; **57**: 269-271 [PMID: 12556803 DOI: 10.1067/mge.2003.79]
- Yadav S, Dutta AK, Sarin SK. Do umbilical vein catheterization and sepsis lead to portal vein thrombosis? A prospective, clinical, and sonographic evaluation. *J Pediatr Gastroenterol Nutr* 1993; **17**: 392-396 [PMID: 8145094 DOI: 10.1097/00005176-199311000-00010]
- Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PE, Plessier A, Roulot D, Chaffaut C, Bourcier V, Trinchet JC, Valla DC. Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study. *Hepatology* 2015; **61**: 660-667 [PMID: 25284616 DOI: 10.1002/hep.27546]
- Zocco MA, Di Stasio E, De Cristofaro R, Novi M, Ainora ME, Ponziani F, Riccardi L, Lancellotti S, Santoliquido A, Flore R, Pompili M, Rapaccini GL, Tondi P, Gasbarrini GB, Landolfi R, Gasbarrini A. Thrombotic risk factors in patients with liver cirrhosis: correlation with MELD scoring system and portal vein thrombosis development. *J Hepatol* 2009; **51**: 682-689 [PMID: 19464747 DOI: 10.1016/j.jhep.2009.03.013]
- Francoz C, Belghiti J, Vilgrain V, Sommacale D, Paradis V, Condat B, Denninger MH, Sauvanet A, Valla D, Durand F. Splanchnic vein thrombosis in candidates for liver transplantation: usefulness of screening and anticoagulation. *Gut* 2005; **54**: 691-697 [PMID: 15831918 DOI: 10.1136/gut.2004.042796]
- Maruyama H, Okugawa H, Takahashi M, Yokosuka O. De novo portal vein thrombosis in virus-related cirrhosis: predictive factors and long-term outcomes. *Am J Gastroenterol* 2013; **108**: 568-574 [PMID: 23381015 DOI: 10.1038/ajg.2012.452]
- Amitrano L, Guardascione MA, Brancaccio V, Margaglione M, Manguso F, Iannaccone L, Grandone E, Balzano A. Risk factors and clinical presentation of portal vein thrombosis in patients with liver cirrhosis. *J Hepatol* 2004; **40**: 736-741 [PMID: 15094219 DOI: 10.1016/j.jhep.2004.01.001]
- Garcia-Pagan JC, Valla DC. Portal vein thrombosis: a predictable milestone in cirrhosis? *J Hepatol* 2009; **51**: 632-634 [PMID: 19660824 DOI: 10.1016/j.jhep.2009.06.009]
- Molmenti EP, Roodhouse TW, Molmenti H, Jaiswal K, Jung G, Marubashi S, Sanchez EQ, Gogel B, Levy MF, Goldstein RM, Fasola CG, Elliott EE, Bursac N, Mulligan D, Gonwa TA, Klintmalm GB. Thrombendvenectomy for organized portal vein thrombosis at the time of liver transplantation. *Ann Surg* 2002; **235**: 292-296 [PMID: 11807371 DOI: 10.1097/00000658-200202000-00019]
- Tao YF, Teng F, Wang ZX, Guo WY, Shi XM, Wang GH, Ding GS, Fu ZR. Liver transplant recipients with portal vein thrombosis: a single center retrospective study. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 34-39 [PMID: 19208512]
- Ravaioli M, Zanella M, Grazi GL, Ercolani G, Cescon M, Del Gaudio M, Cucchetti A, Pinna AD. Portal vein thrombosis and liver transplantation: evolution during 10 years of experience at the University of Bologna. *Ann Surg* 2011; **253**: 378-384 [PMID: 21183851 DOI: 10.1097/SLA.0b013e318206818b]
- Dumortier J, Czyglik O, Poncet G, Blanchet MC, Boucaud C, Henry L, Boillot O. Eversion thrombectomy for portal vein thrombosis during liver transplantation. *Am J Transpl* 2002; **2**: 934-938 [DOI: 10.1034/j.1600-6143.2002.21009.x]
- Yerdel MA, Gunson B, Mirza D, Karayalçın K, Olliff S, Buckels J, Mayer D, McMaster P, Pirenne J. Portal vein thrombosis in adults undergoing liver transplantation: risk factors, screening, management, and outcome. *Transplantation* 2000; **69**: 1873-1881 [PMID: 10830225 DOI: 10.1097/00007890-200005150-00023]
- Francoz C, Valla D, Durand F. Portal vein thrombosis, cirrhosis, and liver transplantation. *J Hepatol* 2012; **57**: 203-212 [PMID: 22446690 DOI: 10.1016/j.jhep.2011.12.034]
- Chawla Y, Duseja A, Dhiman RK. Review article: the modern management of portal vein thrombosis. *Aliment Pharmacol Ther* 2009; **30**: 881-894 [PMID: 19678814 DOI: 10.1111/j.1365-2036.2009.04116.x]
- De Gaetano AM, Laforce M, Patriquin H, De Franco A, Aubin B, Paradis K. Cavernous transformation of the portal vein: patterns of intrahepatic and splanchnic collateral circulation detected with Doppler sonography. *AJR Am J Roentgenol* 1995; **165**: 1151-1155 [PMID: 7572494 DOI: 10.2214/ajr.165.5.7572494]
- Dhiman RK, Behera A, Chawla YK, Dilawari JB, Suri S. Portal hypertensive biliopathy. *Gut* 2007; **56**: 1001-1008 [PMID: 17170017 DOI: 10.1136/gut.2006.103606]
- Tsochatzis EA, Senzolo M, Germani G, Gatt A, Burroughs AK. Systematic review: portal vein thrombosis in cirrhosis. *Aliment Pharmacol Ther* 2010; **31**: 366-374 [PMID: 19863496 DOI: 10.1111/j.1365-2036.2009.04182.x]
- Harki J, Plompen EP, van Noord D, Hoekstra J, Kuipers EJ, Janssen HL, Tjwa ET. Gastrointestinal ischaemia in patients with acute and chronic portal vein thrombosis. *J Hepatol* 2014; **60**: S239-S240 [DOI: 10.1016/S0168-8278(14)60672-3]
- Luca A, Caruso S, Milazzo M, Marrone G, Mamone G, Crinò F, Maruzzelli L, Miraglia R, Floridia G, Vizzini G. Natural course of extrahepatic nonmalignant partial portal vein thrombosis in patients with cirrhosis. *Radiology* 2012; **265**: 124-132 [PMID: 22891357 DOI: 10.1016/S0168-8278(12)60648-5]
- Rajani R, Björnsson E, Bergquist A, Danielsson A, Gustavsson A, Grip O, Melin T, Sangfelt P, Wallerstedt S, Almer S. The epidemiology and clinical features of portal vein thrombosis: a multicentre study. *Aliment Pharmacol Ther* 2010; **32**: 1154-1162 [PMID: 21039677 DOI: 10.1111/j.1365-2036.2010.04454.x]
- Shah V. Molecular mechanisms of increased intrahepatic resistance in portal hypertension. *J Clin Gastroenterol* 2007; **41** Suppl 3: S259-S261 [PMID: 17975474 DOI: 10.1097/MCG.0b013e318150d0e1]
- Amitrano L, Guardascione MA, Ames PR. Coagulation abnormalities in cirrhotic patients with portal vein thrombosis. *Clin Lab* 2007; **53**: 583-589 [PMID: 18257465]

- 26 **Kinjo N**, Kawanaka H, Akahoshi T, Tomikawa M, Yamashita N, Konishi K, Tanoue K, Shirabe K, Hashizume M, Maehara Y. Risk factors for portal venous thrombosis after splenectomy in patients with cirrhosis and portal hypertension. *Br J Surg* 2010; **97**: 910-916 [PMID: 20474001 DOI: 10.1002/bjs.7002]
- 27 **Bosch J**, Masti R, Kravetz D, Bruix J, Gaya J, Rigau J, Rodes J. Effects of propranolol on azygos venous blood flow and hepatic and systemic hemodynamics in cirrhosis. *Hepatology* 1984; **4**: 1200-1205 [PMID: 6500511 DOI: 10.1002/hep.1840040617]
- 28 **Sersté T**, Melot C, Francoz C, Durand F, Rautou PE, Valla D, Moreau R, Lebrech D. Deleterious effects of beta-blockers on survival in patients with cirrhosis and refractory ascites. *Hepatology* 2010; **52**: 1017-1022 [PMID: 20583214 DOI: 10.1002/hep.23775]
- 29 **Ge PS**, Runyon BA. The changing role of beta-blocker therapy in patients with cirrhosis. *J Hepatol* 2014; **60**: 643-653 [PMID: 24076364 DOI: 10.1016/j.jhep.2013.09.016]
- 30 **Leithead JA**, Rajoriya N, Tehami N, Hodson J, Gunson BK, Tripathi D, Ferguson JW. Non-selective  $\beta$ -blockers are associated with improved survival in patients with ascites listed for liver transplantation. *Gut* 2014; Epub ahead of print [PMID: 25281417 DOI: 10.1136/gutjnl-2013-306502]
- 31 **Monroe DM**, Hoffman M. The coagulation cascade in cirrhosis. *Clin Liver Dis* 2009; **13**: 1-9 [PMID: 19150304 DOI: 10.1016/j.cld.2008.09.014]
- 32 **Tripodi A**, Primignani M, Chantarangkul V, Clerici M, Dell'Era A, Fabris F, Salerno F, Mannucci PM. Thrombin generation in patients with cirrhosis: the role of platelets. *Hepatology* 2006; **44**: 440-445 [PMID: 16871542 DOI: 10.1002/hep.21266]
- 33 **Arjal R**, Trotter JF. International normalized ratio of prothrombin time in the model for end-stage liver disease score: an unreliable measure. *Clin Liver Dis* 2009; **13**: 67-71 [PMID: 19150311 DOI: 10.1016/j.cld.2008.09.009]
- 34 **Tripodi A**. Tests of coagulation in liver disease. *Clin Liver Dis* 2009; **13**: 55-61 [PMID: 19150309 DOI: 10.1016/j.cld.2008.09.002]
- 35 **Gulley D**, Teal E, Suvannasankha A, Chalasani N, Liangpunsakul S. Deep vein thrombosis and pulmonary embolism in cirrhosis patients. *Dig Dis Sci* 2008; **53**: 3012-3017 [PMID: 18443906 DOI: 10.1007/s10620-008-0265-3]
- 36 **Northup PG**, McMahon MM, Ruhl AP, Altschuler SE, Volk-Bednarz A, Caldwell SH, Berg CL. Coagulopathy does not fully protect hospitalized cirrhosis patients from peripheral venous thromboembolism. *Am J Gastroenterol* 2006; **101**: 1524-1538; quiz 1680 [PMID: 16863556 DOI: 10.1111/j.1572-0241.2006.00588.x]
- 37 **García-Fuster MJ**, Abdilla N, Fabiá MJ, Fernández C, Oliver V. [Venous thromboembolism and liver cirrhosis]. *Rev Esp Enferm Dig* 2008; **100**: 259-262 [PMID: 18662076]
- 38 **Sogaard KK**, Horváth-Puhó E, Grønbaek H, Jepsen P, Vilstrup H, Sørensen HT. Risk of venous thromboembolism in patients with liver disease: a nationwide population-based case-control study. *Am J Gastroenterol* 2009; **104**: 96-101 [PMID: 19098856 DOI: 10.1038/ajg.2008.34]
- 39 **Tripodi A**, Primignani M, Chantarangkul V, Dell'Era A, Clerici M, de Franchis R, Colombo M, Mannucci PM. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology* 2009; **137**: 2105-2111 [PMID: 19706293 DOI: 10.1053/j.gastro.2009.08.045]
- 40 **Tripodi A**, Primignani M, Lemma L, Chantarangkul V, Mannucci PM. Evidence that low protein C contributes to the procoagulant imbalance in cirrhosis. *J Hepatol* 2013; **59**: 265-270 [PMID: 23583273 DOI: 10.1016/j.jhep.2013.03.036]
- 41 **Tripodi A**, Anstee QM, Sogaard KK, Primignani M, Valla DC. Hypercoagulability in cirrhosis: causes and consequences. *J Thromb Haemost* 2011; **9**: 1713-1723 [PMID: 21729237 DOI: 10.1111/j.1538-7836.2011.04429.x]
- 42 **Janssen HL**, Meinardi JR, Vleggaar FP, van Uum SH, Haagsma EB, van Der Meer FJ, van Hattum J, Chamuleau RA, Adang RP, Vandenbroucke JP, van Hoek B, Rosendaal FR. Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. *Blood* 2000; **96**: 2364-2368 [PMID: 11001884]
- 43 **Pasta L**, Marrone C, D'amico M, Virdone R, D'amico G, Sammarco P, Fabiano C, Pagliaro L. MTHFR C677T mutations in liver cirrhosis with and without portal vein thrombosis. *Liver Int* 2006; **26**: 269-270 [PMID: 16448467 DOI: 10.1111/j.1478-3231.2005.01215.x]
- 44 **Amirano L**, Guardascione MA, Ames PR, Margaglione M, Iannaccone L, Brancaccio V, Balzano A. Increased plasma prothrombin concentration in cirrhotic patients with portal vein thrombosis and prothrombin G20210A mutation. *Thromb Haemost* 2006; **95**: 221-223 [PMID: 16493481]
- 45 **Romero Gómez M**, Suárez García E, López Lacomba D, Marchante I, Grande L, Castro Fernandez M. Antiphospholipid antibodies are related to portal vein thrombosis in patients with liver cirrhosis. *J Clin Gastroenterol* 2000; **31**: 237-240 [PMID: 11034005 DOI: 10.1097/00004836-200010000-00011]
- 46 **Trum JW**, Dominique V, Gil C, Degott C, Rueff B, Santoni P, Ducroix JP, Capron JP, Bousquet O, Opolon P, Jean-Pierre B. Bacteroides bacteraemia of undetermined origin: strong association with portal vein thrombosis and cryptogenic pylephlebitis. *Eur J Gastroenterol Hepatol* 1993; **5**: 655-659 [DOI: 10.1097/00042737-199308000-00018]
- 47 **Lin RS**, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, Hsu WC, Huang CC, Wang SS, Lo KJ. Endotoxemia in patients with chronic liver diseases: relationship to severity of liver diseases, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995; **22**: 165-172 [PMID: 7790704 DOI: 10.1016/0168-8278(95)80424-2]
- 48 **Cirera I**, Bauer TM, Navasa M, Vila J, Grande L, Taurá P, Fuster J, García-Valdecasas JC, Lacy A, Suárez MJ, Rimola A, Rodés J. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001; **34**: 32-37 [PMID: 11211904 DOI: 10.1016/S0168-8278(00)00013-1]
- 49 **Lamps LW**, Hunt CM, Green A, Gray GF, Washington K. Alterations in colonic mucosal vessels in patients with cirrhosis and noncirrhotic portal hypertension. *Hum Pathol* 1998; **29**: 527-535 [PMID: 9596279 DOI: 10.1016/S0046-8177(98)90071-5]
- 50 **Berg RD**. Bacterial translocation from the gastrointestinal tract. *Trends Microbiol* 1995; **3**: 149-154 [PMID: 7613757 DOI: 10.1016/S0966-842X(00)88906-4]
- 51 **Mehta G**, Gustot T, Mookerjee RP, Garcia-Pagan JC, Fallon MB, Shah VH, Moreau R, Jalan R. Inflammation and portal hypertension - the undiscovered country. *J Hepatol* 2014; **61**: 155-163 [PMID: 24657399 DOI: 10.1016/j.jhep.2014.03.014]
- 52 **Bellot P**, García-Pagán JC, Francés R, Abalde JG, Navasa M, Pérez-Mateo M, Such J, Bosch J. Bacterial DNA translocation is associated with systemic circulatory abnormalities and intrahepatic endothelial dysfunction in patients with cirrhosis. *Hepatology* 2010; **52**: 2044-2052 [PMID: 20979050 DOI: 10.1002/hep.23918]
- 53 **Steib CJ**, Hartmann AC, v Hesler C, Benesic A, Hennenberg M, Bilzer M, Gerbes AL. Intraperitoneal LPS amplifies portal hypertension in rat liver fibrosis. *Lab Invest* 2010; **90**: 1024-1032 [PMID: 20212458 DOI: 10.1038/labinvest.2010.60]
- 54 **Violi F**, Ferro D, Basili S, Lionetti R, Rossi E, Merli M, Riggio O, Bezzi M, Capocaccia L. Ongoing prothrombotic state in the portal circulation of cirrhotic patients. *Thromb Haemost* 1997; **77**: 44-47 [PMID: 9031447]
- 55 **Villa E**, Cammà C, Marietta M, Luongo M, Critelli R, Colopi S, Tata C, Zecchini R, Gitto S, Petta S, Lei B, Bernabucci V, Vukotic R, De Maria N, Schepis F, Karampatou A, Caporali C, Simoni L, Del Buono M, Zambotto B, Turola E, Fornaciari G, Schianchi S, Ferrari A, Valla D. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* 2012; **143**: 1253-60.e1-4 [PMID: 22819864 DOI: 10.1053/j.gastro.2012.07.018]
- 56 **Turnes J**, Garcia-Pagan JC, Abalde JG, Hernandez-Guerra M, Dell'Era A, Bosch J. Pharmacological reduction of portal



- pressure and long-term risk of first variceal bleeding in patients with cirrhosis. *Am J Gastroenterol* 2006; **101**: 506-512 [PMID: 16542287 DOI: 10.1111/j.1572-0241.2006.00453.x]
- 57 **Senzolo M**, Cholongitas E, Burra P, Leandro G, Thalheimer U, Patch D, Burroughs AK. beta-Blockers protect against spontaneous bacterial peritonitis in cirrhotic patients: a meta-analysis. *Liver Int* 2009; **29**: 1189-1193 [PMID: 19508620 DOI: 10.1111/j.1478-3231.2009.02038.x]
  - 58 **Okuda K**, Ohnishi K, Kimura K, Matsutani S, Sumida M, Goto N, Musha H, Takashi M, Suzuki N, Shinagawa T. Incidence of portal vein thrombosis in liver cirrhosis. An angiographic study in 708 patients. *Gastroenterology* 1985; **89**: 279-286 [PMID: 4007419]
  - 59 **Subramanyam BR**, Balthazar EJ, Lefleur RS, Horii SC, Hulnick DH. Portal venous thrombosis: correlative analysis of sonography, CT and angiography. *Am J Gastroenterol* 1984; **79**: 773-776 [PMID: 6385690]
  - 60 **Van Gansbeke D**, Avni EF, Delcour C, Engelholm L, Struyven J. Sonographic features of portal vein thrombosis. *AJR Am J Roentgenol* 1985; **144**: 749-752 [PMID: 3883708 DOI: 10.2214/ajr.144.4.749]
  - 61 **Sringeri R**. Incidental Portal Vein Thrombosis: Does It Impact the Surgical Outcomes Following Liver Transplantation? *Liver Transpl* 2013; **19**: S289
  - 62 **Lee HK**, Park SJ, Yi BH, Yeon EK, Kim JH, Hong HS. Portal vein thrombosis: CT features. *Abdom Imaging* 2008; **33**: 72-79 [PMID: 17694406 DOI: 10.1007/s00261-007-9200-x]
  - 63 **Tublin ME**, Dodd GD, Baron RL. Benign and malignant portal vein thrombosis: differentiation by CT characteristics. *AJR Am J Roentgenol* 1997; **168**: 719-723 [PMID: 9057522 DOI: 10.2214/ajr.168.3.9057522]
  - 64 **Wallner B**, Edelman RR, Finn JP, Mattle HP. Bright pleural effusion and ascites on gradient-echo MR images: a potential source of confusion in vascular MR studies. *AJR Am J Roentgenol* 1990; **155**: 1237-1240 [PMID: 2122672 DOI: 10.2214/ajr.155.6.2122672]
  - 65 **Englesbe MJ**, Schaubel DE, Cai S, Guidinger MK, Merion RM. Portal vein thrombosis and liver transplant survival benefit. *Liver Transpl* 2010; **16**: 999-1005 [PMID: 20677291 DOI: 10.1002/lt.22105]
  - 66 **Berry K**, Taylor J, Liou IW, Ioannou GN. Portal vein thrombosis is not associated with increased mortality among patients with cirrhosis. *Clin Gastroenterol Hepatol* 2015; **13**: 585-593 [PMID: 25459555 DOI: 10.1016/j.cgh.2014.10.010]
  - 67 **Dell'Era A**, Iannuzzi F, Fabris FM, Fontana P, Reati R, Grillo P, Aghemo A, de Franchis R, Primignani M. Impact of portal vein thrombosis on the efficacy of endoscopic variceal band ligation. *Dig Liver Dis* 2014; **46**: 152-156 [PMID: 24084343 DOI: 10.1016/j.dld.2013.08.138]
  - 68 **Vianna RM**, Mangus RS, Kubal C, Fridell JA, Beduschi T, Tector AJ. Multivisceral transplantation for diffuse portomesenteric thrombosis. *Ann Surg* 2012; **255**: 1144-1150 [PMID: 22549750 DOI: 10.1097/SLA.0b013e31825429e0]
  - 69 **Rodríguez-Castro KI**, Porte RJ, Nadal E, Germani G, Burra P, Senzolo M. Management of nonneoplastic portal vein thrombosis in the setting of liver transplantation: a systematic review. *Transplantation* 2012; **94**: 1145-1153 [PMID: 23128996 DOI: 10.1097/TP.0b013e31826e8e53]
  - 70 **Englesbe MJ**, Kubus J, Muhammad W, Sonnenday CJ, Welling T, Punch JD, Lynch RJ, Marrero JA, Pelletier SJ. Portal vein thrombosis and survival in patients with cirrhosis. *Liver Transpl* 2010; **16**: 83-90 [PMID: 20035521 DOI: 10.1002/lt.21941]
  - 71 **Paskonis M**, Jurgaitis J, Mehrabi A, Kashfi A, Fonouni H, Strupas K, Büchler MW, Kraus TW. Surgical strategies for liver transplantation in the case of portal vein thrombosis--current role of cavoportal hemitransposition and renoportal anastomosis. *Clin Transplant* 2006; **20**: 551-562 [PMID: 16968480 DOI: 10.1111/j.1399-0012.2006.00560.x]
  - 72 **Selvaggi G**, Weppler D, Nishida S, Moon J, Levi D, Kato T, Tzakis AG. Ten-year experience in porto-caval hemitransposition for liver transplantation in the presence of portal vein thrombosis. *Am J Transplant* 2007; **7**: 454-460 [PMID: 17229075 DOI: 10.1111/j.1600-6143.2006.01649.x]
  - 73 **Hibi T**, Nishida S, Levi DM, Selvaggi G, Tekin A, Fan J, Ruiz P, Tzakis AG. When and why portal vein thrombosis matters in liver transplantation: a critical audit of 174 cases. *Ann Surg* 2014; **259**: 760-766 [PMID: 24299686 DOI: 10.1097/SLA.0000000000000252]
  - 74 **Manzanet G**, Sanjuán F, Orbis P, López R, Moya A, Juan M, Vila J, Asensi J, Sendra P, Ruiz J, Prieto M, Mir J. Liver Transplantation in patients with portal vein thrombosis. *Liver Transpl* 2001; **7**: 125-131 [PMID: 11172396 DOI: 10.1053/jlts.2001.21295]
  - 75 **Bertelli R**, Nardo B, Montalti R, Beltempo P, Puviani L, Cavallari A. Liver transplantation in recipients with portal vein thrombosis: experience of a single transplant center. *Transplant Proc* 2005; **37**: 1119-1121 [PMID: 15848641 DOI: 10.1016/j.transproceed.2005.01.031]
  - 76 **Doria C**, Marino IR. Acute portal vein thrombosis secondary to donor/recipient portal vein diameter mismatch after orthotopic liver transplantation: a case report. *Int Surg* 2003; **88**: 184-187 [PMID: 14717522]
  - 77 **Doenecke A**, Tsui TY, Zuelke C, Scherer MN, Schnitzbauer AA, Schlitt HJ, Obed A. Pre-existent portal vein thrombosis in liver transplantation: influence of pre-operative disease severity. *Clin Transplant* 2010; **24**: 48-55 [PMID: 19236435 DOI: 10.1111/j.1399-0012.2009.00977.x]
  - 78 **Robles R**, Fernandez JA, Hernández Q, Marín C, Ramírez P, Sánchez-Bueno F, Luján JA, Rodríguez JM, Acosta F, Parrilla P. Eversion thromboendovenectomy in organized portal vein thrombosis during liver transplantation. *Clin Transplant* 2004; **18**: 79-84 [PMID: 15108774 DOI: 10.1111/j.1399-0012.2004.00120.x]
  - 79 **Florman S**, Miller CM. Live donor liver transplantation. *Liver Transpl* 2006; **12**: 499-510 [PMID: 16555328 DOI: 10.1002/lt.20754]
  - 80 **Egawa H**, Tanaka K, Kasahara M, Takada Y, Oike F, Ogawa K, Sakamoto S, Kozaki K, Taira K, Ito T. Single center experience of 39 patients with preoperative portal vein thrombosis among 404 adult living donor liver transplantations. *Liver Transpl* 2006; **12**: 1512-1518 [PMID: 17004256 DOI: 10.1002/lt.20777]
  - 81 **Sugawara Y**, Makuuchi M, Tamura S, Matsui Y, Kaneko J, Hasegawa K, Imamura H, Kokudo N, Motomura N, Takamoto S. Portal vein reconstruction in adult living donor liver transplantation using cryopreserved vein grafts. *Liver Transpl* 2006; **12**: 1233-1236 [PMID: 16724339 DOI: 10.1002/lt.20786]
  - 82 **North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices**. Prediction of the first variceal hemorrhage in patients with cirrhosis of the liver and esophageal varices. A prospective multicenter study. *N Engl J Med* 1988; **319**: 983-989 [PMID: 3262200 DOI: 10.1056/NEJM198810133191505]
  - 83 **Amitrano L**, Guardascione MA, Menchise A, Martino R, Scaglione M, Giovine S, Romano L, Balzano A. Safety and efficacy of anticoagulation therapy with low molecular weight heparin for portal vein thrombosis in patients with liver cirrhosis. *J Clin Gastroenterol* 2010; **44**: 448-451 [PMID: 19730112]
  - 84 **Delgado MG**, Seijo S, Yepes I, Achúcar L, Catalina MV, García-Criado A, Abalde JG, de la Peña J, Bañares R, Albillos A, Bosch J, García-Pagán JC. Efficacy and safety of anticoagulation on patients with cirrhosis and portal vein thrombosis. *Clin Gastroenterol Hepatol* 2012; **10**: 776-783 [PMID: 22289875 DOI: 10.1016/j.cgh.2012.01.012]
  - 85 **Senzolo M**, M Sartori T, Rossetto V, Burra P, Cillo U, Boccagni P, Gasparini D, Miotto D, Simioni P, Tsochatzis E, A Burroughs K. Prospective evaluation of anticoagulation and transjugular intrahepatic portosystemic shunt for the management of portal vein thrombosis in cirrhosis. *Liver Int* 2012; **32**: 919-927 [PMID: 22435854 DOI: 10.1111/j.1478-3231.2012.02785.x]
  - 86 **Werner KT**, Sando S, Carey EJ, Vargas HE, Byrne TJ, Douglas DD, Harrison ME, Rakela J, Aql BA. Portal vein thrombosis in patients with end stage liver disease awaiting liver transplantation: outcome of anticoagulation. *Dig Dis Sci* 2013; **58**: 1776-1780

- [PMID: 23314858 DOI: 10.1007/s10620-012-2548-y]
- 87 **Intagliata NM**, Maitland H, Northup PG, Caldwell SH. Treating thrombosis in cirrhosis patients with new oral agents: ready or not? *Hepatology* 2015; **61**: 738-739 [PMID: 24829112 DOI: 10.1002/hep.27225]
  - 88 **Martinez M**, Tandra A, Vuppalanchi R. Treatment of acute portal vein thrombosis by nontraditional anticoagulation. *Hepatology* 2014; **60**: 425-426 [PMID: 24395623 DOI: 10.1002/hep.26998]
  - 89 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]
  - 90 **Heidbuchel H**, Verhamme P, Alings M, Antz M, Hacke W, Oldgren J, Sinnaeve P, Camm AJ, Kirchhof P. European Heart Rhythm Association Practical Guide on the use of new oral anticoagulants in patients with non-valvular atrial fibrillation. *Europace* 2013; **15**: 625-651 [PMID: 23625942 DOI: 10.1093/europace/eut083]
  - 91 **Schulman S**, Kearon C, Kakkar AK, Schellong S, Eriksson H, Baanstra D, Kvamme AM, Friedman J, Mismetti P, Goldhaber SZ. Extended use of dabigatran, warfarin, or placebo in venous thromboembolism. *N Engl J Med* 2013; **368**: 709-718 [PMID: 23425163 DOI: 10.1056/NEJMoa1113697]
  - 92 **Agnelli G**, Buller HR, Cohen A, Curto M, Gallus AS, Johnson M, Masiukiewicz U, Pak R, Thompson J, Raskob GE, Weitz JI. Oral apixaban for the treatment of acute venous thromboembolism. *N Engl J Med* 2013; **369**: 799-808 [PMID: 23808982 DOI: 10.1056/NEJMoa1302507]
  - 93 **Eriksson BI**, Borris LC, Friedman RJ, Haas S, Huisman MV, Kakkar AK, Bandel TJ, Beckmann H, Muehlhofer E, Misselwitz F, Geerts W. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Engl J Med* 2008; **358**: 2765-2775 [PMID: 18579811 DOI: 10.1056/NEJMoa0800374]
  - 94 **Makris M**, Van Veen JJ, Tait CR, Mumford AD, Laffan M. Guideline on the management of bleeding in patients on antithrombotic agents. *Br J Haematol* 2013; **160**: 35-46 [PMID: 23116425 DOI: 10.1111/bjh.12107]
  - 95 **Scaglione F**. New oral anticoagulants: comparative pharmacology with vitamin K antagonists. *Clin Pharmacokinet* 2013; **52**: 69-82 [PMID: 23292752 DOI: 10.1007/s40262-012-0030-9]
  - 96 **Pawlotsky JM**, Aghemo A, Dusheiko G, Fornis X, Puoti M, Sarrazin C. EASL recommendations on treatment of hepatitis C-2014. London, United Kingdom: 49th Annual Meeting of the European Association for the Study of the Liver, 2014: pp. 9-13
  - 97 **Potze W**, Adelmeijer J, Lisman T. Decreased in vitro anticoagulant potency of Rivaroxaban and Apixaban in plasma from patients with cirrhosis. *Hepatology* 2015; **61**: 1435-1436 [PMID: 25088782 DOI: 10.1002/hep.27350]
  - 98 **Martinez M**, Tandra A, Vuppalanchi R. Reply. *Hepatology* 2015; **61**: 2119-2120 [PMID: 25266638 DOI: 10.1002/hep.27351]
  - 99 **Han G**, Qi X, He C, Yin Z, Wang J, Xia J, Yang Z, Bai M, Meng X, Niu J, Wu K, Fan D. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with symptomatic portal hypertension in liver cirrhosis. *J Hepatol* 2011; **54**: 78-88 [PMID: 20932597 DOI: 10.1016/j.jhep.2010.06.029]
  - 100 **Perarnau JM**, Baju A, D'alteroche L, Viguier J, Ayoub J. Feasibility and long-term evolution of TIPS in cirrhotic patients with portal thrombosis. *Eur J Gastroenterol Hepatol* 2010; **22**: 1093-1098 [PMID: 20308910 DOI: 10.1097/MEG.0b013e328338d995]
  - 101 **D'Avola D**, Bilbao JL, Zozaya G, Pardo F, Rotellar F, Iñarrairaegui M, Quiruga J, Sangro B, Herrero JI. Efficacy of transjugular intrahepatic portosystemic shunt to prevent total portal vein thrombosis in cirrhotic patients awaiting for liver transplantation. *Transplant Proc* 2012; **44**: 2603-2605 [PMID: 23146469 DOI: 10.1016/j.transproceed.2012.09.050]
  - 102 **Luca A**, Miraglia R, Caruso S, Milazzo M, Sapere C, Maruzzelli L, Vizzini G, Tuziolino F, Gridelli B, Bosch J. Short- and long-term effects of the transjugular intrahepatic portosystemic shunt on portal vein thrombosis in patients with cirrhosis. *Gut* 2011; **60**: 846-852 [PMID: 21357252 DOI: 10.1136/gut.2010.228023]
  - 103 **Bauer J**, Johnson S, Durham J, Ludkowski M, Trotter J, Bak T, Wachs M. The role of TIPS for portal vein patency in liver transplant patients with portal vein thrombosis. *Liver Transpl* 2006; **12**: 1544-1551 [PMID: 17004250 DOI: 10.1002/lt.20869]
  - 104 **Senzolo M**, Tibbals J, Cholongitas E, Triantos CK, Burroughs AK, Patch D. Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with and without cavernous transformation. *Aliment Pharmacol Ther* 2006; **23**: 767-775 [PMID: 16556179 DOI: 10.1111/j.1365-2036.2006.02820.x]
  - 105 **Wang Z**, Zhao H, Wang X, Zhang H, Jiang M, Tsao J, Luo X, Yang L, Li X. Clinical outcome comparison between TIPS and EBL in patients with cirrhosis and portal vein thrombosis. *Abdom Imaging* 2014; Epub ahead of print [PMID: 25504374 DOI: 10.1007/s00261-014-0320-9]
  - 106 **Van Ha TG**, Hodge J, Funaki B, Lorenz J, Rosenblum J, Straus C, Leef J. Transjugular intrahepatic portosystemic shunt placement in patients with cirrhosis and concomitant portal vein thrombosis. *Cardiovasc Intervent Radiol* 2006; **29**: 785-790 [PMID: 16850140 DOI: 10.1007/s00270-005-0090-4]
  - 107 **Ferral H**, Gamboa P, Postoak DW, Albernaz VS, Young CR, Speeg KV, McMahan CA. Survival after elective transjugular intrahepatic portosystemic shunt creation: prediction with model for end-stage liver disease score. *Radiology* 2004; **231**: 231-236 [PMID: 14990811 DOI: 10.1148/radiol.2311030967]
  - 108 **Clavien PA**, Selzner M, Tuttle-Newhall JE, Harland RC, Suhocki P. Liver transplantation complicated by misplaced TIPS in the portal vein. *Ann Surg* 1998; **227**: 440-445 [PMID: 9527068 DOI: 10.1097/0000658-199803000-00017]
  - 109 **Boyer TD**, Haskal ZJ. The role of transjugular intrahepatic portosystemic shunt in the management of portal hypertension. *Hepatology* 2005; **41**: 386-400 [PMID: 15660434 DOI: 10.1002/hep.20559]
  - 110 **DeLeve LD**, Valla DC, Garcia-Tsao G. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]
  - 111 **Hollingshead M**, Burke CT, Mauro MA, Weeks SM, Dixon RG, Jaques PF. Transcatheter thrombolytic therapy for acute mesenteric and portal vein thrombosis. *J Vasc Interv Radiol* 2005; **16**: 651-661 [PMID: 15872320 DOI: 10.1097/01.RVI.0000156265.79960.86]
  - 112 **Malkowski P**, Pawlak J, Michalowicz B, Szczepan J, Wroblewski T, Leowska E, Krawczyk M. Thrombolytic treatment of portal thrombosis. *Hepatogastroenterology* 2003; **50**: 2098-2100 [PMID: 14696472]
  - 113 **De Santis A**, Moscatelli R, Catalano C, Iannetti A, Gigliotti F, Cristofari F, Trapani S, Attali AF. Systemic thrombolysis of portal vein thrombosis in cirrhotic patients: a pilot study. *Dig Liver Dis* 2010; **42**: 451-455 [PMID: 19819770 DOI: 10.1016/j.dld.2009.08.009]
  - 114 **Blum U**, Haag K, Rössle M, Ochs A, Gabelmann A, Boos S, Langer M. Noncavernomatous portal vein thrombosis in hepatic cirrhosis: treatment with transjugular intrahepatic portosystemic shunt and local thrombolysis. *Radiology* 1995; **195**: 153-157 [PMID: 7892458 DOI: 10.1148/radiology.195.1.7892458]
  - 115 **Krag A**, Wiest R, Albillos A, Gluud LL. The window hypothesis: haemodynamic and non-haemodynamic effects of  $\beta$ -blockers improve survival of patients with cirrhosis during a window in the disease. *Gut* 2012; **61**: 967-969 [PMID: 22234982 DOI: 10.1136/gutjnl-2011-301348]
  - 116 **Rasaratnam B**, Kaye D, Jennings G, Dudley F, Chin-Dusting J. The effect of selective intestinal decontamination on the hyperdynamic circulatory state in cirrhosis. A randomized trial. *Ann Intern Med* 2003; **139**: 186-193 [PMID: 12899586 DOI: 10.7326/0003-4819-139-3-200308050-00008]
  - 117 **Vairappan B**, Sharama V, Winstanley A, Davies N, Shah N, Jalan R. Modulation of the DDAH-ADMA pathway with the Farnesoid X receptor (FXR) agonist INT-747 restores hepatic eNOS activity and lowers portal pressure in cirrhotic rats. *Hepatology* 2009; **50**: 336A-337A
  - 118 **European Association For The Study Of The Liver**; European

Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

119 **Kinjo N**, Kawanaka H, Akahoshi T, Matsumoto Y, Kamori M, Nagao Y, Hashimoto N, Uehara H, Tomikawa M, Shirabe K, Maehara Y. Portal vein thrombosis in liver cirrhosis. *World J Hepatol* 2014; **6**: 64-71 [PMID: 24575165]

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## Somatostatin analogs for gastric carcinoids: For many, but not all

Sara Massironi, Alessandra Zilli, Dario Conte

Sara Massironi, Alessandra Zilli, Dario Conte, Gastroenterology and Endoscopy Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, I-20122 Milan, Italy

Alessandra Zilli, Dario Conte, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, I-20122 Milan, Italy

**Author contributions:** Massironi S designed the paper and wrote the first draft of the manuscript; Zilli A contributed to data acquisition and carried out the literature research; Conte D critically revised the manuscript for relevant intellectual content about neuroendocrine tumors; Massironi S and Zilli A wrote the final version of the manuscript; all the authors finally approved this manuscript.

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**Correspondence to:** Sara Massironi, MD, PhD, Gastroenterology and Endoscopy Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milano, Italy. [sara.massironi@policlinico.mi.it](mailto:sara.massironi@policlinico.mi.it)  
Telephone: +39-2-55033445  
Fax: +39-2-55033644

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### Abstract

Gastric carcinoids (GCs) are classified as: type I, related to hypergastrinemia due to chronic atrophic gastritis (CAG), type II, associated with Zollinger-Ellison syndrome in multiple endocrine neoplasia type 1, and type III, which is normogastrinemic. The management of type- I gastric carcinoids (GC1s) is still debated, because of their relatively benign course. According to the European Neuroendocrine Tumor Society guidelines endoscopic resection is indicated whenever possible; however, it is not often feasible because of the presence of a multifocal disease, large lesions, submucosal invasion or, rarely, lymph node involvement. Therefore, somatostatin analogs (SSAs) have been proposed as treatment for GC1s in view of their antisecretory, antiproliferative and antiangiogenic effects. However, in view of the high cost of this therapy, its possible side effects and the relatively benign course of the disease, SSAs should be reserved to specific subsets of "high risk patients", *i.e.*, those patients with multifocal or recurrent GCs. Indeed, it is reasonable that, after the development of a gastric neuroendocrine neoplasm in patients with a chronic predisposing condition (such as CAG), other enterochromaffin-like cells can undergo neoplastic proliferation, being chronically stimulated by hypergastrinemia. Therefore, definite indications to SSAs treatment should be established in order to avoid the undertreatment or overtreatment of GCs.

**Key words:** Neuroendocrine tumors; Atrophic gastritis; Octreotide; Lanreotide; Enterochromaffin-like cells; Carcinoid tumors

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**Core tip:** The management of type- I gastric carcinoids is still debated because of their relatively benign course against the fact that they can have a heterogeneous



unpredictable biological behavior. Even if in most cases their endoscopic treatment is effective, in some particular cases it may not be sufficient. The potential role of somatostatin analogs (SSAs) has been reported in several recent studies. However, neither a standard indication nor a specific schedule of treatment with defined dosage and duration have been adopted to date. Because of the high cost of SSAs, clear-cut indications for this therapy are required in order to avoid any overtreatment.

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## INTRODUCTION

### Gastric carcinoids - clinical features

**Epidemiology:** Gastric carcinoids (GCs) represent up to 23% of all digestive neuroendocrine neoplasms (NENs). Their annual incidence is of about 0.2/100000, with a marked increase in the last few decades, also owing to the expanding indications for upper gastrointestinal tract endoscopy (UGE) and improved immunohistological identification techniques<sup>[1-4]</sup>. Over the last 35 years a 10 to 15-fold increase has been reported in the United States<sup>[5]</sup> and in the United Kingdom<sup>[6]</sup> respectively.

Modlin reported that the percentage of GCs among all gastric tumors has increased from 0.3% to 1.77%. Notably, the 5-year survival rate for GCs has risen from 51% to 63%<sup>[7]</sup>.

**Classification:** GCs include three different types of gastric neuroendocrine tumors<sup>[8]</sup>: types I (GC1s) and II (GC2s) are related to hypergastrinemia, due to chronic atrophic autoimmune gastritis<sup>[9]</sup> and gastrinoma-related Zollinger-Ellison syndrome (ZES) respectively, while type III (GC3s) are generally sporadic tumors, associated with normal gastrin levels and a worse prognosis<sup>[10]</sup> (Table 1).

GC1s account for 75%-80% of all GCs. They usually occur in women in their 50s and 70s and are usually asymptomatic and non-functioning, thus they are incidentally detected during UGE performed for other reasons, *e.g.*, anemia or dyspepsia<sup>[11,12]</sup>. Most GC1 cases are polypoid with a median diameter of 5 mm  $\phi$ , generally confined to the mucosa-submucosa of the gastric fundus or body and, less frequently, antrum. They are usually well differentiated (*i.e.*, with Ki-67 index < 2%), multiple in 65% of all cases and sometimes present as microcarcinoids, macroscopically undetectable at UGE. Noteworthy, 8%-23% of GC1s may metastasize to regional lymph nodes and seldom even to the liver, mainly when facing with lesions

**Table 1** Characteristics of gastric carcinoids according to the European Neuroendocrine Tumor Society guidelines<sup>[8]</sup>

	GC1	GC2	GC3
Proportion among g-NEN	70%-80%	5%-6%	14%-25%
Grading	NET G1	NET G1-G2	NEC G3
Associated conditions	CAG	Gastrinoma/ MEN-1	None
Serum gastrin values	Elevated	Elevated	Normal
Metastases	2%-5%	10%-30%	50%-100%
Mortality rate	0%	< 10%	25%-30%

GC 1, 2 and 3: Type I, II and III gastric carcinoids; NENs: Neuroendocrine neoplasms; CAG: Chronic atrophic gastritis; MEN-1: Multiple endocrine neoplasia type I.

extended to the deep submucosa or presenting lymph node involvement<sup>[13,14]</sup>. Moreover, the evolution to neuroendocrine carcinoma may occur in 3% of patients<sup>[15]</sup>. Nevertheless, local or distant metastases have been reported also in patients affected by GC1s with low proliferation index (< 2%), small size and exclusive intramucosal invasion<sup>[16-19]</sup>. In addition, GC1s frequently recur (5%-67% of cases after endoscopic treatment)<sup>[20,21]</sup>, with a median recurrence-free time interval of 24 mo after endoscopic resection. Notably, recurrences are independent of polyp diameter, number of polyps or serum gastrin levels.

GC2s generally develop in patients with duodenal or pancreatic gastrinoma causing ZES syndrome in the context of a multiple endocrine neoplasia type I (MEN-1)<sup>[22]</sup>. These are usually small multiple polyps (diameter of 1-2 cm  $\phi$ ), localized in the fundic mucosa, with a low or moderate proliferation grade, but may also exhibit angioinvasion, invasion of muscularis propria and metastases at regional lymph nodes or, less frequently, at distant sites. Unlike GC1s, GC2s are equally frequent in male and female patients. Their local excision is generally recommended, even in the presence of multiple lesions. In addition, in view of their antiproliferative effects, SSAs may be useful in those subgroups of patients presenting slowly progressive carcinoids. Tomassetti *et al.*<sup>[23]</sup> observed GC2 regression in three patients with ZES/MEN-1 after a long-term therapy with SSAs. However, the management of GC2s is complicated by the controversies regarding the treatment of gastrinoma in MEN-1. Indeed, the indication for extensive duodenal-pancreatic surgery in patients with MEN-1, who present pharmacologically controllable ZES and no other clinically symptomatic hormonal excess syndrome, remains debatable, also because SSAs play a role in reducing tumor growth<sup>[24]</sup>.

GC3s are generally large (> 2 cm  $\phi$ ), solitary, ulcerated neoplasms, with infiltrative growth, elevated Ki-67 index and a higher risk of metastatic spreading when compared to GC1s and GC2s (metastatic rate of 50%-100%). GC3s occur mostly in men older than 50 years, presenting pain, anemia and weight



**Table 2** Grading of neuroendocrine neoplasms according to the World Health Organization 2010 classification<sup>[8]</sup>

Histological classification	G1 (low grade)	G2 (intermediate grade)	G3 (high grade)
Mitotic rate ( <i>n</i> /10 HPF)	< 2	2–20	> 20
Ki-67 index	< 3%	3%–20%	> 20%

loss. Unlike GC1s and GC2s, GC3s may be associated with an atypical carcinoid syndrome, characterized by itching, bronchospasm and flushing, mediated by histamine released from enterochromaffin-like (ECL) cells. The management of GC3s is fairly clear and similar to that of gastric adenocarcinoma; it is based on surgery (partial or total gastrectomy with lymph node dissection) and chemotherapy if surgery is not feasible<sup>[8]</sup>.

The treatment of metastatic liver disease includes hepatic resection, embolization of the hepatic artery, radiofrequency ablation and cryoablation.

**Grading and staging:** The histological classification of NENs includes grade (G) and differentiation. The grading system (Table 2) is based on the rate of proliferation, defined by the number of mitoses per 10 high-power microscopic fields or per 2 mm<sup>2</sup> (mitotic rate) and as the percentage of tumor cells positively immuno-labelling for the Ki-67 antigen (Ki-67 index). Differentiation refers to the extent to which neoplastic cells resemble normal cells. Generally, well-differentiated NENs are of low or intermediate grade, whereas poorly differentiated NENs are usually of high grade<sup>[25]</sup>.

The tumor-node-metastasis (TNM) staging system is based on the size and/or extent (reach) of the primary tumor (T), whether cancer cells have spread to nearby (regional) lymph nodes (N), and whether any metastasis (M), or the spread of the cancer to other parts of the body, has occurred. According to the TNM staging, five stages can be considered: 0 for tumors in situ < 0.5 mm  $\phi$ , I for small NENs invading submucosa or lamina propria, II for larger or more invasive neoplasms without metastases, III for tumors invading surrounding structures or spreading to regional node metastases, and IV for NENs with distant metastases (Table 3)<sup>[26]</sup>.

### Type-I gastric carcinoids

GC1s are neuroendocrine tumors of the gastric mucosa originating from ECL cells in response to chronic hypergastrinemia associated with chronic atrophic gastritis (CAG), either autoimmune or due to *Helicobacter pylori* infection. GC1s are the most frequent GCs, accounting for 70%–80% of all gastric NENs.

**Pathogenesis:** The ECL cells constitute the largest endocrine cell population of the gastric body and fundus mucosa; they express CCK-2 (gastrin) receptors,

**Table 3** Scoring staging system according to tumor-node-metastasis<sup>[26]</sup>

T-primary tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	<i>In-situ</i> tumor/dysplasia (< 0.5 mm)
T1	Tumor invades lamina propria or submucosa and < 1 cm
T2	Tumor invades muscularis propria or subserosa or > 1 cm
T3	Tumor penetrates serosa
T4	Tumor penetrates serosa and/or invades adjacent structures
N-regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M-metastases	
MX	Distant metastasis cannot be assessed
M0	No distant metastases
M1	Distant metastases
Stage	
0	Tis N0 M0
1	T1 N0 M0
2A	T2 N0 M0
2B	T3 N0 M0
3A	T4 N0 M0
3B	Any T N1 M0
4	Any T Any N M1

mediating cell growth and histamine secretion, and stimulate acid secretion from the adjacent parietal cells<sup>[27–29]</sup>.

Due to the progressive destruction of gastric parietal cells, achlorhydria causes chronic hypergastrinemia, which in turn is responsible for ECL cell hyperplasia and sometimes dysplasia, which over time may lead to ECL cell carcinoid development<sup>[30,31]</sup>.

Among the factors influencing the trophic sensitivity of ECL cells to hypergastrinemia, the female gender is the most relevant one: the ECL cell hyperplasia is found more frequent in female than in male CAG patients. Furthermore, the decrease in ECL cells density with age increasing does not occur in women<sup>[32]</sup>. GCs associated with hypergastrinemia are more common in female subjects, whereas sporadic GCs, which develop through a gastrin-independent mechanism, occur mostly in males.

ECL cell hyperplasia may disappear when the hypergastrinemia is abolished. SSAs may be used to inhibit gastrin release and thus reduce the ECL cell hyperplasia<sup>[33]</sup>. Some morphometric studies have showed that antrectomy was responsible for a reduction of the ECL cell volume vs the total volume of endocrine cells, while octreotide reduced the volume of all endocrine cells<sup>[34]</sup>.

In addition to chronic autoimmune atrophic gastritis, also the proton pump inhibitors, the use of which has been increasing worldwide, can induce gastric achlorhydria and consequent hypergastrinemia, even if the actual association with an increased risk of GCs has not been demonstrated yet<sup>[35]</sup>. Probably, hypergastrinemia does not represent the only risk factor predisposing to the development

of gastric NENs, as not all GCs are associated with hypergastrinemia (see gastric carcinoid type III) and not all the conditions associated with hypergastrinemia lead to neoplastic proliferation. This may also be promoted by genetic background, intragastric environmental changes related to achlorhydria and/or intramucosal modifications occurring in CAG, which have been implicated in the pathogenesis of gastric adenocarcinoma. Finally, factors such as prostanglandins and lymphokines, locally released during chronic inflammation, as in the case of CAG, may play a role in the pathogenesis of GCs<sup>[36,37]</sup>.

**Clinical and endoscopic features:** These lesions, typically multicentric/multiple, small, usually with polypoid appearance (up to 78% according to Merola<sup>[21]</sup>) and localized in the oxyntic atrophic mucosa, are characterized by slow growth (low proliferative index Ki-67, *i.e.*, G1 according to the World Health Organization classification) and a low tendency to local or distal invasion. Only larger lesions (> 1 cm) are associated with lymph node or other organ spreading (in 3%-8% and 2% of cases, respectively), with a 5-year survival > 95%. These neoplasms only seldom have a functional syndrome, as in almost all cases they do not secrete specific hormones. Their detection is usually incidental or achieved during CAG follow-up. It is estimated that about 2.4%-5% of CAG patients develop GC1s in their lifetime, with an annual incidence of approximately 0.4% to 2.4%<sup>[9,11]</sup>.

Since CAG is associated with chronic hypergastrinemia, GC1s typically occur in the form of recurrent lesions. Moreover, even after endoscopic resection, up to 67% of GC1s recur after a mean time of 24 mo, with a reported trend to evolve in less differentiated lesions.

### **Management of type-I gastric carcinoid - a clinical challenge**

GC1s are usually treated conservatively. The European Neuroendocrine Tumor Society (ENETS) Consensus Guidelines have suggested that annual surveillance is sufficient in case of GC1s with a diameter < 10 mm  $\phi$ <sup>[8]</sup>. This approach is supported by some reports<sup>[24,38-40]</sup> which suggest that careful endoscopic follow-up might represent a reasonable safe option in selected patients. However, further studies evaluating larger cohorts during a longer follow-up period are necessary in order to support this clinical behavior, as some cases of progressive malignant GC1s have already been reported<sup>[17-19,41]</sup>.

Accordingly, the ENETS guidelines recommend endoscopic resection whenever possible, even in the presence of small carcinoids (diameter < 1 cm  $\phi$ ) and for up to 6 polyps not involving the muscularis propria<sup>[8]</sup>. Endoscopic mucosal resection (EMR) and submucosal dissection (ESD), characterized by a high rate of efficacy and a low risk of complications, have been suggested for treating GCs<sup>[42-46]</sup> but the complete

endoscopic eradication is not always feasible because carcinoids may be multifocal or submucosal. Many small studies have shown that the combination of endoscopic resection and surveillance of GC1s was a good treatment for lesions without local or distant spreading. Merola *et al.*<sup>[21]</sup> reported a 100% survival rate and no metastases in endoscopically treated patients; however, the recurrence rate was up to 68% after endoscopic resection. A lower recurrence rate (18% and 0.4%) is reported in recent Turkish<sup>[46]</sup> and Japanese studies<sup>[20]</sup> in which patients with GC1s were treated with EMR or ESD and followed up over a median of five years. Possible differences of the recurrence rates among these studies can depend on the difficulty to determine whether the lesions detected during follow-up endoscopies are true recurrences or simply small lesions missed at the initial endoscopy. Furthermore, more advanced endoscopic techniques, such as ESD and the routine use of chromoendoscopy, have been undertaken in Eastern countries, probably contributing to lower recurrence rates.

Currently, when dealing with the deep invasion of the gastric wall and detection of positive resection margins after endoscopic mucosal resection, local surgical tumor resection is generally performed. On the other hand, as this type of tumors are often multiple and recurrent, antral resection is recommended as it aims to avoid chronic gastrin stimulation of ECL cells and even if the long-term benefits of antrectomy remain uncertain<sup>[47,48]</sup>. Indeed, a further recurrence has been described in up to 20% of all cases and the surgical approach is more invasive and at higher risk of complications. However, in the case of disease progression or recurrence after local surgical resection, partial or total gastrectomy along with lymph node dissection should be carried out as suggested in the current guidelines<sup>[8]</sup>.

Overall, the optimal treatment for GC1s remains controversial in view of their usually benign course, but also of their tendency to recur: a controlled study comparing the outcomes of endoscopic surveillance, endoscopic resection, surgical approach and SSAs treatment of GC1s is yet to be carried out.

### **SSAs**

**Historical perspectives:** The history of SSAs begins in 1973 when Brazeau and Guillemin discovered a GHRH antagonist, which they called somatostatin and which appeared to be widely distributed in the organs and nervous system<sup>[49]</sup> of animals and humans.

Noteworthy, native somatostatin plays an important regulatory role in neurotransmission and secretion, by preventing the release of the growth hormone, thyroid-stimulating hormone, gastrointestinal hormones, pancreatic enzymes and neuropeptides. It is not suitable though for long-term clinical application due to its short half-life (< 3 min) and the impact of rebound hypersecretion. Therefore, synthetic drugs

were developed with improved pharmacokinetic characteristics. SSAs have been used since 1980 to control the symptoms related to gastroenteropancreatic NENs, especially carcinoids and VIPomas, due to their characteristic of expressing somatostatin receptors (SSTR).

Long-acting SSAs have been used as a medical treatment of both functioning and non-functioning neuroendocrine tumors, due to the SSAs capability to inhibit hormone release and neoplastic growth by binding to specific high-affinity SSTR. Two different SSAs, octreotide and lanreotide, which principally bind to receptor subtypes 2 and 5, are clinically used with comparable effectiveness. Recently, pasireotide, a SSA with high affinity to all types of somatostatin receptors, has been introduced, even if its use is still restricted to those patients with partial/nil response to octreotide and lanreotide<sup>[50]</sup>.

In a multicenter study comparing the treatment with lanreotide (30 mg i.m. every 10 d) vs octreotide (200 µg s.c. twice or thrice daily) in 33 patients with carcinoid syndrome, O'Toole *et al.*<sup>[51]</sup> did not evidence any significant differences with regard to symptom control. Again, both these analogs have been reported to have an antiproliferative effect *in vitro*<sup>[52,53]</sup>. Data from uncontrolled prospective and retrospective clinical series<sup>[54-56]</sup> showed tumor shrinkage and disappearance in response to either short-acting SSAs alone or when combined with interferon-α. Further trials have reported tumor stabilization in up to 50% of patients, but without complete regression. More recently, two randomized double-blind placebo-controlled prospective studies<sup>[57,58]</sup> have clearly supported the antiproliferative effects of SSAs with a prolonged progression-free-survival in patients affected by enteropancreatic neuroendocrine tumors and treated with octreotide LAR (long-acting release) and lanreotide. However, there is need for additional studies, including patients with a primary tumor outside the midgut, in order to demonstrate the antiproliferative effects of octreotide. Again, further controlled trials in larger cohorts of patients are strongly required in order to identify the predictive factors of response and to confirm whether such a response may positively influence the patients' survival rate.

**Benefits of SSAs therapy:** The antineoplastic effects of somatostatin and SSAs are both direct and indirect, the former depending on the direct binding to tumor cells, the latter ones mediated through the inhibition of growth factors, angiogenesis and the immune system. The anti-tumour effect of SSAs may include both cytostatic (growth arrest) and cytotoxic (pro-apoptotic) mechanisms<sup>[59-62]</sup>. SSAs may also inhibit the insulin-like growth factor- I secretion, thought to be involved in recurrence, growth and aggressiveness of some endocrine and non-endocrine tumors<sup>[63]</sup>. In a

systematic analysis of data from 62 published studies about antiproliferative effects of SSAs<sup>[64]</sup>, tumor growth stabilization was observed for a period of 8-16 mo in about 50% of the patients, and tumor regression in about 10%-20% of patients.

Unfortunately, tachyphylaxis can develop after months or years on treatment, probably due to the development of resistant cell clones within the tumor, whereas it does not seem related to any down-regulation of the number of somatostatin receptors on the cell surface.

Another relevant antiproliferative effect of SSAs is based on their capability to inhibit the production and secretion of many angiogenic factors, thereby reducing the tumor growth rate<sup>[65]</sup>. Therefore, SSAs may suppress tumor growth either directly, through their effect on SSTR expressing cells, or indirectly, *via* the inhibition of angiogenic factors, such as VEGF<sup>[66]</sup>.

Finally, SSAs have an antisecretory role, as supported by a study by Fykse *et al.*<sup>[67]</sup> in which five patients with hypergastrinemia and GCs were every month treated with octreotide LAR over one year with the consequent significant reduction in tumor burden, ECL cell grade of hyperplasia and the normalization of circulating chromogranin A levels. A further study<sup>[23]</sup> reported a significant reduction in gastrin levels and tumor regression in three patients suffering from Zollinger-Ellison syndrome and treated with lanreotide or octreotide for one year. Despite the small number of patients included in these studies, the results suggest that SSAs both inhibit gastrin secretion and growth of these tumors.

**Side effects of SSAs therapy:** Generally, SSAs are safe, easy to use and well tolerated with infrequent mild side effects, the most common being abdominal discomfort, bloating, diarrhea (related to the inhibition of pancreatic enzymes) usually self-limiting after the first few months of therapy. The development of cholestasis is reported in up to 60% of patients and can be partially prevented by orally administered ursodeoxycholic acid. Impaired glucose tolerance and bradycardia have also been occasionally reported<sup>[68]</sup>.

### **Somatostatin analogs ... for many gastric carcinoids**

Endoscopic resection has proved to represent a safe effective treatment for GC1s as it is associated with a 100% survival rate, even if recurrences have been described with a frequency of up to 67% of all patients endoscopically treated<sup>[21]</sup>. Indeed, GC1s are frequently multiple and can present submucosal invasion; in addition, they are sometimes undetectable at UGE, as they present as microcarcinoids. Therefore, the endoscopic approach is not always effective or adequate and other treatment options are needed for some subgroups of "high risk" patients, *i.e.*, those with a multifocal or recurrent disease. Moreover GC1s can be considered a complication of another disorder

that involves the entire gastric mucosa, so that a locoregional treatment may not be appropriate and a systemic therapy is thus a more pertinent approach.

SSAs have been proposed as a potential treatment in view of their antiproliferative, antisecretory and antiangiogenic effects, although they are not currently recommended according to both the ENETS<sup>[8]</sup> and NANETS guidelines<sup>[69]</sup> for type-1 GCs.

Of interest, Ferraro *et al.*<sup>[70]</sup> observed that octreotide, given daily over six months, decreased fasting gastrin levels and ECL proliferation in patients with CAG and hypergastrinemia. Bakke *et al.*<sup>[71]</sup> have reported a direct antiproliferative effect of octreotide on rat ECL cells.

SSAs have also been occasionally used in tertiary referral centres to treat single or multiple GC1s with diameter < 1 cm  $\phi$  and low proliferative index<sup>[72]</sup>. When administered in a continuous way, SSAs have contributed to the reduction in number and size of GC1s and the suppression of circulating gastrin levels, by inhibiting gastrin release from antral G cells. However, a series by Jianu *et al.*<sup>[73]</sup> has reported GC1 size increase and early recurrence after the SSA withdrawal in patients followed up over a median of 5 years from therapy discontinuation. One should consider though that such findings have not been confirmed by other studies.

Reasonably, after the development of a neuroendocrine neoplasm, other ECL cells can develop neoplastic proliferation in similar conditions (*e.g.*, chronic hypergastrinemia). Therefore, patients with recurrent GC1s should be considered at higher risk of developing other recurrences.

### Somatostatin analogs ... not for all

Not all GC1 patients are to be treated with SSAs on the consideration that such a therapy comes with high costs attached and the 5-year survival rate of GC1 patients when regularly followed up with endoscopy remains good<sup>[74]</sup>. Accordingly, SSAs are better reserved to specific subsets of patients, such as those with recurrent or multifocal GC1s. Actually, no clear guidelines are currently available for patients presenting GC1 recurrence after repeated endoscopic resection. For these patients antrectomy has been proposed in spite of its invasiveness and possibly related complications<sup>[75]</sup>. Furthermore, some morphometric studies have demonstrated that octreotide reduced the overall endocrine cell volume<sup>[76]</sup>, while antrectomy decreased only the volume of ECL cells.

A further treatment option can be that of multiple endoscopic resections; however, in the context of CAG, gastric mucosa is chronically and diffusely altered and GC1s are often multifocal, extended to submucosa and sometimes macroscopically undetectable, making endoscopic resection not always feasible and radical. In those countries where advanced endoscopic techniques are in place, management by endoscopy

can also apply to multifocal GCs and GCs involving the submucosal layer and hence endoscopic resection is preferred as the first-line therapy. The far larger use of standard polypectomies instead of the more effective “*en bloc*” resection and ESD might account for the still high recurrence rates observed in some series<sup>[21]</sup>.

Given the above considerations, we have recently reported on a series of CAG patients with recurrent GC1s, treated with SSAs until carcinoid disappearance and suppression of circulating gastrin values. For such patients SSAs have represented an efficient therapeutic option (Massironi *et al.* submitted). As already reported, SSAs can exert an antiproliferative effect on neoplastic ECL-cells as well as other hyperplastic or dysplastic ECL-cells, which frequently accompanied GC1s, thus reducing the risk of further development<sup>[77]</sup>. Moreover, several studies<sup>[19]</sup> have reported that SSAs might significantly suppress intestinal metaplasia, which may be present in patients with chronic atrophic gastritis and may represent a risk factor for gastric adenocarcinoma<sup>[78,79]</sup>.

Another pharmacological therapeutic option is represented in the near future by netazepide, an orally administered highly selective active gastrin/CCK-2 receptor antagonist, recently described as an effective treatment in animal models of ECL-cell tumours induced by hypergastrinemia<sup>[80]</sup>. Its use over a 12-wk period in a recent non-randomized trial was associated with a significant reduction in both the number of GC1s and the size of the largest tumour, of about 30%, and with the normalization of CgA levels, but not of gastrin values<sup>[81]</sup>. Nevertheless, randomized controlled trials involving longer-term treatment and larger cohorts of patients, are required to confirm these results.

## CONCLUSION

Approval is still to come for specific indications, schedule and duration of therapy to avoid the undertreatment or overtreatment of recurrent GC1s. The duration of a SSA-based therapy may be guided by both the regression of GC and the suppression of gastrin levels. Finally, the comparison between new therapies, such as gastrin receptor antagonists and antibodies against progastrin-releasing peptides, and the traditional ones, such as the endoscopic or surgical approaches, can be useful in order to identify the most effective management approach.

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## REFERENCES

- 1 Niederle MB, Hackl M, Kaserer K, Niederle B. Gastroenteropancreatic neuroendocrine tumours: the current incidence and staging based on the WHO and European Neuroendocrine Tumour



- Society classification: an analysis based on prospectively collected parameters. *Endocr Relat Cancer* 2010; **17**: 909-918 [PMID: 20702725 DOI: 10.1677/ERC-10-0152]
- 2 **Fraenkel M**, Kim M, Faggiano A, de Herder WW, Valk GD; Knowledge NETwork. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer* 2014; **21**: R153-R163 [PMID: 24322304 DOI: 10.1530/ERC-13-0125]
  - 3 **Kidd M**, Gustafsson B, Modlin IM. Gastric carcinoids (neuroendocrine neoplasms). *Gastroenterol Clin North Am* 2013; **42**: 381-397 [PMID: 23639647 DOI: 10.1016/j.gtc.2013.01.009]
  - 4 **Lawrence B**, Gustafsson BI, Chan A, Svejda B, Kidd M, Modlin IM. The epidemiology of gastroenteropancreatic neuroendocrine tumors. *Endocrinol Metab Clin North Am* 2011; **40**: 1-18, vii [PMID: 21349409 DOI: 10.1016/j.ecl.2010.12.005]
  - 5 **Yao JC**, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* 2008; **26**: 3063-3072 [PMID: 18565894 DOI: 10.1200/JCO.2007.15.4377]
  - 6 **Ellis L**, Shale MJ, Coleman MP. Carcinoid tumors of the gastrointestinal tract: trends in incidence in England since 1971. *Am J Gastroenterol* 2010; **105**: 2563-2569 [PMID: 20823835 DOI: 10.1038/ajg.2010.341]
  - 7 **Modlin IM**, Lye KD, Kidd M. A 50-year analysis of 562 gastric carcinoids: small tumor or larger problem? *Am J Gastroenterol* 2004; **99**: 23-32 [PMID: 14687136]
  - 8 **Delle Fave G**, Kwekkeboom DJ, Van Cutsem E, Rindi G, Kos-Kudla B, Knigge U, Sasano H, Tomassetti P, Salazar R, Ruzsniwski P; Barcelona Consensus Conference participants. ENETS Consensus Guidelines for the management of patients with gastroduodenal neoplasms. *Neuroendocrinology* 2012; **95**: 74-87 [PMID: 22262004 DOI: 10.1159/000335595]
  - 9 **Vannella L**, Sbrozzi-Vanni A, Lahner E, Bordi C, Pillozzi E, Corleto VD, Osborn JF, Delle Fave G, Annibale B. Development of type I gastric carcinoid in patients with chronic atrophic gastritis. *Aliment Pharmacol Ther* 2011; **33**: 1361-1369 [PMID: 21492197 DOI: 10.1111/j.1365-2036.2011.04659.x]
  - 10 **Borch K**, Ahrén B, Ahlman H, Falkmer S, Granérus G, Grmelius L. Gastric carcinoids: biologic behavior and prognosis after differentiated treatment in relation to type. *Ann Surg* 2005; **242**: 64-73 [PMID: 15973103]
  - 11 **Marignani M**, Delle Fave G, Mecarocci S, Bordi C, Angeletti S, D'Ambra G, Aprile MR, Corleto VD, Monarca B, Annibale B. High prevalence of atrophic body gastritis in patients with unexplained microcytic and macrocytic anemia: a prospective screening study. *Am J Gastroenterol* 1999; **94**: 766-772 [PMID: 10086664]
  - 12 **Borch K**, Renvall H, Liedberg G. Gastric endocrine cell hyperplasia and carcinoid tumors in pernicious anemia. *Gastroenterology* 1985; **88**: 638-648 [PMID: 2578420]
  - 13 **La Rosa S**, Inzani F, Vanoli A, Klersy C, Dainese L, Rindi G, Capella C, Bordi C, Solcia E. Histologic characterization and improved prognostic evaluation of 209 gastric neuroendocrine neoplasms. *Hum Pathol* 2011; **42**: 1373-1384 [PMID: 21531442 DOI: 10.1016/j.humpath.2011.01.018]
  - 14 **Granberg D**, Wilander E, Stridsberg M, Granerus G, Skogseid B, Oberg K. Clinical symptoms, hormone profiles, treatment, and prognosis in patients with gastric carcinoids. *Gut* 1998; **43**: 223-228 [PMID: 10189848]
  - 15 **Qvigstad G**, Falkmer S, Westre B, Waldum HL. Clinical and histopathological tumour progression in ECL cell carcinoids ("ECLomas"). *APMIS* 1999; **107**: 1085-1092 [PMID: 10660138]
  - 16 **Bordi C**. Gastric carcinoids. *Ital J Gastroenterol Hepatol* 1999; **31** Suppl 2: S94-S97 [PMID: 10604110]
  - 17 **Spampatti MP**, Massironi S, Rossi RE, Conte D, Sciola V, Ciafardini C, Ferrero S, Lodi L, Peracchi M. Unusually aggressive type I gastric carcinoid: a case report with a review of the literature. *Eur J Gastroenterol Hepatol* 2012; **24**: 589-593 [PMID: 22465973 DOI: 10.1097/MEG.0b013e328350fae8]
  - 18 **Tatsuta T**, Yoshimura T, Hasui K, Takasugi K, Sawaya M, Hanabata N, Shimoyama T, Kijima H, Fukuda S. Multiple gastric G1 neuroendocrine tumors with venous and lymphatic invasion. *Intern Med* 2013; **52**: 1697-1701 [PMID: 23903502]
  - 19 **Grozinsky-Glasberg S**, Thomas D, Strosberg JR, Pape UF, Felder S, Tsolakis AV, Alexandraki KI, Fraenkel M, Saiegh L, Reissman P, Kaltsas G, Gross DJ. Metastatic type I gastric carcinoid: a real threat or just a myth? *World J Gastroenterol* 2013; **19**: 8687-8695 [PMID: 24379587 DOI: 10.3748/wjg.v19.i46.8687]
  - 20 **Sato Y**, Imamura H, Kaizaki Y, Koizumi W, Ishido K, Kurahara K, Suzuki H, Fujisaki J, Hirakawa K, Hosokawa O, Ito M, Kaminishi M, Furuta T, Chiba T, Haruma K. Management and clinical outcomes of type I gastric carcinoid patients: retrospective, multicenter study in Japan. *Dig Endosc* 2014; **26**: 377-384 [PMID: 24188531 DOI: 10.1111/den.12197]
  - 21 **Merola E**, Sbrozzi-Vanni A, Panzuto F, D'Ambra G, Di Giulio E, Pillozzi E, Capurso G, Lahner E, Bordi C, Annibale B, Delle Fave G. Type I gastric carcinoids: a prospective study on endoscopic management and recurrence rate. *Neuroendocrinology* 2012; **95**: 207-213 [PMID: 21811050 DOI: 10.1159/000329043]
  - 22 **Gibril F**, Schumann M, Pace A, Jensen RT. Multiple endocrine neoplasia type 1 and Zollinger-Ellison syndrome: a prospective study of 107 cases and comparison with 1009 cases from the literature. *Medicine (Baltimore)* 2004; **83**: 43-83 [PMID: 14747767]
  - 23 **Tomassetti P**, Migliori M, Caletti GC, Fusaroli P, Corinaldesi R, Gullo L. Treatment of type II gastric carcinoid tumors with somatostatin analogues. *N Engl J Med* 2000; **343**: 551-554 [PMID: 10954763]
  - 24 **Ruzsniwski P**, Laucournet H, Elouaer-Blanc L, Mignon M, Bonfils S. Long-acting somatostatin (SMS 201-995) in the management of Zollinger-Ellison syndrome: evidence for sustained efficacy. *Pancreas* 1988; **3**: 145-152 [PMID: 2897687]
  - 25 **Oberg K**, Castellano D. Current knowledge on diagnosis and staging of neuroendocrine tumors. *Cancer Metastasis Rev* 2011; **30** Suppl 1: 3-7 [PMID: 21311954 DOI: 10.1007/s10555-011-9292-1]
  - 26 **Rindi G**, Klöppel G, Alhman H, Caplin M, Couvelard A, de Herder WW, Eriksson B, Falchetti A, Falconi M, Komminoth P, Körner M, Lopes JM, McNicol AM, Nilsson O, Perren A, Scarpa A, Scoazec JY, Wiedenmann B. TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Arch* 2006; **449**: 395-401 [PMID: 16967267 DOI: 10.1007/s00428-006-0250-1]
  - 27 **Solcia E**, Rindi G, Silini E, Villani L. Enterochromaffin-like (ECL) cells and their growths: relationships to gastrin, reduced acid secretion and gastritis. *Baillieres Clin Gastroenterol* 1993; **7**: 149-165 [PMID: 7682874 DOI: 10.1016/0950-3528(93)90035-Q]
  - 28 **Håkanson R**, Chen D, Andersson K, Monstein HJ, Zhao CM, Ryberg B, Sundler F, Mattsson H. The biology and physiology of the ECL cell. *Yale J Biol Med* 1994; **67**: 123-134 [PMID: 7502521]
  - 29 **Bordi C**, D'Adda T, Azzoni C, Pilato FP, Caruana P. Hypergastrinemia and gastric enterochromaffin-like cells. *Am J Surg Pathol* 1995; **19** Suppl 1: S8-19 [PMID: 7762739]
  - 30 **Bordi C**, D'Adda T, Azzoni C, Ferraro G. Pathogenesis of ECL cell tumors in humans. *Yale J Biol Med* 1998; **71**: 273-284 [PMID: 10461358]
  - 31 **Borch K**, Renvall H, Liedberg G, Andersen BN. Relations between circulating gastrin and endocrine cell proliferation in the atrophic gastric fundic mucosa. *Scand J Gastroenterol* 1986; **21**: 357-363 [PMID: 3715400 DOI: 10.3109/00365528609003087]
  - 32 **Green DM**, Bishop AE, Rindi G, Lee FI, Daly MJ, Domin J, Bloom SR, Polak JM. Enterochromaffin-like cell populations in human fundic mucosa: quantitative studies of their variations with age, sex, and plasma gastrin levels. *J Pathol* 1989; **157**: 235-241 [PMID: 2926564 DOI: 10.1002/path.1711570310]
  - 33 **Delle Fave G**, Annibale B. Modulation of growth of human gastric enterochromaffin-like cells. *Digestion* 1996; **57** Suppl 1: 15-16 [PMID: 8813460 DOI: 10.1159/000201386]
  - 34 **D'Adda T**, Annibale B, Delle Fave G, Bordi C. Oxyntic endocrine cells of hypergastrinaemic patients. Differential response to antrectomy or octreotide. *Gut* 1996; **38**: 668-674 [PMID: 8707110]



- DOI: 10.1136/gut.38.5.668]
- 35 **Jianu CS**, Fossmark R, Viset T, Qvigstad G, Sørdal O, Mårvik R, Waldum HL. Gastric carcinoids after long-term use of a proton pump inhibitor. *Aliment Pharmacol Ther* 2012; **36**: 644-649 [PMID: 22861200 DOI: 10.1111/apt.12012]
  - 36 **Solcia E**, Rindi G, Fiocca R, Villani L, Buffà R, Ambrosiani L, Capella C. Distinct patterns of chronic gastritis associated with carcinoid and cancer and their role in tumorigenesis. *Yale J Biol Med* 1992; **65**: 793-804; discussion 827-829 [PMID: 1341079]
  - 37 **Sanui A**, Yotsumoto F, Tsujioka H, Fukami T, Horiuchi S, Shiota K, Yoshizato T, Kawarabayashi T, Kuroki M, Miyamoto S. HB-EGF inhibition in combination with various anticancer agents enhances its antitumor effects in gastric cancer. *Anticancer Res* 2010; **30**: 3143-3149 [PMID: 20871033]
  - 38 **Ravizza D**, Fiori G, Trovato C, Fazio N, Bonomo G, Luca F, Bodei L, Pelosi G, Tamayo D, Crosta C. Long-term endoscopic and clinical follow-up of untreated type 1 gastric neuroendocrine tumours. *Dig Liver Dis* 2007; **39**: 537-543 [PMID: 17433795 DOI: 10.1016/j.dld.2007.01.018]
  - 39 **Rappel S**, Altendorf-Hofmann A, Stolte M. Prognosis of gastric carcinoid tumours. *Digestion* 1995; **56**: 455-462 [PMID: 8536814 DOI: 10.1159/000201276]
  - 40 **Hosokawa O**, Kaizaki Y, Hattori M, Douden K, Hayashi H, Morishita M, Ohta K. Long-term follow up of patients with multiple gastric carcinoids associated with type A gastritis. *Gastric Cancer* 2005; **8**: 42-46 [PMID: 15747174 DOI: 10.1007/s10120-004-0303-6]
  - 41 **Hori K**, Fukui H, Imura J, Kojima T, Fujita M, Kawamata H, Chiba T, Fujimori T. Benign gastric carcinoid tumor with hypergastrinemia followed up for 12 years. *Gastric Cancer* 2000; **3**: 161-164 [PMID: 11984731 DOI: 10.1007/PL00011712]
  - 42 **Ichikawa J**, Tanabe S, Koizumi W, Kida Y, Imaizumi H, Kida M, Saigenji K, Mitomi H. Endoscopic mucosal resection in the management of gastric carcinoid tumors. *Endoscopy* 2003; **35**: 203-206 [PMID: 12584637 DOI: 10.1055/s-2003-37256]
  - 43 **Hopper AD**, Bourke MJ, Hourigan LF, Tran K, Moss A, Swan MP. En-bloc resection of multiple type 1 gastric carcinoid tumors by endoscopic multi-band mucosectomy. *J Gastroenterol Hepatol* 2009; **24**: 1516-1521 [PMID: 19743997 DOI: 10.1111/j.1440-1746.2009.05909.x]
  - 44 **Uygun A**, Kadayifci A, Polat Z, Yilmaz K, Gunal A, Demir H, Bagci S. Long-term results of endoscopic resection for type I gastric neuroendocrine tumors. *J Surg Oncol* 2014; **109**: 71-74 [PMID: 24165913 DOI: 10.1002/jso.23477]
  - 45 **Li QL**, Zhang YQ, Chen WF, Xu MD, Zhong YS, Ma LL, Qin WZ, Hu JW, Cai MY, Yao LQ, Zhou PH. Endoscopic submucosal dissection for foregut neuroendocrine tumors: an initial study. *World J Gastroenterol* 2012; **18**: 5799-5806 [PMID: 23155323 DOI: 10.3748/wjg.v18.i40.5799]
  - 46 **Oshima T**, Okugawa T, Hori K, Kim Y, Tanaka J, Watari J, Miwa H. Successful endoscopic submucosal dissection of gastric carcinoid in a patient with autoimmune gastritis and systemic lupus erythematosus. *Intern Med* 2012; **51**: 1211-1213 [PMID: 22687792]
  - 47 **Lupinacci RM**, Dias AR, Mello ES, Kondo A. Minute type I gastric carcinoid with regional lymph node metastasis. *Int J Surg Pathol* 2013; **21**: 169-172 [PMID: 22923778 DOI: 10.1177/1066896912457201]
  - 48 **Gladly RA**, Strong VE, Coit D, Allen PJ, Gerdes H, Shia J, Klimstra DS, Brennan MF, Tang LH. Defining surgical indications for type I gastric carcinoid tumor. *Ann Surg Oncol* 2009; **16**: 3154-3160 [PMID: 19727959 DOI: 10.1245/s10434-009-0687-y]
  - 49 **Brazeau P**, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 1973; **179**: 77-79 [PMID: 4682131]
  - 50 **Wolin EM**, Hu K, Hughes G, Bouillaud E, Giannone V, Resendiz KH. Safety, tolerability, pharmacokinetics, and pharmacodynamics of a long-acting release (LAR) formulation of pasireotide (SOM230) in patients with gastroenteropancreatic neuroendocrine tumors: results from a randomized, multicenter, open-label, phase I study. *Cancer Chemother Pharmacol* 2013; **72**: 387-395 [PMID: 23765178 DOI: 10.1007/s00280-013-2202-1]
  - 51 **O'Toole D**, Ducreux M, Bommelaer G, Wemeau JL, Bouché O, Catus F, Blumberg J, Ruzsiewicz P. Treatment of carcinoid syndrome: a prospective crossover evaluation of lanreotide versus octreotide in terms of efficacy, patient acceptability, and tolerance. *Cancer* 2000; **88**: 770-776 [PMID: 10679645]
  - 52 **Susini C**, Buscail L. Rationale for the use of somatostatin analogs as antitumor agents. *Ann Oncol* 2006; **17**: 1733-1742 [PMID: 16801334]
  - 53 **Grozinsky-Glasberg S**, Kaltsas G, Gur C, Gal E, Thomas D, Fichman S, Alexandraki K, Barak D, Glaser B, Shimon I, Gross DJ. Long-acting somatostatin analogues are an effective treatment for type 1 gastric carcinoid tumours. *Eur J Endocrinol* 2008; **159**: 475-482 [PMID: 18662970 DOI: 10.1530/EJE-08-0420]
  - 54 **Toumpanakis C**, Caplin ME. Update on the role of somatostatin analogs for the treatment of patients with gastroenteropancreatic neuroendocrine tumors. *Semin Oncol* 2013; **40**: 56-68 [PMID: 23391113 DOI: 10.1053/j.seminoncol.2012.11.006]
  - 55 **Sidéri L**, Dubé P, Rinke A. Antitumor effects of somatostatin analogs in neuroendocrine tumors. *Oncologist* 2012; **17**: 747-755 [PMID: 22628056 DOI: 10.1634/theoncologist.2011-0458]
  - 56 **Palazzo M**, Lombard-Bohas C, Cadiot G, Matysiak-Budnik T, Rebours V, Vullierme MP, Couvelard A, Hentic O, Ruzsiewicz P. Ki67 proliferation index, hepatic tumor load, and pretreatment tumor growth predict the antitumoral efficacy of lanreotide in patients with malignant digestive neuroendocrine tumors. *Eur J Gastroenterol Hepatol* 2013; **25**: 232-238 [PMID: 23108416 DOI: 10.1097/MEG.0b013e328359d1a6]
  - 57 **Caplin ME**, Pavel M, Čwikla JB, Phan AT, Raderer M, Sedláčková E, Cadiot G, Wolin EM, Capdevila J, Wall L, Rindi G, Langley A, Martinez S, Blumberg J, Ruzsiewicz P. Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *N Engl J Med* 2014; **371**: 224-233 [PMID: 25014687 DOI: 10.1056/NEJMoa1316158]
  - 58 **Rinke A**, Müller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Bläker M, Harder J, Arnold C, Gress T, Arnold R. Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumor growth in patients with metastatic neuroendocrine midgut tumors: a report from the PROMID Study Group. *J Clin Oncol* 2009; **27**: 4656-4663 [PMID: 19704057 DOI: 10.1200/JCO.2009.22.8510]
  - 59 **Bousquet C**, Puente E, Buscail L, Vaysse N, Susini C. Antiproliferative effect of somatostatin and analogs. *Chemotherapy* 2001; **47** Suppl 2: 30-39 [PMID: 11275700]
  - 60 **Ferrante E**, Pellegrini C, Bondioni S, Peverelli E, Locatelli M, Gelmini P, Luciani P, Peri A, Mantovani G, Bosari S, Beck-Peccoz P, Spada A, Lania A. Octreotide promotes apoptosis in human somatotroph tumor cells by activating somatostatin receptor type 2. *Endocr Relat Cancer* 2006; **13**: 955-962 [PMID: 16954443]
  - 61 **Pyronnet S**, Bousquet C, Najib S, Azar R, Laklai H, Susini C. Antitumor effects of somatostatin. *Mol Cell Endocrinol* 2008; **286**: 230-237 [PMID: 18359151 DOI: 10.1016/j.mce.2008.02.002]
  - 62 **Bousquet C**, Guillermet J, Vernejoul F, Lahlou H, Buscail L, Susini C. Somatostatin receptors and regulation of cell proliferation. *Dig Liver Dis* 2004; **36** Suppl 1: S2-S7 [PMID: 15077905 DOI: 10.1016/j.dld.2003.11.007]
  - 63 **Furukawa M**, Raffeld M, Mateo C, Sakamoto A, Moody TW, Ito T, Venzon DJ, Serrano J, Jensen RT. Increased expression of insulin-like growth factor I and/or its receptor in gastrinomas is associated with low curability, increased growth, and development of metastases. *Clin Cancer Res* 2005; **11**: 3233-3242 [PMID: 15867218 DOI: 10.1158/1078-0432.CCR-04-1915]
  - 64 **Eriksson B**, Oberg K. Summing up 15 years of somatostatin analog therapy in neuroendocrine tumors: future outlook. *Ann Oncol* 1999; **10** Suppl 2: S31-S38 [PMID: 10399030 DOI: 10.1093/annonc/10.suppl\_2.S31]
  - 65 **Barrie R**, Woltering EA, Hajarizadeh H, Mueller C, Ure T, Fletcher WS. Inhibition of angiogenesis by somatostatin and

- somatostatin-like compounds is structurally dependent. *J Surg Res* 1993; **55**: 446-450 [PMID: 7692142 DOI: 10.1006/jsre.1993.1167]
- 66 **Mentlein R**, Eichler O, Forstreuter F, Held-Feindt J. Somatostatin inhibits the production of vascular endothelial growth factor in human glioma cells. *Int J Cancer* 2001; **92**: 545-550 [PMID: 11304689 DOI: 10.1002/ijc.1223]
  - 67 **Fykse V**, Sandvik AK, Qvigstad G, Falkmer SE, Syversen U, Waldum HL. Treatment of ECL cell carcinoids with octreotide LAR. *Scand J Gastroenterol* 2004; **39**: 621-628 [PMID: 15370681 DOI: 10.1080/00365520410005225]
  - 68 **Öberg K**. Biotherapies for GEP-NETs. *Best Pract Res Clin Gastroenterol* 2012; **26**: 833-841 [PMID: 23582922 DOI: 10.1016/j.bpg.2013.01.001]
  - 69 **Kulke MH**, Anthony LB, Bushnell DL, de Herder WW, Goldsmith SJ, Klimstra DS, Marx SJ, Pasieka JL, Pommier RF, Yao JC, Jensen RT; North American Neuroendocrine Tumor Society (NANETS). NANETS treatment guidelines: well-differentiated neuroendocrine tumors of the stomach and pancreas. *Pancreas* 2010; **39**: 735-752 [PMID: 20664472 DOI: 10.1097/MPA.0b013e3181ebb168]
  - 70 **Ferraro G**, Annibale B, Marignani M, Azzoni C, D'Adda T, D'Ambra G, Bordi C, delle Fave G. Effectiveness of octreotide in controlling fasting hypergastrinemia and related enterochromaffin-like cell growth. *J Clin Endocrinol Metab* 1996; **81**: 677-683 [PMID: 8636288]
  - 71 **Bakke I**, Sandvik AK, Waldum HL. Octreotide inhibits the enterochromaffin-like cell but not peroxisome proliferator-induced hypergastrinemia. *J Mol Endocrinol* 2000; **25**: 109-119 [PMID: 10915223 DOI: 10.1677/jme.0.0250109]
  - 72 **Campana D**, Nori F, Pezzilli R, Piscitelli L, Santini D, Brocchi E, Corinaldesi R, Tomassetti P. Gastric endocrine tumors type I: treatment with long-acting somatostatin analogs. *Endocr Relat Cancer* 2008; **15**: 337-342 [PMID: 18310299 DOI: 10.1677/ERC-07-0251]
  - 73 **Jianu CS**, Fossmark R, Syversen U, Hauso Ø, Fykse V, Waldum HL. Five-year follow-up of patients treated for 1 year with octreotide long-acting release for enterochromaffin-like cell carcinoids. *Scand J Gastroenterol* 2011; **46**: 456-463 [PMID: 21133821 DOI: 10.3109/0365552.2010.539255]
  - 74 **de Bree E**, Papadimitraki E, Skordilis P, Tsiftsis DD. Multiple gastric carcinoids. *J Am Coll Surg* 2004; **199**: 517 [PMID: 15325627 DOI: 10.1016/j.jamcollsurg.2004.05.260]
  - 75 **Guillem P**. [Gastric carcinoid tumours. Is there a place for antrectomy?]. *Ann Chir* 2005; **130**: 323-326 [PMID: 15890310 DOI: 10.1016/j.anchir.2005.03.010]
  - 76 **Bordi C**, Azzoni C, Pilato FP, Robutti F, D'Ambra G, Caruana P, Rindi G, Corleto VD, Annibale B, Delle Fave G. Morphometry of gastric endocrine cells in hypergastrinemic patients treated with the somatostatin analogue octreotide. *Regul Pept* 1993; **47**: 307-318 [PMID: 8234912 DOI: 10.1016/0167-0115(93)90397-Q]
  - 77 **Annibale B**, Azzoni C, Corleto VD, di Giulio E, Caruana P, D'Ambra G, Bordi C, Delle Fave G. Atrophic body gastritis patients with enterochromaffin-like cell dysplasia are at increased risk for the development of type I gastric carcinoid. *Eur J Gastroenterol Hepatol* 2001; **13**: 1449-1456 [PMID: 11742193 DOI: 10.1097/00042737-200112000-00008]
  - 78 **Kokkola A**, Haapiainen R, Laxén F, Puolakkainen P, Kivilaakso E, Virtamo J, Sipponen P. Risk of gastric carcinoma in patients with mucosal dysplasia associated with atrophic gastritis: a follow up study. *J Clin Pathol* 1996; **49**: 979-984 [PMID: 9038734 DOI: 10.1136/jcp.49.12.979]
  - 79 **Siurala M**, Seppala K. Atrophic gastritis as a possible precursor of gastric carcinoma and pernicious anemia. Results of follow-up examinations. *Acta Med Scand* 1960; **166**: 455-474 [PMID: 13831404 DOI: 10.1111/j.0954-6820.1960.tb17401.x]
  - 80 **Fossmark R**, Sørvald Ø, Jianu CS, Qvigstad G, Nordrum IS, Boyce M, Waldum HL. Treatment of gastric carcinoids type 1 with the gastrin receptor antagonist netazepide (YF476) results in regression of tumours and normalisation of serum chromogranin A. *Aliment Pharmacol Ther* 2012; **36**: 1067-1075 [PMID: 23072686 DOI: 10.1111/apt.12090]
  - 81 **Moore AR**, Boyce M, Steele IA, Campbell F, Varro A, Pritchard DM. Netazepide, a gastrin receptor antagonist, normalises tumour biomarkers and causes regression of type 1 gastric neuroendocrine tumours in a nonrandomised trial of patients with chronic atrophic gastritis. *PLoS One* 2013; **8**: e76462 [PMID: 24098507 DOI: 10.1371/journal.pone.0076462]

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## Prognostication and response assessment in liver and pancreatic tumors: The new imaging

Riccardo De Robertis, Paolo Tinazzi Martini, Emanuele Demozzi, Gino Puntel, Silvia Ortolani, Sara Cingarlini, Andrea Ruzzenente, Alfredo Guglielmi, Giampaolo Tortora, Claudio Bassi, Paolo Pederzoli, Mirko D'Onofrio

Riccardo De Robertis, Emanuele Demozzi, Gino Puntel, Mirko D'Onofrio, Department of Radiology, Verona Comprehensive Cancer Network, G.B. Rossi Hospital, University of Verona, 37134 Verona, Italy

Paolo Tinazzi Martini, Department of Radiology, Casa di Cura Pederzoli, 37019 Peschiera del Garda, Italy

Silvia Ortolani, Sara Cingarlini, Giampaolo Tortora, Department of Oncology, Verona Comprehensive Cancer Network, G.B. Rossi Hospital, University of Verona, 37134 Verona, Italy

Andrea Ruzzenente, Alfredo Guglielmi, Department of Hepato-Biliary Surgery, Verona Comprehensive Cancer Network, G.B. Rossi Hospital, University of Verona, 37134 Verona, Italy

Claudio Bassi, Department of Pancreatic Surgery, Verona Comprehensive Cancer Network, G.B. Rossi Hospital, University of Verona, 37134 Verona, Italy

Paolo Pederzoli, Department of Pancreatic Surgery, Casa di Cura Pederzoli, 37019 Peschiera del Garda, Italy

**Author contributions:** All authors contributed equally to this work; Tinazzi Martini P, Ruzzenente A, Guglielmi A, Cingarlini S, Tortora G, Bassi C, Pederzoli P and D'Onofrio M designed the research; Demozzi E, Puntel G and Ortolani S performed the research and analyzed the data; and De Robertis R, Demozzi E and Puntel G wrote the paper.

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**Correspondence to:** Riccardo De Robertis, MD, Department of Radiology, Verona Comprehensive Cancer Network, G.B. Rossi Hospital, University of Verona, Piazzale L.A. Scuro 10, 37134 Verona, Italy. [riccardo.derobertis@hotmail.it](mailto:riccardo.derobertis@hotmail.it)  
Telephone: +39-45-8124301  
Fax: +39-45-8027490

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### Abstract

Diffusion-weighted imaging (DWI), dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and perfusion computed tomography (CT) are technical improvements of morphologic imaging that can evaluate functional properties of hepato-bilio-pancreatic tumors during conventional MRI or CT examinations. Nevertheless, the term "functional imaging" is commonly used to describe molecular imaging techniques, as positron emission tomography (PET) CT/MRI, which still represent the most widely used methods for the evaluation of functional properties of solid neoplasms; unlike PET or single photon emission computed tomography, functional imaging techniques applied to conventional MRI/CT examinations do not require the administration of radiolabeled drugs or specific equipments. Moreover, DWI and DCE-MRI can be performed during the same session, thus providing a comprehensive "one-step" morphological and functional evaluation of hepato-bilio-pancreatic tumors. Literature data reveal that functional imaging techniques could be proposed for the evaluation of these tumors before treatment, given that they may improve staging and predict prognosis or clinical outcome. Microscopic changes within neoplastic tissues induced by treatments can be detected and quantified with functional imaging, therefore these techniques could be used also for post-treatment assessment, even at an early stage. The aim of this editorial is to describe possible applications of new functional imaging techniques apart from

molecular imaging to hepatic and pancreatic tumors through a review of up-to-date literature data, with a particular emphasis on pathological correlations, prognostic stratification and post-treatment monitoring.

**Key words:** Diffusion magnetic resonance imaging; Perfusion imaging; Hepatocellular carcinoma; Liver neoplasms; Pancreatic neoplasms

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**Core tip:** Diffusion-weighted imaging and perfusion imaging could add functional information to the morphological evaluation of hepatic and pancreatic tumors. Diffusion-weighted imaging findings seem to be correlated with pathological features and could predict the clinical outcome of hepatocellular carcinomas and pancreatic tumors, especially neuroendocrine neoplasms. Apparent diffusion coefficient quantification and perfusion techniques can be of value for the evaluation of response to ablative treatments, loco-regional therapies and anti-angiogenic therapies, even at an early stage.

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## INTRODUCTION

Functional imaging techniques include different methods that can detect or measure changes in metabolism, blood flow, and chemical composition. This group included both molecular imaging methods, as positron emission tomography (PET)-computed tomography (CT)/magnetic resonance imaging (MRI) or single photon emission computed tomography (SPECT), and radiological techniques, as diffusion-weighted imaging (DWI), dynamic contrast-enhanced MRI (DCE-MRI) and perfusion CT (pCT). Functional imaging techniques are technical improvements of conventional morphological techniques that can provide both qualitative and quantitative information on hepato-bilio-pancreatic tumors<sup>[1-3]</sup>, being therefore similar to molecular imaging techniques. Functional techniques can be performed during conventional imaging evaluations as CT or MRI, therefore they describe both morphological and functional features of solid tumors; moreover, they do not need radiolabeled agents as fluorodeoxyglucose (<sup>18</sup>F-FDG) or specific equipments.

DWI evaluate the random diffusion of water molecules: biological tissues with high cellular density

or altered cellular membranes will present diffusion restriction, which is depicted as signal hyperintensity areas on high *b*-value DW images and hypointensity on the apparent diffusion coefficient (ADC) maps; ADC measurement can also quantify water molecules' diffusion. As dedifferentiation or therapies may induce microscopic changes in neoplastic tissues that could modify water molecules' diffusion, DWI can distinguish between different degrees of malignancy and can be also proposed for post-treatment monitoring. Moreover, DWI can be performed in a single session with DCE-MRI, thus providing a comprehensive "one-step" morphological and functional evaluation of hepato-bilio-pancreatic tumors.

Perfusion imaging techniques evaluate changes in signal intensity (DCE-MRI) or density (pCT) after contrast medium injection, being therefore able to assess microvascularization through the evaluation of the dynamics of contrast medium distribution from vessels to the neoplastic tissue. Perfusion parameters are therefore theoretically good candidates for the evaluation of microscopic vascular differences between lesions with different pathological grade and for the assessment of treatment response, especially after chemoembolization or during treatments with anti-angiogenic drugs.

This editorial analyzes up-to-date literature data regarding the application of functional imaging techniques, apart from molecular imaging, to hepatic and pancreatic tumors, with particular emphasis on correlations to pathological features, prognostic stratification and therapeutic response assessment.

## FUNCTIONAL IMAGING TECHNIQUES: TECHNICAL BASES

In 1965 Stejskal and Tanner<sup>[4]</sup> developed a modified T2-weighted MR sequence for the detection of water molecules' diffusion. DWI enables the visualization of Brownian random motions of water molecules in the extracellular, intracellular, and intravascular spaces<sup>[5]</sup>. DWI provides information on tissue cellularity and integrity of cell membranes, since the degree of restriction to water diffusion in biological tissues is inversely correlated to these features<sup>[6-9]</sup>. Restricted diffusion is present in tissues with narrowed extracellular spaces as a consequence of a high cellular density, which increases the number of hydrophobic cellular membranes, whereas in cystic or necrotic lesions water diffusion is relatively "free"<sup>[10]</sup>. The *b* value is a technical parameter that regulates the sensitivity of this sequence to water molecules' diffusion. Generally, both low and high *b* values are used for DWI examination; nevertheless, the choice of the *b* value may vary from institution to institution. The intravoxel incoherent motion (IVIM) model is an advanced DWI technique developed by Le Bihan<sup>[11,12]</sup> that enables a separate quantitative assessment of all the microscopic



translational motions that contribute to DWI signal. In biological tissues, these motions are represented by the molecular diffusion of water, expressed by diffusion ( $D$ ) and pseudodiffusion ( $D^*$ ), and the perfusion effect caused by blood circulation in the capillary network (perfusion fraction -  $f$ ). IVIM, therefore, can evaluate perfusion features without the need of contrast medium injection. Multiple  $b$  values must be used for IVIM evaluation.

DCE-MRI was developed to assess myocardial and pulmonary blood flow. This technique requires the intravenous injection of a gadolinium-based contrast agent, followed by rapid serial signal intensity measurements while the contrast agent enters tumor arterioles, passes through capillary beds and washes out of the tumor. Technical improvements have shortened the acquisition time and have led to the development of three-dimensional sequences, which replaced single-section examinations: as a consequence, DCE-MRI can be applied to abdominal imaging. The contrast kinetics features assessed by DCE-MRI reflect tissue perfusion, the concentration-time curve in the arterial input vessel, the capillary surface area, the permeability and the volume of the extracellular extravascular space. As a consequence, several metrics can be derived from DCE-MRI evaluation: the volume transfer constant ( $K^{trans}$ ), the fractional volume of the extravascular-extracellular space ( $v_e$ ), the rate constant ( $K_{ep}$ , where  $K_{ep} = K^{trans}/v_e$ ), the fractional volume of the plasma space ( $v_p$ ), the area under the contrast agent concentration-time curve (AUC)<sup>[13]</sup>. In 1999, a consensus opinion agreed to standardize the terminology of DCE-MRI studies<sup>[13]</sup> and selected AUC<sub>60</sub> and  $K^{trans}$  as the preferred end points in clinical trials involving anti-angiogenic drugs<sup>[13,14]</sup>. Nevertheless, DCE-MRI end points can be tailored to the specific drug involved in the trial.

Perfusion CT has the same physical bases of DCE-MRI, as it is based on the evaluation of temporal changes in tissue density following intravenous administration of iodine contrast medium. By rapid sequential acquisitions during contrast medium passage, pCT allows the quantification of tissues' vascularity. Perfusion can be quantified using mathematical modeling techniques (mainly the compartmental and the deconvolution analysis) that use data derived both from the tissue and the vascular system<sup>[15-17]</sup>. The analytical methods and the acquisition protocols vary from institution to institution and between commercial vendors, leading to poor standardization. Many different metrics can be directly or indirectly derived from pCT studies: blood flow (BF), representing the flow rate through vasculature; blood volume (BV), representing the volume of flowing blood; mean transit time (MTT), representing the average time taken to travel from arteries to veins; perfusion, representing the flow rate through vasculature; permeability surface (PS), representing the total flux from plasma to interstitial space; peak enhancement image (PEI), representing

the maximum enhancement in a tissue region of interest; and time to peak (TTP), defined as the time from the arrival of the contrast medium in major arterial vessels to the peak enhancement). Other than poor standardization, another important drawback of pCT is the high radiation dose, even though technical improvements have recently led to the development of low-dose pCT examination protocols<sup>[18]</sup>.

## CORRELATION WITH PATHOLOGICAL FINDINGS AND PROGNOSTIC STRATIFICATION

### Primary liver tumors

The prognosis and management of hepatocellular carcinoma (HCC) depend on size, degree of dedifferentiation, presence of vascular invasion and intrahepatic metastases<sup>[19]</sup>. As advanced and poorly differentiated HCCs have a significantly worse prognosis than well and moderately differentiated lesions after surgical resection<sup>[20]</sup>, preoperative staging and prognostic prediction play an important role, eventually suggesting wider surgical clearance margins and closer post-treatment surveillance.

As the pathological grade of HCC depends on cellular and structural atypia<sup>[21]</sup>, increasing cellular density, nuclear-to-cytoplasmic ratio, and architectural complexity accompanying dedifferentiation may cause water diffusion restriction. DWI features can be assessed both with a visual (qualitative) and a quantitative analysis through ADC measurement. An *et al*<sup>[22]</sup> reported a linear correlation towards higher grades in HCCs showing diffusion restriction: the combination of absence of diffusion restriction (defined as no hyperintensity on high  $b$ -value DW images) and no arterial enhancement at conventional contrast-enhanced MRI in predicting well differentiated HCCs had a 100% positive predictive value. The multistep nature of HCC dedifferentiation probably necessitates a quantitative approach rather than a simple visual analysis. Details on the main published studies regarding ADC measurement and correlations with the pathological grade of HCCs are reported in Table 1. Overall, literature data show that HCC dedifferentiation tends to be associated with a decrease of the ADC value, despite differences between studies<sup>[23-29]</sup>. Apart from the direct correlation with the pathological grade, Nakanishi *et al*<sup>[29]</sup> found that ADC quantification might have a clinical prognostic value, being significantly lower in patients with early recurrence after surgery than in those without early recurrence. One important aspect dealing with ADC measurement is the choice of the region of interest (ROI), given that a "whole-tumor" ROI can be irrespective of lesion heterogeneity: as previously mentioned, necrotic areas have a relatively free diffusion and should be avoided during ROI placement because they may falsely increase the ADC value; ADC measurement should be therefore per-

**Table 1** Data derived from the main published studies that have tested correlations between apparent diffusion coefficient values and pathological grade of hepatocellular carcinomas

Study	Number of patients	b values (s/mm <sup>2</sup> )	mean ADC value ( $\times 10^{-3}$ mm <sup>2</sup> /s)
Nasu <i>et al</i> <sup>[23]</sup>	99	0, 500	1.45 (WD); 1.46 (MD); 1.36 (PD)
Piana <i>et al</i> <sup>[24]</sup>	99	0, 500	1.29 (WD); 1.22 (MD); 1.21 (PD)
Saito <i>et al</i> <sup>[25]</sup>	32	100, 800	1.25 (WD); 1.12 (MD); 1.13 (PD)
Muhi <i>et al</i> <sup>[26]</sup>	73	500, 800	0.91 (WD); 0.71 (MD); 0.68 (PD)
Nishie <i>et al</i> <sup>[27]</sup>	80	0, 500, 1000	1.21 (WD); 1.14 (MD); 0.76 (PD)
Heo <i>et al</i> <sup>[28]</sup>	27	0, 1000	1.20 (WD); 1.10 (MD); 0.90 (PD)
Nakanishi <i>et al</i> <sup>[29]</sup>	44	0, 50, 1000	1.29 (MD); 1.07 (PD)

ADC: Apparent diffusion coefficient; WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated.

formed only on solid areas showing diffusion restriction.

Studies regarding IVIM imaging reported interesting results. Woo *et al*<sup>[30]</sup> found that the D value (diffusion) quantification had significantly higher AUC than ADC measurement for the differentiation between high-grade and low-grade HCCs. Moreover, the percentage of arterial enhancement depicted at conventional contrast-enhanced MRI, which is directly linked to the degree of dedifferentiation of HCCs, was correlated with IVIM-derived *f* value (perfusion fraction).

As previously mentioned, the prognosis of patients with HCC depends also on other pathological features: DWI has been tested for the detection of malignant features of HCC, as vascular involvement or intra-hepatic metastases. It has been reported that ADC measurement has a high sensitivity and specificity (reaching up to 93.5% and 78.6%, respectively) for the prediction of microvascular involvement<sup>[31,32]</sup>. Portal vein involvement precludes most curative options<sup>[33]</sup>, but its diagnosis may be hampered by the presence of a non-neoplastic thrombus in a cirrhotic liver. Few and controversial papers have been published regarding the ability of DWI in distinguishing malignant from non-malignant thrombi: Catalano *et al*<sup>[34]</sup> reported that most neoplastic thrombi were isointense to the primary tumor on DWI, whereas all bland thrombi were hypointense; it must be noted that blood degradation products present variable T2 signal prolongation and water diffusivity, therefore false-positives may be encountered at DWI<sup>[35]</sup>. Satellite nodules are important determinants of patients' prognosis and influence the therapeutic approach. The high accuracy of DWI in detecting small HCCs, even smaller than 1 cm, may be assumed to be applicable to intrahepatic HCC metastases<sup>[36]</sup>.

Arterial blood supply tends to increase during hepatocarcinogenesis. Perfusion imaging techniques

would be ideal for the prediction of the pathological grade and clinical behavior of HCCs, but literature data at this regard are relatively poor. One single study<sup>[37]</sup> reported a significant negative correlation between the standardized uptake value (SUV) derived from <sup>18</sup>F-FDG PET/CT and K<sup>trans</sup> in advanced HCCs. Some more studies have been conducted with pCT: while Ippolito *et al*<sup>[38]</sup> did not report any significant correlation between pCT-derived parameters and pathological grade, Sahani *et al*<sup>[39]</sup> found that well-differentiated HCCs had significantly higher perfusion values than less differentiated lesions. Yang *et al*<sup>[40]</sup> reported that pCT could quantitatively assess the blood supply and particularly its distribution during hepatocarcinogenesis, with statistically significant correlations between BF, hepatic arterial perfusion (HAP) and microvascular density (MVD).

Few experiences, mainly focused to a qualitative visual assessment of DW images, have been reported regarding cholangiocarcinoma (CCC). Cui *et al*<sup>[41]</sup> found an inverse correlation between the pathological grade and ADC values; Park *et al*<sup>[42]</sup> reported that the addition of DWI to conventional sequences might improve the pre-operative assessment of hilar CCC, increasing the sensitivity for the evaluation of tumor extent along the bile ducts and liver invasion, thus improving T stage, a parameter that is directly related to prognosis.

### Pancreatic tumors

Although the prognosis of patients with pancreatic ductal adenocarcinomas (PDACs) is related to the pathological grade, treatment choice mainly relies on clinical stage. Surgical resection is the only curative treatment for this neoplasm, therefore the pre-operative prediction of the pathological grade may have a smaller importance for PDAC management as compared to other pancreatic tumors.

Some studies have tried to correlate DWI findings with the pathological grade, but results are controversial<sup>[43-45]</sup>. Details on the most relevant published studies are reported in Table 2. Overall, low-grade PDACs tend to present low ADC values<sup>[43-45]</sup>, but it still not clear which histological feature mainly contributes to diffusion restriction. Wang *et al*<sup>[43]</sup> and Muraoka *et al*<sup>[46]</sup> reported that tumors with limited glandular formation and dense fibrosis (*i.e.*, paucicellular tumors) had lower ADC values as compared to well-differentiated lesions characterized by neoplastic tubular structures; moreover, PDACs with dense fibrosis showed significantly lower ADC values than those with loose fibrosis. Fibrosis may be therefore the key factor contributing to diffusion restriction in PDACs, but these findings have not been confirmed by other studies: particularly, Klauss *et al*<sup>[47]</sup> reported that the difference between the IVIM-derived D value (diffusion) of moderate and severe fibrosis PDACs was significant, but the cellular complexes surrounded by fibrosis

**Table 2** Data derived from the main published studies that have correlated apparent diffusion coefficient quantification with the pathological grade of pancreatic ductal adenocarcinomas and neuroendocrine tumors

Study	n	b values (s/mm <sup>2</sup> )	Histotype	mean ADC value ( $\times 10^{-3}$ mm <sup>2</sup> /s)
Wang <i>et al</i> <sup>[43]</sup>	21	0, 500	PDAC	2.10 (WD-MD); 1.46 (PD)
Legrand <i>et al</i> <sup>[44]</sup>	22	Multiple <sup>1</sup>	PDAC	1.43 (WD); 1.94 (MD-PD)
Rosenkrantz <i>et al</i> <sup>[45]</sup>	30	0, 500	PDAC	1.78/1.75 (WD-MD); 1.69/1.62 (PD) <sup>2</sup>
Wang <i>et al</i> <sup>[58]</sup>	18	0, 50, 500	PanNET	1.75 (G1); 1.00 (G3)
Jang <i>et al</i> <sup>[59]</sup>	20	0, 800	PanNET	1.48 (G1-G2); 1.04 (G3)
Hwang <i>et al</i> <sup>[60]</sup>	44	Multiple <sup>3</sup>	PanNET	1.31 (G1); 1.08 (G2-G3)

<sup>1</sup>50, 200, 400, 600, 800 s/mm<sup>2</sup>; <sup>2</sup>Two readers; <sup>3</sup>0, 25, 50, 75, 100, 150, 200, 500, 800, 1000 s/mm<sup>2</sup>. n: Number of patients; ADC: Apparent diffusion coefficient; PDAC: Pancreatic ductal adenocarcinoma; WD: Well differentiated; PanNET: Pancreatic neuroendocrine tumor; MD: Moderately differentiated; PD: Poorly differentiated.

might provide more structural limitations than fibrosis alone. Legrand *et al*<sup>[44]</sup> reported that mean ADC values did not significantly differ between tumors having < 50% and those having > 50% of fibrotic stroma, or between tumors containing dense fibrosis and those containing loose fibrosis. Similarly, Rosenkrantz *et al*<sup>[45]</sup> reported no associations between ADC values and “adverse” pathological features as poor differentiation. Some authors have proposed a more practical role for functional imaging, testing correlations with clinical features as tumor stage or survival. Hayano *et al*<sup>[48]</sup> reported a significant negative correlation between ADC values, size and number of metastatic lymphnodes; PDACs with low ADC values presented also a high tendency to show portal system and extra-pancreatic nerve plexus invasion. The comparison of CT and DWI performed by Fukukura *et al*<sup>[49]</sup>, instead, reported that only CT findings might be associated with the clinical behavior of PDACs. Some studies focused on the application of DWI to the detection and characterization of liver metastases from PDAC and reported high sensitivity and specificity using DWI alone<sup>[50]</sup> or DWI plus other sequences<sup>[51-53]</sup>; imaging features derived from conventional MR sequences should be always taken into account because of the possible presence of DWI false-positives.

Well-differentiated PDACs have a higher micro-vascular density as compared to less differentiated tumors<sup>[54]</sup>; perfusion imaging should therefore theoretically be able to identify well-differentiated PDACs, which have better prognosis than poorly differentiated lesions. It has been reported that pCT-derived PEI and BV values could identify high grade PDACs with 100% specificity and 75% accuracy<sup>[55]</sup>. Ueno *et al*<sup>[56]</sup> reported that DCE-MRI might predict the survival of patients with advanced PDAC: all patients included in this study

showed transient decreases in signal intensity [signal ratio (SR): 6.9%-55.7%]; high SR (cut-off 22%) significantly correlated with higher disease stage and presence of nodal metastases; patients with high SR had significantly short overall survival.

Pancreatic neuroendocrine tumors (PanNETs) can be divided into well/moderately differentiated and poorly differentiated lesions and their mitotic rate based on the quantification of the mitotic index (Ki67%) can distinguish three categories: G1, with a Ki67  $\leq$  2%, G2 (Ki67 3%-20%), and G3 (Ki67 > 20%)<sup>[57]</sup>. Several treatment options, ranging from surgery to systemic therapy or loco-regional treatments, have been proposed for PanNETs according to their grade of differentiation. The histological grade plays therefore a key role in the clinical management of PanNETs; many studies have been conducted regarding the application of functional imaging techniques to PanNETs, apart from nuclear imaging techniques. Details regarding ADC measurements correlations with the grade of differentiation are reported in Table 2. Overall, it seems that ADC values are correlated with the Ki67 labeling index: G3 PanNETs tend to present lower mean ADC values compared to well-differentiated PanNETs<sup>[58-60]</sup>. As for PDACs, staging plays a fundamental for treatment planning and prognostication of PanNETs. In most cases, liver metastases from PanNETs are hypervascular; nevertheless, in some cases they can be iso- or hypovascular and therefore difficult to detect and correctly characterize using conventional imaging techniques. Moreover, heterogeneity of liver metastases has been reported<sup>[61]</sup>. DWI is a good functional technique for the detection of PanNET liver metastases, with 71% sensitivity and 85%-100% specificity, equal or even higher than T2-weighted and contrast-enhanced images<sup>[62,63]</sup>. In a study that evaluated the role of DWI in the differentiation of hemangiomas from other hypervascular liver lesions, the mean ADC value of NETs metastases was found to be  $1.43 \times 10^{-3} \pm 0.39 \times 10^{-3}$  mm<sup>2</sup>/s<sup>[64]</sup>, slightly higher than that reported by Schmid-Tannwald ( $1.23 \times 10^{-3} \pm 0.31 \times 10^{-3}$  mm<sup>2</sup>/s)<sup>[65]</sup>. Nevertheless, MR features derived from conventional sequences should be always taken into account, due to a wide overlap in ADC values among different liver lesions. DWI can also obtain images from the entire body in one single acquisition (whole-body diffusion-weighted imaging - WBDWI). Cossetti *et al*<sup>[66]</sup> reported two cases of NETs with distant metastases (bone and mediastinal lymphnodes) discovered by WBDWI and confirmed by Octreoscan. Etchebehere *et al*<sup>[67]</sup> compared WBDWI with <sup>68</sup>Ga-DOTATATE PET-CT and <sup>99m</sup>Tc-HYNIC-Octreotide SPECT-CT: WBDWI had a similar accuracy when compared to molecular imaging techniques for lung and liver lesions, while showed a higher false-negative rate for bone lesions. Schraml *et al*<sup>[68]</sup> reported that PET-CT and WBDWI had comparable overall detection rates for NETs metastases but

significantly differed in organ-based detection rates with superiority of PET-CT for lymph node and pulmonary lesions and of WBDWI for liver and bone metastases.

Experimental applications of pCT to PanNETs reported interesting results. Rodallec *et al.*<sup>[69]</sup> and D'Assignies *et al.*<sup>[70]</sup> reported that pCT features were correlated with MVD; moreover, BF values of benign PanNETs were higher than those of uncertain behavior tumors and carcinomas, and significant correlations were reported between BF, MTT and proliferation index, microscopic vascular neoplastic involvement and presence of nodal or liver metastases. Regarding staging, Ng *et al.*<sup>[71]</sup> reported that BF and hepatic arterial fraction were significantly higher in liver metastases from PanNETs than in healthy liver, thus reflecting an increased arterial blood supply to metastatic lesions; opposite relationships were found for MTT and PS. Guyennon *et al.*<sup>[72]</sup> reported that pCT could provide additional information in respect to conventional CT; particularly, despite both hypervascular and hypovascular metastases presented higher hepatic arterial perfusion index as compared to the background liver, mean BF and BV values were higher in hyperdense metastases compared with hypodense lesions. All liver metastases showed higher BF, BV, PS and hepatic arterial perfusion index as compared to the background liver.

Functional parameters derived from pCT may therefore be useful for the characterization of suspect PanNET liver metastases when they present atypical morphological features, as hypovascularity.

Well- and poorly-differentiated PanNETs present different DCE-MRI features. Bali *et al.*<sup>[73]</sup> reported that a signal intensity - time curve similar to that of the aorta was typical of well-differentiated PanNETs, while a curve characterized by a slow enhancement was present in non well-differentiated PanNETs, but also in PDACs. Moreover, a positive correlation was observed between the MVD and the distribution factor, which reflects the volume fraction of the tissue that is accessible to the contrast agent (*i.e.*, the plasma and the extravascular extracellular space). Kim *et al.*<sup>[74]</sup> found a significant difference in the perfusion characteristics of well-differentiated PanNETs and neuroendocrine carcinomas:  $K^{trans}$  values, representing tissue blood flow, were significantly lower in G3 tumors. Interestingly, the mean  $K^{trans}$  of neuroendocrine carcinomas was higher than that of PDACs, thus reflecting the true histological features of PanNETs: even if poorly differentiated, they present higher MVD as compared to PDACs.

DCE-MRI has been tested for PanNETs staging. Koh *et al.*<sup>[75]</sup> found three different patterns of contrast enhancement for neuroendocrine hepatic metastases, with specific perfusional parameters. DCE-MRI is therefore potentially able to categorize metastases on the basis of their vascular characteristics, with prognostic and therapeutic consequences. Armbruster *et al.*<sup>[76]</sup> reported that arterial flow fraction and intracellular uptake fraction have a high diagnostic

accuracy for the distinction between NET liver metastases and normal hepatic tissue. DCE-MRI parameters are also partially correlated to SUVs derived from  $^{18}\text{F}$ -FDG- and  $^{68}\text{Ga}$ -DOTA-Tyr(3)-octreotate ( $^{68}\text{Ga}$ -DOTATATE-) PET/CT<sup>[77]</sup>.

## RESPONSE TO TREATMENTS

### Primary liver tumors

Loco-regional therapies as percutaneous or intra-operative ablation techniques [radiofrequency ablation (RFA), microwaves, irreversible electroporation or transarterial chemoembolization (TACE) and radioembolization (TARE)], have significantly contributed to the control of unresectable localized HCCs<sup>[78]</sup>. As these therapies may be repeated and interchangeably applied, early assessment of treatment response is crucial. Response Evaluation Criteria In Solid Tumors (RECIST) criteria are not applicable to HCC, as both loco-regional treatments and systemic therapy generally result in tumor necrosis rather than shrinkage. The European Association for the Study of Liver Diseases has proposed to assess response to loco-regional treatments by assessing the decrease in viable tumor volume, seen as a decrease in contrast-enhancing areas at conventional contrast-enhanced CT/MRI<sup>[79]</sup>. However, the differentiation of viable tissue from treatment-induced changes as inflammation or granulation tissue can be difficult, as these non-tumoral changes can present contrast enhancement<sup>[80,81]</sup>. DWI and perfusion imaging techniques may have a potential role in the differentiation of viable tumor from treatment-induced necrosis. Viable neoplastic areas present high cellularity with intact cell membranes and show high vascularization; conversely, treatment-induced necrotic and inflammatory changes present a reduced cellular density, an increased membrane permeability and poor vascularization.

Radiofrequency ablation (RFA) induces coagulative necrosis in tumor tissues. Lu *et al.*<sup>[82]</sup> reported that the ADC values of HCCs successfully treated with RFA showed a predictable evolution and might help radiologists to monitor tumor response, being significantly high starting from 1 mo after RFA. Ippolito *et al.*<sup>[83]</sup> reported that pCT enabled the assessment of HCC vascularity after RFA, providing quantitative information about the presence of arterial vessels within viable residual neoplastic tissues: in this study, a significant difference in perfusion, arterial perfusion (AP), and hepatic perfusion index (HPI) values was found between treated lesions with residual tumor and those successfully treated. Eccles *et al.*<sup>[84]</sup> reported statistically significant changes in ADC values of HCCs treated with radiotherapy (RT): in their study, the baseline median ADC of  $1.56 \times 10^{-3} \text{ mm}^2/\text{s}$  increased to  $1.89 \times 10^{-3} \text{ mm}^2/\text{s}$  at RT week one, to  $1.91 \times 10^{-3} \text{ mm}^2/\text{s}$  during week two and to  $2.01 \times 10^{-3} \text{ mm}^2/\text{s}$  one month following treatment; early increases of ADC values were correlated with sustained tumor response. Kim *et al.*<sup>[85]</sup>



**Table 3** Data derived from the main published studies that have evaluated apparent diffusion coefficient values before and after trans-arterial treatments of primary and metastatic liver tumors

Study	n	b values (s/mm <sup>2</sup> )	ADC before treatment (× 10 <sup>-3</sup> mm <sup>2</sup> /s)	ADC after treatment (× 10 <sup>-3</sup> mm <sup>2</sup> /s)	Histotype	Treatment
Kamel <i>et al</i> <sup>[86]</sup>	38	0, 500	1.51	1.70	HCC	TACE
Sahin <i>et al</i> <sup>[87]</sup>	74	0, 50, 400, 800	1.10	1.27	HCC	TACE
Kamel <i>et al</i> <sup>[88]</sup>	24	0, 50, 750	1.86	2.13	HCC	TACE
Chen <i>et al</i> <sup>[89]</sup>	20	0, 500	1.56	2.09	HCC	TACE
Yuan <i>et al</i> <sup>[90]</sup>	41	0, 500	2.22	1.42	HCC	TACE
Deng <i>et al</i> <sup>[98]</sup>	6	0, 500	1.30	2.23	HCC	TARE
Kamel <i>et al</i> <sup>[99]</sup>	13	0, 500	1.65	1.95	HCC	TARE
Rhee <i>et al</i> <sup>[100]</sup>	20	0, 500	1.64	1.82	HCC	TARE
Mannelli <i>et al</i> <sup>[102]</sup>	36	0, 50, 500	1.64	1.92	HCC	TACE
Kubota <i>et al</i> <sup>[103]</sup>	25	0, 500	1.271	1.357 <sup>1</sup> /1.222 <sup>2</sup>	HCC	TACE
Liapi <i>et al</i> <sup>[120]</sup>	26	0, 500	1.51	1.79	Metastases (PanNET)	TACE
Li <i>et al</i> <sup>[121]</sup>	26	0, 750	1.31	1.59	Metastases (PanNET)	TACE

<sup>1</sup>No disease relapse; <sup>2</sup>Disease relapse. ADC values are presented as means. n: Number of patients; ADC: Apparent diffusion coefficient; HCC: Hepatocellular carcinoma; PanNET: Pancreatic neuroendocrine tumor; TACE: Trans-arterial chemoembolization; TARE: Trans-arterial radioembolization.

reported that ADC values and DCE-MRI parameters acquired before concurrent chemoradiotherapy correlated with progression-free survival (PFS) and were valuable in the prediction of the clinical outcome. The best cutoff values for response prediction of ADC,  $K^{trans}$ ,  $K_{ep}$ , and extravascular extracellular volume fraction ( $v_e$ ) were  $1.008 \times 10^{-3}$  mm<sup>2</sup>/s, 0.108 /min, 0.570 min<sup>-1</sup>, and 0.298%, respectively.

Many studies have been conducted on the application of functional imaging techniques for HCCs treated with TACE and TARE; the most significant results are reported in Table 3. Overall, ADC values tend to increase after TACE, even at an early evaluation<sup>[86-89]</sup>. DWI can reliably assess the efficacy of trans-arterial treatments: Yuan *et al*<sup>[90]</sup> reported differences in the mean ADC values of the necrotic and vital tumor tissues after TACE ( $2.22 \times 10^{-3} \pm 0.31 \times 10^{-3}$  mm<sup>2</sup>/s and  $1.42 \times 10^{-3} \pm 0.25 \times 10^{-3}$  mm<sup>2</sup>/s, respectively); a significant linear correlation was identified between the ADC value of the entire area of the treated mass and the extent of tumor necrosis ( $r = 0.58$ ;  $P < 0.001$ ). Mannelli *et al*<sup>[91]</sup> did not report differences between conventional MRI sequences and DWI for the assessment of post-TACE necrosis, although enhancement decrease on MRI subtraction images was more significantly correlated with pathological findings than ADC increase. Although quantitative analysis of diffusion restriction appears to be of value in assessing response to TACE, visual analysis seems to be less accurate: Goshima *et al*<sup>[92]</sup> reported that DW images were significantly less sensitive than contrast-enhanced images in detecting residual/recurrent tumor after TACE, and Yu *et al*<sup>[93]</sup> reported that the addition of DW images to contrast-enhanced images reduced specificity and diagnostic accuracy in detecting perilesional recurrence. Probably, the presence of treatment-induced granulation tissue is the cause of DWI false positives.

Regarding pCT, Yang *et al*<sup>[94]</sup> reported a significant decrease of the HAP, total liver perfusion (TLP), and

hepatic arterial perfusion index (HAPI) values 4 wk after TACE.

Braren *et al*<sup>[95]</sup> reported strong correlation between the extravascular extracellular volume fraction assessed with DCE-MRI and the percentage of residual tumor after TACE. Taouli *et al*<sup>[96]</sup> reported that untreated HCCs had higher arterial fraction and lower portal/venous hepatic blood flow values than chemoembolized HCCs.

Trans-arterial yttrium-90 (<sup>90</sup>Y) radioembolization (TARE) aims to deliver a high radiation dose to HCCs<sup>[97]</sup>. Although a small study reported a 60% increase in the mean ADC value after TARE<sup>[98]</sup>, other studies reported less conspicuous ADC increases (approximately 10%-20%)<sup>[99]</sup>. Rhee *et al*<sup>[100]</sup> reported that 1-mo response to TARE assessed with DWI significantly preceded size changes: the mean baseline ADC value ( $1.64 \times 10^{-3} \pm 0.30 \times 10^{-3}$  mm<sup>2</sup>/s) increased to  $1.81 \times 10^{-3} \pm 0.37 \times 10^{-3}$  mm<sup>2</sup>/s at 1 mo ( $P < 0.05$ ), and to  $1.82 \times 10^{-3} \pm 0.23 \times 10^{-3}$  mm<sup>2</sup>/s at 3 mo ( $P < 0.05$ ), while the mean tumor size did not significantly modify at 1 or 3 mo. Functional imaging techniques may be helpful for response prediction to trans-arterial treatments. Park *et al*<sup>[101]</sup> reported that IVIM imaging could predict lipiodol uptake: the D\* (pseudodiffusion) value was significantly higher in a "lipiodol-good uptake" HCC group than in a "lipiodol-poor uptake" group. Mannelli *et al*<sup>[102]</sup> reported that ADC quantification could predict response to TACE: HCCs with poor/incomplete response (< 50% necrosis) had significantly lower pre- and post-TACE ADC values than lesions with good/complete response. Kubota *et al*<sup>[103]</sup> reported that the percent ADC value modification after therapy was significantly higher in non-relapsed HCCs ( $85.2\% \pm 12.4\%$ ) as compared to lesions with disease relapse ( $8.0\% \pm 56.7\%$ ,  $P < 0.001$ ). Konstantinidis *et al*<sup>[104]</sup> reported that DCE-MRI could predict treatment outcome after hepatic arterial infusion (HAI) of floxuridine and dexamethasone (with or without bevacizumab) in advanced intra-hepatic

**Table 4** Data derived from the main published studies that have correlated functional radiological techniques with response to systemic therapies of hepatic and pancreatic tumors

Study	n	Technique	Imaging biomarker	Histotype	Treatment
Lewin <i>et al</i> <sup>[107]</sup>	12	IVIM	f increase	HCC	sorafenib
Vouche <i>et al</i> <sup>[108]</sup>	15	DWI	ADC increase	HCC	<sup>90</sup> Y TARE ± sorafenib
Hsu <i>et al</i> <sup>[109]</sup>	31	DCE-MRI	K <sup>trans</sup> decrease	HCC	sorafenib+metronomic tegafur/uracil
Yopp <i>et al</i> <sup>[111]</sup>	17	DCE-MRI	AUC <sub>90</sub> /AUC <sub>180</sub> /K <sup>trans</sup> decrease	HCC	bevacizumab
Jiang <i>et al</i> <sup>[112]</sup>	23	pCT	BF/BV/PS decrease	HCC	bevacizumab + cytotoxic agents
Kim <i>et al</i> <sup>[113]</sup>	10	DCE-MRI/DWI	MTT increase K <sup>trans</sup> /K <sub>ep</sub> decrease	HCC	sunitinib
Sahani <i>et al</i> <sup>[114]</sup>	23	DCE-MRI/DWI	ADC increase K <sup>trans</sup> /K <sub>ep</sub> decrease	HCC	sunitinib
Kim <i>et al</i> <sup>[123]</sup>	35	pCT	ADC decrease	CRC metastases	XELOX, FOLFOX, FOLFIRI
Schlemmer <i>et al</i> <sup>[124]</sup>	24	pCT	BF decrease	PanNET metastases	Tyrosine-kinase inhibitors
Anzidei <i>et al</i> <sup>[125]</sup>	18	pCT, DWI	Perfusion decrease CP decrease	CRC metastases	Oxaliplatinum, capecitabine, bevacizumab
De Bruyne <i>et al</i> <sup>[127]</sup>	19	DCE-MRI	ADC increase	CRC metastases	Bevacizumab
Vriens <i>et al</i> <sup>[129]</sup>	23	DCE-MRI	AUC decrease	CRC metastases	Cytotoxic therapy
Coenegrachts <i>et al</i> <sup>[130]</sup>	10	DCE-MRI	K <sup>trans</sup> decrease	CRC metastases	Bevacizumab + FOLFIRI
Deckers <i>et al</i> <sup>[126]</sup>	20	DWI	K <sub>ep</sub> increase	CRC metastases	Chemotherapy
Niwa <i>et al</i> <sup>[133]</sup>	63	DWI	ADC decrease	CRC metastases	Gemcitabine
Cuneo <i>et al</i> <sup>[134]</sup>	12	DWI	ADC decrease	PDAC	Chemoradiation
Yao <i>et al</i> <sup>[135]</sup>	39	pCT	ADC increase	PDAC	Bevacizumab ± everolimus
Miyazaki <i>et al</i> <sup>[132]</sup>	20	DCE-MRI	BF decrease	PanNET	<sup>90</sup> Y-octreotide
			Distribution volume increase	PanNET metastases	

n: Number of patients; IVIM: Intravoxel incoherent-motion diffusion-weighted imaging; f: Perfusion fraction; HCC: Hepatocellular carcinoma; <sup>90</sup>Y TARE: <sup>90</sup>Yttrium trans-arterial radioembolization; DWI: Diffusion-weighted imaging; ADC: Apparent diffusion coefficient; DCE-MRI: Dynamic contrast-enhanced MRI; K<sup>trans</sup>: Volume transfer constant; AUC<sub>90</sub>, AUC<sub>180</sub>: Area under the curve at 90 and 180 s; pCT: Perfusion computed tomography; BF: Blood flow; BV: Blood volume; PS: Permeability surface; MTT: Mean transit time; K<sub>ep</sub>: Rate constant; CP: Capillary permeability.

CCCs: AUC<sub>90</sub> and AUC<sub>180</sub> were significantly higher in ≥ 3-year survivors than < 3-year survivors.

The advent of anti-angiogenic therapies, including sorafenib and bevacizumab, greatly expanded treatment options for HCCs. As anti-angiogenic drugs frequently do not induce tumor shrinkage but acts on tumor vascularization, functional imaging techniques may be suitable for the evaluation of patients treated with these agents. Details regarding the most relevant studies on treatment assessment by means of functional radiological techniques are reported in Table 4.

Sorafenib, an oral multikinase inhibitor that suppresses tumor cell proliferation and angiogenesis, is so far the only drug that has shown overall survival benefit in patients with advanced HCC<sup>[105]</sup> and represents the standard systemic therapy for patients with advanced (unresectable and/or metastatic) HCCs with well-preserved liver function and for intermediate-stage HCCs with disease progression after local treatments<sup>[106]</sup>. As sorafenib inhibits neovascularization and decreases tumor vascularity, perfusion parameters should decrease in responding patients. Nevertheless, literature data are controversial. Lewin *et al*<sup>[107]</sup> reported a significant f (perfusion fraction) increase in responders at 2 wk and at 2 mo of sorafenib therapy, whereas a decrease was noted in non-responders at the same time intervals. Vouche *et al*<sup>[108]</sup> reported that ADC values did not change 1 and 3 mo after <sup>90</sup>Y TARE or <sup>90</sup>Y TARE plus sorafenib treatments. These results may be explained by the pleiotropic anti-angiogenic

actions of sorafenib, which destroys tumor vessels and improves the integrity of basement membranes of the remaining microvessels, thus leading to less “water leakage” from the perfusion pool. Modifications during sorafenib treatment can be assessed also using perfusion-imaging techniques. Hsu *et al*<sup>[109]</sup> found good correlations between K<sup>trans</sup> values and survival in patients who received sorafenib plus metronomic tegafur/uracil therapy: baseline K<sup>trans</sup> was higher in patients with RECIST partial response (PR) or stable disease (SD) than in those with progressive disease (PD). Frampas *et al*<sup>[110]</sup> reported a non-significant decrease in all pCT-derived values between RECIST non-progressors and progressors treated with sorafenib.

Although not routinely used in clinical practice, other anti-angiogenic therapies are on study for HCCs, including bevacizumab (a monoclonal antibody directed against vascular endothelial growth factor - VEGF) and sunitinib (an oral multikinase inhibitor with VEGF-receptor as one of its targets). Yopp *et al*<sup>[111]</sup> reported a significant decrease of AUC<sub>90</sub>, AUC<sub>180</sub>, and K<sup>trans</sup> in HCCs treated with bevacizumab; time to progression inversely correlated with AUC<sub>90</sub> and AUC<sub>180</sub> changes ( $P < 0.05$  and  $P < 0.001$ ). In one study focused on locally advanced HCCs receiving bevacizumab and cytotoxic therapy, high pretreatment K<sup>trans</sup> identified patients with RECIST response to therapy<sup>[112]</sup>. Sunitinib seems to induce K<sup>trans</sup> and K<sub>ep</sub> decrease and ADC increase<sup>[113,114]</sup>; these modifications can be assessed even at a very early stage (after 2 wk of treatment).

Moreover, patients with larger  $K^{\text{trans}}$  and  $K_{\text{ep}}$  decrease might have a favorable clinical outcome; high baseline  $K^{\text{trans}}$  and large decreases of the extracellular volume fraction were correlated with longer PFS. No significant changes at DCE-MRI have been reported after vandetanib treatment<sup>[115,116]</sup>.

### Liver metastases

Lu *et al.*<sup>[82]</sup> reported that metastatic liver lesions successfully treated by RFA showed a predictable evolution of ADC values, with an up-and-down variation during follow-up. Szurowska *et al.*<sup>[117]</sup> reported that low pre-treatment ADC values could predict complete response of colorectal adenocarcinoma (CRC) liver metastases treated with RFA. Meijerink *et al.*<sup>[118]</sup> reported that pCT-derived BF distribution fully paralleled PET/CT images in showing either the absence or presence of local recurrence after RFA: high hepatic arterial perfusion ( $> 50$  mL/min per 100 g) and low portal venous perfusion ( $< 10$  mL/min per 100 g) areas represented viable neoplastic tissue. Marugami *et al.*<sup>[119]</sup> reported that ADC quantification might be helpful for the early detection of response in CRC liver metastases treated with HAI chemotherapy with 5-fluorouracil: ADC increase was significantly greater in responders than in non-responders.

Chemoembolization induces an increase of ADC values in PanNET liver metastases<sup>[120-122]</sup>; response to TACE can be assessed even at an early stage, starting from three weeks after treatment<sup>[122]</sup>. Details on the main published studies regarding functional imaging applications after trans-arterial treatments are reported in Table 3. Functional radiological techniques have been tested for follow-up evaluations during systemic treatment of liver metastases; details are reported in Table 4. Kim *et al.*<sup>[123]</sup> reported that pCT-derived BF and flow extraction product (FEP) could be used as early response predictors in patients with liver metastases from CRC, being both significantly different between responders and non-responders to XELOX, FOLFOX or FOLFIRI chemotherapy regimens.

Schlemmer *et al.*<sup>[124]</sup> reported that metastatic NETs with good response to tyrosine kinase inhibitors showed a significant tendency towards lower perfusion values assessed by pCT as compared to poor responders. Anzidei *et al.*<sup>[125]</sup> reported that both pCT and DWI could detect therapy-induced (oxaliplatin, capecitabine and bevacizumab) modifications in CRC liver metastases vascularization before significant size changes became evident: capillary permeability was significantly higher in lesions with complete and partial response; moreover, ADC values were significantly higher in partial response lesions than in patients with stable disease. Deckers *et al.*<sup>[126]</sup> reported that the increase of ADC values in responding liver metastases could occur even within days after the start of chemotherapy; unfortunately, as these changes were of smaller magnitude than the variability of ADC

measurement, ADC quantification was not reliable enough to predict final response at such an early time point in individual lesions.

Many studies have been conducted with DCE-MRI, probably as a consequence of the standardization of DCE-MRI-derived endpoints. De Bruyne *et al.*<sup>[127]</sup> reported that bevacizumab therapy could decrease DCE-MRI-derived AUC in patients with CRC liver metastases. Vriens *et al.*<sup>[128]</sup> reported a large reduction in DCE-MRI-derived perfusion parameters and glucose metabolic rate at  $^{18}\text{F}$ -FDG PET/CT in CRC metastases treated with bevacizumab. The same author<sup>[129]</sup> reported also that cytotoxic chemotherapy did not alter DCE-MRI-derived properties of tumor vasculature. Coenegrachts *et al.*<sup>[130]</sup> reported that a decrease of  $K_{\text{ep}}$  allowed early identification of response after 6 wk of FOLFIRI and bevacizumab treatment. O'Connor *et al.*<sup>[131]</sup> reported that the variance of CRC liver metastases shrinkage after bevacizumab and FOLFOX-6 treatment was mainly explained by the median values of  $v_e$ , tumor enhancing fraction (EF), and microvascular uniformity. Miyazaki *et al.*<sup>[132]</sup> reported that DCE-MRI-derived liver distribution volume and tumor distribution volume were significantly increased in liver metastases with good response to radiolabeled octreotide; low pretreatment values of liver distribution volume and high tumor arterial flow fraction were associated with better response.

### Pancreatic tumors

DWI has been tested for treatment response evaluation of pancreatic tumors: therapy seems to increase ADC values. Niwa *et al.*<sup>[133]</sup> reported ADC differences among patients with advanced pancreatic cancer treated with gemcitabine: in particular, significant differences between patients with progressive disease and those with stable disease were found at 3- and 6-mo follow-up. Tumor progression rate was significantly higher in patients with low ADC values than in those with higher values. Cuneo *et al.*<sup>[134]</sup> reported a significant correlation between pre-treatment mean ADC values of surgically resected PDACs and the amount of tumor cell destruction after chemoradiation evaluated on surgical specimens, with a Pearson correlation coefficient of 0.94 ( $P = 0.001$ ): the mean pre-treatment ADC value was  $1.61 \times 10^{-3} \text{ mm}^2/\text{s}$  in responding patients ( $> 90\%$  tumor cell destruction) compared to  $1.25 \times 10^{-3} \text{ mm}^2/\text{s}$  in non-responding patients ( $> 10\%$  viable tumor).

Regarding PanNETs, Yao *et al.*<sup>[135]</sup> reported that bevacizumab was associated with a 44% decrease in BF in patients with low-to intermediate grade tumors; the addition of everolimus induced a further 29% BF decrease. Everolimus alone was associated with 13% increase in MTT. Pretreatment PS ( $P = 0.009$ ), post-treatment MTT ( $P = 0.003$ ), percent reduction in BF ( $P = 0.03$ ), and percent reduction in BV ( $P = 0.002$ ) were associated with high percent reduction in tumor diameters. Such perfusion changes occurred early

after treatment start and might be used as functional biomarkers of response to bevacizumab or everolimus treatment.

## CONCLUSION

Literature data reveal that DWI can provide prognostic stratification of HCCs and PDACs, as DWI findings may reflect “adverse” pathological features associated with poor clinical outcome and prognosis. ADC values are generally low in poorly differentiated lesions, although different results have been reported regarding the direct correlation of ADC values and the pathological grade. Perfusion imaging techniques can theoretically depict microvascular changes related to dedifferentiation of HCCs and PDACs, but poor and controversial results have been reported.

Overall, it seems that functional radiological techniques find their most important applications in PanNETs: both DWI and perfusion imaging methods provide indirect information on their clinical behavior and improve their staging.

Functional imaging techniques can predict treatment outcome and assess response of primary and metastatic hepatic tumors to loco-regional therapies, particularly TACE: ADC increase seems to be associated with good clinical outcome.

Perfusion imaging can be of value in the post-treatment assessment of patients treated with tyrosine kinases inhibitors: DCE-MRI and pCT can distinguish responders from non-responders using DCE-imaging.

Functional radiological techniques are therefore reliable and useful to evaluate patients with hepatic and pancreatic tumors; these “new imaging” techniques could be therefore considered and -whenever possible- adopted as a part of CT/MRI examination protocols.

## REFERENCES

- 1 **Lim KS.** Diffusion-weighted MRI of hepatocellular carcinoma in cirrhosis. *Clin Radiol* 2014; **69**: 1-10 [PMID: 24034549 DOI: 10.1016/j.crad.2013.07.022]
- 2 **Wang Y,** Miller FH, Chen ZE, Merrick L, Mortelet KJ, Hoff FL, Hammond NA, Yaghami V, Nikolaidis P. Diffusion-weighted MR imaging of solid and cystic lesions of the pancreas. *Radiographics* 2011; **31**: E47-E64 [PMID: 21721197 DOI: 10.1148/rg.313105174]
- 3 **Pandharipande PV,** Krinsky GA, Rusinek H, Lee VS. Perfusion imaging of the liver: current challenges and future goals. *Radiology* 2005; **234**: 661-673 [PMID: 15734925 DOI: 10.1148/radiol.2343031362]
- 4 **Stejskal EO,** Tanner JE. Spin Diffusion Measurements: Spin Echoes in the Presence of a Time Dependent Field Gradient. *J Chem Phys* 1965; **42**: 288 [DOI: 10.1063/1.1695690]
- 5 **Koh DM,** Collins DJ. Diffusion-weighted MRI in the body: applications and challenges in oncology. *AJR Am J Roentgenol* 2007; **188**: 1622-1635 [PMID: 17515386 DOI: 10.2214/AJR.06.1403]
- 6 **Guo Y,** Cai YQ, Cai ZL, Gao YG, An NY, Ma L, Mahankali S, Gao JH. Differentiation of clinically benign and malignant breast lesions using diffusion-weighted imaging. *J Magn Reson Imaging* 2002; **16**: 172-178 [PMID: 12203765 DOI: 10.1002/jmri.10140]
- 7 **Gauvain KM,** McKinsty RC, Mukherjee P, Perry A, Neil JJ, Kaufman BA, Hayashi RJ. Evaluating pediatric brain tumor cellularity with diffusion-tensor imaging. *AJR Am J Roentgenol* 2001; **177**: 449-454 [PMID: 11461881 DOI: 10.2214/ajr.177.2.1770449]
- 8 **Sugahara T,** Korogi Y, Kochi M, Ikushima I, Shigematu Y, Hirai T, Okuda T, Liang L, Ge Y, Komohara Y, Ushio Y, Takahashi M. Usefulness of diffusion-weighted MRI with echo-planar technique in the evaluation of cellularity in gliomas. *J Magn Reson Imaging* 1999; **9**: 53-60 [PMID: 10030650]
- 9 **Lang P,** Wendland MF, Saeed M, Gindele A, Rosenau W, Mathur A, Gooding CA, Genant HK. Osteogenic sarcoma: noninvasive in vivo assessment of tumor necrosis with diffusion-weighted MR imaging. *Radiology* 1998; **206**: 227-235 [PMID: 9423677 DOI: 10.1148/radiology.206.1.9423677]
- 10 **Taouli B,** Koh DM. Diffusion-weighted MR imaging of the liver. *Radiology* 2010; **254**: 47-66 [PMID: 20032142 DOI: 10.1148/radiol.09090021]
- 11 **Le Bihan D,** Breton E, Lallemand D, Grenier P, Cabanis E, Laval-Jeantet M. MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. *Radiology* 1986; **161**: 401-407 [PMID: 3763909 DOI: 10.1148/radiology.161.2.3763909]
- 12 **Le Bihan D,** Breton E, Lallemand D, Aubin ML, Vignaud J, Laval-Jeantet M. Separation of diffusion and perfusion in intravoxel incoherent motion MR imaging. *Radiology* 1988; **168**: 497-505 [PMID: 3393671 DOI: 10.1148/radiology.168.2.3393671]
- 13 **Tofts PS,** Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV, Larsson HB, Lee TY, Mayr NA, Parker GJ, Port RE, Taylor J, Weisskoff RM. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. *J Magn Reson Imaging* 1999; **10**: 223-232 [PMID: 10508281 DOI: 10.1002/(SICI)1522-2586(199909)10:3]
- 14 **Leach MO,** Brindle KM, Evelhoch JL, Griffiths JR, Horsman MR, Jackson A, Jayson GC, Judson IR, Knopp MV, Maxwell RJ, McIntyre D, Padhani AR, Price P, Rathbone R, Rustin GJ, Tofts PS, Tozer GM, Vennart W, Waterton JC, Williams SR, Workman P. The assessment of antiangiogenic and antivascular therapies in early-stage clinical trials using magnetic resonance imaging: issues and recommendations. *Br J Cancer* 2005; **92**: 1599-1610 [PMID: 15870830 DOI: 10.1038/sj.bjc.6602550]
- 15 **Miles KA.** Functional computed tomography in oncology. *Eur J Cancer* 2002; **38**: 2079-2084 [PMID: 12387833 DOI: 10.1016/S0959-8049(02)00386-6]
- 16 **Miles KA.** Perfusion CT for the assessment of tumour vascularity: which protocol? *Br J Radiol* 2003; **76** Spec No 1: S36-S42 [PMID: 15456712 DOI: 10.1259/bjr/18486642]
- 17 **Cao J,** Yang A, Long XY, Liu H, Cao JN, Li H. [CT hepatic volume measurement combined with CT perfusion imaging in evaluating the hepatic functional reserve]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2007; **32**: 422-426 [PMID: 17611318]
- 18 **Li HO,** Sun C, Xu ZD, Miao F, Zhang DJ, Chen JH, Li X, Wang XM, Liu C, Zhao B. Low-dose whole organ CT perfusion of the pancreas: preliminary study. *Abdom Imaging* 2014; **39**: 40-47 [PMID: 24258077 DOI: 10.1007/s00261-013-0045-1]
- 19 **Haratake J,** Takeda S, Kasai T, Nakano S, Tokui N. Predictable factors for estimating prognosis of patients after resection of hepatocellular carcinoma. *Cancer* 1993; **72**: 1178-1183 [PMID: 7687921 DOI: 10.1002/1097-0142(19930815)72:4<1178>]
- 20 **Lauwers GY,** Terris B, Balis UJ, Batts KP, Regimbeau JM, Chang Y, Graeme-Cook F, Yamabe H, Ikai I, Cleary KR, Fujita S, Flejou JF, Zuberberg LR, Nagorney DM, Belghiti J, Yamaoka Y, Vauthey JN. Prognostic histologic indicators of curatively resected hepatocellular carcinomas: a multi-institutional analysis of 425 patients with definition of a histologic prognostic index. *Am J Surg Pathol* 2002; **26**: 25-34 [PMID: 11756766 DOI: 10.1097/00000478-200201000-00003]
- 21 **Coleman WB.** Mechanisms of human hepatocarcinogenesis. *Curr Mol Med* 2003; **3**: 573-588 [PMID: 14527088 DOI: 10.2174/1566524033479546]
- 22 **An C,** Park MS, Jeon HM, Kim YE, Chung WS, Chung YE,



- Kim MJ, Kim KW. Prediction of the histopathological grade of hepatocellular carcinoma using qualitative diffusion-weighted, dynamic, and hepatobiliary phase MRI. *Eur Radiol* 2012; **22**: 1701-1708 [PMID: 22434421 DOI: 10.1007/s00330-012-2421-6]
- 23 **Nasu K**, Kuroki Y, Tsukamoto T, Nakajima H, Mori K, Minami M. Diffusion-weighted imaging of surgically resected hepatocellular carcinoma: imaging characteristics and relationship among signal intensity, apparent diffusion coefficient, and histopathologic grade. *AJR Am J Roentgenol* 2009; **193**: 438-444 [PMID: 19620441 DOI: 10.2214/AJR.08.1424]
  - 24 **Piana G**, Trinquart L, Meskine N, Barrau V, Beers BV, Vilgrain V. New MR imaging criteria with a diffusion-weighted sequence for the diagnosis of hepatocellular carcinoma in chronic liver diseases. *J Hepatol* 2011; **55**: 126-132 [PMID: 21145857 DOI: 10.1016/j.jhep.2010.10.023]
  - 25 **Saito K**, Moriyasu F, Sugimoto K, Nishio R, Saguchi T, Akata S, Tokuyue K. Histological grade of differentiation of hepatocellular carcinoma: comparison of the efficacy of diffusion-weighted MRI with T2-weighted imaging and angiography-assisted CT. *J Med Imaging Radiat Oncol* 2012; **56**: 261-269 [PMID: 22697322 DOI: 10.1111/j.1754-9485.2012.02374.x]
  - 26 **Muhi A**, Ichikawa T, Motosugi U, Sano K, Matsuda M, Kitamura T, Nakazawa T, Araki T. High-b-value diffusion-weighted MR imaging of hepatocellular lesions: estimation of grade of malignancy of hepatocellular carcinoma. *J Magn Reson Imaging* 2009; **30**: 1005-1011 [PMID: 19856432 DOI: 10.1002/jmri.21931]
  - 27 **Nishie A**, Tajima T, Asayama Y, Ishigami K, Kakiyama D, Nakayama T, Takayama Y, Okamoto D, Fujita N, Taketomi A, Yoshimitsu K, Honda H. Diagnostic performance of apparent diffusion coefficient for predicting histological grade of hepatocellular carcinoma. *Eur J Radiol* 2011; **80**: e29-e33 [PMID: 20619566 DOI: 10.1016/j.ejrad.2010.06.019]
  - 28 **Heo SH**, Jeong YY, Shin SS, Kim JW, Lim HS, Lee JH, Koh YS, Cho CK, Kang HK. Apparent diffusion coefficient value of diffusion-weighted imaging for hepatocellular carcinoma: correlation with the histologic differentiation and the expression of vascular endothelial growth factor. *Korean J Radiol* 2010; **11**: 295-303 [PMID: 20461183 DOI: 10.3348/kjr.2010.11.3.295]
  - 29 **Nakanishi M**, Chuma M, Hige S, Omatsu T, Yokoo H, Nakanishi K, Kamiyama T, Kubota K, Haga H, Matsuno Y, Onodera Y, Kato M, Asaka M. Relationship between diffusion-weighted magnetic resonance imaging and histological tumor grading of hepatocellular carcinoma. *Ann Surg Oncol* 2012; **19**: 1302-1309 [PMID: 21927976 DOI: 10.1245/s10434-011-2066-8]
  - 30 **Woo S**, Lee JM, Yoon JH, Joo I, Han JK, Choi BI. Intravoxel incoherent motion diffusion-weighted MR imaging of hepatocellular carcinoma: correlation with enhancement degree and histologic grade. *Radiology* 2014; **270**: 758-767 [PMID: 24475811 DOI: 10.1148/radiol.13130444]
  - 31 **Suh YJ**, Kim MJ, Choi JY, Park MS, Kim KW. Preoperative prediction of the microvascular invasion of hepatocellular carcinoma with diffusion-weighted imaging. *Liver Transpl* 2012; **18**: 1171-1178 [PMID: 22767394 DOI: 10.1002/lt.23502]
  - 32 **Xu P**, Zeng M, Liu K, Shan Y, Xu C, Lin J. Microvascular invasion in small hepatocellular carcinoma: is it predictable with preoperative diffusion-weighted imaging? *J Gastroenterol Hepatol* 2014; **29**: 330-336 [PMID: 24033853 DOI: 10.1111/jgh.12358]
  - 33 **Salem R**, Lewandowski R, Roberts C, Goin J, Thurston K, Abouljoud M, Courtney A. Use of Yttrium-90 glass microspheres (TheraSphere) for the treatment of unresectable hepatocellular carcinoma in patients with portal vein thrombosis. *J Vasc Interv Radiol* 2004; **15**: 335-345 [PMID: 15064336 DOI: 10.1097/01.RVI.0000123319.20705.92]
  - 34 **Catalano OA**, Choy G, Zhu A, Hahn PF, Sahani DV. Differentiation of malignant thrombus from bland thrombus of the portal vein in patients with hepatocellular carcinoma: application of diffusion-weighted MR imaging. *Radiology* 2010; **254**: 154-162 [PMID: 20032150 DOI: 10.1148/radiol.09090304]
  - 35 **Yu JS**, Chung JJ, Kim JH, Kim KW. Limited value of diffusion-weighted MR imaging for differentiating bland from malignant portal venous thrombi. *Radiology* 2010; **256**: 673-674; author reply 674 [PMID: 20656851 DOI: 10.1148/radiol.100277]
  - 36 **Yu JS**, Chung JJ, Kim JH, Cho ES, Kim DJ, Ahn JH, Kim KW. Detection of small intrahepatic metastases of hepatocellular carcinomas using diffusion-weighted imaging: comparison with conventional dynamic MRI. *Magn Reson Imaging* 2011; **29**: 985-992 [PMID: 21616624 DOI: 10.1016/j.mri.2011.04.010]
  - 37 **Ahn SJ**, Park MS, Kim KA, Park JY, Kim I, Kang WJ, Lee SK, Kim MJ. <sup>18</sup>F-FDG PET metabolic parameters and MRI perfusion and diffusion parameters in hepatocellular carcinoma: a preliminary study. *PLoS One* 2013; **8**: e71571 [PMID: 23940769 DOI: 10.1371/journal.pone.0071571]
  - 38 **Ippolito D**, Capraro C, Casiraghi A, Cestari C, Sironi S. Quantitative assessment of tumour associated neovascularisation in patients with liver cirrhosis and hepatocellular carcinoma: role of dynamic-CT perfusion imaging. *Eur Radiol* 2012; **22**: 803-811 [PMID: 22086560 DOI: 10.1007/s00330-011-2307-z]
  - 39 **Sahani DV**, Holalkere NS, Mueller PR, Zhu AX. Advanced hepatocellular carcinoma: CT perfusion of liver and tumor tissue-initial experience. *Radiology* 2007; **243**: 736-743 [PMID: 17517931 DOI: 10.1148/radiol.2433052020]
  - 40 **Yang HF**, Du Y, Ni JX, Zhou XP, Li JD, Zhang Q, Xu XX, Li Y. Perfusion computed tomography evaluation of angiogenesis in liver cancer. *Eur Radiol* 2010; **20**: 1424-1430 [PMID: 20179942 DOI: 10.1007/s00330-009-1693-y]
  - 41 **Cui XY**, Chen HW, Cai S, Bao J, Tang QF, Wu LY, Fang XM. Diffusion-weighted MR imaging for detection of extrahepatic cholangiocarcinoma. *Eur J Radiol* 2012; **81**: 2961-2965 [PMID: 22285604 DOI: 10.1016/j.ejrad.2011.12.040]
  - 42 **Park MJ**, Kim YK, Lim S, Rhim H, Lee WJ. Hilar cholangiocarcinoma: value of adding DW imaging to gadoxetic acid-enhanced MR imaging with MR cholangiopancreatography for preoperative evaluation. *Radiology* 2014; **270**: 768-776 [PMID: 24475800 DOI: 10.1148/radiol.13130009]
  - 43 **Wang Y**, Chen ZE, Nikolaidis P, McCarthy RJ, Merrick L, Sternick LA, Horowitz JM, Yaghamai V, Miller FH. Diffusion-weighted magnetic resonance imaging of pancreatic adenocarcinomas: association with histopathology and tumor grade. *J Magn Reson Imaging* 2011; **33**: 136-142 [DOI: 10.1002/jmri.22414]
  - 44 **Legrand L**, Duchatelle V, Molinié V, Boulay-Coletta I, Sibilleau E, Zins M. Pancreatic adenocarcinoma: MRI conspicuity and pathologic correlations. *Abdom Imaging* 2015; **40**: 85-94 [PMID: 25030776 DOI: 10.1007/s00261-014-0196-8]
  - 45 **Rosenkrantz AB**, Matza BW, Sabach A, Hajdu CH, Hindman N. Pancreatic cancer: lack of association between apparent diffusion coefficient values and adverse pathological features. *Clin Radiol* 2013; **68**: e191-e197 [PMID: 23312674 DOI: 10.1016/j.crad.2012.11.006]
  - 46 **Muraoka N**, Uematsu H, Kimura H, Imamura Y, Fujiwara Y, Murakami M, Yamaguchi A, Itoh H. Apparent diffusion coefficient in pancreatic cancer: characterization and histopathological correlations. *J Magn Reson Imaging* 2008; **27**: 1302-1308 [PMID: 18504750 DOI: 10.1002/jmri.21340]
  - 47 **Klauss M**, Lemke A, Grünberg K, Simon D, Re TJ, Wente MN, Laun FB, Kauczor HU, Delorme S, Grenacher L, Stieltjes B. Intravoxel incoherent motion MRI for the differentiation between mass forming chronic pancreatitis and pancreatic carcinoma. *Invest Radiol* 2011; **46**: 57-63 [PMID: 21139505 DOI: 10.1097/RLI.0b013e3181fb3bf2]
  - 48 **Hayano K**, Miura F, Amano H, Toyota N, Wada K, Kato K, Sano K, Takeshita K, Aoyagi T, Shuto K, Matsubara H, Asano T, Takada T. Correlation of apparent diffusion coefficient measured by diffusion-weighted MRI and clinicopathologic features in pancreatic cancer patients. *J Hepatobiliary Pancreat Sci* 2013; **20**: 243-248 [PMID: 22311389 DOI: 10.1007/s00534-011-0491-5]
  - 49 **Fukukura Y**, Takumi K, Higashi H, Shinichi H, Kamimura K, Yoneyama T, Tateyama A. Contrast-enhanced CT and diffusion-weighted MR imaging: performance as a prognostic factor in patients with pancreatic ductal adenocarcinoma. *Eur J Radiol* 2014; **83**: 612-619 [PMID: 24418286 DOI: 10.1016/j.ejrad.2013.12.016]

- 50 **Holzappel K**, Reiser-Erkan C, Fingerle AA, Erkan M, Eiber MJ, Rummeny EJ, Friess H, Kleeff J, Gaa J. Comparison of diffusion-weighted MR imaging and multidetector-row CT in the detection of liver metastases in patients operated for pancreatic cancer. *Abdom Imaging* 2011; **36**: 179-184 [PMID: 20563868 DOI: 10.1007/s00261-010-9633-5]
- 51 **Kim YK**, Kim CS, Han YM, Lee YH. Detection of liver malignancy with gadoteric acid-enhanced MRI: is addition of diffusion-weighted MRI beneficial? *Clin Radiol* 2011; **66**: 489-496 [PMID: 21367403 DOI: 10.1016/j.crad.2010.09.007]
- 52 **Kenis C**, Deckers F, De Foer B, Van Mieghem F, Van Laere S, Pouillon M. Diagnosis of liver metastases: can diffusion-weighted imaging (DWI) be used as a stand alone sequence? *Eur J Radiol* 2012; **81**: 1016-1023 [PMID: 21377305 DOI: 10.1016/j.ejrad.2011.02.019]
- 53 **Miller FH**, Hammond N, Siddiqi AJ, Shroff S, Khatri G, Wang Y, Merrick LB, Nikolaidis P. Utility of diffusion-weighted MRI in distinguishing benign and malignant hepatic lesions. *J Magn Reson Imaging* 2010; **32**: 138-147 [PMID: 20578020 DOI: 10.1002/jmri.22235]
- 54 **Barão A**, Ruiz-Sauri A, Valencia G, Gómez-Mateo Mdel C, Sabater L, Ferrandez A, Llobart-Bosch A. High microvessel density in pancreatic ductal adenocarcinoma is associated with high grade. *Virchows Arch* 2013; **462**: 541-546 [PMID: 23579431 DOI: 10.1007/s00428-013-1409-1]
- 55 **D'Onofrio M**, Gallotti A, Mantovani W, Crosara S, Manfrin E, Falconi M, Ventriglia A, Zamboni GA, Manfredi R, Pozzi Mucelli R. Perfusion CT can predict tumoral grading of pancreatic adenocarcinoma. *Eur J Radiol* 2013; **82**: 227-233 [PMID: 23127804 DOI: 10.1016/j.ejrad.2012.09.023]
- 56 **Ueno M**, Niwa T, Ohkawa S, Amano A, Masaki T, Miyakawa K, Yoshida T. The usefulness of perfusion-weighted magnetic resonance imaging in advanced pancreatic cancer. *Pancreas* 2009; **38**: 644-648 [PMID: 19546836 DOI: 10.1097/MPA.0b013e3181ac1b27]
- 57 **Bosman FT**, World Health Organization, International Agency for Research on Cancer. WHO classification of tumours of the digestive system. 4th ed. Lyon, France: IARC press, 2010
- 58 **Wang Y**, Chen ZE, Yaghami V, Nikolaidis P, McCarthy RJ, Merrick L, Miller FH. Diffusion-weighted MR imaging in pancreatic endocrine tumors correlated with histopathologic characteristics. *J Magn Reson Imaging* 2011; **33**: 1071-1079 [PMID: 21509863 DOI: 10.1002/jmri.22541]
- 59 **Jang KM**, Kim SH, Lee SJ, Choi D. The value of gadoteric acid-enhanced and diffusion-weighted MRI for prediction of grading of pancreatic neuroendocrine tumors. *Acta Radiol* 2014; **55**: 140-148 [PMID: 23897307 DOI: 10.1177/0284185113494982]
- 60 **Hwang EJ**, Lee JM, Yoon JH, Kim JH, Han JK, Choi BI, Lee KB, Jang JY, Kim SW, Nickel MD, Kiefer B. Intravoxel incoherent motion diffusion-weighted imaging of pancreatic neuroendocrine tumors: prediction of the histologic grade using pure diffusion coefficient and tumor size. *Invest Radiol* 2014; **49**: 396-402 [PMID: 24500090 DOI: 10.1097/RLI.0000000000000028]
- 61 **Couvelard A**, Deschamps L, Ravaud P, Baron G, Sauvanet A, Hentic O, Colnot N, Paradis V, Belghiti J, Bedossa P, Ruszniewski P. Heterogeneity of tumor prognostic markers: a reproducibility study applied to liver metastases of pancreatic endocrine tumors. *Mod Pathol* 2009; **22**: 273-281 [PMID: 18997736 DOI: 10.1038/modpathol.2008.177]
- 62 **d'Assignies G**, Fina P, Bruno O, Vullierme MP, Tubach F, Paradis V, Sauvanet A, Ruszniewski P, Vilgrain V. High sensitivity of diffusion-weighted MR imaging for the detection of liver metastases from neuroendocrine tumors: comparison with T2-weighted and dynamic gadolinium-enhanced MR imaging. *Radiology* 2013; **268**: 390-399 [PMID: 23533288 DOI: 10.1148/radiol.13121628]
- 63 **Singh N**, Telles S. High frequency yoga breathing can increase alveolar dead space. Comment to: Gastroesophageal reflux disease and pulmonary function: a potential role of the dead space extension, Damir Bonacin, Damir Fabijanić, Mislav Radić, Željko Puljiz, Gorana Trgo, Andre Bratanić, Izet Hozo, Jadranka Tocilj, Med Sci Monit, 2012; 18(5): CR271-275. *Med Sci Monit* 2012; **18**: LE5-L6; author reply LE5-L6; [PMID: 22739742 DOI: 10.12659/MSM.882719]
- 64 **Vossen JA**, Buijs M, Liapi E, Eng J, Bluemke DA, Kamel IR. Receiver operating characteristic analysis of diffusion-weighted magnetic resonance imaging in differentiating hepatic hemangioma from other hypervascular liver lesions. *J Comput Assist Tomogr* 2008; **32**: 750-756 [PMID: 18830105 DOI: 10.1097/RCT.0b013e31816a6823]
- 65 **Schmid-Tannwald C**, Thomas S, Ivancevic MK, Dahi F, Rist C, Sethi I, Oto A. Diffusion-weighted MRI of metastatic liver lesions: is there a difference between hypervascular and hypovascular metastases? *Acta Radiol* 2014; **55**: 515-523 [PMID: 23986455 DOI: 10.1177/0284185113501493]
- 66 **Cossetti RJ**, Bezerra RO, Gumz B, Telles A, Costa FP. Whole body diffusion for metastatic disease assessment in neuroendocrine carcinomas: comparison with OctreoScan® in two cases. *World J Surg Oncol* 2012; **10**: 82 [PMID: 22591909 DOI: 10.1186/1477-7819-10-82]
- 67 **Etchebehere EC**, de Oliveira Santos A, Gumz B, Vicente A, Hoff PG, Corradi G, Ichiki WA, de Almeida Filho JG, Cantoni S, Camargo EE, Costa FP. 68Ga-DOTATATE PET/CT, 99mTc-HYNIC-octreotide SPECT/CT, and whole-body MR imaging in detection of neuroendocrine tumors: a prospective trial. *J Nucl Med* 2014; **55**: 1598-1604 [PMID: 25168627 DOI: 10.2967/jnumed.114.144543]
- 68 **Schraml C**, Schwenzer NF, Sperling O, Aschoff P, Lichy MP, Müller M, Brendle C, Werner MK, Claussen CD, Pfannenbergl C. Staging of neuroendocrine tumours: comparison of [<sup>68</sup>Ga]DOTATOC multiphase PET/CT and whole-body MRI. *Cancer Imaging* 2013; **13**: 63-72 [PMID: 23466785 DOI: 10.1102/1470-7330.2013.0007]
- 69 **Rodallec M**, Vilgrain V, Couvelard A, Rufat P, O'Toole D, Barrau V, Sauvanet A, Ruszniewski P, Menu Y. Endocrine pancreatic tumours and helical CT: contrast enhancement is correlated with microvascular density, histoprognostic factors and survival. *Pancreatol* 2006; **6**: 77-85 [PMID: 16327283 DOI: 10.1159/000090026]
- 70 **d'Assignies G**, Couvelard A, Bahrami S, Vullierme MP, Hammel P, Hentic O, Sauvanet A, Bedossa P, Ruszniewski P, Vilgrain V. Pancreatic endocrine tumors: tumor blood flow assessed with perfusion CT reflects angiogenesis and correlates with prognostic factors. *Radiology* 2009; **250**: 407-416 [PMID: 19095784 DOI: 10.1148/radiol.2501080291]
- 71 **Ng CS**, Hobbs BP, Chandler AG, Anderson EF, Herron DH, Charnsangavej C, Yao J. Metastases to the liver from neuroendocrine tumors: effect of duration of scan acquisition on CT perfusion values. *Radiology* 2013; **269**: 758-767 [PMID: 23824990 DOI: 10.1148/radiol.13122708]
- 72 **Guyennon A**, Mihaila M, Palma J, Lombard-Bohas C, Chayvialle JA, Pilleul F. Perfusion characterization of liver metastases from endocrine tumors: Computed tomography perfusion. *World J Radiol* 2010; **2**: 449-454 [PMID: 21179313 DOI: 10.4329/wjr.v2.i11.449]
- 73 **Bali MA**, Metens T, Denolin V, Delhaye M, Demetter P, Closset J, Matos C. Tumoral and nontumoral pancreas: correlation between quantitative dynamic contrast-enhanced MR imaging and histopathologic parameters. *Radiology* 2011; **261**: 456-466 [PMID: 21852570 DOI: 10.1148/radiol.11103515]
- 74 **Kim JH**, Lee JM, Park JH, Kim SC, Joo I, Han JK, Choi BI. Solid pancreatic lesions: characterization by using timing bolus dynamic contrast-enhanced MR imaging assessment—a preliminary study. *Radiology* 2013; **266**: 185-196 [PMID: 23192779 DOI: 10.1148/radiol.12120111]
- 75 **Koh TS**, Thng CH, Hartono S, Kwek JW, Khoo JB, Miyazaki K, Collins DJ, Orton MR, Leach MO, Lewington V, Koh DM. Dynamic contrast-enhanced MRI of neuroendocrine hepatic metastases: A feasibility study using a dual-input two-compartment model. *Magn Reson Med* 2011; **65**: 250-260 [PMID: 20860001 DOI: 10.1002/mrm.22596]
- 76 **Armbruster M**, Zech CJ, Sourbron S, Ceelen F, Auernhammer CJ, Rist C, Haug A, Singnurkar A, Reiser MF, Sommer WH.

- Diagnostic accuracy of dynamic gadoteric-acid-enhanced MRI and PET/CT compared in patients with liver metastases from neuroendocrine neoplasms. *J Magn Reson Imaging* 2014; **40**: 457-466 [PMID: 24347148 DOI: 10.1002/jmri.24363]
- 77 **Armbruster M**, Sourbron S, Haug A, Zech CJ, Ingris M, Auernhammer CJ, Nikolaou K, Paprottka PM, Rist C, Reiser MF, Sommer WH. Evaluation of neuroendocrine liver metastases: a comparison of dynamic contrast-enhanced magnetic resonance imaging and positron emission tomography/computed tomography. *Invest Radiol* 2014; **49**: 7-14 [PMID: 24002080 DOI: 10.1097/RLI.0b013e3182a4eb4a]
  - 78 **Ramsey DE**, Kernagis LY, Soulen MC, Geschwind JF. Chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2002; **13**: S211-S221 [PMID: 12354839 DOI: 10.1016/S1051-0443(07)61789-8]
  - 79 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]
  - 80 **Vossen JA**, Buijs M, Kamel IR. Assessment of tumor response on MR imaging after locoregional therapy. *Tech Vasc Interv Radiol* 2006; **9**: 125-132 [PMID: 17561215 DOI: 10.1053/j.tvir.2007.02.004]
  - 81 **Hwang SH**, Yu JS, Chung J, Chung JJ, Kim JH, Kim KW. Transient hepatic attenuation difference (THAD) following transcatheter arterial chemoembolization for hepatic malignancy: changes on serial CT examinations. *Eur Radiol* 2008; **18**: 1596-1603 [PMID: 18351345 DOI: 10.1007/s00330-008-0916-y]
  - 82 **Lu TL**, Becce F, Bize P, Denys A, Meuli R, Schmidt S. Assessment of liver tumor response by high-field (3 T) MRI after radiofrequency ablation: short- and mid-term evolution of diffusion parameters within the ablation zone. *Eur J Radiol* 2012; **81**: e944-e950 [PMID: 22817977 DOI: 10.1016/j.ejrad.2012.06.011]
  - 83 **Ippolito D**, Bonaffini PA, Capraro C, Leni D, Corso R, Sironi S. Viable residual tumor tissue after radiofrequency ablation treatment in hepatocellular carcinoma: evaluation with CT perfusion. *Abdom Imaging* 2013; **38**: 502-510 [PMID: 22743839 DOI: 10.1007/s00261-012-9924-0]
  - 84 **Eccles CL**, Haider EA, Haider MA, Fung S, Lockwood G, Dawson LA. Change in diffusion weighted MRI during liver cancer radiotherapy: preliminary observations. *Acta Oncol* 2009; **48**: 1034-1043 [PMID: 19634060 DOI: 10.1080/02841860903099972]
  - 85 **Kim KA**, Park MS, Ji HJ, Park JY, Han KH, Kim MJ, Kim KW. Diffusion and perfusion MRI prediction of progression-free survival in patients with hepatocellular carcinoma treated with concurrent chemoradiotherapy. *J Magn Reson Imaging* 2014; **39**: 286-292 [PMID: 24302545 DOI: 10.1002/jmri.24161]
  - 86 **Kamel IR**, Bluemke DA, Eng J, Liapi E, Messersmith W, Reyes DK, Geschwind JF. The role of functional MR imaging in the assessment of tumor response after chemoembolization in patients with hepatocellular carcinoma. *J Vasc Interv Radiol* 2006; **17**: 505-512 [PMID: 16567675 DOI: 10.1097/01.RVI.00002000-52.02183.92]
  - 87 **Sahin H**, Harman M, Cinar C, Bozkaya H, Parildar M, Elmas N. Evaluation of treatment response of chemoembolization in hepatocellular carcinoma with diffusion-weighted imaging on 3.0-T MR imaging. *J Vasc Interv Radiol* 2012; **23**: 241-247 [PMID: 22019180 DOI: 10.1016/j.jvir.2011.08.030]
  - 88 **Kamel IR**, Liapi E, Reyes DK, Zahurak M, Bluemke DA, Geschwind JF. Unresectable hepatocellular carcinoma: serial early vascular and cellular changes after transarterial chemoembolization as detected with MR imaging. *Radiology* 2009; **250**: 466-473 [PMID: 19188315 DOI: 10.1148/radiol.2502072222]
  - 89 **Chen CY**, Li CW, Kuo YT, Jaw TS, Wu DK, Jao JC, Hsu JS, Liu GC. Early response of hepatocellular carcinoma to transcatheter arterial chemoembolization: choline levels and MR diffusion constants--initial experience. *Radiology* 2006; **239**: 448-456 [PMID: 16569781 DOI: 10.1148/radiol.2392042202]
  - 90 **Yuan Z**, Li WT, Ye XD, Peng WJ, Xiao XS. Utility of diffusion-weighted imaging to assess hepatocellular carcinoma viability following transarterial chemoembolization. *Oncol Lett* 2014; **8**: 831-836 [PMID: 25013505 DOI: 10.3892/ol.2014.2228]
  - 91 **Mannelli L**, Kim S, Hajdu CH, Babb JS, Clark TW, Taouli B. Assessment of tumor necrosis of hepatocellular carcinoma after chemoembolization: diffusion-weighted and contrast-enhanced MRI with histopathologic correlation of the explanted liver. *AJR Am J Roentgenol* 2009; **193**: 1044-1052 [PMID: 19770328 DOI: 10.2214/AJR.08.1461]
  - 92 **Goshima S**, Kanematsu M, Kondo H, Yokoyama R, Kajita K, Tsuge Y, Watanabe H, Shiratori Y, Onozuka M, Moriyama N. Diffusion-weighted imaging of the liver: optimizing b value for the detection and characterization of benign and malignant hepatic lesions. *J Magn Reson Imaging* 2008; **28**: 691-697 [PMID: 18777553 DOI: 10.1002/jmri.21467]
  - 93 **Yu JS**, Kim JH, Chung JJ, Kim KW. Added value of diffusion-weighted imaging in the MRI assessment of perilesional tumor recurrence after chemoembolization of hepatocellular carcinomas. *J Magn Reson Imaging* 2009; **30**: 153-160 [PMID: 19557734 DOI: 10.1002/jmri.21818]
  - 94 **Yang L**, Zhang XM, Tan BX, Liu M, Dong GL, Zhai ZH. Computed tomographic perfusion imaging for the therapeutic response of chemoembolization for hepatocellular carcinoma. *J Comput Assist Tomogr* 2012; **36**: 226-230 [PMID: 22446364 DOI: 10.1097/RCT.0b013e318245c23c]
  - 95 **Braren R**, Altomonte J, Settles M, Neff F, Esposito I, Ebert O, Schwaiger M, Rummeny E, Steingoetter A. Validation of preclinical multiparametric imaging for prediction of necrosis in hepatocellular carcinoma after embolization. *J Hepatol* 2011; **55**: 1034-1040 [PMID: 21354233 DOI: 10.1016/j.jhep.2011.01.049]
  - 96 **Taouli B**, Johnson RS, Hajdu CH, Oei MT, Merad M, Yee H, Rusinek H. Hepatocellular carcinoma: perfusion quantification with dynamic contrast-enhanced MRI. *AJR Am J Roentgenol* 2013; **201**: 795-800 [PMID: 24059368 DOI: 10.2214/AJR.12.9798]
  - 97 **Goin JE**, Salem R, Carr BI, Dancy JE, Soulen MC, Geschwind JF, Goin K, Van Buskirk M, Thurston K. Treatment of unresectable hepatocellular carcinoma with intrahepatic yttrium 90 microspheres: factors associated with liver toxicities. *J Vasc Interv Radiol* 2005; **16**: 205-213 [PMID: 15713921 DOI: 10.1097/01.RVI.00001142592.89564.F9]
  - 98 **Deng J**, Miller FH, Rhee TK, Sato KT, Mulcahy MF, Kulik LM, Salem R, Omary RA, Larson AC. Diffusion-weighted MR imaging for determination of hepatocellular carcinoma response to yttrium-90 radioembolization. *J Vasc Interv Radiol* 2006; **17**: 1195-1200 [PMID: 16868174 DOI: 10.1097/01.RVI.0000227234.81718.EB]
  - 99 **Kamel IR**, Reyes DK, Liapi E, Bluemke DA, Geschwind JF. Functional MR imaging assessment of tumor response after 90Y microsphere treatment in patients with unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2007; **18**: 49-56 [PMID: 17296704 DOI: 10.1016/j.jvir.2006.10.005]
  - 100 **Rhee TK**, Naik NK, Deng J, Atassi B, Mulcahy MF, Kulik LM, Ryu RK, Miller FH, Larson AC, Salem R, Omary RA. Tumor response after yttrium-90 radioembolization for hepatocellular carcinoma: comparison of diffusion-weighted functional MR imaging with anatomic MR imaging. *J Vasc Interv Radiol* 2008; **19**: 1180-1186 [PMID: 18656011 DOI: 10.1016/j.jvir.2008.05.002]
  - 101 **Park YS**, Lee CH, Kim JH, Kim IS, Kiefer B, Seo TS, Kim KA, Park CM. Using intravoxel incoherent motion (IVIM) MR imaging to predict lipiodol uptake in patients with hepatocellular carcinoma following transcatheter arterial chemoembolization: a preliminary result. *Magn Reson Imaging* 2014; **32**: 638-646 [PMID: 24703575 DOI: 10.1016/j.mri.2014.03.003]
  - 102 **Mannelli L**, Kim S, Hajdu CH, Babb JS, Taouli B. Serial diffusion-weighted MRI in patients with hepatocellular carcinoma: Prediction and assessment of response to transarterial chemoembolization. Preliminary experience. *Eur J Radiol* 2013; **82**: 577-582 [PMID: 23246330 DOI: 10.1016/j.ejrad.2012.11.026]
  - 103 **Kubota K**, Yamanishi T, Itoh S, Murata Y, Miyatake K,



- Yasunami H, Morio K, Hamada N, Nishioka A, Ogawa Y. Role of diffusion-weighted imaging in evaluating therapeutic efficacy after transcatheter arterial chemoembolization for hepatocellular carcinoma. *Oncol Rep* 2010; **24**: 727-732 [PMID: 20664980 DOI: 10.3892/or.00000914]
- 104 **Konstantinidis IT**, Do RK, Gultekin DH, Gönen M, Schwartz LH, Fong Y, Allen PJ, D'Angelica MI, DeMatteo RP, Klimstra DS, Kemeny NE, Jarnagin WR. Regional chemotherapy for unresectable intrahepatic cholangiocarcinoma: a potential role for dynamic magnetic resonance imaging as an imaging biomarker and a survival update from two prospective clinical trials. *Ann Surg Oncol* 2014; **21**: 2675-2683 [PMID: 24664624 DOI: 10.1245/s10434-014-3649-y]
- 105 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
- 106 **Verslype C**, Rosmorduc O, Rougier P. Hepatocellular carcinoma: ESMO-ESDO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23** Suppl 7: vii41-vii48 [PMID: 22997453 DOI: 10.1093/annonc/mds225]
- 107 **Lewin M**, Fartoux L, Vignaud A, Arrivé L, Menu Y, Rosmorduc O. The diffusion-weighted imaging perfusion fraction *f* is a potential marker of sorafenib treatment in advanced hepatocellular carcinoma: a pilot study. *Eur Radiol* 2011; **21**: 281-290 [PMID: 20683597 DOI: 10.1007/s00330-010-1914-4]
- 108 **Vouche M**, Kulik L, Atassi R, Memon K, Hickey R, Ganger D, Miller FH, Yaghami V, Abecassis M, Baker T, Mulcahy M, Nayar R, Lewandowski RJ, Salem R. Radiological-pathological analysis of WHO, RECIST, EASL, mRECIST and DWI: Imaging analysis from a prospective randomized trial of Y90 ± sorafenib. *Hepatology* 2013; **58**: 1655-1666 [PMID: 23703789 DOI: 10.1002/hep.26487]
- 109 **Hsu CY**, Shen YC, Yu CW, Hsu C, Hu FC, Hsu CH, Chen BB, Wei SY, Cheng AL, Shih TT. Dynamic contrast-enhanced magnetic resonance imaging biomarkers predict survival and response in hepatocellular carcinoma patients treated with sorafenib and metronomic tegafur/uracil. *J Hepatol* 2011; **55**: 858-865 [PMID: 21338641 DOI: 10.1016/j.jhep.2011.01.032]
- 110 **Frampas E**, Lassau N, Zappa M, Vullierme MP, Koscielny S, Vilgrain V. Advanced Hepatocellular Carcinoma: early evaluation of response to targeted therapy and prognostic value of Perfusion CT and Dynamic Contrast Enhanced-Ultrasound. Preliminary results. *Eur J Radiol* 2013; **82**: e205-e211 [PMID: 23273822 DOI: 10.1016/j.ejrad.2012.12.004]
- 111 **Yopp AC**, Schwartz LH, Kemeny N, Gultekin DH, Gönen M, Bamboat Z, Shia J, Haviland D, D'Angelica MI, Fong Y, DeMatteo RP, Allen PJ, Jarnagin WR. Antiangiogenic therapy for primary liver cancer: correlation of changes in dynamic contrast-enhanced magnetic resonance imaging with tissue hypoxia markers and clinical response. *Ann Surg Oncol* 2011; **18**: 2192-2199 [PMID: 21286939 DOI: 10.1245/s10434-011-1570-1]
- 112 **Jiang T**, Kambadakone A, Kulkarni NM, Zhu AX, Sahani DV. Monitoring response to antiangiogenic treatment and predicting outcomes in advanced hepatocellular carcinoma using image biomarkers, CT perfusion, tumor density, and tumor size (RECIST). *Invest Radiol* 2012; **47**: 11-17 [PMID: 21512396 DOI: 10.1097/RLL.0b013e3182199bb5]
- 113 **Kim H**, Keene KS, Sarver DB, Lee SK, Beasley TM, Morgan DE, Posey JA. Quantitative perfusion- and diffusion-weighted magnetic resonance imaging of gastrointestinal cancers treated with multikinase inhibitors: a pilot study. *Gastrointest Cancer Res* 2014; **7**: 75-81 [PMID: 25276260]
- 114 **Sahani DV**, Jiang T, Hayano K, Duda DG, Catalano OA, Ancukiewicz M, Jain RK, Zhu AX. Magnetic resonance imaging biomarkers in hepatocellular carcinoma: association with response and circulating biomarkers after sunitinib therapy. *J Hematol Oncol* 2013; **6**: 51 [PMID: 23842041 DOI: 10.1186/1756-8722-6-51]
- 115 **Hsu C**, Yang TS, Huo TI, Hsieh RK, Yu CW, Hwang WS, Hsieh TY, Huang WT, Chao Y, Meng R, Cheng AL. Vandetanib in patients with inoperable hepatocellular carcinoma: a phase II, randomized, double-blind, placebo-controlled study. *J Hepatol* 2012; **56**: 1097-1103 [PMID: 22245891 DOI: 10.1016/j.jhep.2011.12.013]
- 116 **Mross K**, Fasol U, Frost A, Benkelmann R, Kuhlmann J, Büchert M, Unger C, Blum H, Hennig J, Milenkova TP, Tessier J, Krebs AD, Ryan AJ, Fischer R. DCE-MRI assessment of the effect of vandetanib on tumor vasculature in patients with advanced colorectal cancer and liver metastases: a randomized phase I study. *J Angiogenesis Res* 2009; **1**: 5 [PMID: 19946413 DOI: 10.1186/2040-2384-1-5]
- 117 **Szurowska E**, Nowicki TK, Izycka-Swieszevska E, Zadrozny D, Markiet K, Studniarek M. Predictive value of apparent diffusion coefficient in evaluation of colorectal carcinoma hepatic metastases' response to radiofrequency ablation. *J Magn Reson Imaging* 2013; **38**: 1027-1032 [PMID: 23526807 DOI: 10.1002/jmri.24089]
- 118 **Meijerink MR**, van Waesberghe JH, van der Weide L, van den Tol P, Meijer S, Comans EF, Golding RP, van Kuijk C. Early detection of local RFA site recurrence using total liver volume perfusion CT initial experience. *Acad Radiol* 2009; **16**: 1215-1222 [PMID: 19524457 DOI: 10.1016/j.acra.2009.03.023]
- 119 **Marugami N**, Tanaka T, Kitano S, Hirohashi S, Nishiofuku H, Takahashi A, Sakaguchi H, Matsuoka M, Otsuji T, Takahama J, Higashiura W, Kichikawa K. Early detection of therapeutic response to hepatic arterial infusion chemotherapy of liver metastases from colorectal cancer using diffusion-weighted MR imaging. *Cardiovasc Intervent Radiol* 2009; **32**: 638-646 [PMID: 19238482 DOI: 10.1007/s00270-009-9532-8]
- 120 **Liapi E**, Geschwind JF, Vossen JA, Buijs M, Georgiades CS, Blumke DA, Kamel IR. Functional MRI evaluation of tumor response in patients with neuroendocrine hepatic metastasis treated with transcatheter arterial chemoembolization. *AJR Am J Roentgenol* 2008; **190**: 67-73 [PMID: 18094295 DOI: 10.2214/AJR.07.2550]
- 121 **Li Z**, Bonekamp S, Halappa VG, Corona-Villalobos CP, Pawlik T, Bhagat N, Reyes D, Lai H, Geschwind JF, Kamel IR. Islet cell liver metastases: assessment of volumetric early response with functional MR imaging after transarterial chemoembolization. *Radiology* 2012; **264**: 97-109 [PMID: 22627602 DOI: 10.1148/radiol.12112161]
- 122 **Gowdra Halappa V**, Corona-Villalobos CP, Bonekamp S, Li Z, Reyes D, Cosgrove D, Pawlik TM, Diaz LA, Bhagat N, Eng J, Geschwind JF, Kamel IR. Neuroendocrine liver metastasis treated by using intraarterial therapy: volumetric functional imaging biomarkers of early tumor response and survival. *Radiology* 2013; **266**: 502-513 [PMID: 23192780 DOI: 10.1148/radiol.12120495]
- 123 **Kim DH**, Kim SH, Im SA, Han SW, Goo JM, Willmann JK, Lee ES, Eo JS, Paeng JC, Han JK, Choi BI. Intermodality comparison between 3D perfusion CT and <sup>18</sup>F-FDG PET/CT imaging for predicting early tumor response in patients with liver metastasis after chemotherapy: preliminary results of a prospective study. *Eur J Radiol* 2012; **81**: 3542-3550 [PMID: 22459347 DOI: 10.1016/j.ejrad.2012.02.012]
- 124 **Schlemmer M**, Sourbron SP, Schinwald N, Nikolaou K, Becker CR, Reiser MF, Berger F. Perfusion patterns of metastatic gastrointestinal stromal tumor lesions under specific molecular therapy. *Eur J Radiol* 2011; **77**: 312-318 [PMID: 19720488 DOI: 10.1016/j.ejrad.2009.07.031]
- 125 **Anzidei M**, Napoli A, Zaccagna F, Cartocci G, Saba L, Menichini G, Cavallo Marincola B, Marotta E, Di Mare L, Catalano C, Passariello R. Liver metastases from colorectal cancer treated with conventional and antiangiogenic chemotherapy: evaluation with liver computed tomography perfusion and magnetic resonance diffusion-weighted imaging. *J Comput Assist Tomogr* 2011; **35**: 690-696 [PMID: 22082538 DOI: 10.1097/RCT.0b013e318230d905]
- 126 **Deckers F**, De Foer B, Van Mieghem F, Botelberge T, Weytjens R, Padhani A, Pouillon M. Apparent diffusion coefficient



- measurements as very early predictive markers of response to chemotherapy in hepatic metastasis: a preliminary investigation of reproducibility and diagnostic value. *J Magn Reson Imaging* 2014; **40**: 448-456 [PMID: 24924334 DOI: 10.1002/jmri.24359]
- 127 **De Bruyne S**, Van Damme N, Smeets P, Ferdinande L, Ceelen W, Mertens J, Van de Wiele C, Troisi R, Libbrecht L, Laurent S, Geboes K, Peeters M. Value of DCE-MRI and FDG-PET/CT in the prediction of response to preoperative chemotherapy with bevacizumab for colorectal liver metastases. *Br J Cancer* 2012; **106**: 1926-1933 [PMID: 22596235 DOI: 10.1038/bjc.2012.184]
  - 128 **Vriens D**, de Geus-Oei LF, Heerschap A, van Laarhoven HW, Oyen WJ. Vascular and metabolic response to bevacizumab-containing regimens in two patients with colorectal liver metastases measured by dynamic contrast-enhanced MRI and dynamic 18F-FDG-PET. *Clin Colorectal Cancer* 2011; **10**: E1-E5 [PMID: 21609927 DOI: 10.3816/CCC.2011.n.010]
  - 129 **Vriens D**, van Laarhoven HW, van Asten JJ, Krabbe PF, Visser EP, Heerschap A, Punt CJ, de Geus-Oei LF, Oyen WJ. Chemotherapy response monitoring of colorectal liver metastases by dynamic Gd-DTPA-enhanced MRI perfusion parameters and 18F-FDG PET metabolic rate. *J Nucl Med* 2009; **50**: 1777-1784 [PMID: 19837750 DOI: 10.2967/jnumed.109.064790]
  - 130 **Coenegrachts K**, Bols A, Haspelslagh M, Rigauts H. Prediction and monitoring of treatment effect using T1-weighted dynamic contrast-enhanced magnetic resonance imaging in colorectal liver metastases: potential of whole tumour ROI and selective ROI analysis. *Eur J Radiol* 2012; **81**: 3870-3876 [PMID: 22944331 DOI: 10.1016/j.ejrad.2012.07.022]
  - 131 **O'Connor JP**, Rose CJ, Jackson A, Watson Y, Cheung S, Maders F, Whitcher BJ, Roberts C, Buonaccorsi GA, Thompson G, Clamp AR, Jayson GC, Parker GJ. DCE-MRI biomarkers of tumour heterogeneity predict CRC liver metastasis shrinkage following bevacizumab and FOLFOX-6. *Br J Cancer* 2011; **105**: 139-145 [PMID: 21673686 DOI: 10.1038/bjc.2011.191]
  - 132 **Miyazaki K**, Orton MR, Davidson RL, d'Arcy JA, Lewington V, Koh TS, Thng CH, Leach MO, Collins DJ, Koh DM. Neuroendocrine tumor liver metastases: use of dynamic contrast-enhanced MR imaging to monitor and predict radiolabeled octreotide therapy response. *Radiology* 2012; **263**: 139-148 [PMID: 22344403 DOI: 10.1148/radiol.12110770]
  - 133 **Niwa T**, Ueno M, Ohkawa S, Yoshida T, Doiuchi T, Ito K, Inoue T. Advanced pancreatic cancer: the use of the apparent diffusion coefficient to predict response to chemotherapy. *Br J Radiol* 2009; **82**: 28-34 [PMID: 19095814 DOI: 10.1259/bjr/43911400]
  - 134 **Cuneo KC**, Chenevert TL, Ben-Josef E, Feng MU, Greenon JK, Hussain HK, Simeone DM, Schipper MJ, Anderson MA, Zalupski MM, Al-Hawary M, Galban CJ, Rehemtulla A, Feng FY, Lawrence TS, Ross BD. A pilot study of diffusion-weighted MRI in patients undergoing neoadjuvant chemoradiation for pancreatic cancer. *Transl Oncol* 2014; **7**: 644-649 [PMID: 25389460 DOI: 10.1016/j.tranon.2014.07.005]
  - 135 **Yao JC**, Phan AT, Hess K, Fogelman D, Jacobs C, Dagohoy C, Leary C, Xie K, Ng CS. Perfusion computed tomography as functional biomarker in randomized run-in study of bevacizumab and everolimus in well-differentiated neuroendocrine tumors. *Pancreas* 2015; **44**: 190-197 [PMID: 25426617]

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## Exclusive enteral nutrition in children with Crohn's disease

Andrew S Day, Robert N Lopez

Andrew S Day, Robert N Lopez, Department of Paediatrics, University of Otago, Christchurch 8140, New Zealand  
Andrew S Day, Robert N Lopez, Paediatrics Department, Christchurch Hospital, Christchurch 8140, New Zealand

**Author contributions:** Day AS and Lopez RN contributed equally to this paper.

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**Correspondence to:** Andrew S Day, Professor, Department of Paediatrics, University of Otago, P.O. Box 4345, Christchurch, 8140, New Zealand. [andrew.day@otago.ac.nz](mailto:andrew.day@otago.ac.nz)  
**Telephone:** +64-3-3640747  
**Fax:** +64-3-3640919

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### Abstract

Exclusive enteral nutrition involves the use of a complete liquid diet, with the exclusion of normal dietary components for a defined period of time, as a therapeutic measure to induce remission in active Crohn's disease (CD). This very efficacious approach leads to high rates of remission, especially in children and adolescents newly diagnosed with CD. This intervention also results in mucosal healing,

nutritional improvements and enhanced bone health. Whilst several recent studies have provided further elaboration of the roles of exclusive enteral nutrition in the management of CD, other reports have provided new understanding of the mechanisms by which this intervention acts.

**Key words:** Children; Crohn's disease; Exclusive enteral nutrition; Nutrition; Outcomes; Mechanisms

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**Core tip:** Exclusive enteral nutrition is well-established as a key therapy in children with active Crohn's disease. Recent studies increasingly support this role, whilst other data has illustrated key mechanisms of action.

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### INTRODUCTION

Crohn's disease (CD), one of the inflammatory bowel diseases (IBD), is a chronic inflammatory condition that may involve any part of the gastrointestinal tract (GIT) commonly leading to symptoms such as abdominal pain, diarrhoea and nutritional impairments<sup>[1]</sup>. CD may present at any age, with up to one quarter of cases being diagnosed during childhood. In recent years, rates of CD have been increasing in many countries. Recent Australian data, for instance, shows a ten-fold increase in the incidence of CD over the first decade of the 21<sup>st</sup> century<sup>[2]</sup>. Other reports also demonstrate increasing incidence of CD in various countries and indicate that this condition is often presenting at younger ages<sup>[3]</sup>.

The best accepted hypothesis for the pathogenesis of CD is that uncontrolled inflammation in the GIT follows a dysregulated immune response to environmental triggers in individuals with a genetic susceptibility<sup>[1]</sup>. Environmental factors implicated include diet and the intestinal microflora. Some dietary factors, such as breast-feeding in infancy are protective, whilst others (for example, a high fat diet) are associated with increased risk.

Onset of CD in children and adolescents is commonly associated with weight loss, and may also lead to impaired linear growth and pubertal delay. Consequently, the management of CD in this age group requires close attention to nutrition, with frequent assessment of weight, height and weight for height measurements<sup>[1,4]</sup>. Exclusive enteral nutrition (EEN) provides a way to induce remission and optimise nutrition following diagnosis. EEN involves the administration of a liquid diet formulation for a defined period of time as the sole intervention to induce remission<sup>[4]</sup>. EEN has essentially no side-effects and is associated with high rates of mucosal healing. However, similar to all other currently-available therapies for CD, EEN is not curative. This article focuses upon the roles of EEN in paediatric CD, with particular regards to new understandings of the role of EEN as well as the mechanisms of this therapy.

## CD AND NUTRITIONAL CONSEQUENCES

CD is characterised by the finding of acute and chronic inflammatory changes in any section of the GIT. CD can be distinguished from ulcerative colitis (another form of IBD) by disease location, extent of involvement through the bowel wall, its patchy nature and the finding of non-caseating granuloma<sup>[5]</sup>. CD in childhood is typically extensive with pan-enteric involvement commonly seen; more than half of children have disease involvement proximal to the terminal ileum<sup>[1,6]</sup>. The initial features of CD also may include perianal, perioral or extra-intestinal features.

Many children diagnosed with CD present with classical symptoms of diarrhoea, abdominal pain and weight loss. Others, however, may present with less obvious symptoms such as lethargy, isolated joint symptoms, or oral findings.

Many reports indicate that almost all children presenting with CD have a history of either weight loss or plateauing of weight gain: in some studies more than 85% of children have these features<sup>[1,7]</sup>. This likely reflects early satiety or post-prandial abdominal discomfort. Circulating pro-inflammatory cytokines [for example, tumour necrosis factor (TNF)- $\alpha$ ] also contribute to anorexia<sup>[8]</sup>. Consequent to changes in weight, a number of children may also have impaired linear growth. These changes in normal growth patterns may exist for many months prior to diagnosis, sometimes preceding any specific gastrointestinal symptoms<sup>[9]</sup>. Measurement of weight, height and

weight for height assessments is essential at diagnosis. Review of historical growth patterns and interpretation of linear growth in the context of familial growth patterns are also important. Subsequent to diagnosis close attention to growth patterns and to the velocity of linear growth is required to ensure that adequate growth is attained and then maintained.

In addition to altered weight and height, impaired nutrition and uncontrolled inflammation also leads to delayed pubertal development<sup>[8]</sup>. In past generations, the combined effects of these growth impairments commonly resulted in reduced final adult height. Consequently, assessment of pubertal status in adolescents and calculation of bone age are important aspects of the ongoing care of children with CD, from diagnosis onwards.

Micronutrient deficiencies are also seen in children with CD. Although deficiencies of iron and vitamin D are seen most commonly, vitamin B12, zinc and selenium may also be low<sup>[10]</sup>. Although the nutritional impacts of CD may be most pronounced at diagnosis (when inflammation is uncontrolled prior to the commencement of therapy), these adverse effects also can occur at any subsequent stage.

Given that CD can have adverse nutritional consequences in children, it is not surprising that close ongoing attention to nutrition is a critical aspect of patient management. The use of EEN provides an important and vital role in managing many of the negative nutritional impacts of CD in children as well as inducing remission<sup>[4]</sup>. Although the main focus of EEN is upon the induction of remission and initial control of disease, further benefits may follow. Additionally, ongoing maintenance enteral nutrition (MEN) after initial EEN may assist in the maintenance of remission<sup>[11,12]</sup>.

## EFFICACY OF EEN IN INDUCTION OF REMISSION

A number of studies published over the last 15 years have demonstrated the efficacy of EEN in children with active CD. Generally EEN leads to the induction of remission in approximately 85% of patients. A meta-analysis of paediatric studies indicated that EEN had equivalent response to corticosteroids in children with active CD<sup>[13]</sup>. Although still involving relatively small cohorts, studies published in the last year or so confirm and build upon previous data.

A prospective Australian study involving 34 children demonstrated clinical remission in 84% and biochemical remission in 76%, whilst 58% had early endoscopic response<sup>[14]</sup>. A subset of this group also had small bowel imaging (magnetic resonance enterography) before and after EEN: 3 of these 14 children had complete transmural healing.

A recent Spanish study evaluated the outcomes of 40 children treated with EEN<sup>[15]</sup>. On an intention to

treat basis, 80% entered remission after 6-8 wk of EEN. When the investigators evaluated the outcomes in the 34 children who had completed the full period of EEN, 32 (92.1%) entered remission.

A retrospective study conducted in the Netherlands assessed the outcomes of EEN in 77 children<sup>[16]</sup>. Of the children who completed a course of EEN, 71% had complete remission whilst 26% had partial remission. The investigators noted that ileal or ileo-colonic disease location and poor nutritional status at baseline were important determinants of outcome in this series.

The impact of disease location upon outcomes has been variable in reported studies.

One study conducted in the United Kingdom showed a marked disparity between colonic disease (50% response rate) and ileal or ileo-colonic disease (remission rate between 92% and 83% respectively)<sup>[17]</sup>. In contrast, a subsequent report from Scotland involving 114 children showed that those with isolated terminal ileal disease had a lower remission rate, but that location did not otherwise influence outcome<sup>[18]</sup>. When evaluated in a Cochrane analysis, there was felt to be insufficient evidence to clearly elucidate the impact of location upon outcome<sup>[19]</sup>.

Exclusivity is an important determinant of efficacy for EEN as illustrated in two separate reports. Johnson *et al.*<sup>[20]</sup> demonstrated that almost three times as many children managed with EEN entered remission compared to a group of children managed with Partial EN (half of daily calories provided with normal diet and half as formula). More recently, Gupta *et al.*<sup>[21]</sup> reported that 65% of 23 children entered remission when managed with a novel Partial EN regimen (comprising 90% of intake as overnight enteral feeds and 10% as normal diet during the day).

To date there has been little consideration of how factors such as disease location or disease severity might influence outcomes in an individual patient. One study demonstrates that an early fall in faecal calprotectin levels corresponded with response to EEN one month later<sup>[22]</sup>. Further evaluation of faecal markers or other specific indicators might permit the development of predictive algorithms that lead to a more individualised application of EEN in children.

EEN may have roles in the perioperative period in children with CD. Preoperative nutritional support may enhance weight and improve nutritional parameters (*e.g.*, serum albumin) leading to enhanced operative outcomes. Extensive data from clinical trials conducted in Japan also show the benefits of enhanced nutritional support post-operatively. These studies demonstrated that the use of maintenance enteral nutrition to provide up to 50% of caloric requirements in adults with surgically-induced remission delayed recurrence in these individuals<sup>[23,24]</sup>.

some children may have nausea or loose motions initially, others may have constipation. Transient elevation of hepatic transaminases were noted in one case series<sup>[25]</sup>, but was not observed in a second series<sup>[26]</sup>. Refeeding syndrome has also been reported following EEN<sup>[27,28]</sup>. The three children reported in these reports had moderate/severe malnutrition, placing them at increased risk of refeeding syndrome. Consequently, when commencing EEN in children with significant malnutrition, a routine approach should be utilised to identify those at greater risk and to commence enteral nutrition slowly and carefully along with close monitoring of electrolytes.

## EEN AND MUCOSAL HEALING

Evidence in recent years has emphasised the importance of mucosal healing as a primary outcome measure in children with CD. The disconnect between clinical improvement in patients with active CD and lack of endoscopic change, particularly following corticosteroid treatment, has been shown in a number of studies<sup>[14,29,30]</sup>. While improved patient well-being is a useful and satisfying marker of disease control, the role of mucosal healing as a predictor for long-term CD burden has become very clear<sup>[14,31]</sup>.

The inherent difficulty in documenting mucosal response to therapy is the need to repeat endoscopy, often times in patients who have noted a significant clinical improvement in their symptoms following treatment. A few studies in recent years have managed to document important endoscopic, histologic and biological findings before and after treatment with EEN in children with CD - invaluable evidence to further encourage this therapy as the mainstay towards achieving disease remission.

An English case-controlled study documented a 79% rate of clinical remission in children with CD following an eight-week course of EEN<sup>[31]</sup>. Further to that, they showed improvements in median endoscopic and histologic scores in both ileal and colonic disease following treatment. Of particular significance, this study showed a fall in ileal interferon (INF)- $\gamma$  mRNA and a rise in transforming growth factor (TGF)  $\beta$ 1 mRNA, whereas in the colon interleukin (IL)-8 mRNA fell following treatment.

A number of studies have shown early mucosal healing with EEN at 8-10 wk following commencement of therapy<sup>[14,29,30]</sup>. In addition, EEN has consistently outperformed corticosteroids in achieving mucosal healing in cases of active CD when the two different therapies have been directly compared. Perhaps most encouragingly, early mucosal healing as a result of EEN has been shown to result in improved outcomes at one year - specifically, in terms of reduced rates of endoscopic relapse, hospitalisation and need for anti-TNF agents<sup>[14]</sup>. As a corollary however, poor initial, mucosal response following a course of EEN can be viewed as an indicator of more severe disease course,

## SIDE-EFFECTS OF EEN

Few significant side-effects are seen with EEN<sup>[4]</sup>. Whilst



which may warrant earlier introduction of other medical therapies.

Grover *et al*<sup>[14]</sup> extended the impact of EEN on mucosal healing to further demonstrate that EEN is also able to lead to resolution of transmural inflammation. Follow-up magnetic resonance imaging was utilised to demonstrate improvements and resolution of inflammatory changes in this series of children with newly diagnosed CD.

What is clear is that EEN is the superior therapy for inducing mucosal healing - the gold standard for disease remission. Achievement of this standard is likely to result in improved disease control for children with IBD - a cohort in whom disease phenotype is typically more aggressive.

## EEN ENHANCES GROWTH AND NUTRITION

In addition to anti-inflammatory benefits, EEN also leads to important improvements in nutritional parameters. Early changes after starting EEN include rapid improvement in circulating levels of Insulin-like Growth Factor (IGF)-1, with prompt return to control values<sup>[32]</sup>. Weight is enhanced, with weight gain typically corresponding with efficacy. Some reports have also shown early height catch up during the full period of EEN.

Gerasimidis *et al*<sup>[33]</sup> recently evaluated the influence of EEN upon body composition parameters in 17 children. Body impedance analysis demonstrated marked improvement in lean mass ( $P = 0.0001$ ) but not fat mass ( $P > 0.05$ ) during eight weeks of EEN. During this period, levels of a number of micronutrients also improved.

An earlier report showed improved weight, lean body mass, and skin fold thickness measurements after 3 and 6 wk of EEN<sup>[34]</sup>. Similarly, a subsequent study from Toronto, Canada, showed improvements in weight and lean body mass subsequent to EEN<sup>[35]</sup>. Interestingly, this report also showed improved linear growth in comparison to height gains seen in ten children treated with corticosteroids over the same period of time.

## EEN AND BONE HEALTH

It is well established that active CD negatively impacts upon bone health. Reduced bone mineral density and increased fracture risk are known complications of poorly-controlled CD<sup>[36]</sup>. Future fracture risk is associated with peak bone mass, which is acquired primarily during childhood and adolescence<sup>[37]</sup>. Among the factors leading to reduced bone mass in children with IBD are vitamin D deficiency, corticosteroid therapy, reduced sunlight exposure, decreased physical activity and uncontrolled intestinal inflammation, which directly and indirectly contributes to malnutrition<sup>[36-39]</sup>.

Vitamin D deficiency is a prevalent issue amongst children with IBD. Levin *et al*<sup>[37]</sup> found that the majority of their cohort of Australian children with IBD were either vitamin D deficient ( $< 51$  nmol/L) or insufficient (51-75 nmol/L). This report also showed that children with vitamin D deficiency had greater corticosteroid exposure than those with normal vitamin D levels. As may have been expected however, the mean serum vitamin D concentration was higher in the group treated with EEN after diagnosis compared to the group treated with corticosteroids.

An eight-week course of EEN in children newly-diagnosed with IBD has been shown to result in normalisation of bone markers indicating more new bone formation and less bone resorption<sup>[38]</sup>. Furthermore, a six-week course of EEN (followed immediately by a two-week course of partial EN) resulted in better improvements in z-scores (on DEXA scans) when compared to a group of Canadian children treated with corticosteroids<sup>[39]</sup>.

EEN has also been shown to have more direct benefits on bone mineral density. A German study elegantly demonstrated that bone metabolism and muscle mass improved within 12 wk of commencement with EEN in children with CD<sup>[36]</sup>. The peripheral quantitative computed tomography method used in this study demonstrated improved trabecular density z-scores, normalisation of initially high cortical density z-scores and improved muscle cross-sectional area.

## VARIATIONS IN EEN PRACTICE AND PROTOCOLS

Although EEN is well established as a standard and safe therapy to induce remission in active CD, there are marked differences in the application of EEN, as well as variations in individual protocols.

A trans-Atlantic study published in 2003 reported that EEN was used regularly by 62% of European paediatric gastroenterologists, whilst only 5% of practitioners in the United States used this therapy<sup>[40]</sup>. Subsequent studies have indicated that EEN was used regularly by 12% of North American and 38% of Australian paediatric gastroenterologists<sup>[41,42]</sup>. A recent Swedish report found that 96% of paediatric units in Sweden used EEN as a treatment option in active CD, whilst 68% of those respondents routinely used EEN as initial therapy in newly-diagnosed CD<sup>[43]</sup>.

The reasons for these marked differences in the routine application of EEN are not fully characterised. Two studies shed some light on what influences the choice of EEN as a preferred option to treat CD in childhood. The routine use of EEN by Australian paediatric gastroenterologists appeared to be closely related to their awareness of this therapy during their training<sup>[42]</sup>. Those who were not routinely using EEN reported concerns about adherence, cost and resource requirements. When asked similar questions, North

American respondents reported similar concerns<sup>[43]</sup>. Again the routine recommendation of EEN was associated with the practitioners' previous experience with EEN.

In addition, EEN protocols vary in many regards between units and countries<sup>[44]</sup>. Variations include the duration of EEN course, the type or brand of formulation used, and the inclusion of other oral intake (such as other fluids or boiled lollies) during EEN. A number of studies demonstrate no differences between outcomes seen with elemental or polymeric enteral formulae<sup>[4]</sup>, and indicate that polymeric formulae have superior taste and acceptability<sup>[45]</sup>. However, the impact of any other differences upon comparative outcomes has not yet been evaluated.

A typical regimen involves the use of a polymeric formula administered exclusively over eight weeks<sup>[4,44,46]</sup>. Formula is introduced gradually over the first three days of the course of therapy, until required daily amounts are reached (specific details of calculating caloric requirements and further practical aspects of EEN are included in reference<sup>[4]</sup>). Whilst most children are able to take the required volumes orally, some will require placement of a nasogastric tube to facilitate compliance. During the period of EEN, children are encouraged to take additional water orally and to chew small amounts of sugar-less chewing gum. At the completion of the eight week period of EEN, one small meal would typically be reintroduced every three days, whilst reducing the daily volume of formula with each added meal.

## MECHANISMS OF EEN IN CD

Although EEN has been used for many years, it is only in the last few years that an understanding of the mechanisms of this therapy has emerged. Evidence now indicates three primary components to the actions of EEN: alteration of the intestinal microflora, enhancement of barrier function and direct anti-inflammatory effects. However, the relationships between these events and the triggers for these changes have yet to be elucidated.

### **EEN and the intestinal microbiota**

Initial support for modulation of the intestinal flora by EEN came from three earlier studies, using molecular tools<sup>[47-49]</sup>. The most comprehensive of these studies used denaturing gel gradient electrophoresis to examine changes in the flora during and following a course of EEN in children<sup>[49]</sup>. In this report, EEN resulted in a marked and prolonged reduction of bacterial diversity across all bacterial groups. In particular, variations in the composition of the Bacteroides group correlated closely with reducing disease activity and inflammatory proteins.

A more recent study used a similar molecular technique (temperature gradient gel electrophoresis)

to ascertain changes during and following EEN<sup>[50]</sup>. The results arising from this work again showed a reduction in diversity during EEN, with a divergence from the control setting seen in the children with CD. Interestingly, the concentrations of the putative protective *Faecalibacterium prausnitzii* species fell after 1 mo of EEN. This study also demonstrated an increase in faecal pH and a reduction in butyrate levels during EEN. In contrast, an increase in faecal butyrate levels was seen in a Swedish study of 18 children managed with EEN<sup>[51]</sup>. In these patients the induction of remission was associated with increased butyrate and decreased acetic acid in faecal samples.

A new report has employed high-throughput sequencing to delineate changes in the flora during and following EEN<sup>[52]</sup>. The number of operational taxonomic units (OTU) reduced markedly upon commencing EEN, and this correlated closely with the successful induction of remission. Furthermore, subsequent disease exacerbations occurred in conjunction with an increase in OTUs. A particular finding was that families within the Firmicutes correlated with disease activity.

Together these reports indicate that EEN leads to pronounced changes in the intestinal flora, and variations in faecal metabolic activity. The relationships between these changes and coincident improvements in mucosal inflammation are yet to be ascertained.

### **EEN and barrier function**

The intestinal mucosa provides an essential barrier to the outside world: intestinal permeability is a central component of this activity. Increased permeability occurs secondary to active inflammation in the gut and may also be present in preclinical CD.

In elegant *in vitro* studies Nahidi *et al.*<sup>[53]</sup> showed that a polymeric formula (as used in EEN) resulted in normalisation of altered permeability and migration of key tight junction proteins back to the cell membrane. This report was further supported by studies employing a murine model of gut inflammation<sup>[54]</sup>. In this model, the administration of a polymeric formula in animals with gut inflammation reversed impaired intestinal permeability and altered tight junction protein localisation, in conjunction with normalisation of inflammatory changes. Although this work is consistent and clear, these findings have not yet been complemented by *in vivo* studies in individuals with CD.

### **Direct anti-inflammatory effects of EEN**

Numerous studies have demonstrated that the use of EEN results in reductions in mucosal levels of pro-inflammatory cytokines. Meister *et al.*<sup>[55]</sup> used *ex vivo* mucosal biopsies to demonstrate that an elemental formula resulted in an increased ratio of IL-1Ra to IL-1 $\beta$  compared to the ratio in control samples. Using an *in vitro* model of inflammation de Jong *et al.*<sup>[56]</sup> showed that polymeric formula led to a reduction in cellular production of IL-8 following stimulation

with TNF- $\alpha$ . Preliminary data indicated that this anti-inflammatory effect of the polymeric formula was mediated by disruption of intracellular nuclear factor (NF)- $\kappa$ B signalling. Subsequent experiments have demonstrated that the administration of polymeric formula results in inhibition of specific kinases in the NF- $\kappa$ B signal transduction pathway (unpublished observations). These findings suggest that as yet unidentified active elements within formulae used for EEN interact directly with epithelial cells leading to anti-inflammatory effects. Again, further work is required to definitively elucidate these processes.

## CONCLUSION

EEN is now firmly established as a first line therapy for the induction of remission in children with active CD. Recent studies further support and clearly substantiate the added benefits of EEN. However, additional collaborative studies are now required to further progress the use and application of EEN. Hopefully, ways to optimise and individualise EEN protocols should arise from such endeavours.

A number of investigators have provided intriguing data on the mechanisms by which EEN exerts its clinical benefits. Although these include *in vitro* and animal methods, the clear implication is that EEN interacts directly with the inflamed gut. The relevant importance of the various mechanisms is not yet clarified - further elaboration of these aspects may prompt the development of novel enteral nutrition formulations for EEN that enhance outcomes. In addition, these data should further enhance our understanding of the roles that these mucosal events play in the pathogenesis of CD. In turn, these avenues of research promise to result in novel approaches that may lead to better outcomes for children with CD.

## REFERENCES

- 1 **Lemberg DA**, Day AS. Crohn disease and ulcerative colitis in children: an update for 2014. *J Paediatr Child Health* 2015; **51**: 266-270 [PMID: 25039307 DOI: 10.1111/jpc.12685]
- 2 **Phavichitr N**, Cameron DJ, Catto-Smith AG. Increasing incidence of Crohn's disease in Victorian children. *J Gastroenterol Hepatol* 2003; **18**: 329-332 [PMID: 12603535]
- 3 **Benchimol EI**, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflamm Bowel Dis* 2011; **17**: 423-439 [PMID: 20564651 DOI: 10.1002/ibd.21349]
- 4 **Critch J**, Day AS, Otley A, King-Moore C, Teitelbaum JE, Shashidhar H. Use of enteral nutrition for the control of intestinal inflammation in pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 2012; **54**: 298-305 [PMID: 22002478 DOI: 10.1097/MPG.0b013e318235b397]
- 5 **IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition**. Inflammatory bowel disease in children and adolescents: recommendations for diagnosis—the Porto criteria. *J Pediatr Gastroenterol Nutr* 2005; **41**: 1-7 [PMID: 15990620]
- 6 **Van Limbergen J**, Russell RK, Drummond HE, Aldhous MC, Round NK, Nimmo ER, Smith L, Gillett PM, McGrogan P, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Satsangi J, Wilson DC. Definition of phenotypic characteristics of childhood-onset inflammatory bowel disease. *Gastroenterology* 2008; **135**: 1114-1122 [PMID: 18725221 DOI: 10.1053/j.gastro.2008.06.081]
- 7 **Vasseur F**, Gower-Rousseau C, Vernier-Massouille G, Dupas JL, Merle V, Merlin B, Lerebours E, Savoye G, Salomez JL, Cortot A, Colombel JF, Turck D. Nutritional status and growth in pediatric Crohn's disease: a population-based study. *Am J Gastroenterol* 2010; **105**: 1893-1900 [PMID: 20145606 DOI: 10.1038/ajg.2010.20]
- 8 **Walters TD**, Griffiths AM. Mechanisms of growth impairment in pediatric Crohn's disease. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 513-523 [PMID: 19713986 DOI: 10.1038/nrgastro.2009.124]
- 9 **Kanof ME**, Lake AM, Bayless TM. Decreased height velocity in children and adolescents before the diagnosis of Crohn's disease. *Gastroenterology* 1988; **95**: 1523-1527 [PMID: 3181677]
- 10 **Gerasimidis K**, McGrogan P, Edwards CA. The aetiology and impact of malnutrition in paediatric inflammatory bowel disease. *J Hum Nutr Diet* 2011; **24**: 313-326 [PMID: 21564345 DOI: 10.1111/j.1365-277X.2011.01171.x]
- 11 **Wilschanski M**, Sherman P, Pencharz P, Davis L, Corey M, Griffiths A. Supplementary enteral nutrition maintains remission in paediatric Crohn's disease. *Gut* 1996; **38**: 543-548 [PMID: 8707085]
- 12 **Cameron FL**, Gerasimidis K, Papangelou A, Missiou D, Garrick V, Cardigan T, Buchanan E, Barclay AR, McGrogan P, Russell RK. Clinical progress in the two years following a course of exclusive enteral nutrition in 109 paediatric patients with Crohn's disease. *Aliment Pharmacol Ther* 2013; **37**: 622-629 [PMID: 23360085 DOI: 10.1111/apt.12230]
- 13 **Heuschkel RB**, Menache CC, Megerian JT, Baird AE. Enteral nutrition and corticosteroids in the treatment of acute Crohn's disease in children. *J Pediatr Gastroenterol Nutr* 2000; **31**: 8-15 [PMID: 10896064]
- 14 **Grover Z**, Muir R, Lewindon P. Exclusive enteral nutrition induces early clinical, mucosal and transmural remission in paediatric Crohn's disease. *J Gastroenterol* 2014; **49**: 638-645 [PMID: 23636735 DOI: 10.1007/s00535-013-0815-0]
- 15 **Navas-López VM**, Blasco-Alonso J, Lacasa Maseri S, Girón Fernández-Crehuet F, Serrano Nieto MJ, Vicioso Recio MI, Sierra Salinas C. [Exclusive enteral nutrition continues to be first line therapy for pediatric Crohn's disease in the era of biologics]. *An Pediatr (Barc)* 2014; Epub ahead of print [PMID: 24704330 DOI: 10.1016/j.anpedi.2014.02.027]
- 16 **de Bie C**, Kindermann A, Escher J. Use of exclusive enteral nutrition in paediatric Crohn's disease in The Netherlands. *J Crohns Colitis* 2013; **7**: 263-270 [PMID: 22820027 DOI: 10.1016/j.crohns.2012.07.001]
- 17 **Afzal NA**, Davies S, Paintin M, Arnaud-Battandier F, Walker-Smith JA, Murch S, Heuschkel R, Fell J. Colonic Crohn's disease in children does not respond well to treatment with enteral nutrition if the ileum is not involved. *Dig Dis Sci* 2005; **50**: 1471-1475 [PMID: 16110838]
- 18 **Buchanan E**, Gaunt WW, Cardigan T, Garrick V, McGrogan P, Russell RK. The use of exclusive enteral nutrition for induction of remission in children with Crohn's disease demonstrates that disease phenotype does not influence clinical remission. *Aliment Pharmacol Ther* 2009; **30**: 501-507 [PMID: 19549288 DOI: 10.1111/j.1365-2036.2009.04067.x]
- 19 **Zachos M**, Tondeur M, Griffiths AM. Enteral nutritional therapy for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2007; **(1)**: CD000542 [PMID: 17253452]
- 20 **Johnson T**, Macdonald S, Hill SM, Thomas A, Murphy MS. Treatment of active Crohn's disease in children using partial enteral nutrition with liquid formula: a randomised controlled trial. *Gut* 2006; **55**: 356-361 [PMID: 16162683]
- 21 **Gupta K**, Noble A, Baldassano R, Sreedharan R, Grossman A, Kachelries K. A Novel Enteral Nutritional Therapy Protocol for the Treatment of Pediatric Crohn's Disease. *J Pediatr Gastroenterol*

- Nutr* 2010; **51** (Suppl 2): E87
- 22 **Gerasimidis K**, Nikolau CK, Edwards CA, McGrogan P. Serial fecal calprotectin changes in children with Crohn's disease on treatment with exclusive enteral nutrition: associations with disease activity, treatment response, and prediction of a clinical relapse. *J Clin Gastroenterol* 2011; **45**: 234-239 [PMID: 20871409 DOI: 10.1097/MCG.0b013e3181f39af5]
  - 23 **Yamamoto T**, Nakahigashi M, Umegae S, Kitagawa T, Matsumoto K. Impact of long-term enteral nutrition on clinical and endoscopic recurrence after resection for Crohn's disease: A prospective, non-randomized, parallel, controlled study. *Aliment Pharmacol Ther* 2007; **25**: 67-72 [PMID: 17229221]
  - 24 **Esaki M**, Matsumoto T, Hizawa K, Nakamura S, Jo Y, Mibu R, Iida M. Preventive effect of nutritional therapy against postoperative recurrence of Crohn disease, with reference to findings determined by intra-operative enteroscopy. *Scand J Gastroenterol* 2005; **40**: 1431-1437 [PMID: 16316891]
  - 25 **Schatorjé E**, Hoekstra H. Transient hypertransaminasemia in paediatric patients with Crohn disease undergoing initial treatment with enteral nutrition. *J Pediatr Gastroenterol Nutr* 2010; **51**: 336-340 [PMID: 20601906 DOI: 10.1097/MPG.0b013e3181d94f63]
  - 26 **Lemberg DA**, Leach ST, Day AS. Transient hypertransaminasemia in pediatric patients with Crohn disease. *J Pediatr Gastroenterol Nutr* 2011; **53**: 229 [PMID: 21788771 DOI: 10.1097/MPG.0b013e31821c6497]
  - 27 **Afzal NA**, Addai S, Fagbemi A, Murch S, Thomson M, Heuschkel R. Refeeding syndrome with enteral nutrition in children: a case report, literature review and clinical guidelines. *Clin Nutr* 2002; **21**: 515-520 [PMID: 12468372]
  - 28 **Akobeng AK**, Thomas AG. Refeeding syndrome following exclusive enteral nutritional treatment in Crohn disease. *J Pediatr Gastroenterol Nutr* 2010; **51**: 364-366 [PMID: 20639770 DOI: 10.1097/MPG.0b013e3181e712d6]
  - 29 **Borrelli O**, Cordischi L, Cirulli M, Paganelli M, Labalestra V, Uccini S, Russo PM, Cucchiara S. Polymeric diet alone versus corticosteroids in the treatment of active pediatric Crohn's disease: a randomized controlled open-label trial. *Clin Gastroenterol Hepatol* 2006; **4**: 744-753 [PMID: 16682258]
  - 30 **Berni Canani R**, Terrin G, Borrelli O, Romano MT, Manguso F, Coruzzo A, D'Armiento F, Romeo EF, Cucchiara S. Short- and long-term therapeutic efficacy of nutritional therapy and corticosteroids in paediatric Crohn's disease. *Dig Liver Dis* 2006; **38**: 381-387 [PMID: 16301010]
  - 31 **Fell JM**, Paintin M, Arnaud-Battandier F, Beattie RM, Hollis A, Kitching P, Donnet-Hughes A, MacDonald TT, Walker-Smith JA. Mucosal healing and a fall in mucosal pro-inflammatory cytokine mRNA induced by a specific oral polymeric diet in paediatric Crohn's disease. *Aliment Pharmacol Ther* 2000; **14**: 281-289 [PMID: 10735920]
  - 32 **Day AS**, Aurangzeb B, Leach ST, Lemberg DA, Walker J. The effects of Exclusive Enteral Nutrition upon the Insulin-like Growth Factor pathway in children with Crohn disease. *AGW* October 2008. *J Gastro Hepatol* 2008; **23**: A303
  - 33 **Gerasimidis K**, Talwar D, Duncan A, Moyes P, Buchanan E, Hassan K, O'Reilly D, McGrogan P, Edwards CA. Impact of exclusive enteral nutrition on body composition and circulating micronutrients in plasma and erythrocytes of children with active Crohn's disease. *Inflamm Bowel Dis* 2012; **18**: 1672-1681 [PMID: 22069243 DOI: 10.1002/ibd.21916]
  - 34 **Khoshoo V**, Reifen R, Neuman MG, Griffiths A, Pencharz PB. Effect of low- and high-fat, peptide-based diets on body composition and disease activity in adolescents with active Crohn's disease. *JPEN J Parenter Enteral Nutr* 1996; **20**: 401-405 [PMID: 8950740]
  - 35 **Azcue M**, Rashid M, Griffiths A, Pencharz PB. Energy expenditure and body composition in children with Crohn's disease: effect of enteral nutrition and treatment with prednisolone. *Gut* 1997; **41**: 203-208 [PMID: 9301499]
  - 36 **Werkstetter KJ**, Schatz SB, Alberer M, Filipiak-Pittroff B, Koletzko S. Influence of exclusive enteral nutrition therapy on bone density and geometry in newly diagnosed pediatric Crohn's disease patients. *Ann Nutr Metab* 2013; **63**: 10-16 [PMID: 23867548 DOI: 10.1159/000350369]
  - 37 **Levin AD**, Wadhwa V, Leach ST, Woodhead HJ, Lemberg DA, Mendoza-Cruz AC, Day AS. Vitamin D deficiency in children with inflammatory bowel disease. *Dig Dis Sci* 2011; **56**: 830-836 [PMID: 21222159 DOI: 10.1007/s10620-010-1544-3]
  - 38 **Whitten KE**, Leach ST, Bohane TD, Woodhead HJ, Day AS. Effect of exclusive enteral nutrition on bone turnover in children with Crohn's disease. *J Gastroenterol* 2010; **45**: 399-405 [PMID: 19957194 DOI: 10.1007/s00535-009-0165-0]
  - 39 **Soo J**, Malik BA, Turner JM, Persad R, Wine E, Siminoski K, Huynh HQ. Use of exclusive enteral nutrition is just as effective as corticosteroids in newly diagnosed pediatric Crohn's disease. *Dig Dis Sci* 2013; **58**: 3584-3591 [PMID: 24026403 DOI: 10.1007/s10620-013-2855-y]
  - 40 **Levine A**, Milo T, Buller H, Markowitz J. Consensus and controversy in the management of pediatric Crohn disease: an international survey. *J Pediatr Gastroenterol Nutr* 2003; **36**: 464-469 [PMID: 12658036]
  - 41 **Stewart M**, Day AS, Otley A. Physician attitudes and practices of enteral nutrition as primary treatment of paediatric Crohn disease in North America. *J Pediatr Gastroenterol Nutr* 2011; **52**: 38-42 [PMID: 20975582 DOI: 10.1097/MPG.0b013e3181e2c724]
  - 42 **Day AS**, Stephenson T, Stewart M, Otley AR. Exclusive enteral nutrition for children with Crohn's disease: use in Australia and attitudes of Australian paediatric gastroenterologists. *J Paediatr Child Health* 2009; **45**: 337-341 [PMID: 19490411 DOI: 10.1111/j.1440-1754.2009.01498.x]
  - 43 **Gråfors JM**, Casswall TH. Exclusive enteral nutrition in the treatment of children with Crohn's disease in Sweden: a questionnaire survey. *Acta Paediatr* 2011; **100**: 1018-1022 [PMID: 21272070 DOI: 10.1111/j.1651-2227.2011.02178.x]
  - 44 **Whitten KE**, Rogers P, Ooi CY, Day AS. International survey of enteral nutrition protocols used in children with Crohn's disease. *J Dig Dis* 2012; **13**: 107-112 [PMID: 22257479 DOI: 10.1111/j.1751-2980.2011.00558.x]
  - 45 **Wall CL**, Day AS, Gearry RB. Use of exclusive enteral nutrition in adults with Crohn's disease: a review. *World J Gastroenterol* 2013; **19**: 7652-7660 [PMID: 24282355 DOI: 10.3748/wjg.v19.i43.7652]
  - 46 **Day AS**, Whitten KE, Lemberg DA, Clarkson C, Vitug-Sales M, Jackson R, Bohane TD. Exclusive enteral feeding as primary therapy for Crohn's disease in Australian children and adolescents: a feasible and effective approach. *J Gastroenterol Hepatol* 2006; **21**: 1609-1614 [PMID: 16928225]
  - 47 **Pryce-Millar E**, Murch SH, Heuschkel RB. Enteral nutrition therapy in Crohn's disease changes the mucosal flora. *J Pediatr Gastroenterol Nutr* 2004; **39** (Suppl 1): 289
  - 48 **Lionetti P**, Callegari ML, Ferrari S, Cavicchi MC, Pozzi E, de Martino M, Morelli L. Enteral nutrition and microflora in pediatric Crohn's disease. *JPEN J Parenter Enteral Nutr* 2005; **29**: S173-5; discussion S175-8, S184-8 [PMID: 15980280]
  - 49 **Leach ST**, Mitchell HM, Eng WR, Zhang L, Day AS. Sustained modulation of intestinal bacteria by exclusive enteral nutrition used to treat children with Crohn's disease. *Aliment Pharmacol Ther* 2008; **28**: 724-733 [PMID: 19145728]
  - 50 **Gerasimidis K**, Bertz M, Hanske L, Junick J, Biskou O, Aguilera M, Garrick V, Russell RK, Blaut M, McGrogan P, Edwards CA. Decline in presumptively protective gut bacterial species and metabolites are paradoxically associated with disease improvement in pediatric Crohn's disease during enteral nutrition. *Inflamm Bowel Dis* 2014; **20**: 861-871 [PMID: 24651582 DOI: 10.1097/MIB.0000000000000023]
  - 51 **Tjellström B**, Högberg L, Stenhammar L, Magnusson KE, Midtvedt T, Norin E, Sundqvist T. Effect of exclusive enteral nutrition on gut microflora function in children with Crohn's disease. *Scand J Gastroenterol* 2012; **47**: 1454-1459 [PMID: 23016828 DOI: 10.3109/00365521.2012.703234]
  - 52 **Kaakoush NO**, Day AS, Leach ST, Lemberg DA, Nielsen



- S, Mitchell HM. Effect of exclusive enteral nutrition on the microbiota of children with newly diagnosed Crohn's disease. *Clin Transl Gastroenterol* 2015; **6**: e71 [PMID: 25588524 DOI: 10.1038/ctg.2014.21]
- 53 **Nahidi L**, Day AS, Lemberg DA, Leach ST. Differential effects of nutritional and non-nutritional therapies on intestinal barrier function in an in vitro model. *J Gastroenterol* 2012; **47**: 107-117 [PMID: 21953313 DOI: 10.1007/s00535-011-0471-1]
- 54 **Nahidi L**, Leach ST, Mitchell HM, Kaakoush NO, Lemberg DA, Munday JS, Huinao K, Day AS. Inflammatory bowel disease therapies and gut function in a colitis mouse model. *Biomed Res Int* 2013; **2013**: 909613 [PMID: 24027765 DOI: 10.1155/2013/909613]
- 55 **Meister D**, Bode J, Shand A, Ghosh S. Anti-inflammatory effects of enteral diet components on Crohn's disease-affected tissues in vitro. *Dig Liver Dis* 2002; **34**: 430-438 [PMID: 12132791]
- 56 **de Jong NS**, Leach ST, Day AS. Polymeric formula has direct anti-inflammatory effects on enterocytes in an in vitro model of intestinal inflammation. *Dig Dis Sci* 2007; **52**: 2029-2036 [PMID: 17406842]

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## What are the effects of proton pump inhibitors on the small intestine?

Shunji Fujimori

Shunji Fujimori, Department of Gastroenterology, Nippon Medical School, Graduate School of Medicine, Tokyo 113-8603, Japan

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Correspondence to: Shunji Fujimori, MD, PhD, Department of Gastroenterology, Nippon Medical School, Graduate School of Medicine, 1-1-5, Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan. [s-fujimori@nms.ac.jp](mailto:s-fujimori@nms.ac.jp)  
Telephone: +81-3-38222131  
Fax: +81-3-56851793

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### Abstract

Generally, proton-pump inhibitors (PPIs) have great benefit for patients with acid related disease with less frequently occurring side effects. According to a recent report, PPIs provoke dysbiosis of the small intestinal bacterial flora, exacerbating nonsteroidal anti-inflammatory drug-induced small intestinal injury.

Several meta-analyses and systematic reviews have reported that patients treated with PPIs, as well as post-gastrectomy patients, have a higher frequency of small intestinal bacterial overgrowth (SIBO) compared to patients who lack the aforementioned conditions. Furthermore, there is insufficient evidence that these conditions induce *Clostridium difficile* infection. At this time, PPI-induced dysbiosis is considered a type of SIBO. It now seems likely that intestinal bacterial flora influence many diseases, such as inflammatory bowel disease, diabetes mellitus, obesity, non-alcoholic fatty liver disease, and autoimmune diseases. When attempting to control intestinal bacterial flora with probiotics, prebiotics, and fecal microbiota transplantation, *etc.*, the influence of acid suppression therapy, especially PPIs, should not be overlooked.

**Key words:** Proton-pump inhibitors; Nonsteroidal anti-inflammatory drug; Small intestine; Dysbiosis; Small intestinal bacterial overgrowth

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**Core tip:** Proton-pump inhibitor (PPI) administration provokes dysbiosis of small intestinal bacterial flora, which exacerbates nonsteroidal anti-inflammatory drug-induced small intestinal injury. Dysbiosis is considered part of small intestinal bacterial overgrowth. Both PPI administration and gastrectomy increase the frequency of small intestinal bacterial overgrowth. Intestinal bacterial flora influence a number of systematic diseases. The influence of acid suppression therapy, especially PPIs, on small intestinal bacterial flora is worth noting.

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Proton-pump inhibitors (PPIs) induce strong acid suppression in the stomach and are used to treat upper gastrointestinal ulcerative lesions, including reflux esophagitis. Generally, PPIs are highly beneficial for patients with acid-related disease, and side effects from PPIs are infrequent. Recently, Wallace *et al*<sup>[1]</sup> reported that PPIs exacerbate nonsteroidal anti-inflammatory drug (NSAID)-induced small intestine injury in rats. In their study, omeprazole and lansoprazole caused “dysbiosis”, or a microbial imbalance, which exacerbated NSAID-induced small intestinal injury. These results are important for researchers who investigate NSAID-associated small intestinal injuries. Because PPIs are co-administered with NSAIDs to prevent NSAID-induced gastroduodenal injury, many studies have evaluated NSAID-induced small intestinal injury in healthy volunteers<sup>[2-4]</sup>. Until recently, most studies on NSAID-associated injury have focused on the upper gastrointestinal tract because the stomach and duodenum are common sites of major morbidity and mortality in the clinical setting. As a result, PPIs and prostaglandin analogs are the currently established treatments against NSAID-induced gastroduodenal injuries<sup>[5]</sup>. However, PPIs can affect the small intestinal bacterial flora. On the other hand, the examination protocol for evaluating NSAID-associated small intestinal injuries has not changed since the Wallace *et al* study was published.

As mentioned above, many studies have evaluated healthy volunteers who were treated with the combination of NSAIDs and PPIs. These studies have shown that the preventive effect of PPIs does not extend to the small intestine<sup>[4]</sup>; after taking NSAIDs and PPIs for two weeks, > 50% of subjects had small intestine injuries. If PPIs do indeed exacerbate small intestinal injury, most capsule endoscopy studies that evaluated NSAID-induced small intestinal injury with the concomitant administration of PPIs likely overestimated the frequency of NSAID-induced small intestinal injury. Accordingly, it is very important that we appropriately interpret the results of these studies by evaluating NSAID-associated small intestinal injury while considering whether or not PPIs have been co-administered.

Recently, the concept of dysbiosis, also called dysbacteriosis, has received substantial attention in various biomedical fields. Since the 1960s, the word “dysbiosis” has been used in research on intestinal bacteria flora in infants and post-gastrectomy patients in Germany. “Dysbiosis” was first used in a manuscript written in English in 1985<sup>[6]</sup>. Afterwards, the term was used in a 1987 study of patients with ulcerative colitis whose intestinal flora had increased levels of *Proteus*, a genus of Gram-negative proteobacteria<sup>[7]</sup>. Since then, “dysbiosis” has become established, and its use in publications written in English has increased. However,

previous publications only evaluated approximately 10 species of bacteria, as closely as possible, using the available technology<sup>[8]</sup>. In the beginning, “dysbiosis” was mostly used in research on inflammatory bowel disease<sup>[9,10]</sup>. However, it has recently been posited that intestinal bacterial flora influence many diseases, such as diabetes mellitus<sup>[11]</sup>, obesity, non-alcoholic fatty liver disease<sup>[12,13]</sup>, and autoimmune diseases<sup>[14]</sup>. While 150 manuscripts that were published between 1960 and 2009 and included in PubMed used the word “dysbiosis”, in the period from 2010 to 2014, an additional 800 manuscripts of that type were published. Clearly, the field of dysbiosis has attracted attention in recent years.

What does it mean for PPIs to induce dysbiosis? The first report of a possible association between PPIs and small intestinal bacterial overgrowth (SIBO) was published in 2008<sup>[15]</sup>. Recently, a meta-analysis reported on the statistical relationship between PPIs and SIBO<sup>[16]</sup>. In addition, numerous reports have stated that the number of patients with SIBO increases after total gastrectomy<sup>[17]</sup>. According to one report, the frequency of patients with *Clostridium difficile* (*C. difficile*) diarrhea increased during PPI administration in 2003<sup>[18]</sup>. Several other reports describe an association between PPI administration and *C. difficile* infection. However, there was no clear association between PPI administration and *C. difficile* infection in a recent systematic review<sup>[19]</sup>. Additionally, there are no reports of an increase in the number of patients with *C. difficile* diarrhea following total gastrectomy. Therefore, at this time, it is doubtful that PPI administration triggers *C. difficile* infection, while the frequency of SIBO is likely increased by PPI administration. Presumably, SIBO is closely related to dysbiosis. Currently, PPI-induced dysbiosis is considered part of SIBO.

In the near future, intestinal bacterial flora will be controlled by probiotics (viable micro-organisms with beneficial physiologic or therapeutic properties) and prebiotics (dietary components that foster the growth of beneficial bacteria). In addition, fecal microbiota transplantation is a promising therapy for controlling intestinal bacterial flora; its effectiveness has been reported in patients with inflammatory disease as well as in patients with *C. difficile* infection<sup>[20,21]</sup>. When attempting to control intestinal bacterial flora, the influence of acid suppression therapy, especially PPIs, should not be overlooked.

## REFERENCES

- 1 **Wallace JL**, Syer S, Denou E, de Palma G, Vong L, McKnight W, Jury J, Bolla M, Bercik P, Collins SM, Verdu E, Ongini E. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. *Gastroenterology* 2011; **141**: 1314-1322, 1322.e1-5 [PMID: 21745447 DOI: 10.1053/j.gastro.2011.06.075]
- 2 **Goldstein JL**, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. *Clin Gastroenterol Hepatol* 2005; **3**: 133-141 [PMID: 15704047]

- 3 **Fujimori S**, Seo T, Gudis K, Ehara A, Kobayashi T, Mitsui K, Yonezawa M, Tanaka S, Tatsuguchi A, Sakamoto C. Prevention of nonsteroidal anti-inflammatory drug-induced small-intestinal injury by prostaglandin: a pilot randomized controlled trial evaluated by capsule endoscopy. *Gastrointest Endosc* 2009; **69**: 1339-1346 [PMID: 19243767 DOI: 10.1016/j.gie.2008.08.017]
- 4 **Fujimori S**, Takahashi Y, Seo T, Gudis K, Ehara A, Kobayashi T, Mitsui K, Yonezawa M, Tanaka S, Tatsuguchi A, Sakamoto C. Prevention of traditional NSAID-induced small intestinal injury: recent preliminary studies using capsule endoscopy. *Digestion* 2010; **82**: 167-172 [PMID: 20588029 DOI: 10.1159/000308361]
- 5 **Lanas A**, Ferrandez A. NSAID-induced gastrointestinal damage: current clinical management and recommendations for prevention. *Chin J Dig Dis* 2006; **7**: 127-133 [PMID: 16808792]
- 6 **Bai K**. On the mechanism of cereobiogen readjustment to dysbiosis. *Prog Clin Biol Res* 1985; **181**: 169-170 [PMID: 3927307]
- 7 **Kanareykina SK**, Misautova AA, Zlatkina AR, Levina EN. Proteus dysbioses in patients with ulcerative colitis. *Nahrung* 1987; **31**: 557-561 [PMID: 3657933]
- 8 **Knoke M**, Bernhardt H. Clinical significance of changes of flora in the upper digestive tract. *Infection* 1989; **17**: 255-258 [PMID: 2767770]
- 9 **Ruseler-van Embden JG**, Schouten WR, van Lieshout LM. Pouchitis: result of microbial imbalance? *Gut* 1994; **35**: 658-664 [PMID: 8200561]
- 10 **Tamboli CP**, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut* 2004; **53**: 1-4 [PMID: 14684564]
- 11 **Tilg H**, Moschen AR. Microbiota and diabetes: an evolving relationship. *Gut* 2014; **63**: 1513-1521 [PMID: 24833634 DOI: 10.1136/gutjnl-2014-306928]
- 12 **Wong VW**, Tse CH, Lam TT, Wong GL, Chim AM, Chu WC, Yeung DK, Law PT, Kwan HS, Yu J, Sung JJ, Chan HL. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis--a longitudinal study. *PLoS One* 2013; **8**: e62885 [PMID: 23638162 DOI: 10.1371/journal.pone.0062885]
- 13 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammation-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]
- 14 **McLean MH**, Dieguez D, Miller LM, Young HA. Does the microbiota play a role in the pathogenesis of autoimmune diseases? *Gut* 2015; **64**: 332-341 [PMID: 25416067 DOI: 10.1136/gutjnl-2014-308514]
- 15 **Spiegel BM**, Chey WD, Chang L. Bacterial overgrowth and irritable bowel syndrome: unifying hypothesis or a spurious consequence of proton pump inhibitors? *Am J Gastroenterol* 2008; **103**: 2972-2976 [PMID: 19086951 DOI: 10.1111/j.1572-0241.2008.01992]
- 16 **Lo WK**, Chan WW. Proton pump inhibitor use and the risk of small intestinal bacterial overgrowth: a meta-analysis. *Clin Gastroenterol Hepatol* 2013; **11**: 483-490 [PMID: 23270866 DOI: 10.1016/j.cgh.2012.12.011]
- 17 **Paik CN**, Choi MG, Lim CH, Park JM, Chung WC, Lee KM, Jun KH, Song KY, Jeon HM, Chin HM, Park CH, Chung IS. The role of small intestinal bacterial overgrowth in postgastrectomy patients. *Neurogastroenterol Motil* 2011; **23**: e191-e196 [PMID: 21324050 DOI: 10.1111/j.1365-2982.2011.01686]
- 18 **Cunningham R**, Dale B, Undy B, Gaunt N. Proton pump inhibitors as a risk factor for Clostridium difficile diarrhoea. *J Hosp Infect* 2003; **54**: 243-245 [PMID: 12855243]
- 19 **Tleyjeh IM**, Bin Abdulhak AA, Riaz M, Alasmari FA, Garbati MA, AlGhamdi M, Khan AR, Al Tannir M, Erwin PJ, Ibrahim T, Allehibi A, Baddour LM, Sutton AJ. Association between proton pump inhibitor therapy and clostridium difficile infection: a contemporary systematic review and meta-analysis. *PLoS One* 2012; **7**: e50836 [PMID: 23236397 DOI: 10.1371/journal.pone.0050836]
- 20 **Khoruts A**, Dicksved J, Jansson JK, Sadowsky MJ. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. *J Clin Gastroenterol* 2010; **44**: 354-360 [PMID: 20048681 DOI: 10.1097/MCG.0b013e3181c87e02]
- 21 **Petrof EO**, Khoruts A. From stool transplants to next-generation microbiota therapeutics. *Gastroenterology* 2014; **146**: 1573-1582 [PMID: 24412527 DOI: 10.1053/j.gastro.2014.01.004]

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## 2015 Advances in Nonalcoholic Fatty Liver Disease

# Cardiovascular risk across the histological spectrum and the clinical manifestations of non-alcoholic fatty liver disease: An update

Vasilios G Athyros, Konstantinos Tziomalos, Niki Katsiki, Michael Doumas, Asterios Karagiannis, Dimitri P Mikhailidis

Vasilios G Athyros, Niki Katsiki, Michael Doumas, Asterios Karagiannis, Second Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, Hippokration Hospital, 55132 Thessaloniki, Greece  
Konstantinos Tziomalos, First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, AHEPA Hospital, 55132 Thessaloniki, Greece  
Dimitri P Mikhailidis, Department of Clinical Biochemistry (Vascular Prevention Clinic), Royal Free Hospital Campus, University College London Medical School, University College London, London NW3 2QG, United Kingdom

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**Correspondence to:** Dimitri P Mikhailidis, MD, FFPM, FRCP, FRCPath, Academic Head, Department of Clinical Biochemistry (Vascular Prevention Clinic), Royal Free Hospital

Campus, University College London Medical School, University College London, Pond Street, London NW3 2QG, United Kingdom. [mikhailidis@aol.com](mailto:mikhailidis@aol.com)  
Telephone: +44-20-78302258  
Fax: +44-20-78302235

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## Abstract

Non-alcoholic fatty liver disease (NAFLD) is considered to be an independent cardiovascular disease (CVD) risk factor. However, simple steatosis has a benign clinical course without excess mortality. In contrast, the advanced form of NAFLD, non-alcoholic steatohepatitis (NASH) with liver fibrosis increases mortality by approximately 70%, due to an increase in CVD mortality by approximately 300%. Chronic kidney disease (CKD) may be caused by NAFLD/NASH and it substantially increases CVD risk, especially in the presence of type 2 diabetes mellitus. Moreover, CKD may trigger NAFLD/NASH deterioration in a vicious cycle. NAFLD/NASH is also related to increased arterial stiffness (AS), an independent CVD risk factor that further raises CVD risk. Diagnosis of advanced liver fibrosis (mainly by simple non-invasive tests), CKD, and increased AS should be made early in the course of NAFLD and treated appropriately. Lifestyle measures and statin treatment may help resolve NAFLD/NASH and beneficially affect the CVD risk factors mentioned

above.

**Key words:** Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Cardiovascular disease; Liver fibrosis; Statins; Chronic kidney disease; Arterial stiffness; Inflammation

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**Core tip:** Non-alcoholic fatty liver disease (NAFLD) is an independent cardiovascular disease (CVD) risk factor. However, simple steatosis has a rather benign clinical course, while its advanced form, non-alcoholic steatohepatitis (NASH) substantially increases total mortality, mainly due to increased CVD events. In this review we propose the use of statin treatment for NASH, given its beneficial effect on NAFLD/NASH and CVD risk. There are data suggesting biopsy proven amelioration of NASH and normalization in liver ultrasonography and enzyme values as well as improvement of chronic kidney disease and arterial stiffness that usually accompany NASH and exacerbate CVD risk.

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), a term describing the most common liver disease (affects approximately 15%-30% of the general population in Western Countries), is characterized by accumulation of fat (> 5%) in liver cells in the absence of excessive alcohol intake, chronic viral hepatitis or other liver disease<sup>[1,2]</sup>.

NAFLD has a high prevalence (75%-100%) in populations with pre-existing metabolic conditions characterized by insulin resistance (IR) such as obesity, metabolic syndrome (MetS) or type 2 diabetes mellitus (T2DM)<sup>[1,2]</sup>. NAFLD prevalence continues to increase due to the obesity and T2DM pandemic<sup>[1]</sup>.

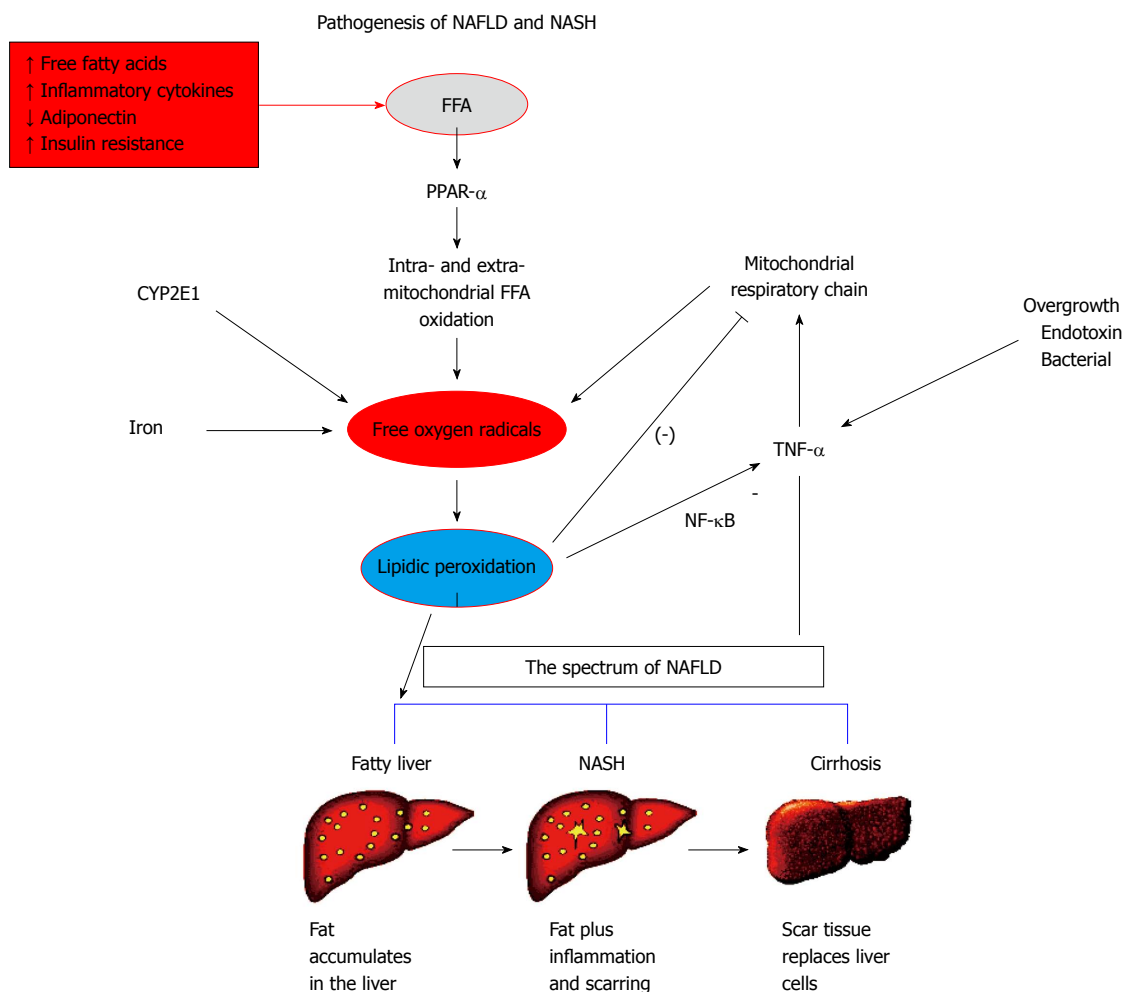
Histological manifestations of NAFLD range from simple steatosis, non-alcoholic steatohepatitis (NASH; characterized by hepatocellular necroinflammation and ballooning), liver fibrosis and cirrhosis, which in some cases may progress to hepatocellular carcinoma<sup>[3]</sup>. Despite its high prevalence, the pathogenesis of steatosis and the progression to NASH with fibrosis/cirrhosis, and the natural history of NAFLD are not yet entirely clear (Figure 1)<sup>[4]</sup>. The evidence<sup>[3]</sup> suggests

that NASH prevalence ranges from 3%-5% (> 20% of NAFLD cases) in the general population, however this rises to 37% in the morbidly obese<sup>[5]</sup>.

NAFLD/NASH is considered as the hepatic manifestation of MetS, and are closely related to cardiovascular disease (CVD)<sup>[6]</sup>, to the extent that NAFLD/NASH and CVD are viewed as two aspects of a shared disease<sup>[6]</sup>. More patients with NASH die from CVD than from liver disease<sup>[6,7]</sup>. Nevertheless, CVD risk level is not the same across the entire histological and clinical spectrum of NAFLD<sup>[8]</sup>. It seems that simple steatosis and NASH are considered disease states of different CVD risk involvement, each with different consequences, linked to environmental and genetic factors<sup>[9,10]</sup>. The multiple parallel hit theory suggests that NASH may occur directly in many individuals without previous simple steatosis<sup>[9,10]</sup>. It is possible that NASH can happen in the absence of simple steatosis because inflammation related to NASH pathogenesis might originate in the gut microbiota, in response to the prime neutrophil chemokines and macrophage-inflammatory protein-2, inflamed adipose tissue and to circulating inflammatory cells. In any case, the identification and management of high CVD risk patients with NAFLD/NASH remains a clinical challenge<sup>[8]</sup>. This narrative review considers this key issue, referring only to recent advances in diagnosis, risk stratification and treatment of NAFLD/NASH<sup>[9,10]</sup>.

## DEGREE OF LIVER FIBROSIS AND CVD RISK

The United States National Health and Nutrition Examination Survey (NHANES) was conducted in 1988-1994 and followed-up 11154 participants until the end of 2006 (mean follow up: 14.5 years). The findings suggest that the degree of liver fibrosis is related to clinical outcome in NAFLD/NASH patients<sup>[7]</sup>. The diagnosis of NAFLD was based on liver ultrasonography and the degree of liver fibrosis in NAFLD patients was determined without a biopsy by the NAFLD fibrosis score (NFS), the AST-platelet ratio index (APRI) and the FIB-4 score<sup>[7]</sup>. The ultrasonographic prevalence of NAFLD was 34% (if projected to the entire United States adult population this represents 43.2 million Americans), however, if only people with moderate to severe steatosis were included in NAFLD diagnosis its prevalence falls to 20.2%, projecting to 25.6 million United States adults<sup>[7]</sup>. The majority of NAFLD patients (71.7%) had simple steatosis, while 28.3% had NFS values suggesting an intermediate (25.1%) or high (3.2%) level of liver fibrosis<sup>[7]</sup>. These data project to 10.8 million United States adults with NAFLD with some evidence of advanced fibrosis, 9.4 million with an intermediate probability and 1.4 million with a high probability of fibrosis<sup>[7]</sup>. The 15 years follow-up showed that NAFLD in the form of steatosis was not related with higher total mortality compared with those without NAFLD (adjusted HR = 1.05, 95%CI: 0.93-1.19, *P* =



**Figure 1** Factors that contribute to the pathogenesis of non-alcoholic fatty liver disease or non-alcoholic steatohepatitis. NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; CYP2E1: Cytochrome P450 2E1; FFA: Free fatty acid; NF- $\kappa$ B: Nuclear factor kappa-light-chain-enhancer.

NS)<sup>[7]</sup>. In contrast, there was a progressive increase in total mortality with increasing levels of liver fibrosis scores [adjusted HR = 1.69, 95%CI: 1.09-2.63 for the NFS, 1.85 (1.02-3.37) for the APRI, and 1.66 (0.98-2.82) for the FIB-4], as compared with subjects without fibrosis<sup>[7]</sup>. The increase in mortality was mainly due to increased CVD mortality (HR = 3.46, 95%CI: 1.91-6.25 for NFS; 2.53, 1.33-3.83 for APRI; 2.68, 1.44-4.99 for FIB-4)<sup>[7]</sup>. Thus, prospective data from this US representative cohort suggest that steatosis per se, does not increase total and CVD mortality, while NASH, with advanced fibrosis as evaluated by non-invasive markers, was related to increased total and (mainly) CVD mortality<sup>[7]</sup>.

The fact that NAFLD at the stage of simple steatosis has a benign clinical course without excess mortality has been reported 10 years ago<sup>[11]</sup>. Also, the fact that NASH with liver fibrosis was related to increased overall and CVD mortality was established by studies with a long-term follow-up (up to 28 years)<sup>[12,13]</sup>. Moreover, fibrosis or even cirrhosis was found in 15%-50% of NASH patients on their index biopsy, suggesting that a good portion of NASH patients

develop progressive liver disease that could lead to liver-related mortality<sup>[14,15]</sup>. A study reporting survival of biopsy proven NAFLD or alcoholic fatty liver disease (AFLD) after 24 and 20 years of follow-up, respectively, showed that CVD was the most common cause of death (48%) in NAFLD patients, while liver-related death was recorded in 7% of these patients<sup>[16]</sup>. In contrast, AFLD patients had a liver-related death rate of 36% and CVD death rate of 32%<sup>[16]</sup>. NASH patients with moderate or severe fibrosis at baseline showed a worse survival rate than patients with none or mild fibrosis at baseline (adjusted HR = 2.09,  $P = 0.01$ )<sup>[16]</sup>.

Liver biopsy is the gold standard for the diagnosis of NAFLD or NASH<sup>[16]</sup>. The classification in liver biopsy is as follow: Steatosis should have a > 5% fat content in the liver biopsy. Fibrosis stage was defined as follows: 0 = none, 1 = perisinusoidal or periportal fibrosis, 1A = mild perisinusoidal fibrosis, 1B = moderate perisinusoidal fibrosis, 1C = portal/periportal fibrosis, 2 = perisinusoidal - portal/periportal fibrosis, 3 = bridging fibrosis, and, 4 = cirrhosis<sup>[17]</sup>. However, it is not possible to perform a liver biopsy in all patients with NAFLD (a considerable proportion of the general

population); not all patients need a liver biopsy and many will not consent. Thus, non-invasive tests can be used instead to evaluate the stage of fibrosis and consequently the overall risk of these patients<sup>[18]</sup>. A recent study evaluated the usefulness of 4 validated non-invasive scoring systems that were originally designed to distinguish patients from those without advanced liver fibrosis in comparison with the results of liver biopsy<sup>[18]</sup>. Thus, 310 biopsy proven NAFLD/NASH patients were followed for median period of 105 mo. The 4 tests were the 3 used in the United States National Health and Nutrition Examination Survey<sup>[7]</sup>, NFS, APRI, and FIB-4<sup>[19,20]</sup>, plus the more recently introduced BARD score<sup>[21]</sup>. These are calculated using the original published formulas<sup>[19-21]</sup>. The score of these simple non-invasive tests were useful for the identification of NAFLD patients in this study who are at increased risk for total and liver-related mortality and predicted clinical outcome successfully<sup>[18]</sup>. These results were confirmed by a meta-analysis of 32 studies evaluating the diagnostic accuracy of these 4 non-invasive tests in comparison with the results of liver biopsy<sup>[22]</sup>. Results of this evaluation suggested an excellent sensitivity and specificity of these tests<sup>[22]</sup>. Thus, with the use of these 4 simple tests NAFLD patients can be evaluated for both CVD risk and progressive liver disease risk<sup>[22]</sup>. These tests can also divide NASH patients into those with and those without advanced fibrosis and therefore increased overall risk, indicating which patients need more intensive therapy<sup>[22]</sup>. It seems that these data were lost in the large number of papers on NAFLD, which has dramatically increased during the last 5 years. The vast majority of the papers or the majority of studies analyzed in a review on the NAFLD and CVD association, include NAFLD patients diagnosed by ultrasonography or alanine aminotransferase (ALT) levels<sup>[23,24]</sup>. These usually report an increased CVD risk in NAFLD patients, however it is not clear which NAFLD patients shape this risk: all NAFLD patients or those with (advanced) fibrosis? Only a few studies identify this association and also indicate NAFLD patients at risk of systemic complications, based on the 4 simple tests mentioned above to distinguish NAFLD patient with liver fibrosis<sup>[25]</sup>. One of these studies used the NFS and the FIB-4 scores to separate 1559 NAFLD patients that included those with high possibility of liver fibrosis (group 1) and those with a low probability (group 2)<sup>[25]</sup>. In group 1 the prevalence of CVD at baseline was 7.7% vs 2.3% (NFS,  $P = 0.002$ ) and 9.0% vs 2.3% (FIB-4,  $P = 0.0012$ )<sup>[25]</sup>. The prevalence of T2DM at baseline was in group 1 in comparison with group 2, 31.5% vs 3.1% (NFS,  $P < 0.0001$ ) and 17.0% vs 4.7% (FIB-4,  $P < 0.0001$ )<sup>[25]</sup>. New onset diabetes (NOD) prevalence was 4.5% vs 1.2% (NFS,  $P = 0.034$ ) and 3.6% vs 1.2% (FIB-4,  $P = 0.11$ ), and of CVD in these patients was 5.0% vs 0.9% (NFS,  $P = 0.0019$ ) and 5.4% vs 0.9% (FIB-4,  $P = 0.0034$ )<sup>[25]</sup>. This study brings

forward another issue: that of pre-existing T2DM and the risk of NOD according to the degree of liver fibrosis in NAFLD patients; diabetes further increases the risk of CVD morbidity and mortality in NAFLD and MetS patients<sup>[26]</sup>. This can also be seen from the other way around: in T2DM patients the prevalence of NAFLD is 75%-100%, of NASH 63%-87%, liver fibrosis 22%-60%, and advanced liver fibrosis (4%-9%)<sup>[27]</sup>, according to the number of MetS components, visceral obesity, older age, increased duration of T2DM, and the presence of family history of T2DM<sup>[27]</sup>. This is a vicious cycle leading from NAFLD/NASH to T2DM and vice versa. In any case the presence of NAFLD, and especially with (advanced) liver fibrosis, in T2DM patients is associated with increased overall (CVD and liver-related) mortality<sup>[27]</sup>. Given that an increase in the adherence to multiple interventions in patients with T2DM is feasible and effective in better controlling the disease, as shown in a best practice study<sup>[28]</sup>, and that similar measures may improve NAFLD/NASH<sup>[26]</sup> this is an interesting implication for the treatment of both diseases<sup>[28-31]</sup>.

We need to abandon the idea that elevated liver enzymes and steatosis on ultrasonography alone could define CVD and liver-related risk; we need to calculate the overall risk of NAFLD/NASH patients with the use of the 4 simple tests, routinely available in clinical practice<sup>[32]</sup>. We need a pragmatic approach to diagnosis and staging of NAFLD so that patients at risk of complications can be identified<sup>[32]</sup>. This approach has implications for both diagnosis and treatment<sup>[33]</sup>.

### NAFLD, chronic kidney disease and CVD risk

The prevalence of chronic kidney disease (CKD) among the general population in the United States is 13% (that of early stages of CKD, including stage 3, is 11%)<sup>[34]</sup>, in Europe it is similar<sup>[35]</sup>, while in Japan it is much higher; 20% of the adult population is estimated to have CKD stage 3-5 [glomerular filtration rate (GFR)  $< 60 \text{ mL/min per } 1.73 \text{ m}^2$ ]<sup>[36]</sup>. In total, there seem to be more than 1.1 million patients with end-stage-renal disease (ESRD) worldwide<sup>[37]</sup>. CKD is an independent CVD risk factor substantially increasing its morbidity and mortality, to the degree that it is considered as a coronary heart disease (CHD) risk equivalent<sup>[38]</sup>. CVD is the leading cause of morbidity (40% of hospitalizations) and mortality (50% of deaths) in CKD patients<sup>[37,38]</sup>. Less than a half of CKD patients have develop ESRD, because most of them die from CVD before they develop ESRD<sup>[37,38]</sup>.

NAFLD is considered to be a risk factor for CKD and is associated with an increased prevalence and incidence of CKD<sup>[37,39]</sup>. In a community-based study involving 2103 patients with T2DM the prevalence of stage 3 or higher of CKD was 15% among patients with ultrasound-diagnosed NAFLD vs 9% ( $P < 0.001$ ) among T2DM patients without NAFLD, after adjustment for numerous baseline confounding factors and



independent traditional CKD risk factors<sup>[40]</sup>. Another study evaluated 1361 subjects with an abnormal oral glucose tolerance test (OGTT) on routine screening<sup>[41]</sup>. Participants with ultrasound-diagnosed NAFLD had a higher prevalence of microalbuminuria compared with patients with impaired glucose tolerance who did not have NAFLD (19% vs 6.3% in abnormal OGTT subjects; 32.6% vs 4.5% in newly diagnosed T2DM patients;  $P < 0.0001$ ) after adjusting for several classical risk factors<sup>[41]</sup>. These results suggest that NAFLD is a predictor of another CKD manifestation, microalbuminuria, in patients with prediabetes or T2DM<sup>[41]</sup>.

Two studies from the NHANES 2001 through 2006 investigated the association between serum surrogate markers of NAFLD, gamma-glutamyl-transpeptidase (GGT) and bilirubin concentrations, and CKD in a (United States) nation-wide representative sample of 13188 adults<sup>[42,43]</sup>. Serum GGT elevation was associated with an increased odds of CKD (OR = 2.38, 95%CI: 2.02-2.80,  $P < 0.0001$ )<sup>[42]</sup>, while total bilirubin levels were independently related with both decreasing estimated-GFR and increasing albuminuria in United States adults<sup>[43]</sup>.

A recent review suggests that NAFLD, and mainly NASH, is related with an increased and independent risk of developing CVD, T2DM, CKD, and colorectal cancers<sup>[44]</sup>. Finally, in a meta-analysis, which included 33 studies with 63902 participants, NAFLD was associated with an increased risk of prevalent (OR = 2.12, 95%CI: 1.69-2.66) and incident (HR = 1.79, 95%CI: 1.65-1.95) CKD<sup>[45]</sup>. NASH was related to a higher prevalence (OR = 2.53, 95%CI: 1.58-4.05) and incidence (HR = 2.12, 95%CI: 1.42-3.17) of CKD than simple steatosis<sup>[45]</sup>, and advanced fibrosis in NASH was associated with an even higher prevalence (OR = 5.20, 95%CI: 3.14-8.61) and incidence (HR = 3.29, 95%CI: 2.30-4.71) of CKD than NASH without advanced fibrosis<sup>[45]</sup>. These data suggest that the presence and the severity of NAFLD/NASH and advanced fibrosis are clearly related with an increased risk and the severity of CKD<sup>[45]</sup>.

Recent studies suggest the NAFLD/NASH is characterized by inflammation of the liver which may secrete proinflammatory, pro-fibrogenic, and anti-fibrinolytic substances, including fetuin-A, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and plasminogen activator inhibitor-1 (PAI-1), all causing kidney injury<sup>[46]</sup>. The above mentioned meta-analysis also found that even mild renal impairment may promote NAFLD generation, within a vicious cycle, with unfavourable CVD and metabolic consequences<sup>[45]</sup>, suggesting that CKD *per se* may contribute to the pathogenesis of NAFLD<sup>[45,47]</sup>. It has been shown in male Sprague-Dawley rats that nephrectomy results in a substantial dysregulation of hepatic fatty acid metabolism, steatohepatitis, IR, glucose and lipid metabolism aberrations, early even prior to glomerulosclerosis and

CKD development<sup>[47]</sup>.

Moreover, in liver transplant recipients the odds of developing CKD is high, while the risk of death in these patients increases exponentially when GFR is  $< 30$  mL/min per  $1.73 \text{ m}^2$  (HR = 2.67 and 5.47; 95%CI: 1.80-9.65, for stage 4 and 5, respectively)<sup>[48]</sup>. Given that available therapeutic options to reverse CKD are restricted, it should be emphasized that there is a need for hepatologists to diagnose CKD early in patients with chronic liver disease, mainly NAFLD, to optimize management aimed at delaying CKD progression<sup>[49]</sup>. In a large registry including 1120295 adults it was estimated that the adjusted HR for hospitalizations is high and for death in stage 5 CKD patients reaches 5.9 (95%CI: 5.4-6.5), while that for overt atherosclerotic CVD is 3.4 (95%CI: 3.1-3.8) compared with subjects with normal renal function<sup>[50]</sup>, suggesting a low quality of life and a reduced life expectancy<sup>[50]</sup>. It has also been shown that stage 3 or higher diabetic nephropathy is related to an annual mortality rate of 20%, similar only to that of cancer<sup>[51]</sup>. If a patient has T2DM and CKD plus NAFLD/NASH (clinical practice suggests that almost all patients with diabetic nephropathy have NAFLD) the CVD burden is even greater<sup>[45,52,53]</sup>.

Thus, NAFLD and CKD share some key cardiometabolic risk factors and it is suggested that they have common pathophysiological mechanisms; one can lead to the other and their co-existence leads to a geometrically increased CVD risk in patients that have both conditions, especially in those with metabolic disorders such as T2DM<sup>[37,39,45,52,53]</sup>. Given that there is available treatment of NAFLD<sup>[54-56]</sup> and CKD (up to stage 3)<sup>[57]</sup>, that also prevents CVD or its complications<sup>[58,59]</sup>, especially in patients with T2DM with increased CVD risk<sup>[60,61]</sup>, hepatologists, nephrologists, and cardiologists should place additional emphasis on the early diagnosis of both NAFLD and CKD<sup>[62,63]</sup>.

Another issue is that elevated serum uric acid (SUA) levels, related to renal function and T2DM<sup>[64-66]</sup>, may play a role in the pathogenesis of NAFLD. There seems to be a link between elevated SUA levels and MetS/NAFLD<sup>[67-69]</sup>. Given that NAFLD, CKD, and elevated SUA levels are implicated in increased CVD risk, attention should be given to SUA within the treatment of NAFLD<sup>[68-71]</sup>. It has been reported that some lipid and blood pressure lowering treatments that decrease CVD risk and improve/preserve renal function, while improving NAFLD, reduce SUA levels with their off-target effects<sup>[68-73]</sup>. These specific drugs should be preferred for the treatment of NAFLD risk factors, such as arterial hypertension or dyslipidaemia.

## NAFLD, ARTERIAL STIFFNESS AND CVD RISK

NAFLD was found to be an independent predictor

of faster progression of arterial stiffness (AS), even after adjusting for other CVD risk factors, thus further increasing CVD risk. Pulse Wave Velocity (PWV), a measure of AS, is independently associated with increased CVD risk across several patient groups and even in the general population, in both genders<sup>[74-76]</sup>. NAFLD is associated with AS, as evaluated by PWV, even in a non-obese, non-hypertensive, and non-diabetic young and middle-aged (Chinese) population<sup>[77]</sup>. NAFLD patients with severe liver fibrosis have the higher increase in AS<sup>[78]</sup>. Moreover, in patients with stage 3-5 CKD, PWV is much higher than in patients with CKD stage 1-2 and constitutes a major clinical determinant of CVD event rate and severity, independently of traditional CVD risk factors<sup>[79,80]</sup>. The pathogenesis of AS in NAFLD is not clear. One mechanism might be related to the systemic inflammation linked to NAFLD, and mainly NASH with fibrosis<sup>[78]</sup>, which seems to have an adverse effect on arterial compliance; high-sensitivity C-reactive protein (hsCRP) and pro-inflammatory cytokines may have adverse effects on the elastic properties of the wall of large arteries<sup>[81]</sup>. Arterial compliance was low in NAFLD patients with elevated hsCRP, while NAFLD with low hsCRP had no effect on arterial compliance<sup>[81]</sup>. Central obesity was a vital determinant for both increased AS and elevated hsCRP levels in NAFLD patients<sup>[81]</sup>. Another issue is whole blood viscosity (WBV), a predictor of CVD events<sup>[82]</sup>. WBV was shown to be increased in NAFLD and to be independently related with AS, even after adjusting for other CVD risk factors<sup>[82]</sup>. This association between WBV and AS has been shown in T2DM patients also, which comprise a great portion of NAFLD patients<sup>[83]</sup>. Thus, detection of abnormal WBV and AS should be performed early within risk stratification in NAFLD patients<sup>[82]</sup>.

There are data suggesting that the older theory on AS pathogenesis (AS is a result of large artery atherosclerosis) is not correct<sup>[84]</sup>. Stiff arteries suffer from arteriosclerosis, which is different from atherosclerosis, and this is also verified by the fact that there is little or no association between PWV and traditional CVD risk factors, except for age and arterial hypertension<sup>[84]</sup>. Additionally, PWV does not increase during the early stages of large artery atherosclerosis, but it is increased during the advanced atherosclerotic plaque period, probably due to arterial (aortic) calcification (AC)<sup>[84]</sup>. AC is a common complication of CKD and ESRD, and the extent of AC in the general population and in patients with CKD is predictive of subsequent CVD mortality beyond traditional CVD risk factors<sup>[85]</sup>. Thus, late atherosclerosis and CKD both promote AC and increase AS<sup>[84,85]</sup>.

There is evidence that lifestyle changes, antidiabetic drugs, inducible nitric oxide synthesis, antihypertensive agents [mainly inhibitors of the renin-angiotensin-aldosterone system (RAAS)], and statins improve arterial elasticity in patients with a wide spectrum of diseases, such as CVD, CKD, T2DM, MetS, obesity, primary biliary cirrhosis, NAFLD, heart failure

with preserved ejection fraction, arterial hypertension and dyslipidaemia<sup>[86-100]</sup>. Moreover, anti-inflammatory drugs, such as corticosteroids and anti-TNF- $\alpha$  therapy have been shown to improve arterial compliance in patients with chronic inflammatory conditions<sup>[101]</sup>. Overall a multifactorial approach appears to be the optimal solution for the management of increased AS due to NAFLD<sup>[26-31,89,90,93,97]</sup>. However, differences appear to exist within classes of agents, with some statins and RAAS inhibitors having a more favourable effects on AS<sup>[94-96,99,100]</sup>.

## NAFLD, STATINS (AND OTHER HYPOLIPIDAEMIC AGENTS) AND CVD RISK

### *Safety of statins in patients with NAFLD*

Data from the Third NHANES (1988-1994) that included 15676 subjects from the United States suggest that the prevalence of ALT elevation was high in the United States, ranging from 7%-15% according to race<sup>[102]</sup>. In the majority of those with increased ALT levels this could be attributed to NAFLD, in the absence of alcohol abuse, viral hepatitis or hemochromatosis<sup>[102]</sup>. Moreover, unexplained ALT elevation (61% of cases) was strongly associated with adiposity and other features of MetS<sup>[102]</sup>. NHANES data collected from 1988 to 2008 showed that the prevalence of major causes of chronic liver disease remained stable, except for NAFLD, which increased steadily, alongside with the increase of the prevalence of metabolic diseases (MetS, T2DM)<sup>[103]</sup>. Given the increasing rates of obesity, NAFLD prevalence is expected to contribute substantially to the increased burden of chronic liver disease (and CVD) in the United States<sup>[103]</sup>.

An observational study included 342 hyperlipidaemic patients with elevated transaminases who were prescribed a statin, 1437 hyperlipidaemic patients with normal transaminases who were prescribed a statin and 2245 patients with elevated liver enzymes but who were not prescribed a statin<sup>[104]</sup>. Patients with elevated transaminase levels were given atorvastatin or simvastatin at a median dose of 10 and 20 mg/d<sup>[104]</sup>. The incidence of further elevation in transaminase levels was similar in the two groups<sup>[104]</sup>. Among patients treated with a statin, the incidence of mild-moderate elevation in transaminase levels [ $< 10$  times the upper limit of normal (ULN) or  $< 10$  times the baseline transaminase levels] was higher in patients with elevated transaminases at baseline than in those with normal transaminase levels ( $n = 1437$ )<sup>[104]</sup>. However, the incidence of more marked elevation in transaminase levels ( $> 10$  times the ULN) did not differ between the two groups<sup>[104]</sup>. Another observational study ( $n = 3399$ ) in patients with elevated transaminase levels who were treated with lovastatin reported similar findings<sup>[105]</sup>. Moreover,  $>$

112000 person-years of 5 year-exposure in double-blind randomized trials comparing placebo and pravastatin (40 mg once daily) showed low risk for hepatotoxicity<sup>[106]</sup>.

### Effect of statins on NAFLD

In clinical practice up to 10 years ago it was “forbidden” to prescribe statins, even in high CVD risk patients, if they had a chronic liver disease, including NAFLD, and modestly elevated serum transaminases.

Eight years ago, given that there was no proven effective therapy for NAFLD, atorvastatin (10-80 mg/d) was tested in 25 NAFLD patients for the treatment of their dyslipidaemia; 22 patients completed the study<sup>[107]</sup>. After 6 mo of atorvastatin treatment, 8 patients (36.3%) had normal transaminase levels, while the remaining patients continued treatment for 12 more months<sup>[107]</sup>. During that period 20% of patients presented with normal transaminase levels, while the rest of the patients demonstrated a 10% reduction in baseline levels<sup>[107]</sup>. These results suggested that treatment with atorvastatin in NAFLD patients with dyslipidaemia resulted in a normalization of lipid profile and a significant reduction in serum ALT and this treatment was both effective and safe<sup>[107]</sup>.

Seven years ago a study evaluated the efficacy of atorvastatin (10 mg daily) for 24 mo in the treatment of 31 patients with biopsy proven NASH and dyslipidaemia<sup>[108]</sup>. Follow-up liver biopsy was performed in 17 patients<sup>[108]</sup>. After treatment, 23 patients (74.2%) had normal transaminase levels, adiponectin levels were noticeably increased, and the levels of TNF- $\alpha$  were significantly reduced<sup>[108]</sup>. These results suggest that atorvastatin may have acted *via* a reduction in markers of systemic inflammation, such as TNF- $\alpha$ , as well as increased adiponectin levels<sup>[108]</sup>, while another study suggested a beneficial effect of atorvastatin on NASH due to the reduction in serum levels of advanced glycosylated end products (AGEs), implicated in the pathogenesis of NASH<sup>[109]</sup>. However, 4/17 patients had progression of fibrosis in the repeat biopsy after 2-years and 3 of them progressed to stage 3<sup>[108]</sup>. The authors could not explain these divergent results and attributed them to sampling error, heterogeneity of the population, or untreated postprandial rise in triglyceride (TG) levels<sup>[108]</sup>.

Six years ago a pilot study with 16 NASH patients showed that monotherapy with simvastatin does not seem to be an effective treatment for NASH<sup>[110]</sup>. From the 16 patients with biopsy proven NASH, 14 completed the study and 10 underwent follow-up biopsy after one year. Although there was a 26% low-density lipoprotein cholesterol (LDL-C) reduction in the simvastatin group as compared with placebo, there was no significant improvement in serum transaminases, hepatic steatosis, necroinflammatory activity or stage of fibrosis<sup>[110]</sup>.

Five years ago, the *post hoc* analysis of the Greek

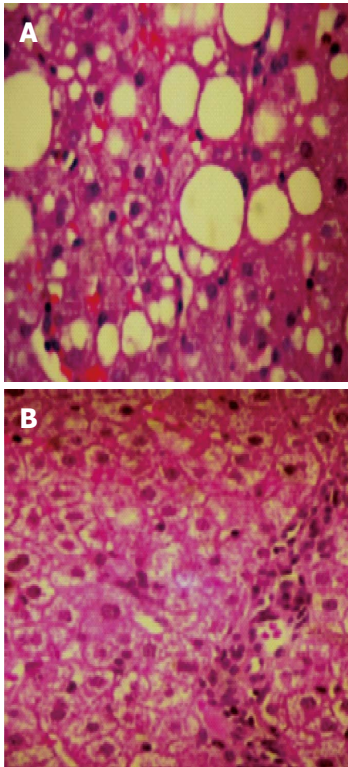
Atorvastatin and Coronary Heart Disease Evaluation (GREACE) survival study ( $n = 1600$ ; 437 patients had moderately abnormal liver tests at baseline probably due to NAFLD as indicated by liver ultrasonography and after exclusion of other liver diseases)<sup>[111]</sup> showed that 227 participants who were treated with statins (mainly atorvastatin, mean dose 24 mg/d) had a substantial improvement in liver tests, ALT, aspartate aminotransferase (AST), and GGT ( $P < 0.0001$ ), whereas 210 not treated with a statin had a further increase of liver enzyme concentrations<sup>[111]</sup>. Statin treatment was safe in patients with CVD and NAFLD; only 1% discontinued treatment<sup>[110]</sup>. Thus, atorvastatin did not have any adverse effect on liver enzymes; on the contrary, it reduced them substantially and improved liver ultrasonography within the 3-year duration of the study<sup>[111]</sup>.

Four years ago a study with pitavastatin (2 mg/d for 12 mo) in 20 patients with biopsy-proven NASH with dyslipidaemia was reported<sup>[112]</sup>. Liver enzymes and lipid profile were significantly improved, however NAFLD/NASH activity score and fibrosis stage did not change significantly in all patients (they improved in 54% and 42%, respectively) and 3 of the 13 patients with a repeat biopsy had progression of fibrosis during the treatment<sup>[112]</sup>.

Three years ago a study included 42 biopsy-proven NASH patients treated with atorvastatin 10 mg/d for 12 mo. Atorvastatin significantly decreased liver transaminase, GGT, LDL-C, TGs, type IV collagen, and TNF- $\alpha$  levels, while it improved NAFLD activity score and increased liver to spleen density ratio<sup>[113]</sup>.

During the same year a prospective study investigated the effect 2.5 mg/d rosuvastatin for 24 mo in 19 patients with biopsy-proven NASH with dyslipidaemia<sup>[114]</sup>. Transaminase levels, relatively low at the beginning, were not significantly changed during the treatment, while the lipid profile was significantly improved<sup>[114]</sup>. At the same time NAFLD activity score and fibrotic stage did not change significantly in all patients; they were improved in 33% and 33% of patients, and remained stable in 33% and 56% of patients, respectively, while 1 of 9 patients had progression of fibrosis during rosuvastatin treatment<sup>[114]</sup>. This result was attributed to the very low dose of rosuvastatin administered (2.5 mg/d)<sup>[114]</sup>. During the same year a *post hoc* analysis of the survival study Assessing the Treatment Effect in Metabolic Syndrome Without Perceptible Diabetes (ATTEMPT)<sup>[115]</sup> with an atorvastatin- (34 or 24 mg/d) based multifactorial treatment approach in patients and MetS and NAFLD showed that attaining multiple treatment targets is safe and beneficial in primary prevention patients with MetS and NAFLD<sup>[115]</sup>. Lipid levels and liver enzymes were normalized and ultrasonographic evidence of NAFLD resolved during the 42 mo duration of the study in both intensive (mean dose 34 mg/d) and standard (mean dose 24 mg/d) atorvastatin treatment groups<sup>[115]</sup>.





**Figure 2** Liver biopsy of a patients with non-alcoholic steatohepatitis at baseline (A) and one year after 10 mg/d of rosuvastatin monotherapy (B) [unpublished data from personal archive].

In 2013 the *post hoc* analysis of the Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) trial ( $n = 8863$ ) showed that high dose atorvastatin treatment (80 mg) in 1081 (12.2%) patients, who had an ALT  $\geq$  ULN resulted in normalisation of ALT values<sup>[116]</sup>. The higher the atorvastatin dose (80 mg/d) the greater the reduction in liver enzyme levels<sup>[116]</sup>.

In 2014 a pilot study ( $n = 6$ ) with 10 mg/d of rosuvastatin monotherapy in biopsy proven NASH patients with MetS, showed within one year of treatment a normalisation of lipid profile, all liver enzymes, and complete resolution of NASH in the repeat biopsy (fibrosis, necroinflammation, ballooning, and steatosis were totally absent and histology revealed a normal liver tissue) in 5 out of 6 patients<sup>[117]</sup>. In 2015 we completed the study, for which the pilot was designed (unpublished data), with 20 biopsy proven (repeat biopsy after 12 mo of treatment) NASH patients with MetS treated with rosuvastatin 10 mg/d as monotherapy. The results remained as impressive as in the pilot study (Figure 2) and this study confirmed that the patients did not have MetS any longer, due to the reduction in TGs, the increase in high density lipoprotein cholesterol (HDL-C), and a paradoxical (substantial by 20 mg/dL) reduction in fasting plasma glucose. Waist circumference and body mass index did not change, thus, the improvement could not be attributed to reduction of (abdominal) obesity.

As stated in the above data there has been a gradual and hesitant attempt during the last 8 years to investigate the efficacy of statins in the treatment NAFLD/NASH. The results of these studies suggest that the effect of statins on NAFLD/NASH is intensity of compound and dose dependent<sup>[107-117]</sup>. The *post hoc* analyses of GREACE, ATTEMPT and IDEAL studies are hypothesis generating and there is a need for randomized controlled prospective studies on this issue. However, with all statins, except rosuvastatin, out of patent no pharmaceutical company is eager to finance a prospective study on this issue. This has to be done by independent researchers. We did our part, let others continue. In any case, we all know that several years are needed in order for guidelines to state a new indication for a drug. However, the information provided may help clinicians make decisions for patients with a highly prevalent disease.

From all the above the conclusion with practical implications was described by a Hepatologist: "Yes! Statins can be given to liver patients"<sup>[118]</sup>.

#### **Effect of other hypolipidaemic drugs on NAFLD/NASH**

Ezetimibe can be added to statin treatment in patients who cannot achieve LDL-C targets despite treatment with the maximal tolerated dose of a high intensity statin<sup>[119]</sup>. The effect of ezetimibe on NAFLD was studied in a few, mainly uncontrolled studies with rather small number of patients. Serum ALT levels significantly decreased within 6 mo and in 4 patients levels reached the normal range ( $< 30$  U/L), which was accompanied with at least a 10% decrease in serum total cholesterol and LDL-C. However, ezetimibe had no effect on liver steatosis as assessed with ultrasonography<sup>[120]</sup>. In another uncontrolled study in 10 patients with NASH, treatment with ezetimibe for 6 mo<sup>[121]</sup>, NASH score and steatosis grade were also significantly improved in the repeat liver biopsy. The fibrosis stage did not change significantly, but 6 of the 10 patients exhibited an improvement in their fibrosis stage<sup>[121]</sup>. In another uncontrolled study 45 patients with liver biopsy-proven NAFLD were treated with ezetimibe for 24 mo<sup>[122]</sup>. Histological features of steatosis and necroinflammation improved, but fibrosis stage was not significantly changed<sup>[122]</sup>. In a recent randomized controlled study in NAFLD patients (16 on ezetimibe and 12 controls), ezetimibe improved hepatic fibrosis but increased hepatic long-chain fatty acids and HbA1c in NAFLD patients<sup>[123]</sup>. Thus, it is not clear yet which is the long term effect of ezetimibe on NAFLD/NASH (Table 1).

Omega-3 fatty acid supplementation has also been used for the treatment of NAFLD/NASH. Omega-3 fatty acid administration is safe and effective for NAFLD patients with dyslipidaemia and can improve their ALT, serum lipid (mainly TG) levels and normalize the ultrasonographic image of the liver<sup>[124]</sup>. A recent meta-analysis, that included 9 studies (5 randomized



**Table 1 Studies evaluating the effect of hypolipidaemic drug treatment on non-alcoholic fatty liver disease or non-alcoholic steatohepatitis**

Ref.	Year	<i>n</i> of all	<i>n</i> with NAFLD/NASH	Method of diagnosis of NAFLD/NASH	Drug	Dose/d	Duration of treatment	Results-main findings
Gómez-Domínguez <i>et al</i> <sup>[107]</sup>	2006	25	22	ALT levels	Atorvastatin	10-80 mg	12 mo	Normalization of ALT and lipid profile
Hyogo <i>et al</i> <sup>[108]</sup>	2008	31	31	Biopsy-proven NASH	Atorvastatin	10 mg	24 mo	Liver steatosis and NAFLD activity score were significantly improved; in 4 increase in fibrosis stage.
Kimura <i>et al</i> <sup>[109]</sup>	2010	45	45	Biopsy-proven NASH	Atorvastatin	10 mg	12 mo	The steatosis grade and NAFLD activity score were significantly improved.
Nelson <i>et al</i> <sup>[110]</sup>	2009	16	10	Biopsy-proven NASH	Simvastatin	20 mg	12 mo	No statistically significant improvement in serum ALT, hepatic steatosis, necroinflammatory activity or stage of fibrosis within or between groups.
Athyros <i>et al</i> <sup>[111]</sup>	2010	1600	437	ALT levels -ultrasonography	Atorvastatin	24 mg	3 yr	Improved ALT, AST, GGT, AP, and ultrasonography. Reduced cardiovascular events more than those without NAFLD ( <i>P</i> = 0.007).
Hyogo <i>et al</i> <sup>[112]</sup>	2011	20	13	Biopsy-proven NASH	Pitavastatin	2 mg	12 mo	Improved NAFLD activity score and fibrosis stage. In 54% and 42%, respectively, improved histology, however, 3/13 patients progression of fibrosis.
Hyogo <i>et al</i> <sup>[113]</sup>	2012	42	42	Biopsy-proven NASH	Atorvastatin	10 mg	12 mo	Improved NAFLD activity score, normalization of liver enzymes and TNF- $\alpha$ , and increased liver to spleen density ratio.
Nakahara <i>et al</i> <sup>[114]</sup>	2012	19	19	Biopsy-proven NASH	Rosuvastatin	2.5 mg	24 mo	NAFLD activity score and fibrotic stage did not change significantly in all patients, they were improved in 33.3% and 33.3% of patients, and stayed stable in 33.3% and 55.6%, respectively.
Athyros <i>et al</i> <sup>[115]</sup>	2011	1123	326	ALT levels -ultrasonography	Atorvastatin	20-30 mg	42 mo	Improved ALT, AST, GGT, AP, and ultrasonography. Eradicated cardiovascular events in high dose group.
Tikkanen <i>et al</i> <sup>[116]</sup>	2013	8863	1081	ALT levels	Atorvastatin Simvastatin	80 mg 20-40 mg	5 yr	Normalization of liver enzyme values and greater CVD benefit with atorvastatin in the elevated ALT group.
Kargiotis <i>et al</i> <sup>[117]</sup>	2014	6	6	Biopsy-proven NASH	Rosuvastatin Monotherapy	10 mg	12 mo	Complete resolution of NASH in 5 patients. Normalization of liver enzyme values in all patients.
Park <i>et al</i> <sup>[122]</sup>	2011	45	45	Biopsy-proven NASH	Ezetimibe	10 mg	24 mo	Steatosis grade, necroinflammatory grade, ballooning, and NAFLD activity score were significantly improved from baseline. Fibrosis stage was not significantly changed.
Zhu <i>et al</i> <sup>[124]</sup>	2008	72	72	ALT levels -ultrasonography	n-3 PUFA	6 g	6 mo	ALT was reduced but other liver enzymes not. Ultrasonography improved in 53% of patients

NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; AP: Alkaline phosphatase; CVD: Cardiovascular disease; GGT: Gamma-glutamyltransferase.

controlled trials- RCTs), involving 355 NAFLD patients, tested omega-3 vs control treatment for a period from 8 wk to 12 mo<sup>[125]</sup>. Results showed that omega-3 fatty acids were beneficial vs placebo in reducing liver fat and decreasing AST, but not ALT activity<sup>[125]</sup>. Sub-analyses of only the 5 RCTs showed a significant benefit for omega-3 fatty acids vs control on liver fat, but not for ALT or AST<sup>[125]</sup>. The pooled data suggest that omega-3 polyunsaturated fatty acid (PUFA) supplementation may decrease liver fat, however, the optimal dose could not be found<sup>[125]</sup>.

Recently, several high quality reviews on NAFLD/NASH and CVD risk have been published<sup>[126-129]</sup>. These

recent reviews focused on the relation of NAFLD/NASH or other ectopic fat deposition with CVD risk or even more specific with the risk of arrhythmic complications in these patients, focusing mainly to pathophysiological mechanisms and dedicated a rather small portion to treatment<sup>[126-129]</sup>. In contrast, our review focused on specific NASH characteristics (degree of liver fibrosis and CVD risk) or confounding factors (NAFLD-chronic kidney disease and CVD risk as well as NAFLD-arterial stiffness and CVD risk) that could all be treated with a single drug, a statin. However, given that improvement of NAFLD/NASH with concomitant reduction in CVD risk may not be a drug class effect, we had to analyze the

effect of specific regimens at specific doses for specific treatment periods on the progression, amelioration or even reversal of NAFLD/NASH. This is the main difference between our review and the excellent recent analyses on this issue mentioned above.

### Effect of statins on CVD morbidity and mortality in NAFLD patients

There are no prospective RCTs that investigated the effect of statins on CVD morbidity and mortality in NAFLD/NASH patients. Existing data come from *post hoc* analyses of 3 survival trials: GREACE<sup>[111]</sup>, ATTEMPT<sup>[115]</sup>, and IDEAL<sup>[116]</sup>.

The GREACE *post hoc* analysis showed that in patients with mild to moderate elevations of serum transaminases, most probably due to NAFLD as indicated by liver ultrasonography, in the absence of alcohol abuse history and the exclusion of other liver diseases, 24 mg/d of atorvastatin induced substantial reductions in CVD events (68% vs usual care) during the 3-year follow-up period compared with the participants with CHD and normal liver enzymes (39% vs usual care);  $P = 0.007^{[111]}$ . This confirmed the pattern of the higher the CVD risk, the greater the benefit from effective therapy. Moreover, the study results showed that those patients with overt CVD and NAFLD that were at very high risk for recurrent CVD events or CVD death were those who were deprived of statin therapy, up to 10 years ago, because the fear of a transaminase increase with a statin.

The ATTEMPT study was a primary prevention study in patients with MetS without overt CVD or T2DM and its *post hoc* analysis investigated the effect of atorvastatin-based multifactorial intervention on ultrasonography verified NAFLD<sup>[115]</sup>. There were no CVD events in patients with LDL-C levels < 100 mg/dL (atorvastatin dose 34 mg/dL) for the 42 mo of the duration of the study, while there were 5 non-fatal events occurring in the lower atorvastatin dose (24 mg/d) group (log-rank- $P = 0.024$ ).

The IDEAL *post hoc* analysis showed a substantially reduced 5-year CVD event rate with atorvastatin 80 mg/d in patients with elevated transaminases levels (probably due to NAFLD) as compared with 20-40 mg of simvastatin, a less effective dose of a less effective hypolipidaemic agent (11.5% for simvastatin and 6.5% for atorvastatin, HR = 0.556; 95%CI: 0.367-0.842;  $P = 0.0056^{[116]}$ ). This totally confirmed the findings of GREACE and ATTEMPT studies.

Thus, it seems that statin treatment is safe in NAFLD/NASH patients<sup>[102-105]</sup>, may contribute to the normalization of liver function and structure<sup>[107-117]</sup>, and reduces CVD morbidity and mortality in these high CVD risk patients<sup>[111,115,116]</sup>.

## CONCLUSION

NAFLD has been considered as an independent CVD

risk factor. However, it seems that simple steatosis is not related with higher total, CVD or liver-related mortality compared with the general population. In contrast, increasing values of liver fibrosis scores (in NASH patients) is linked to a progressive increase in total mortality by approximately 70% compared with subjects without fibrosis. The increase in overall mortality was mainly due to a higher CVD mortality. Besides fibrosis, the development of CKD substantially increases total and CVD mortality. NAFLD/NASH is considered to be a risk factor for CKD and CKD contributes to NAFLD pathogenesis within a vicious cycle. Liver and kidney disease progress in parallel substantially reducing life expectancy. NAFLD/NASH increase AS which is independently associated with increased CVD risk across many different patient groups and even in the general population. Early detection of NAFLD/NASH, advanced fibrosis, CKD and increased AS could lead to the treatment of this cluster of CVD risk factors with lifestyle measures and multifactorial drug intervention, mainly based on high intensity statins at moderate to high doses. These seem to be safe, resolve NAFLD/NASH and could contribute to the primary or secondary prevention of CVD<sup>[118,119,130,131]</sup>.

## REFERENCES

- 1 Baran B, Akyüz F. Non-alcoholic fatty liver disease: what has changed in the treatment since the beginning? *World J Gastroenterol* 2014; **20**: 14219-14229 [PMID: 25339808 DOI: 10.3748/wjg.v20.i39.14219]
- 2 Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]
- 3 Jiang CM, Pu CW, Hou YH, Chen Z, Alanazy M, Hebbard L. Non alcoholic steatohepatitis a precursor for hepatocellular carcinoma development. *World J Gastroenterol* 2014; **20**: 16464-16473 [PMID: 25469014 DOI: 10.3748/wjg.v20.i44.16464]
- 4 Eguchi A, Povero D, Alkhouri N, Feldstein AE. Novel therapeutic targets for nonalcoholic fatty liver disease. *Expert Opin Ther Targets* 2013; **17**: 773-779 [PMID: 23600493 DOI: 10.1517/14728222.2013.789502]
- 5 Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. *J Hepatol* 2006; **45**: 600-606 [PMID: 16899321]
- 6 Fargion S, Porzio M, Fracanzani AL. Nonalcoholic fatty liver disease and vascular disease: state-of-the-art. *World J Gastroenterol* 2014; **20**: 13306-13324 [PMID: 25309067 DOI: 10.3748/wjg.v20.i37.13306]
- 7 Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology* 2013; **57**: 1357-1365 [PMID: 23175136 DOI: 10.1002/hep.26156]
- 8 Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2011; **33**: 525-540 [PMID: 21198708 DOI: 10.1111/j.1365-2036.2010.04556.x]
- 9 Takaki A, Kawai D, Yamamoto K. Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int J Mol Sci* 2013; **14**: 20704-20728 [PMID: 24132155 DOI: 10.3390/ijms141020704]

- 10 **Farrell GC**, van Rooyen D, Gan L, Chitturi S. NASH is an Inflammatory Disorder: Pathogenic, Prognostic and Therapeutic Implications. *Gut Liver* 2012; **6**: 149-171 [PMID: 22570745 DOI: 10.5009/gnl.2012.6.2.149]
- 11 **Dam-Larsen S**, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sørensen TI, Becker U, Bendtsen F. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut* 2004; **53**: 750-755 [PMID: 15082596 DOI: 10.1136/gut.2003.019984]
- 12 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]
- 13 **Söderberg C**, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, Hulcrantz R. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010; **51**: 595-602 [PMID: 20014114 DOI: 10.1002/hep.23314]
- 14 **Day CP**. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? *Gut* 2002; **50**: 585-588 [PMID: 11950797]
- 15 **James OF**, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol* 1998; **29**: 495-501 [PMID: 9765002]
- 16 **Haflidadottir S**, Jonasson JG, Norland H, Einarsdottir SO, Kleiner DE, Lund SH, Björnsson ES. Long-term follow-up and liver-related death rate in patients with non-alcoholic and alcoholic related fatty liver disease. *BMC Gastroenterol* 2014; **14**: 166 [PMID: 25260964 DOI: 10.1186/1471-230X-14-166]
- 17 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]
- 18 **Angulo P**, Bugianesi E, Björnsson ES, Charatcharoenwithaya P, Mills PR, Barrera F, Haflidadottir S, Day CP, George J. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 782-9.e4 [PMID: 23860502 DOI: 10.1053/j.gastro.2013.06.057]
- 19 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Thorneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]
- 20 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ; Nash Clinical Research Network. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]
- 21 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]
- 22 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]
- 23 **Liu H**, Lu HY. Nonalcoholic fatty liver disease and cardiovascular disease. *World J Gastroenterol* 2014; **20**: 8407-8415 [PMID: 25024598 DOI: 10.3748/wjg.v20.i26.8407]
- 24 **Luo J**, Xu L, Li J, Zhao S. Nonalcoholic fatty liver disease as a potential risk factor of cardiovascular disease. *Eur J Gastroenterol Hepatol* 2015; **27**: 193-199 [PMID: 25563143 DOI: 10.1097/MEG.0000000000000254]
- 25 **Takahashi Y**, Kurosaki M, Tamaki N, Yasui Y, Hosokawa T, Tsuchiya K, Nakanishi H, Itakura J, Izumi N. Non-alcoholic fatty liver disease fibrosis score and FIB-4 scoring system could identify patients at risk of systemic complications. *Hepatol Res* 2014; Epub ahead of print [PMID: 25145976 DOI: 10.1111/hepr.12405]
- 26 **Athyros VG**, Elisaf MS, Alexandrides T, Achimastos A, Ganotakis E, Bilianou E, Karagiannis A, Liberopoulos EN, Tziomalos K, Mikhailidis DP; Assessing the Treatment Effect in Metabolic Syndrome Without Perceptible Diabetes (ATTEMPT) Collaborative Group. Long-term impact of multifactorial treatment on new-onset diabetes and related cardiovascular events in metabolic syndrome: a post hoc ATTEMPT analysis. *Angiology* 2012; **63**: 358-366 [PMID: 22007026 DOI: 10.1177/0003319711421341]
- 27 **Doycheva I**, Patel N, Peterson M, Loomba R. Prognostic implication of liver histology in patients with nonalcoholic fatty liver disease in diabetes. *J Diabetes Complications* 2013; **27**: 293-300 [PMID: 23312215 DOI: 10.1016/j.jdiacomp.2012.10.008]
- 28 **Athyros VG**, Hatzitolios A, Karagiannis A, Didangelos TP, Iliadis F, Dolgyras S, Vosnakidis T, Vasiliadis P, Malias I, Tziomalos K, Samouilidou M, Mikhailidis DP; INDEED Collaborative Group. Initiative for a new diabetes therapeutic approach in a Mediterranean country: the INDEED study. *Curr Med Res Opin* 2009; **25**: 1931-1940 [PMID: 19558210 DOI: 10.1185/03007990903073035]
- 29 **Katsiki N**, Athyros VG, Karagiannis A, Mikhailidis DP. The role of statins in the treatment of type 2 diabetes mellitus: an update. *Curr Pharm Des* 2014; **20**: 3665-3674 [PMID: 24040875 DOI: 10.2174/13816128113196660673]
- 30 **Tziomalos K**, Athyros VG, Karagiannis A. Non-alcoholic fatty liver disease in type 2 diabetes: pathogenesis and treatment options. *Curr Vasc Pharmacol* 2012; **10**: 162-172 [PMID: 22239625 DOI: 10.2174/157016112799305012]
- 31 **Duvnjak M**, Lerotić I, Barsić N, Tomasić V, Virović Jukić L, Velagić V. Pathogenesis and management issues for non-alcoholic fatty liver disease. *World J Gastroenterol* 2007; **13**: 4539-4550 [PMID: 17729403 DOI: 10.3748/wjg.v13.i34.4539]
- 32 **Dyson JK**, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. *Frontline Gastroenterol* 2014; **5**: 211-218 [PMID: 25018867 DOI: 10.1136/flgastro-2013-100403]
- 33 **Athyros VG**, Katsiki N, Karagiannis A. Comment on: Novel therapeutic targets for non-alcoholic fatty liver disease. *Expert Opin Ther Targets* 2013; **17**: 861-862 [PMID: 23763554 DOI: 10.1517/14728222.2013.811024]
- 34 **Levey AS**, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G; National Kidney Foundation. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003; **139**: 137-147 [PMID: 12859163 DOI: 10.7326/0003-4819-139-2-200307150-00013]
- 35 **Locatelli F**, Pozzoni P, Tentori F, del Vecchio L. Epidemiology of cardiovascular risk in patients with chronic kidney disease. *Nephrol Dial Transplant* 2003; **18** Suppl 7: vii2-vii9 [PMID: 12953023 DOI: 10.1093/ndt/gfg1072]
- 36 **Iseki K**. Chronic kidney disease in Japan. *Intern Med* 2008; **47**: 681-689 [PMID: 18421182 DOI: 10.2169/internalmedicine.47.0906]
- 37 **Orlić L**, Mikolasevic I, Bagic Z, Racki S, Stimac D, Milic S. Chronic kidney disease and nonalcoholic Fatty liver disease-is there a link? *Gastroenterol Res Pract* 2014; **2014**: 847539 [PMID: 24729784 DOI: 10.1155/2014/847539]
- 38 **Vanholder R**, Massy Z, Argiles A, Spasovski G, Verbeke F, Lameire N; European Uremic Toxin Work Group. Chronic kidney disease as cause of cardiovascular morbidity and mortality. *Nephrol Dial Transplant* 2005; **20**: 1048-1056 [PMID: 15814534 DOI: 10.1093/ndt/gfh813]
- 39 **Targher G**, Chonchol M, Zoppini G, Abaterusso C, Bonora E. Risk of chronic kidney disease in patients with non-alcoholic fatty liver disease: is there a link? *J Hepatol* 2011; **54**: 1020-1029 [PMID: 21145850 DOI: 10.1016/j.jhep.2010.11.007]
- 40 **Targher G**, Bertolini L, Rodella S, Zoppini G, Lippi G, Day C, Muggeo M. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic

- patients. *Diabetologia* 2008; **51**: 444-450 [PMID: 18058083 DOI: 10.1007/s00125-007-0897-4]
- 41 **Hwang ST**, Cho YK, Yun JW, Park JH, Kim HJ, Park DI, Sohn CI, Jeon WK, Kim BI, Rhee EJ, Oh KW, Lee WY, Jin W. Impact of non-alcoholic fatty liver disease on microalbuminuria in patients with prediabetes and diabetes. *Intern Med J* 2010; **40**: 437-442 [PMID: 19460054 DOI: 10.1111/j.1445-5994.2009.01979.x]
- 42 **Targher G**, Kendrick J, Smits G, Chonchol M. Relationship between serum gamma-glutamyltransferase and chronic kidney disease in the United States adult population. Findings from the National Health and Nutrition Examination Survey 2001-2006. *Nutr Metab Cardiovasc Dis* 2010; **20**: 583-590 [PMID: 19699624 DOI: 10.1016/j.numecd.2009.05.012]
- 43 **Targher G**, Bosworth C, Kendrick J, Smits G, Lippi G, Chonchol M. Relationship of serum bilirubin concentrations to kidney function and albuminuria in the United States adult population. Findings from the National Health and Nutrition Examination Survey 2001-2006. *Clin Chem Lab Med* 2009; **47**: 1055-1062 [PMID: 19634983 DOI: 10.1515/CCLM.2009.244]
- 44 **Armstrong MJ**, Adams LA, Canbay A, Syn WK. Extrahepatic complications of nonalcoholic fatty liver disease. *Hepatology* 2014; **59**: 1174-1197 [PMID: 24002776 DOI: 10.1002/hep.26717]
- 45 **Musso G**, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, Hultcrantz R, Hagström H, Yoon SK, Charatcharoenwitthaya P, George J, Barrera F, Hafliðadóttir S, Björnsson ES, Armstrong MJ, Hopkins LJ, Gao X, Francque S, Verrijken A, Yilmaz Y, Lindor KD, Charlton M, Haring R, Lerch MM, Rettig R, Völzke H, Ryu S, Li G, Wong LL, Machado M, Cortez-Pinto H, Yasui K, Cassader M. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. *PLoS Med* 2014; **11**: e1001680 [PMID: 25050550 DOI: 10.1371/journal.pmed.1001680]
- 46 **Dogru T**, Genc H, Tapan S, Aslan F, Ercin CN, Ors F, Kara M, Sertoglu E, Karslioglu Y, Bagci S, Kurt I, Sonmez A. Plasma fetuin-A is associated with endothelial dysfunction and subclinical atherosclerosis in subjects with nonalcoholic fatty liver disease. *Clin Endocrinol (Oxf)* 2013; **78**: 712-717 [PMID: 22676641 DOI: 10.1111/j.1365-2265.2012.04460.x]
- 47 **Jin K**, Norris K, Vaziri ND. Dysregulation of hepatic fatty acid metabolism in chronic kidney disease. *Nephrol Dial Transplant* 2013; **28**: 313-320 [PMID: 23045433 DOI: 10.1093/ndt/gfs350]
- 48 **Allen AM**, Kim WR, Therneau TM, Larson JJ, Heimbach JK, Rule AD. Chronic kidney disease and associated mortality after liver transplantation—a time-dependent analysis using measured glomerular filtration rate. *J Hepatol* 2014; **61**: 286-292 [PMID: 24713190 DOI: 10.1016/j.jhep.2014.03.034]
- 49 **Musso G**, Tabibian JH, Charlton M. Chronic kidney disease (CKD) and NAFLD: time for awareness and screening. *J Hepatol* 2015; **62**: 983-984 [PMID: 25529627 DOI: 10.1016/j.jhep.2014.11.044]
- 50 **Go AS**, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**: 1296-1305 [PMID: 15385656 DOI: 10.1056/NEJMoa041031]
- 51 **Foley RN**, Murray AM, Li S, Herzog CA, McBean AM, Eggers PW, Collins AJ. Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999. *J Am Soc Nephrol* 2005; **16**: 489-495 [PMID: 15590763 DOI: 10.1681/ASN.2004030203]
- 52 **Lai YC**, Cheng BC, Hwang JC, Lee YT, Chiu CH, Kuo LC, Chen JB. Association of fatty liver disease with nonfatal cardiovascular events in patients undergoing maintenance hemodialysis. *Nephron Clin Pract* 2013; **124**: 218-223 [PMID: 24503573 DOI: 10.1159/000357952]
- 53 **Targher G**, Byrne CD. Clinical Review: Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab* 2013; **98**: 483-495 [PMID: 23293330 DOI: 10.1210/jc.2012-3093]
- 54 **Athyros VG**, Katsiki N, Karagiannis A, Mikhailidis DP. Statins and nonalcoholic fatty liver disease: a bright future? *Expert Opin Investig Drugs* 2013; **22**: 1089-1093 [PMID: 23889731 DOI: 10.1517/13543784.2013.824423]
- 55 **Athyros VG**, Katsiki N, Karagiannis A, Mikhailidis DP. Are statins 'IDEAL' for non-alcoholic fatty liver disease? *Curr Med Res Opin* 2014; **30**: 229-231 [PMID: 24127654 DOI: 10.1185/03007995.2013.855192]
- 56 **Kostapanos MS**, Athyros VG, Karagiannis A, Mikhailidis DP. Mechanisms linking nonalcoholic fatty liver disease with coronary artery disease. *Dig Dis Sci* 2012; **57**: 1109 [PMID: 22311368 DOI: 10.1007/s10620-012-2066-y]
- 57 **Athyros VG**, Mikhailidis DP, Papageorgiou AA, Symeonidis AN, Pehlivanidis AN, Bouloukos VI, Elisaf M. The effect of statins versus untreated dyslipidaemia on renal function in patients with coronary heart disease. A subgroup analysis of the Greek atorvastatin and coronary heart disease evaluation (GREACE) study. *J Clin Pathol* 2004; **57**: 728-734 [PMID: 15220366 DOI: 10.1136/jcp.2003.012989]
- 58 **Athyros VG**, Papageorgiou AA, Mercouris BR, Athyrou VV, Symeonidis AN, Basayannis EO, Demetriadis DS, Kontopoulos AG. Treatment with atorvastatin to the National Cholesterol Educational Program goal versus 'usual' care in secondary coronary heart disease prevention. The GREEK Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study. *Curr Med Res Opin* 2002; **18**: 220-228 [PMID: 12201623 DOI: 10.1185/030079902125000787]
- 59 **Athyros VG**, Papageorgiou AA, Elisaf M, Mikhailidis DP; GREACE Study Collaborative Group. Statins and renal function in patients with diabetes mellitus. *Curr Med Res Opin* 2003; **19**: 615-617 [PMID: 14606984 DOI: 10.1185/030079903125002315]
- 60 **Zoppini G**, Fedeli U, Gennaro N, Saugo M, Targher G, Bonora E. Mortality from chronic liver diseases in diabetes. *Am J Gastroenterol* 2014; **109**: 1020-1025 [PMID: 24890439 DOI: 10.1038/ajg.2014.132]
- 61 **Athyros VG**, Papageorgiou AA, Symeonidis AN, Didangelos TP, Pehlivanidis AN, Bouloukos VI, Mikhailidis DP; GREACE Study Collaborative Group. Early benefit from structured care with atorvastatin in patients with coronary heart disease and diabetes mellitus. *Angiology* 2003; **54**: 679-690 [PMID: 14666956 DOI: 10.1177/000331970305400607]
- 62 **Lonardo A**, Ballestri S, Targher G, Loria P. Diagnosis and management of cardiovascular risk in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 629-650 [PMID: 25327387 DOI: 10.1586/17474124.2015.965143]
- 63 **Targher G**, Chonchol MB, Byrne CD. CKD and nonalcoholic fatty liver disease. *Am J Kidney Dis* 2014; **64**: 638-652 [PMID: 25085644 DOI: 10.1053/j.ajkd.2014.05.019]
- 64 **Athyros VG**, Mikhailidis DP. Uric acid, chronic kidney disease and type 2 diabetes: a cluster of vascular risk factors. *J Diabetes Complications* 2014; **28**: 122-123 [PMID: 24388550 DOI: 10.1016/j.jdiacomp.2013.11.012]
- 65 **Katsiki N**, Karagiannis A, Athyros VG, Mikhailidis DP. Hyperuricaemia: more than just a cause of gout? *J Cardiovasc Med (Hagerstown)* 2013; **14**: 397-402 [PMID: 23032963 DOI: 10.2459/JCM.0b013e3283595adc]
- 66 **Athyros VG**, Karagiannis A, Ganotakis ES, Paletas K, Nicolaou V, Bacharoudis G, Tziomalos K, Alexandrides T, Liberopoulos EN, Mikhailidis DP; Assessing The Treatment Effect in Metabolic syndrome without Perceptible diabeTes (ATTEMPT) Collaborative Group. Association between the changes in renal function and serum uric acid levels during multifactorial intervention and clinical outcome in patients with metabolic syndrome. A post hoc analysis of the ATTEMPT study. *Curr Med Res Opin* 2011; **27**: 1659-1668 [PMID: 21714711 DOI: 10.1185/03007995.2011.595782]
- 67 **Cardoso AS**, Gonzaga NC, Medeiros CC, Carvalho DF. Association of uric acid levels with components of metabolic syndrome and non-alcoholic fatty liver disease in overweight or obese children and adolescents. *J Pediatr (Rio J)* 2013; **89**: 412-418 [PMID: 23791233 DOI: 10.1016/j.jped.2012.12.008]
- 68 **Tsouli SG**, Liberopoulos EN, Mikhailidis DP, Athyros VG, Elisaf MS. Elevated serum uric acid levels in metabolic syndrome: an



- active component or an innocent bystander? *Metabolism* 2006; **55**: 1293-1301 [PMID: 16979398]
- 69 **Katsiki N**, Doumas M, Athyros VG, Karagiannis A. Hyperuricemia as a risk factor for cardiovascular disease. *Expert Rev Cardiovasc Ther* 2015; **13**: 19-20 [PMID: 25428565 DOI: 10.1586/14779072.2015.987129]
- 70 **Athyros VG**, Mikhailidis DP, Liberopoulos EN, Kakafika AI, Karagiannis A, Papageorgiou AA, Tziomalos K, Ganotakis ES, Elisaf M. Effect of statin treatment on renal function and serum uric acid levels and their relation to vascular events in patients with coronary heart disease and metabolic syndrome: a subgroup analysis of the GREek Atorvastatin and Coronary heart disease Evaluation (GREACE) Study. *Nephrol Dial Transplant* 2007; **22**: 118-127 [PMID: 16998214 DOI: 10.1093/ndt/gfl538]
- 71 **Daskalopoulou SS**, Athyros VG, Elisaf M, Mikhailidis D. The impact of serum uric acid on cardiovascular outcomes in the LIFE study. *Kidney Int* 2004; **66**: 1714-1715 [PMID: 15458477 DOI: 10.1111/j.1523-1755.2004.938\_8.x]
- 72 **Athyros VG**, Elisaf M, Papageorgiou AA, Symeonidis AN, Pehlivanidis AN, Bouloukos VI, Milionis HJ, Mikhailidis DP, GREACE Study Collaborative Group. Effect of statins versus untreated dyslipidemia on serum uric acid levels in patients with coronary heart disease: a subgroup analysis of the GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study. *Am J Kidney Dis* 2004; **43**: 589-599 [PMID: 15042535 DOI: 10.1053/j.ajkd.2003.12.023]
- 73 **Daskalopoulou SS**, Tzavaras V, Mikhailidis DP, Elisaf M. Effect on serum uric acid levels of drugs prescribed for indications other than treating hyperuricaemia. *Curr Pharm Des* 2005; **11**: 4161-4175 [PMID: 16375738 DOI: 10.2174/138161205774913309]
- 74 **Li N**, Zhang GW, Zhang JR, Jin D, Li Y, Liu T, Wang RT. Non-alcoholic fatty liver disease is associated with progression of arterial stiffness. *Nutr Metab Cardiovasc Dis* 2015; **25**: 218-223 [PMID: 25456154 DOI: 10.1016/j.numecd.2014.10.002]
- 75 **Vlachopoulos C**, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; **55**: 1318-1327 [PMID: 20338492 DOI: 10.1016/j.jacc.2009.10.061]
- 76 **Lee YJ**, Shim JY, Moon BS, Shin YH, Jung DH, Lee JH, Lee HR. The relationship between arterial stiffness and nonalcoholic fatty liver disease. *Dig Dis Sci* 2012; **57**: 196-203 [PMID: 21750929 DOI: 10.1007/s10620-011-1819-3]
- 77 **Yu XY**, Zhao Y, Song XX, Song ZY. Association between non-alcoholic fatty liver disease and arterial stiffness in the non-obese, non-hypertensive, and non-diabetic young and middle-aged Chinese population. *J Zhejiang Univ Sci B* 2014; **15**: 879-887 [PMID: 25294377 DOI: 10.1631/jzus.B1400028]
- 78 **Sunbul M**, Agirbasli M, Durmus E, Kivrak T, Akin H, Aydin Y, Ergelen R, Yilmaz Y. Arterial stiffness in patients with non-alcoholic fatty liver disease is related to fibrosis stage and epicardial adipose tissue thickness. *Atherosclerosis* 2014; **237**: 490-493 [PMID: 25463079 DOI: 10.1016/j.atherosclerosis.2014.10.004]
- 79 **Wang MC**, Tsai WC, Chen JY, Huang JJ. Stepwise increase in arterial stiffness corresponding with the stages of chronic kidney disease. *Am J Kidney Dis* 2005; **45**: 494-501 [PMID: 15754271 DOI: 10.1053/j.ajkd.2004.11.011]
- 80 **Haydar AA**, Covic A, Colhoun H, Rubens M, Goldsmith DJ. Coronary artery calcification and aortic pulse wave velocity in chronic kidney disease patients. *Kidney Int* 2004; **65**: 1790-1794 [PMID: 15086918 DOI: 10.1111/j.1523-1755.2004.00581.x]
- 81 **Chen JY**, Chou CH, Tsai WC, Wang MC, Ho CS, Li YH, Tsai YS, Tsai LM. Effects of increased systemic inflammation and central obesity on arterial stiffness in patients with nonalcoholic fatty liver disease. *J Am Soc Hypertens* 2012; **6**: 253-260 [PMID: 22651911 DOI: 10.1016/j.jash.2012.04.003]
- 82 **Yu KJ**, Zhang MJ, Li Y, Wang RT. Increased whole blood viscosity associated with arterial stiffness in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2014; **29**: 540-544 [PMID: 23981121 DOI: 10.1111/jgh.12368]
- 83 **Li Y**, Tian XX, Liu T, Wang RT. Association between whole blood viscosity and arterial stiffness in patients with type 2 diabetes mellitus. *Endocrine* 2015; **49**: 148-154 [PMID: 25312690 DOI: 10.1007/s12020-014-451-03]
- 84 **Cecelja M**, Chowienzyk P. Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovasc Dis* 2012; **1**: [PMID: 24175067 DOI: 10.1258/cvd.2012.012016]
- 85 **London GM**. Mechanisms of arterial calcifications and consequences for cardiovascular function. *Kidney Int Suppl* (2011) 2013; **3**: 442-445 [PMID: 25019027 DOI: 10.1038/kisup.2013.92]
- 86 **Liu B**, Che W, Yan H, Zhu W, Wang H. Effects of rosuvastatin vs. simvastatin/ezetimibe on arterial wall stiffness in patients with coronary artery disease. *Intern Med* 2013; **52**: 2715-2719 [PMID: 24334573 DOI: 10.2169/internalmedicine.52.0731]
- 87 **Georgianos PI**, Sarafidis PA, Lasaridis AN. Arterial stiffness: a novel cardiovascular risk factor in kidney disease patients. *Curr Vasc Pharmacol* 2015; **13**: 229-238 [PMID: 24007427]
- 88 **Dahlén EM**, Bjarnegård N, Länne T, Nystrom FH, Ostgren CJ. Sagittal abdominal diameter is a more independent measure compared with waist circumference to predict arterial stiffness in subjects with type 2 diabetes—a prospective observational cohort study. *Cardiovasc Diabetol* 2013; **12**: 55 [PMID: 23536999 DOI: 10.1186/1475-2840-12-55]
- 89 **Katsiki N**, Koumaras C, Athyros VG, Karagiannis A. Thinking beyond traditional cardiovascular risk factors: the role of arterial stiffness in targeting residual risk. *Angiology* 2012; **63**: 9-11 [PMID: 22144689 DOI: 10.1177/0003319711406256]
- 90 **Tziomalos K**, Athyros VG, Karagiannis A. Treating Arterial Stiffness in Young and Elderly Patients with the Metabolic Syndrome. *Curr Pharm Des* 2014; Epub ahead of print [PMID: 24745924]
- 91 **Katsiki N**, Athyros VG, Karagiannis A, Mikhailidis DP. Metabolic syndrome and non-cardiac vascular diseases: an update from human studies. *Curr Pharm Des* 2014; **20**: 4944-4952 [PMID: 24320038 DOI: 10.2174/1381612819666131206100750]
- 92 **Chen TH**, Liao FT, Yang YC, Wang JJ. Inhibition of inducible nitric oxide synthesis ameliorates liver ischemia and reperfusion injury induced transient increase in arterial stiffness. *Transplant Proc* 2014; **46**: 1112-1116 [PMID: 24815141 DOI: 10.1016/j.transproceed.2014.01.002]
- 93 **Athyros VG**, Pagourelas ED, Gossios TD, Vasilikos VG. Treating Heart Failure with Preserved Ejection Fraction Related to Arterial Stiffness. Can we kill Two Birds with One Stone? *Curr Vasc Pharmacol* 2014; Epub ahead of print [PMID: 25426732]
- 94 **Kanaki AI**, Sarafidis PA, Georgianos PI, Kanavos K, Tziolas IM, Zebekakis PE, Lasaridis AN. Effects of low-dose atorvastatin on arterial stiffness and central aortic pressure augmentation in patients with hypertension and hypercholesterolemia. *Am J Hypertens* 2013; **26**: 608-616 [PMID: 23449607 DOI: 10.1093/ajh/hps098]
- 95 **Tousoulis D**, Oikonomou E, Siasos G, Chrysoshoou C, Zaromitidou M, Kioufis S, Maniatis K, Dilaveris P, Miliou A, Michalea S, Papavassiliou AG, Stefanadis C. Dose-dependent effects of short term atorvastatin treatment on arterial wall properties and on indices of left ventricular remodeling in ischemic heart failure. *Atherosclerosis* 2013; **227**: 367-372 [PMID: 23433403 DOI: 10.1016/j.atherosclerosis.2013.01.015]
- 96 **Fassett RG**, Robertson IK, Ball MJ, Geraghty DP, Sharman JE, Coombes JS. Effects of atorvastatin on arterial stiffness in chronic kidney disease: a randomised controlled trial. *J Atheroscler Thromb* 2010; **17**: 235-241 [PMID: 20032570 DOI: 10.5551/jat.2683]
- 97 **Papademetriou V**, Katsiki N, Doumas M, Faselis C. Halting arterial aging in patients with cardiovascular disease: hypolipidemic and antihypertensive therapy. *Curr Pharm Des* 2014; **20**: 6339-6349 [PMID: 24953392 DOI: 10.2174/1381612820666140620162157]
- 98 **Koumaras C**, Katsiki N, Athyros VG, Karagiannis A. Metabolic syndrome and arterial stiffness: the past, the present and the future. *J Cardiovasc Med (Hagerstown)* 2013; **14**: 687-689 [PMID: 24335882 DOI: 10.2459/JCM.0b013e3283657c96]

- 99 **Koumaras C**, Tziomalos K, Stavrinou E, Katsiki N, Athyros VG, Mikhailidis DP, Karagiannis A. Effects of renin-angiotensin-aldosterone system inhibitors and beta-blockers on markers of arterial stiffness. *J Am Soc Hypertens* 2014; **8**: 74-82 [PMID: 24139833 DOI: 10.1016/j.jash.2013.09.001]
- 100 **Stojakovic T**, Claudel T, Putz-Bankuti C, Fauler G, Scharnagl H, Wagner M, Sourij H, Stauber RE, Winkler K, März W, Wascher TC, Trauner M. Low-dose atorvastatin improves dyslipidemia and vascular function in patients with primary biliary cirrhosis after one year of treatment. *Atherosclerosis* 2010; **209**: 178-183 [PMID: 19782361 DOI: 10.1016/j.atherosclerosis.2009.08.052]
- 101 **Mäki-Petäjä KM**, Wilkinson IB. Anti-inflammatory drugs and statins for arterial stiffness reduction. *Curr Pharm Des* 2009; **15**: 290-303 [PMID: 19149619 DOI: 10.2174/138161209787354221]
- 102 **Clark JM**, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; **98**: 960-967 [PMID: 12809815 DOI: 10.1111/j.1572-0241.2003.07486.x]
- 103 **Younossi ZM**, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, Srishord M. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol* 2011; **9**: 524-530.e1; quiz e60 [PMID: 21440669 DOI: 10.1016/j.cgh.2011.03.020]
- 104 **Chalasani N**, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. *Gastroenterology* 2004; **126**: 1287-1292 [PMID: 15131789 DOI: 10.1053/j.gastro.2004.02.015]
- 105 **Vuppalaanchi R**, Teal E, Chalasani N. Patients with elevated baseline liver enzymes do not have higher frequency of hepatotoxicity from lovastatin than those with normal baseline liver enzymes. *Am J Med Sci* 2005; **329**: 62-65 [PMID: 15711421 DOI: 10.1097/00000441-200502000-00002]
- 106 **Pfeffer MA**, Keech A, Sacks FM, Cobbe SM, Tonkin A, Byington RP, Davis BR, Friedman CP, Braunwald E. Safety and tolerability of pravastatin in long-term clinical trials: prospective Pravastatin Pooling (PPP) Project. *Circulation* 2002; **105**: 2341-2346 [PMID: 12021218 DOI: 10.1161/01.CIR.0000017634.00171.24]
- 107 **Gómez-Domínguez E**, Gisbert JP, Moreno-Monteaugado JA, García-Buey L, Moreno-Otero R. A pilot study of atorvastatin treatment in dyslipidemic, non-alcoholic fatty liver patients. *Aliment Pharmacol Ther* 2006; **23**: 1643-1647 [PMID: 16696815 DOI: 10.1111/j.1365-2036.2006.02926.x]
- 108 **Hyogo H**, Tazuma S, Arihiro K, Iwamoto K, Nabeshima Y, Inoue M, Ishitobi T, Nonaka M, Chayama K. Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism* 2008; **57**: 1711-1718 [PMID: 19013295 DOI: 10.1016/j.metabol.2008.07.030]
- 109 **Kimura Y**, Hyogo H, Yamagishi S, Takeuchi M, Ishitobi T, Nabeshima Y, Arihiro K, Chayama K. Atorvastatin decreases serum levels of advanced glycation endproducts (AGEs) in nonalcoholic steatohepatitis (NASH) patients with dyslipidemia: clinical usefulness of AGEs as a biomarker for the attenuation of NASH. *J Gastroenterol* 2010; **45**: 750-757 [PMID: 20112031 DOI: 10.1007/s00535-010-0203-y]
- 110 **Nelson A**, Torres DM, Morgan AE, Fincke C, Harrison SA. A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *J Clin Gastroenterol* 2009; **43**: 990-994 [PMID: 19448566 DOI: 10.1097/MCG.0b013e31819c392e]
- 111 **Athyros VG**, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, Pagourelas ED, Theocharidou E, Karagiannis A, Mikhailidis DP. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. *Lancet* 2010; **376**: 1916-1922 [PMID: 21109302 DOI: 10.1016/S0140-6736(10)61272-X]
- 112 **Hyogo H**, Ikegami T, Tokushige K, Hashimoto E, Inui K, Matsuzaki Y, Tokumo H, Hino F, Tazuma S. Efficacy of pitavastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: An open-label, pilot study. *Hepatol Res* 2011; **41**: 1057-1065 [PMID: 21951922 DOI: 10.1111/j.1872-034X.2011.00849.x]
- 113 **Hyogo H**, Yamagishi S, Maeda S, Kimura Y, Ishitobi T, Chayama K. Atorvastatin improves disease activity of nonalcoholic steatohepatitis partly through its tumour necrosis factor- $\alpha$ -lowering property. *Dig Liver Dis* 2012; **44**: 492-496 [PMID: 22265683 DOI: 10.1016/j.dld.2011.12.013]
- 114 **Nakahara T**, Hyogo H, Kimura Y, Ishitobi T, Arihiro K, Aikata H, Takahashi S, Chayama K. Efficacy of rosuvastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: An open-label, pilot study. *Hepatol Res* 2012; **42**: 1065-1072 [PMID: 22583925 DOI: 10.1111/j.1872-034X.2012.01034.x]
- 115 **Athyros VG**, Gioulema O, Ganotakis ES, Elisaf M, Tziomalos K, Vassiliadis T, Liberopoulos EN, Theocharidou E, Karagiannis A, Mikhailidis DP. Safety and impact on cardiovascular events of long-term multifactorial treatment in patients with metabolic syndrome and abnormal liver function tests: a post hoc analysis of the randomised ATTEMPT study. *Arch Med Sci* 2011; **7**: 796-805 [PMID: 22291824 DOI: 10.5114/aoms.2011.25554]
- 116 **Tikkanen MJ**, Fayyad R, Faergeman O, Olsson AG, Wun CC, Laskey R, Kastelein JJ, Holme I, Pedersen TR. Effect of intensive lipid lowering with atorvastatin on cardiovascular outcomes in coronary heart disease patients with mild-to-moderate baseline elevations in alanine aminotransferase levels. *Int J Cardiol* 2013; **168**: 3846-3852 [PMID: 24001698 DOI: 10.1016/j.ijcard.2013.06.024]
- 117 **Kargiotis K**, Katsiki N, Athyros VG, Gioulema O, Patsiaoura K, Katsiki E, Mikhailidis DP, Karagiannis A. Effect of rosuvastatin on non-alcoholic steatohepatitis in patients with metabolic syndrome and hypercholesterolaemia: a preliminary report. *Curr Vasc Pharmacol* 2014; **12**: 505-511 [PMID: 24805248 DOI: 10.2174/1570161113119990009]
- 118 **Bader T**. Yes! Statins can be given to liver patients. *J Hepatol* 2012; **56**: 305-307 [PMID: 21963520 DOI: 10.1016/j.jhep.2011.08.016]
- 119 **Mikhailidis DP**, Sibbring GC, Ballantyne CM, Davies GM, Catapano AL. Meta-analysis of the cholesterol-lowering effect of ezetimibe added to ongoing statin therapy. *Curr Med Res Opin* 2007; **23**: 2009-2026 [PMID: 17659159 DOI: 10.1185/030079907X210507]
- 120 **Enjoji M**, Machida K, Kohjima M, Kato M, Kotoh K, Matsunaga K, Nakashima M, Nakamura M. NPC1L1 inhibitor ezetimibe is a reliable therapeutic agent for non-obese patients with nonalcoholic fatty liver disease. *Lipids Health Dis* 2010; **9**: 29 [PMID: 20222991 DOI: 10.1186/1476-511X-9-29]
- 121 **Yoneda M**, Fujita K, Nozaki Y, Endo H, Takahashi H, Hosono K, Suzuki K, Mawatari H, Kirikoshi H, Inamori M, Saito S, Iwasaki T, Terauchi Y, Kubota K, Maeyama S, Nakajima A. Efficacy of ezetimibe for the treatment of non-alcoholic steatohepatitis: An open-label, pilot study. *Hepatol Res* 2010; **40**: 566-573 [PMID: 20412324 DOI: 10.1111/j.1872-034X.2010.00644.x]
- 122 **Park H**, Shima T, Yamaguchi K, Mitsuyoshi H, Minami M, Yasui K, Itoh Y, Yoshikawa T, Fukui M, Hasegawa G, Nakamura N, Ohta M, Obayashi H, Okanoue T. Efficacy of long-term ezetimibe therapy in patients with nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 101-107 [PMID: 20658156 DOI: 10.1007/s00535-010-0291-8]
- 123 **Takeshita Y**, Takamura T, Honda M, Kita Y, Zen Y, Kato K, Misu H, Ota T, Nakamura M, Yamada K, Sunagozaka H, Arai K, Yamashita T, Mizukoshi E, Kaneko S. The effects of ezetimibe on non-alcoholic fatty liver disease and glucose metabolism: a randomised controlled trial. *Diabetologia* 2014; **57**: 878-890 [PMID: 24407920 DOI: 10.1007/s00125-013-3149-9]
- 124 **Zhu FS**, Liu S, Chen XM, Huang ZG, Zhang DW. Effects of n-3 polyunsaturated fatty acids from seal oils on nonalcoholic fatty liver disease associated with hyperlipidemia. *World J Gastroenterol* 2008; **14**: 6395-6400 [PMID: 19009658]
- 125 **Parker HM**, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012;

- 56: 944-951 [PMID: 22023985 DOI: 10.1016/j.jhep.2011.08.018]
- 126 **Subasi CF**, Aykut UE, Yilmaz Y. Comparison of noninvasive scores for the detection of advanced fibrosis in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2015; **27**: 137-141 [PMID: 25486027 DOI: 10.1097/MEG.0000000000000255]
- 127 **Karbasi-Afshar R**, Saburi A, Khedmat H. Cardiovascular disorders in the context of non-alcoholic Fatty liver disease: a literature review. *J Tehran Heart Cent* 2014; **9**: 1-8 [PMID: 25561963]
- 128 **Byrne CD**, Targher G. Ectopic fat, insulin resistance, and nonalcoholic fatty liver disease: implications for cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2014; **34**: 1155-1161 [PMID: 24743428 DOI: 10.1161/ATVBAHA.114.303034]
- 129 **Ballestri S**, Lonardo A, Bonapace S, Byrne CD, Loria P, Targher G. Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 1724-1745 [PMID: 24587651 DOI: 10.3748/wjg.v20.i7.1724]
- 130 **Cholesterol Treatment Trialists' (CTT) Collaboration**; Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhalra N, Peto R, Barnes EH, Keech A, Simes J, Collins R. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; **376**: 1670-1681 [PMID: 21067804 DOI: 10.1016/S0140-6736(10)61350-5]
- 131 **Cholesterol Treatment Trialists' (CTT) Collaborators**; Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, Barnes EH, Voysey M, Gray A, Collins R, Baigent C. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet* 2012; **380**: 581-590 [PMID: 22607822 DOI: 10.1016/S0140-6736(12)60367-5]

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## 2015 Advances in Liver Transplantation

# Predictive roles of intraoperative blood glucose for post-transplant outcomes in liver transplantation

Chul Soo Park

Chul Soo Park, Department of Anesthesiology and Pain Medicine, Seoul St. Mary's Hospital, College of Medicine, the Catholic University of Korea, Seoul 137-701, South Korea

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**Correspondence to:** Chul Soo Park, MD, PhD, Department of Anesthesiology and Pain Medicine, Seoul St. Mary's Hospital, College of Medicine, the Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, South Korea. [p6c8s17@yahoo.co.kr](mailto:p6c8s17@yahoo.co.kr)  
Telephone: +82-10-88746817  
Fax: +82-2-5371951

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## Abstract

Diabetogenic traits in patients undergoing liver transplantation (LT) are exacerbated intraoperatively by exogenous causes, such as surgical stress, steroids, blood transfusions, and catecholamines, which lead

to intraoperative hyperglycemia. In contrast to the strict glucose control performed in the intensive care unit, no systematic protocol has been developed for glucose management during LT. Intraoperative blood glucose concentrations typically exceed 200 mg/dL in LT, and extreme hyperglycemia (> 300 mg/dL) is common during the neohepatic phase. Only a few retrospective studies have examined the relationship between intraoperative hyperglycemia and post-transplant complications, with reports of infectious complications or mortality. However, no prospective studies have been conducted regarding the influence of intraoperative hyperglycemia in LT on post-transplant outcome. In addition to absolute blood glucose values, the temporal patterns in blood glucose levels during LT may serve as prognostic features. Persistent neohepatic hyperglycemia (without a decline) throughout LT is a useful indicator of early graft dysfunction. Moreover, intraoperative variability in glucose levels may predict the need for reoperation for hemorrhage after LT. Thus, there is an urgent need for guidelines for glucose control in these patients, as well as prospective studies on the impact of glucose control on various post-transplant complications. This report highlights some of the recent studies related to perioperative blood glucose management focused on LT and liver disease.

**Key words:** Blood glucose; Intraoperative; Liver transplantation; Outcome; Prediction

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**Core tip:** Despite the fact that blood glucose control is essential in critically ill patients, glucose levels are typically not managed effectively in patients undergoing liver transplantation. Currently, there are insufficient data from clinical studies on intraoperative glucose in liver transplantation to establish guidelines for glucose management of these patients. Intraoperative



features of blood glucose levels may be related to immediate and deleterious outcomes after liver transplantation. Identification of these associations will help to emphasize the prognostic role of intraoperative glucose, and stimulate the establishment of a standard protocol for intraoperative glucose management in liver transplantation.

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## INTRODUCTION

Patients with end-stage liver disease have impaired glucose metabolism, which can manifest as glucose intolerance or full diabetes mellitus<sup>[1,2]</sup>. Blood glucose status in these patients often worsens considerably during liver transplantation (LT). Sudden increases in intraoperative blood glucose can result from intrinsic diabetogenic traits and from a variety of exogenous factors, such as surgical stress, corticosteroids, glucose-containing fluid solutions, blood transfusions, and catecholamine vasopressors<sup>[1,3,4]</sup>.

Despite the strict guidelines used for blood glucose control in intensive care unit (ICU) patients<sup>[5-7]</sup>, intraoperative criteria for blood glucose control during LT have not yet been established. Although more than 20 units of insulin are typically administered during LT<sup>[3,8]</sup>, it is almost impossible to maintain blood glucose levels within a preoperatively sustained range. In fact, extreme hyperglycemia with blood glucose > 300 mg/dL is not uncommon during the neohepatic phase after reperfusion to the liver graft<sup>[1,9]</sup>.

Hyperglycemia can increase morbidity and mortality in critically ill patients<sup>[10-12]</sup>. Clinical studies on the impact of perioperative blood glucose have been conducted in major surgical fields, particularly cardiac surgery<sup>[13-15]</sup>. However, severe hyperglycemia in LT has not been rigorously investigated in terms of post-transplant sequelae. Therefore, this review examines the associations of intraoperative blood glucose with clinical outcomes during the immediate period after LT in order to encourage clinicians to pay more attention to the importance of blood glucose management in LT.

## GLUCOSE DYSREGULATION

### **Insulin resistance in liver disease**

Approximately 30%-60% of cirrhotic patients suffer from metabolic disorders related to blood glucose, known as "hepatogenic diabetes"<sup>[16]</sup>. Its pathophysiologic bases include insulin resistance in muscle, hepatic, and adipose tissues, as well

as hyperinsulinemia. Whereas patients with liver cirrhosis show essentially normal hepatic production of glucose<sup>[17,18]</sup>, hypoglycemia can develop in cases of acute decompensated liver failure<sup>[19]</sup>. Hypersecretion of glucagon can often compensate for this decrease in hepatic glucose production<sup>[17]</sup>.

Insulin resistance is associated with endothelial dysfunction in patients with cirrhosis, which increases hepatic vascular resistance and promotes portal hypertension<sup>[20]</sup>. In addition, it contributes to the development of various complications, such as hepatic fibrosis, steatosis, hepatic carcinoma, and resistance to anti-viral treatments<sup>[21]</sup>. Chronic hepatitis C virus infection, which is the leading cause for LT in Western countries, can lead to insulin resistance via upregulation of inflammatory cytokines, such as tumor necrosis factor- $\alpha$ <sup>[22]</sup>, phosphorylation of insulin-receptor substrate-1<sup>[23]</sup>, upregulation of gluconeogenic genes, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase 2<sup>[24]</sup>, and the accumulation of lipid droplets<sup>[25]</sup>. Such insulin resistance results in hypersecretion of insulin in approximately 80% of patients with chronic liver disease, and oral glucose tolerance tests universally reveal high sensitivity for hyperinsulinemia in non-diabetic patients with nonalcoholic fatty liver disease<sup>[26]</sup>.

### **LT**

During orthotopic LT, blood glucose concentrations typically increase abruptly from  $110 \pm 46$  mg/dL to  $204 \pm 60$  mg/dL in the preanhepatic phase (phase I), followed by a further increase to  $384 \pm 72$  mg/dL during the neohepatic phase (phase III)<sup>[27]</sup>. There are few data on blood glucose trends in living-donor LT. Figure 1 shows the perioperative blood glucose trend beginning from the preoperative day to post-transplant day 30 in patients who recently underwent living-donor LT at our center. Blood glucose concentrations peaked during the neohepatic phase, began to decline 3 h post-reperfusion near the end of LT, and then decreased abruptly two days post-transplantation.

**Renal contributions:** Although plasma insulin concentrations increase concomitantly with blood glucose, hyperinsulinemia does not effectively reduce hyperglycemia during the neohepatic phase<sup>[4]</sup>. This may be because post-reperfusion hyperglycemia is not mainly due to insulin hyposecretion, but to peripheral insulin resistance in glucose metabolism, which is exacerbated by the hepatectomy. As the kidney and gut are also involved in gluconeogenesis<sup>[28]</sup>, the risk for hypoglycemia is increased with chronic renal failure, due to the suppression of renal glucose release and decreased glycogen storage<sup>[29]</sup>. Thus, patients with renal failure usually have hyperinsulinemia due to decreased renal clearance and the effects of uremic toxin on the liver<sup>[30]</sup>. Additionally, the subsequent malnutrition or muscle wasting may be more severe,

which decreases their hepatic glycogen stores and gluconeogenic capacity<sup>[31]</sup>. Moreover, acidosis would limit the ability of the liver to compensate *via* hepatorenal reciprocity to maintain normoglycemia<sup>[32]</sup>. As a result, blood glucose must be monitored closely during the anhepatic phase in patients with severely damaged renal function undergoing LT because of the risk of hypoglycemia.

**Exogenous contributions:** Corticosteroid administration can exacerbate preexisting insulin resistance<sup>[33]</sup>, and cause increased release of counterregulatory hormones, such as glucagon, adrenaline, noradrenaline, growth hormone, and cortisol. Therapeutic infusion of vasoactive drugs, such as epinephrine, norepinephrine, and dopamine, can also contribute to the increased blood glucose levels. Other sources include glucose within blood transfusions, and hepatic glucose released by the graft, which is accelerated during rewarming and after perfusion<sup>[34]</sup>. Indeed, the abrupt aggravation of hyperglycemia during the neohepatic phase is mainly caused by glucose influx from the grafted liver<sup>[4]</sup>.

## MANAGEMENT OF BLOOD GLUCOSE

### *Target levels in critically ill patients*

Until the early 2000s, strict blood glucose control (targeting 80-110 mg/dL) was recommended as standard practice in ICUs<sup>[5-7]</sup>. This protocol decreased patient morbidity and mortality compared to conventional management of blood glucose (targeting 180-200 mg/dL). However, the intensive insulin therapy was accompanied by a high risk of hypoglycemia<sup>[10]</sup>. Updated guidelines for regulating blood glucose now advise treating ICU patients to achieve levels  $\leq 180$  mg/dL<sup>[35,36]</sup>. This guideline was formulated based largely on the results of the multicenter NICE-SUGAR trial conducted in Australia, New Zealand, and Canada, which reported a lower incidence of hypoglycemia and hospital mortality compared to stricter blood glucose control<sup>[37]</sup>.

### *Blood glucose during LT*

Strict glucose control is not likely to be achieved during LT due to progressive hyperglycemia induced by exacerbated insulin resistance and exogenous intraoperative factors. Park *et al*<sup>[9]</sup> found that an intraoperative blood glucose criterion threshold of  $> 200$  mg/dL was associated with post-transplant surgical site infection, whereas Ammori *et al*<sup>[3]</sup> used a blood glucose criterion of  $< 150$  mg/dL, which appears to be a more reasonable goal<sup>[38]</sup>. However, due to the lack of any specialized standard protocol, blood glucose control in LT is maintained in accordance with inpatient glycemic management guidelines. The Consensus Statement by the American Association of Clinical Endocrinologists and the American Diabetes

Association recommends initiating insulin infusions in critically ill patients at a blood glucose level no greater than 180 mg/dL<sup>[39]</sup>. The target glucose level is 140-180 mg/dL, and levels below 110 mg/dL should be avoided, even if a lower target may be beneficial in some patient groups.

## BLOOD GLUCOSE AND POST-TRANSPLANT OUTCOMES

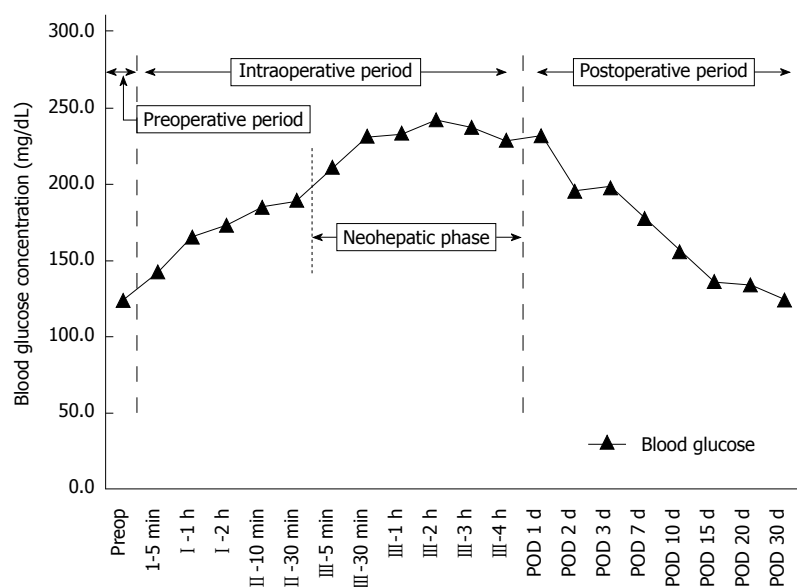
### *Hyperglycemia and post-transplant infection*

Two retrospective studies have documented an association between intraoperative hyperglycemia during LT and post-transplant infectious complications<sup>[3,9]</sup>. In the study by Ammori *et al*<sup>[3]</sup> in 2007 that included 184 patients undergoing LT, the overall infection rate (including superficial skin infection, pneumonia, blood stream infections, peritonitis, urinary tract infection) during the first 30 d post-transplant was significantly higher (48%) in the group with poorly controlled hyperglycemia compared to those with well-controlled blood glucose ( $< 150$  mg/dL; 30%). In the study of 680 LT patients by Park *et al*<sup>[9]</sup> in 2009, severe hyperglycemia ( $\geq 200$  mg/dL) increased the risk of surgical site infection during the immediate post-transplant period by more than twofold (RR = 2.25, 95%CI: 1.26-4.03)<sup>[9]</sup>.

Hyperglycemia influences major components of the immune system that combat infection. For example, early inflammatory responses to tissue injury are suppressed as a result of elevated expression of adhesion molecules, impaired complement activation, interference with the kininogen-bradykinin system, dysregulation of endothelial nitric oxide production<sup>[40-42]</sup>, and increased levels of proinflammatory cytokines, such as interleukins 1 $\beta$  and 18 and tumor necrosis factor- $\alpha$ <sup>[43]</sup>. Hyperglycemia weakens macrophage phagocytosis, and reduces neutrophil adherence<sup>[44,45]</sup>, chemotaxis, and reactive oxygen species production<sup>[46]</sup>. In addition, hyperglycemia interferes with the glycosylation of immune proteins and collagen<sup>[47]</sup>.

### *Glucose variability and post-surgical complications*

Variability in blood glucose levels has been studied in association with mortality in the hospital or ICU<sup>[48,49]</sup>. However, there are few studies examining surgical consequences with respect to perioperative variability in serial blood glucose measurements. A recent single-center, prospective cohort study of 1461 patients undergoing cardiac surgery found that postoperative glycemic variability increases the risk of major adverse events, such as death, myocardial infarction, reoperation, sternal infection, cardiac tamponade, pneumonia, stroke, and renal failure (RR = 1.3, 95%CI: 1.1-1.5)<sup>[50]</sup>. Another retrospective study in 2013 revealed that large variability in preoperative blood glucose was associated with an increased rate of



**Figure 1** Perioperative trend of blood glucose during living-donor liver transplantation. Blood glucose concentrations were measured in 210 patients undergoing living-donor liver transplantation at St. Mary's Hospital (Seoul, South Korea) between 2009 and 2013. I : Preanhepatic phase; II : Anhepatic phase; III : Neohepatic phase; POD: Postoperative day; Preop: Preoperative.

reoperation (RR = 4.14, 95%CI: 1.30-13.33)<sup>[51]</sup>.

Only one study evaluated intraoperative glycemic variability in LT. In this retrospective study of 668 LT patients in 2010, intraoperative variability in blood glucose (SD  $\geq$  55.0 mg/dL) nearly doubled the risk of reoperation for hemorrhage (RR = 1.9, 95%CI: 1.2-3.0)<sup>[52]</sup>. However, the reason for this increased risk remains unclear. According to Hendriks *et al*<sup>[53]</sup>, surgical re-intervention in LT patients is related to intraoperative blood loss. It is possible that the turnover in body fluids due to massive blood loss results in an instability in blood glucose concentrations. *In vitro* studies indicate that rapid, wide swings in glucose levels can adversely affect normal cellular defenses and coagulation/fibrinolytic systems<sup>[54,55]</sup>.

### Persistent neohepatic hyperglycemia and graft dysfunction

As graft-related problems are the most important determinant of initial prognosis after LT<sup>[56]</sup>, early detection of graft-related factors is important for improving post-transplant outcome. Since the early 1990s, blood glucose monitoring has been used as a sensitive indicator of early liver-graft function<sup>[57]</sup>. In animals receiving partial liver allografts, graft function was predicted by intraoperative balance of glucose production and utilization in the liver, measured as the difference between hepatic glucose inflow (at the portal vein and hepatic artery) and outflow (from the hepatic vein, to the liver)<sup>[58]</sup>. Impaired glucose uptake and continuous glycogenolysis causes persistent reperfusion hyperglycemia, which may be an early sign of impaired graft function<sup>[59]</sup>. Therefore, blood glucose levels during the neohepatic phase can be associated with post-transplant liver function.

Moreover, a recent retrospective study found that hyperglycemia (> 200 mg/dL) maintained until the immediate post-transplant period was associated with liver allograft rejection within one year<sup>[60]</sup>. The decline in intraoperative blood glucose that we observed near the end of the neohepatic phase during LT (Figure 1) has not been previously described. Future study is needed to determine whether this decline is associated with functional recovery of the grafted liver.

Prior reports indicate that pronounced insulin insensitivity and hyperinsulinemia in liver failure are attributable to pancreatic hypersecretion and reduced hepatic insulin clearance, secondary to hyperglucagonemia<sup>[61]</sup>. These metabolic abnormalities disappear after successful LT<sup>[62]</sup>. Thus, resolution of hyperglycemia would be expected at the end and immediately following LT upon recovery of liver graft function.

### Mortality rate

The effect of intraoperative glucose management on mortality has primarily been examined in patients undergoing cardiac surgery. Such studies identified a benefit in patients with myocardial infarction who received intraoperative infusions of insulin and potassium<sup>[63,64]</sup>. Additional studies have evaluated the effects of intensive glycemic control on mortality following coronary artery bypass grafting<sup>[65-67]</sup>. The retrospective study by Ammori *et al*<sup>[3]</sup> also reported one-year mortality rates, which were higher in patients with poorly controlled hyperglycemia compared to those with well-controlled glucose levels (21.9% vs 8.8%). A prospective clinical study of the relationship between intraoperative blood glucose and post-transplant mortality is thus warranted.

## CONCLUSION

Patients with end-stage liver disease exhibit hepatogenic diabetes, which manifests as peripheral insulin resistance and hyperinsulinemia. During LT, additional exogenous factors lead to intraoperative refractory hyperglycemia, with peak blood glucose levels occurring after reperfusion. As there are no specific guidelines, conventional methods from other clinical fields are used for the intraoperative management of hyperglycemia in these patients. Retrospective studies demonstrate that intraoperative hyperglycemia is associated with increased risk for infection and one-year mortality. Furthermore, the variability in blood glucose level during LT may predict post-transplant outcomes. Diabetogenic traits return after successful LT, so persistent neohepatic hyperglycemia in association with early indicators of graft dysfunction should be examined in future studies, including prospective clinical studies on the influence of intraoperative blood glucose on post-transplant outcomes.

## REFERENCES

- 1 **Shangraw RE**. Metabolic issues in liver transplantation. *Int Anesthesiol Clin* 2006; **44**: 1-20 [PMID: 16832203 DOI: 10.1097/0004311-200604430-00003]
- 2 **Ahmadieh H**, Azar ST. Liver disease and diabetes: association, pathophysiology, and management. *Diabetes Res Clin Pract* 2014; **104**: 53-62 [PMID: 24485856 DOI: 10.1016/j.diabres.2014.01.003]
- 3 **Ammori JB**, Sigakis M, Englesbe MJ, O'Reilly M, Pelletier SJ. Effect of intraoperative hyperglycemia during liver transplantation. *J Surg Res* 2007; **140**: 227-233 [PMID: 17509267 DOI: 10.1016/j.jss.2007.02.019]
- 4 **Shangraw RE**, Hexem JG. Glucose and potassium metabolic responses to insulin during liver transplantation. *Liver Transpl Surg* 1996; **2**: 443-454 [PMID: 9346691 DOI: 10.1002/lt.500020607]
- 5 **Van den Berghe G**, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006; **354**: 449-461 [PMID: 16452557 DOI: 10.1056/NEJMoa052521]
- 6 **van den Berghe G**, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001; **345**: 1359-1367 [PMID: 11794168 DOI: 10.1056/NEJMoa011300]
- 7 **Van Herpe T**, De Brabanter J, Beullens M, De Moor B, Van den Berghe G. Glycemic penalty index for adequately assessing and comparing different blood glucose control algorithms. *Crit Care* 2008; **12**: R24 [PMID: 18302732 DOI: 10.1186/cc6800]
- 8 **Xia VW**, Obaidi R, Park C, Braunfeld M, Neelakanta G, Nourmand H, Hu KQ, Steadman RH. Insulin therapy in divided doses coupled with blood transfusion versus large bolus doses in patients at high risk for hyperkalemia during liver transplantation. *J Cardiothorac Vasc Anesth* 2010; **24**: 80-83 [PMID: 19362017 DOI: 10.1053/j.jvca.2009.01.032]
- 9 **Park C**, Hsu C, Neelakanta G, Nourmand H, Braunfeld M, Wray C, Steadman RH, Hu KQ, Cheng RT, Xia VW. Severe intraoperative hyperglycemia is independently associated with surgical site infection after liver transplantation. *Transplantation* 2009; **87**: 1031-1036 [PMID: 19352123 DOI: 10.1097/TP.0b013e31819cc3e6]
- 10 **Vanhorebeek I**, Langouche L, Van den Berghe G. Tight blood glucose control with insulin in the ICU: facts and controversies. *Chest* 2007; **132**: 268-278 [PMID: 17625087 DOI: 10.1378/chest.06-3121]
- 11 **Van den Berghe G**. How does blood glucose control with insulin save lives in intensive care? *J Clin Invest* 2004; **114**: 1187-1195 [PMID: 15520847 DOI: 10.1172/jci23506]
- 12 **Wintergerst KA**, Foster MB, Sullivan JE, Woods CR. Association of hyperglycemia, glucocorticoids, and insulin use with morbidity and mortality in the pediatric intensive care unit. *J Diabetes Sci Technol* 2012; **6**: 5-14 [PMID: 22401317 DOI: 10.1177/193229681200600102]
- 13 **Duncan AE**, Abd-Elseyed A, Maheshwari A, Xu M, Soltesz E, Koch CG. Role of intraoperative and postoperative blood glucose concentrations in predicting outcomes after cardiac surgery. *Anesthesiology* 2010; **112**: 860-871 [PMID: 20216389 DOI: 10.1097/ALN]
- 14 **Ouattara A**, Lecomte P, Le Manach Y, Landi M, Jacqueminet S, Platonov I, Bonnet N, Riou B, Coriat P. Poor intraoperative blood glucose control is associated with a worsened hospital outcome after cardiac surgery in diabetic patients. *Anesthesiology* 2005; **103**: 687-694 [PMID: 16192760 DOI: 10.1097/0000542-20051000-00006]
- 15 **Song JW**, Shim JK, Yoo KJ, Oh SY, Kwak YL. Impact of intraoperative hyperglycaemia on renal dysfunction after off-pump coronary artery bypass. *Interact Cardiovasc Thorac Surg* 2013; **17**: 473-478 [PMID: 23690431 DOI: 10.1093/icvts/ivt209]
- 16 **García-Compeán D**, Jaquez-Quintana JO, Maldonado-Garza H. Hepatogenous diabetes. Current views of an ancient problem. *Ann Hepatol* 2009; **8**: 13-20 [PMID: 19221528]
- 17 **Keller U**, Sonnenberg GE, Burckhardt D, Perruchoud A. Evidence for an augmented glucagon dependence of hepatic glucose production in cirrhosis of the liver. *J Clin Endocrinol Metab* 1982; **54**: 961-968 [PMID: 6120952 DOI: 10.1210/jcem-54-5-961]
- 18 **Shangraw RE**, Jahoor F. Effect of liver disease and transplantation on urea synthesis in humans: relationship to acid-base status. *Am J Physiol* 1999; **276**: G1145-G1152 [PMID: 10330005]
- 19 **Pfortmueller CA**, Wiemann C, Funk GC, Leichtle AB, Fiedler GM, Exadaktylos AK, Lindner G. Hypoglycemia is associated with increased mortality in patients with acute decompensated liver cirrhosis. *J Crit Care* 2014; **29**: 316.e7-316.12 [PMID: 24332992 DOI: 10.1016/j.jcrc.2013.11.002]
- 20 **Erice E**, Llop E, Berzigotti A, Abalde JG, Conget I, Seijo S, Reverter E, Albillos A, Bosch J, García-Pagán JC. Insulin resistance in patients with cirrhosis and portal hypertension. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G1458-G1465 [PMID: 22492691]
- 21 **El-Zayadi AR**, Anis M. Hepatitis C virus induced insulin resistance impairs response to anti viral therapy. *World J Gastroenterol* 2012; **18**: 212-224 [PMID: 22294824 DOI: 10.3748/wjg.v18.i3.212]
- 22 **Shintani Y**, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840-848 [PMID: 14988838 DOI: 10.1053/j.gastro.2003.11.056]
- 23 **Banerjee S**, Saito K, Ait-Goughoulte M, Meyer K, Ray RB, Ray R. Hepatitis C virus core protein upregulates serine phosphorylation of insulin receptor substrate-1 and impairs the downstream akt/protein kinase B signaling pathway for insulin resistance. *J Virol* 2008; **82**: 2606-2612 [PMID: 18160431 DOI: 10.1128/jvi.01672-07]
- 24 **Deng L**, Shoji I, Ogawa W, Kaneda S, Soga T, Jiang DP, Ide YH, Hotta H. Hepatitis C virus infection promotes hepatic gluconeogenesis through an NNSA-mediated, FoxO1-dependent pathway. *J Virol* 2011; **85**: 8556-8568 [PMID: 21697492 DOI: 10.1128/jvi.00146-11]
- 25 **Kim KH**, Hong SP, Kim K, Park MJ, Kim KJ, Cheong J. HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPARgamma. *Biochem Biophys Res Commun* 2007; **355**: 883-888 [PMID: 17331464 DOI: 10.1016/j.bbrc.2007.02.044]
- 26 **Manchanayake J**, Chitturi S, Nolan C, Farrell GC. Postprandial hyperinsulinemia is universal in non-diabetic patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2011; **26**: 510-516 [PMID: 21155882 DOI: 10.1111/j.1440-1746.2010.06528.



- x]
- 27 **Atchison SR**, Rettke SR, Fromme GA, Janossy TA, Kunkel SE, Williamson KR, Perkins JD, Rakela J. Plasma glucose concentrations during liver transplantation. *Mayo Clin Proc* 1989; **64**: 241-245 [PMID: 2646482 DOI: 10.1016/S0025-6196(12)65679-1]
  - 28 **Stumvoll M**, Meyer C, Mitrakou A, Gerich JE. Important role of the kidney in human carbohydrate metabolism. *Med Hypotheses* 1999; **52**: 363-366 [PMID: 10416940 DOI: 10.1054/mehy]
  - 29 **Cano N**. Inter-relationships between renal metabolism (both in physiology and renal dysfunction) and the liver. *Curr Opin Clin Nutr Metab Care* 2001; **4**: 279-285 [PMID: 11458021 DOI: 10.1097/00075197-200107000-00006]
  - 30 **Mak RH**, DeFronzo RA. Glucose and insulin metabolism in uremia. *Nephron* 1992; **61**: 377-382 [PMID: 1501732 DOI: 10.1159/000186953]
  - 31 **Garber AJ**, Bier DM, Cryer PE, Pagliara AS. Hypoglycemia in compensated chronic renal insufficiency. Substrate limitation of gluconeogenesis. *Diabetes* 1974; **23**: 982-986 [PMID: 4435312 DOI: 10.2337/diab.23.12.982]
  - 32 **Woerle HJ**, Meyer C, Popa EM, Cryer PE, Gerich JE. Renal compensation for impaired hepatic glucose release during hypoglycemia in type 2 diabetes: further evidence for hepatorenal reciprocity. *Diabetes* 2003; **52**: 1386-1392 [PMID: 12765948 DOI: 10.2337/diabetes.52.6.1386]
  - 33 **Rizza RA**, Mandarino LJ, Gerich JE. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action. *J Clin Endocrinol Metab* 1982; **54**: 131-138 [PMID: 7033265 DOI: 10.1210/jcem-54-1-131]
  - 34 **Nowak G**, Ungerstedt J, Wernerman J, Ungerstedt U, Ericzon BG. Metabolic changes in the liver graft monitored continuously with microdialysis during liver transplantation in a pig model. *Liver Transpl* 2002; **8**: 424-432 [PMID: 12004341 DOI: 10.1053/jlts.2002.32943]
  - 35 **Dellinger RP**, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; **39**: 165-228 [PMID: 23361625 DOI: 10.1007/s00134-012-2769-8]
  - 36 **Orban JC**, Scarlatti A, Lefrant JY, Molinari N, Leone M, Jaber S, Constantini JM, Allaouchiche B, Ichai C. [Management of glycemia: an audit in 66 ICUs]. *Ann Fr Anesth Reanim* 2013; **32**: 84-88 [PMID: 23337340 DOI: 10.1016/j.annfar.2012.12.002]
  - 37 **Finfer S**, Chittock DR, Su SY, Blair D, Foster D, Dhingra V, Bellomo R, Cook D, Dodek P, Henderson WR, Hébert PC, Heritier S, Heyland DK, McArthur C, McDonald E, Mitchell I, Myburgh JA, Norton R, Potter J, Robinson BG, Ronco JJ. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med* 2009; **360**: 1283-1297 [PMID: 19318384 DOI: 10.1056/NEJMoa0810625]
  - 38 **Liu LL**, Niemann CU. Intraoperative management of liver transplant patients. *Transplant Rev (Orlando)* 2011; **25**: 124-129 [PMID: 21514137 DOI: 10.1016/j.tre.2010.10.006]
  - 39 **Moghissi ES**, Korytkowski MT, DiNardo M, Einhorn D, Hellman R, Hirsch IB, Inzucchi SE, Ismail-Beigi F, Kirkman MS, Umpierrez GE; American Association of Clinical Endocrinologists; American Diabetes Association. American Association of Clinical Endocrinologists and American Diabetes Association consensus statement on inpatient glycemic control. *Endocr Pract* 2009; **15**: 353-369 [PMID: 19454396 DOI: 10.4158/ep09102.ra]
  - 40 **Turina M**, Fry DE, Polk HC. Acute hyperglycemia and the innate immune system: clinical, cellular, and molecular aspects. *Crit Care Med* 2005; **33**: 1624-1633 [PMID: 16003073 DOI: 10.1097/01.CCM.0000170106.61978.D8]
  - 41 **Chen NG**, Azhar S, Abbasi F, Carantoni M, Reaven GM. The relationship between plasma glucose and insulin responses to oral glucose, LDL oxidation, and soluble intercellular adhesion molecule-1 in healthy volunteers. *Atherosclerosis* 2000; **152**: 203-208 [PMID: 10996356 DOI: 10.1016/S0021-9150(99)00460-8]
  - 42 **Santilli F**, Cipollone F, Mezzetti A, Chiarelli F. The role of nitric oxide in the development of diabetic angiopathy. *Horm Metab Res* 2004; **36**: 319-335 [PMID: 15156413 DOI: 10.1055/s-2004-814489]
  - 43 **Pickup JC**, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci* 2000; **67**: 291-300 [PMID: 10983873 DOI: 10.1016/S0024-3205(00)00622-6]
  - 44 **Bagdade JD**, Root RK, Bulger RJ. Impaired leukocyte function in patients with poorly controlled diabetes. *Diabetes* 1974; **23**: 9-15 [PMID: 4809622 DOI: 10.2337/diab.23.1.9]
  - 45 **Sima AA**, O'Neill SJ, Naimark D, Yagihashi S, Klass D. Bacterial phagocytosis and intracellular killing by alveolar macrophages in BB rats. *Diabetes* 1988; **37**: 544-549 [PMID: 3360215 DOI: 10.2337/diab.37.5.544]
  - 46 **Alexiewicz JM**, Kumar D, Smogorzewski M, Klin M, Massry SG. Polymorphonuclear leukocytes in non-insulin-dependent diabetes mellitus: abnormalities in metabolism and function. *Ann Intern Med* 1995; **123**: 919-924 [PMID: 7486486 DOI: 10.7326/0003-4819-123-12-199512150-00004]
  - 47 **Black CT**, Hennessey PJ, Andrassy RJ. Short-term hyperglycemia depresses immunity through nonenzymatic glycosylation of circulating immunoglobulin. *J Trauma* 1990; **30**: 830-832; discussion 832-833 [PMID: 2380999 DOI: 10.1097/00005373-199007000-00012]
  - 48 **Egi M**, Bellomo R, Stachowski E, French CJ, Hart G. Variability of blood glucose concentration and short-term mortality in critically ill patients. *Anesthesiology* 2006; **105**: 244-252 [PMID: 16871057 DOI: 10.1097/0000542-200608000-00006]
  - 49 **Ali NA**, O'Brien JM, Dungan K, Phillips G, Marsh CB, Lemeshow S, Connors AF, Preiser JC. Glucose variability and mortality in patients with sepsis. *Crit Care Med* 2008; **36**: 2316-2321 [PMID: 18596625 DOI: 10.1097/CCM.0b013e3181810378]
  - 50 **Subramaniam B**, Lerner A, Novack V, Khabbaz K, Paryente-Wiesmann M, Hess P, Talmor D. Increased glycemic variability in patients with elevated preoperative HbA1C predicts adverse outcomes following coronary artery bypass grafting surgery. *Anesth Analg* 2014; **118**: 277-287 [PMID: 24445629 DOI: 10.1213/ane.0000000000000100]
  - 51 **Endara M**, Masden D, Goldstein J, Gondek S, Steinberg J, Attinger C. The role of chronic and perioperative glucose management in high-risk surgical closures: a case for tighter glycemic control. *Plast Reconstr Surg* 2013; **132**: 996-1004 [PMID: 23783058 DOI: 10.1097/PRS]
  - 52 **Park C**, Huh M, Steadman RH, Cheng R, Hu KQ, Farmer DG, Hong J, Duffy J, Busuttill RW, Xia VW. Extended criteria donor and severe intraoperative glucose variability: association with reoperation for hemorrhage in liver transplantation. *Transplant Proc* 2010; **42**: 1738-1743 [PMID: 20620513 DOI: 10.1016/j.transproceed.2009.12.066]
  - 53 **Hendriks HG**, van der Meer J, de Wolf JT, Peeters PM, Porte RJ, de Jong K, Lip H, Post WJ, Slooff MJ. Intraoperative blood transfusion requirement is the main determinant of early surgical re-intervention after orthotopic liver transplantation. *Transpl Int* 2005; **17**: 673-679 [PMID: 15717214 DOI: 10.1007/s00147-004-0793-5]
  - 54 **Quagliaro L**, Piconi L, Assaloni R, Martinelli L, Motz E, Ceriello A. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. *Diabetes* 2003; **52**: 2795-2804 [PMID: 14578299 DOI: 10.2337/diabetes.52.11.2795]
  - 55 **Slaughter TF**. Hemostasis and glycemic control in the cardiac surgical patient. *Semin Cardiothorac Vasc Anesth* 2006; **10**: 176-179 [PMID: 16959746 DOI: 10.1177/1089253206288993]
  - 56 **Vogt DP**, Henderson JM, Carey WD, Barnes D. The long-term survival and causes of death in patients who survive at least 1 year after liver transplantation. *Surgery* 2002; **132**: 775-780; discussion

- 780 [PMID: 12407365 DOI: 10.1067/msy.2002.128343]
- 57 **Steltzer H**, Tüchy G, Hiesmayr M, Müller C, Germann P, Zimpfer M. Peri-operative liver graft function: monitoring using the relationship between blood glucose and oxygen consumption during anaesthesia. *Anaesthesia* 1992; **47**: 955-958 [PMID: 1466435 DOI: 10.1111/j.1365-2044.1992.tb03197.x]
  - 58 **Shiba H**, Zhu X, Arakawa Y, Irefin S, Wang B, Trenti L, Sanchez IP, Fung JJ, Kelly DM. Glucose balance of porcine liver allograft is an important predictor of outcome. *J Surg Res* 2011; **171**: 851-858 [PMID: 20828723 DOI: 10.1016/j.jss.2010.05.067]
  - 59 **Mallett SV**, Kang Y, Freeman JA, Aggarwal S, Gasior T, Fortunato FL. Prognostic significance of reperfusion hyperglycemia during liver transplantation. *Anesth Analg* 1989; **68**: 182-185 [PMID: 2643890 DOI: 10.1213/00000539-198902000-00021]
  - 60 **Wallia A**, Parikh ND, Molitch ME, Mahler E, Tian L, Huang JJ, Levitsky J. Posttransplant hyperglycemia is associated with increased risk of liver allograft rejection. *Transplantation* 2010; **89**: 222-226 [PMID: 20098286 DOI: 10.1097/TP.0b013e3181c3c2ff]
  - 61 **Vilstrup H**, Iversen J, Tygstrup N. Glucoregulation in acute liver failure. *Eur J Clin Invest* 1986; **16**: 193-197 [PMID: 3089815 DOI: 10.1111/j.1365-2362.1986.tb01328.x]
  - 62 **Konrad T**, Golling M, Vicini P, Toffolo G, Wittman M, Mahon A, Klar E, Cobelli C, Usadel K. Insulin sensitivity and beta-cell secretion after liver transplantation in patients with acute liver failure. *Transplant Proc* 2001; **33**: 2576-2579 [PMID: 11406252 DOI: 10.1016/S0041-1345(01)02102-9]
  - 63 **Lazar HL**, Philippides G, Fitzgerald C, Lancaster D, Shemin RJ, Apstein C. Glucose-insulin-potassium solutions enhance recovery after urgent coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 1997; **113**: 354-360; discussion 360-362 [PMID: 9040630 DOI: 10.1016/S0022-5223(97)70333-7]
  - 64 **Rao V**, Christakis GT, Weisel RD, Ivanov J, Borger MA, Cohen G. The insulin cardioplegia trial: myocardial protection for urgent coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 2002; **123**: 928-935 [PMID: 12019378 DOI: 10.1067/mtc.2002.121686]
  - 65 **Furnary AP**, Gao G, Grunkemeier GL, Wu Y, Zerr KJ, Bookin SO, Floten HS, Starr A. Continuous insulin infusion reduces mortality in patients with diabetes undergoing coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 2003; **125**: 1007-1021 [PMID: 12771873 DOI: 10.1067/mtc.2003.181]
  - 66 **D'Alessandro C**, Leprince P, Golmard JL, Ouattara A, Aubert S, Pavie A, Gandjbakhch I, Bonnet N. Strict glycemic control reduces EuroSCORE expected mortality in diabetic patients undergoing myocardial revascularization. *J Thorac Cardiovasc Surg* 2007; **134**: 29-37 [PMID: 17599483 DOI: 10.1016/j.jtcvs.2007.02.028]
  - 67 **Lazar HL**, Chipkin SR, Fitzgerald CA, Bao Y, Cabral H, Apstein CS. Tight glycemic control in diabetic coronary artery bypass graft patients improves perioperative outcomes and decreases recurrent ischemic events. *Circulation* 2004; **109**: 1497-1502 [PMID: 15006999 DOI: 10.1161/01.cir.0000121747.71054.79]

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## Endoscopic treatment of gastroparesis

Thomas R McCarty, Tarun Rustagi

Thomas R McCarty, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520-8019, United States

Tarun Rustagi, Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520-8019, United States

**Author contributions:** McCarty TR and Rustagi T equally contributed to this paper.

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**Correspondence to:** Tarun Rustagi, MD, Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, 333 Cedar Street, 1080 LMP, New Haven, CT 06520-8019, United States. [tarunrustagi06@gmail.com](mailto:tarunrustagi06@gmail.com)  
Telephone: +1-860-2214034  
Fax: +1-203-7371755

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### Abstract

Gastroparesis has traditionally been a largely medically managed disease with refractory symptoms typically falling under the umbrella of the surgical domain. Surgical options include, but are not limited to, gastrectomy, jejunostomy, pyloromyotomy, or pyloroplasty, and the Food and Drug Administration approved

gastric electrical stimulation implantation. Endoscopic management of gastroparesis most commonly involves intrapyloric botulinum toxin injection; however, there exists a variety of endoscopic approaches on the horizon that have the potential to radically shift standard of care. Endoscopic management of gastroparesis seeks to treat delayed gastric emptying with a less invasive approach compared to the surgical approach. This review will serve to highlight such innovative and potentially transformative, endoscopic interventions available to gastroenterologists in the management of gastroparesis.

**Key words:** Botulinum; Gastrojejunostomy; Transpyloric; Pyloromyotomy; Gastric stimulator; Gastric pacemaker; Stenting

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**Core tip:** Although a majority of gastroparesis patients respond to medical treatment, patients with refractory symptoms pose a therapeutic challenge and are often referred for surgical management. Endoscopic management of gastroparesis seeks to treat delayed gastric emptying with a less invasive approach compared to the surgical approach. Endoscopic treatment of gastroparesis most commonly involves intrapyloric botulinum toxin injection; however, there exists a variety of endoscopic approaches on the horizon that have the potential to radically shift the standard of care for refractory patients.

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### INTRODUCTION

Gastric emptying is a highly regulated, carefully choreographed process that demands a harmony

of synchronized impulses working together as an impetus for which food can be delivered distally down the gastrointestinal tract. These propulsive forces are generated by proximal fundic tone, the interstitial cells of Cajal, distal antral contractions, and relaxation of the pyloric sphincter to create a scripted peristalsis<sup>[1]</sup>. Gastroparesis, or delayed gastric emptying, is characterized by physiologic disturbances in antral hypomotility, increased gastric outlet resistance, and pyloric dysfunction without evidence of obstruction<sup>[2,3]</sup>. Patients typically present with dyspepsia-like symptoms including early satiety, postprandial fullness, bloating, nausea, vomiting, and abdominal pain<sup>[2,4-6]</sup>. The most common etiologies of gastroparesis are idiopathic, diabetic, or post-surgical<sup>[2,7]</sup>.

The epidemiology of gastroparesis is not well known though a small population-based study showed a prevalence in men and women of 9.6 and 37.8 per 100000 persons and an age-adjusted incidence of 2.4 and 9.8 per 100000 person-years, respectively<sup>[8]</sup>. Gastric-emptying scintigraphy is the gold standard for the diagnosis of gastroparesis and consensus recommendations for the procedure involve 99-m technetium sulfur-colloid labeled low fat, egg-white meal with imaging at 0, 1, 2, and 4 h<sup>[9]</sup>. The diagnosis of delayed gastric emptying is confirmed if there is > 90% gastric retention at 1 h, > 60% at 2 h, > 10% at 4 h<sup>[9,10]</sup>. Small, frequent meals along with medical therapy including prokinetic agents, such as dopaminergic (D2) antagonists - metoclopramide and domperidone, or motilin-analogue - erythromycin, are the first line of treatment.

The majority of data regarding the efficacy of conventional prokinetic agents for the treatment of gastroparesis is outdated<sup>[11-15]</sup>. It has been approximately 30 years since the first randomized controlled trials of the conventional prokinetic agents, metoclopramide, domperidone, and erythromycin, have been published, and despite this, they are still first-line agents for the treatment of gastroparesis<sup>[16]</sup>. Metoclopramide has been the most extensively studied and has been associated with less improvement in gastric emptying when compared to erythromycin<sup>[15]</sup>. A meta-analysis assessing benefits of four different drugs in 514 patients in 36 clinical trials reported the macrolide antibiotic erythromycin as the most potent stimulant of gastric emptying, while erythromycin and the dopamine receptor antagonist domperidone (not available in the United States) are best at reducing symptoms of gastroparesis<sup>[17]</sup>. Currently, several novel pharmacotherapies such as ghrelin receptor agonists (TZP-101, TZP-102, RM-131), mitemincin, prucalopride, velusetrag, levosulpiride are in development, though their clinical efficacy and safety remains to be established<sup>[16,18,19]</sup>. While it is generally accepted that a significant percentage of patients require additional therapy beyond prokinetic agents, no clear data exists to determine the percentage of patients who fail medical management.

Patients with symptoms refractory to medical

therapy pose a therapeutic dilemma. Patients are often referred for surgical treatment including a variety of potential procedures not limited to gastrostomy, jejunostomy, pyloromyotomy, pyloroplasty, and gastrectomy to improve gastric emptying. Poor and sometimes unpredictable response, in addition to the morbidity and mortality of surgical interventions, has led to the emergence of endoscopic therapies in management of gastroparesis (Table 1). While frequently under-utilized, endoscopic treatment strategies are at the cusp of altering traditional standard of care with exciting new developments that have the potential to radically shift the preferred management of refractory gastroparesis.

## BOTULINUM TOXIN INJECTION

Endoscopic intrapyloric botulinum toxin injection (IPBI) is the most studied and perhaps the most commonly employed endoscopic treatment for those suffering from refractory gastroparesis, though this therapy still remains highly controversial. Botulinum toxin is a potent inhibitor of neuromuscular transmission used more commonly for the treatment of spasm in gastrointestinal sphincters. Botulinum toxin injection into the lower esophageal sphincter (LES), with or without endoscopic ultrasound (EUS)-guidance, is an established safe and effective therapy for management of achalasia in high-risk surgical patients. Low dose injection of botulinum toxin in the pylorus muscle decreases contractility secondary to decreased acetylcholine release by irreversibly binding to cholinergic receptors and directly affects muscle tone at higher doses<sup>[20]</sup>. The toxin also reduces substance P immuno-reactivity and disrupts pyloric myoelectric activity<sup>[21,22]</sup>.

The procedure involves endoscopic access to the patient's pylorus with injection typically in a radial pattern at or within 2 cm of the pylorus, with a total dose of 100 to 200 units<sup>[23]</sup>. There is an alternative hypothesis that antroduodenal or pyloroduodenal manometry may be helpful to evaluate the baseline pylorus muscle tone to determine the best site for IPBI to predict response; however, this warrants further investigation<sup>[24]</sup>. The usual duration of benefit from IPBI ranges from 1 to 5 mo at which time worsening symptoms can be re-treated with repeated injections<sup>[23,25]</sup>. It is important to note however, that the effect of IPBI may not be limited simply to the pyloric muscle as there have been rare reports of gastric and intestinal absorption leading to peripheral neuromuscular blockade<sup>[26]</sup>. Despite this, the procedure is typically well tolerated and safe. There are a multitude of open-label studies in adults with gastroparesis (regardless of cause - idiopathic, diabetic, and post-surgical) reporting an improvement in symptoms and gastric emptying after endoscopic IPBI<sup>[25,27-31]</sup>. The largest observational study including 63 patients by Bromer *et al.*<sup>[25]</sup> documented a 43% response rate to botulinum toxin treatment lasting



**Table 1 Summary of endoscopic therapies for refractory gastroparesis**

Endoscopic therapies	Technique/mechanism	Advantages	Disadvantages
Intrapyloric botulinum toxin injection	Radial or direct injection of 100-200 U of toxin around the pylorus Toxin binds to cholinergic receptors resulting in decreased acetylcholine release	Safe and well tolerated  Easy to perform  Observational studies report high response rate	No clear benefit in RCTs  Need for repeat treatments
Gastric electric stimulation	Miniature wireless device placed through over-tube and secured to the gastric mucosa with endoclips Device stimulates gastric muscle resulting in more regular, constant amplitudes	Proof of concept design with proven benefit  Currently used prior to definitive surgical placement Less invasive compared to surgical placement	Lack of human studies  No comparative data to surgically placed gastric pacers
Transpyloric stenting	Through-the-scope self-expandable metal stents placed across the pyloric channel	Small case series demonstrating a proven benefit in symptoms	Limited data Potential for stent migration
Endoscopic pyloromyotomy	Submucosal dissection and tunneling with full separation of the pyloric ring (myotomy)	Less invasive alternative to traditional surgical pyloromyotomy	Limited data Technically challenging with limited expertise Potential for complications:
Endoscopic decompression or bypass	Percutaneous endoscopic jejunostomy (PEJ) and direct PEJ Direct post-pyloric enteral nutritional support	Safe and effective	Limited success  Technical difficulty
endoscopic ultrasound-guided gastrojejunostomy	Transluminal anastomosis using self-expanding, lumen-apposing metal stents	Decreased morbidity and mortality compared to surgical approach	Lack of human trials Unknown long-term safety and patency issues

a mean of approximately 5 mo.

While observational data suggests that botulinum toxin injections reduce symptoms and accelerate gastric emptying in both idiopathic and diabetic gastroparesis, 2 independent, double-blinded, randomized controlled studies have shown little to no improvement in gastric emptying and no symptomatic improvement compared with placebo<sup>[32]</sup>. Friedenber *et al*<sup>[33]</sup>, using a randomized, double-blind, placebo-controlled trial, explored whether botulinum toxin improves symptoms to a significantly greater extent than placebo. In this study, 32 patients were randomized to botulinum toxin or placebo with 1-mo follow-up post endoscopic procedure measuring gastric retention at 2 and 4 h and symptoms based upon 2 validated scoring systems - the Gastroparesis Cardinal Symptom Index (GCSI) and the Gastroparesis Visual Analog Scale (GVAS). While endoscopic botulinum toxin injection did improve gastric emptying rates, the benefit was not superior to placebo at 1 mo (67% vs 64% at 2 h,  $P = 0.56$  and 29% vs 28% at 4 h,  $P = 0.86$ , for IPBI vs placebo respectively). Additionally, there was no significant difference or improvement of symptoms between IPBI compared to the placebo (GCSI - 34.4 vs 36.4,  $P = 0.21$ ; GVAS - 603 vs 584,  $P = 0.68$ , respectively). Another randomized-controlled crossover study including 23 patients, predominantly with idiopathic gastroparesis, also reported similar results, with no significant benefit of endoscopic injection of botulinum toxin over placebo in improving either symptoms or rate of gastric emptying<sup>[34]</sup>.

This discrepancy between open-label and rando-

mized controlled studies may be related to dose of toxin injected and patient population selected. In a retrospective cohort study of 179 patients by Coleski *et al*<sup>[23]</sup>, patients treated with 200 units achieved a greater improvement in gastroparetic symptoms 1 to 4 mo post intervention compared to those treated with 100 units (76.7% vs 54.2%,  $P = 0.02$ ). On multivariate analysis female gender, age < 50 years, and a non-diabetic or post-surgical etiology of gastroparesis were found to be associated with a significant response to therapy in this study.

While the use of botulinum toxin remains highly controversial, the American Gastroenterological Association (AGA) currently does not recommend the use of endoscopic IPBI for patients with gastroparesis<sup>[35]</sup>. However, given the small sample size of existing studies with conflicting data, there is an eminent need for larger randomized trials in the future before a definitive decision or treatment guidelines can be concluded.

## ENDOSCOPIC GASTRIC STIMULATOR IMPLANTATION

In 2000, gastric electrical stimulation (GES) was approved by the United States Food and Drug Administration (FDA) as a humanitarian device exemption in patients with refractory symptoms of diabetic or idiopathic gastroparesis<sup>[36]</sup>. Often referred to as a gastric pacer, GES uses an implantable device consisting of a pulse generator that allows for electrical

stimulation at a variety of frequencies. Permanent GES for gastroparesis, typically 6 cm x 5.5 cm x 1 cm, requires a lengthy surgical implantation under general anesthesia. Several case series and small randomized controlled trials, the most important being the Worldwide Anti-Vomiting Electrical Stimulation Study (WAVESS), have shown clinical benefit from GES<sup>[37-43]</sup>. A subsequent meta-analysis by Chu *et al.*<sup>[44]</sup> in 2012 confirmed significant improvement in symptom severity and gastric emptying times, though many of the analyzed studies were low quality observational studies lacking control groups. A more recent study by McCallum *et al.*<sup>[40]</sup> also demonstrated improvement in weekly vomiting frequency amongst all patients with idiopathic gastroparesis with a median reduction of 61.2%. The National Institute of Health and Care Excellence issued guidelines in 2014 that stated current evidence is adequate to support the use of GES<sup>[45]</sup>.

As of 2012, surgery was the only means available to implant the GES device. Although endoscopic placement of temporary gastric stimulators has been proven as a concept and is often used to determine whether a patient will respond to GES before undergoing a permanent implant surgery, the lack of a permanent endoscopic solution and the reliance on future surgery for symptomatic improvement has limited further endoscopic utilization at present<sup>[46,47]</sup>. However, Deb *et al.*<sup>[48]</sup> has designed 5 innovative endoscopic gastric implantation techniques and developed a novel, wirelessly powered miniature gastrostimulator. While this early model was conducted in pig studies with no human data or patient trials, the study provides an important prototype for other dysmotility treatment paradigms and provides exciting new options that may translate in the future to less invasive endoscopic placement in gastroparetic patients. This miniature wireless GES device for endoscopic implantation can be easily inserted into the stomach through an over-tube with 2 GES electrodes endoscopically attached to the gastric mucosa and secured with endoclips<sup>[49]</sup>. Electro-gastrogram recordings have demonstrated that gastric slow waves become more regular with constant amplitudes when stomach tissues are stimulated, in comparison with no stimulation. The frequency-to-amplitude ratio also changes significantly with stimulation<sup>[49]</sup>.

The miniature gastrostimulator and its attachment techniques have the potential to fundamentally shift the approach to refractory gastroparesis and provide a means for endoscopic implantation of gastric stimulator. Although further studies are required to prove the efficacy of such device, if shown to be effective, the possibility of FDA approval given a similar precedent set by GES would provide a clear indication for endoscopic management. The endoscopic gastric implantation device and technique may decrease the need for surgical implantation of GES and revolutionize the

preferred management of refractory gastroparesis.

## TRANSPYLORIC STENTING

A novel approach recently described by Clarke *et al.*<sup>[50]</sup> at Johns Hopkins involves the use of through-the-scope transpyloric stent placement as a treatment for gastroparesis. In this small case series ( $n = 3$ ), double-layered, fully-covered Niti-S self-expandable metallic stents (TaeWoong Medical, Seoul, South Korea) were used to successfully improve symptoms of gastroparesis. The procedure entails the placement of a self-expandable stent across the pyloric channel, deployed under endoscopic guidance without fluoroscopy. The stent is then fully deployed in the transpyloric position with its proximal end in the gastric antrum. Case 1 involved a 23-year-old woman with diabetic gastroparesis; case 2, a 15-year-old boy with chronic nausea and vomiting with markedly abnormal gastric emptying study; and case 3, a 45-year-old man with idiopathic gastroparesis. In all 3 cases, patient symptoms markedly improved or became asymptomatic at 115 d, 122 d, and 174 d follow-up respectively. While this includes only a case series of 3 patients, the stark improvement and lasting results at follow-up after the procedure suggest that transpyloric stent placement improves symptoms associated with impaired gastric emptying<sup>[50]</sup>.

A major concern with transpyloric stenting is stent migration leading to recurrence of symptoms. Several stent securing methods such as endoscopic clips [through-the-scope clip (TTSC) and over-the-scope clip (OTSC)] and endoscopic suturing (ES) have been described to reduce stent migration. Despite the numerous options available, the question remains which stent securing method is superior. In a small case series by Saxena *et al.*<sup>[51]</sup>, transpyloric stent placement and fixation was performed in patients with refractory gastroparesis. The stent was anchored to the antral mucosa with either no device, TTSC, OTSC, or ES. A total of 17 patients underwent 28 transpyloric stent placements with 100% success rate regardless of method. Stent migration occurred as expected in 100% of those with no device; however stent migration was significantly lower in the ES vs TTSC group (16.7% vs 100%,  $P = 0.02$ ). Stent migration occurred more frequently in the OTSC placement group as compared to the ES group (52.9% vs 16.7%,  $P = 0.075$ ). With this data, albeit limited due to the number of the patients studied, there is evidence for concern regarding stent migration with transpyloric stent placement; however, it appears this can be minimized with OTSC and endoscopic suturing.

Currently, future studies are required to truly ascertain the long-term durability, utility, and preferred method for transpyloric stenting and fixation. Until that time, transpyloric stenting will remain a limited option for endoscopists in the management of patients with

refractory gastroparesis.

## ENDOSCOPIC PYLOROMYOTOMY

Rao *et al*<sup>[52]</sup> demonstrated that phasic motor activity in the antrum and duodenum can be stimulated by fundic balloon distention. While there are no such studies to determine the effect of pyloric channel distention on the interstitial cells of Cajal in the stomach or gastric emptying, endoscopic pyloromyotomy and manipulation of the pylorus may improve gastroparesis refractory to medical management. With this notion of distention or disruption of the pylorus to improve gastroparetic symptoms, Khashab *et al*<sup>[53]</sup> demonstrated the feasibility and efficacy of this approach with a case report of the first human gastric peroral endoscopic myotomy (G-POEM) in a patient with severe refractory gastroparesis. The procedure was well tolerated with vast improvement in gastroparetic symptoms noted at 12-wk follow-up.

This technique is similar in principle to the submucosal dissection and myotomy performed for the treatment of achalasia<sup>[54]</sup>. Techniques through a submuscular tunnel were first described in animal models by Pasricha *et al*<sup>[55]</sup> in 2007. Endoscopy is performed and involves myotomy of the inner circular and oblique muscle bundles 2-5 cm proximal to the pylorus on the anterior wall of the stomach, preserving the longitudinal muscle layers with larger vessels in the submucosa coagulated. This is then followed by endoscopic pyloromyotomy by dissecting the pylorus until deeper layers become evident with full separation of the pyloric ring<sup>[54,56]</sup>.

In another study by Shlomovitz *et al*<sup>[56]</sup>, endoscopic pyloromyotomy was performed in 7 female patients with early follow-up suggesting promising symptomatic improvement in 6 of the 7 and normal gastric emptying studies at 4 h noted in 4 of the 5 patients. One patient that did not respond subsequently underwent laparoscopic pyloroplasty. Complications included gastrointestinal bleeding in one patient 2 wk after the procedure and pneumonia. Despite these complications, this endoluminal pyloromyotomy technique could provide an incision-less, less invasive alternative with similar functional outcome as compared to standard laparoscopic pyloroplasty<sup>[56]</sup>. While the small number of cases certainly limits the ability to determine the true impact of this procedure in the management of gastroparesis, with more frequent use, increasing technical experience, and more data, endoscopic pyloromyotomy has exciting potential to be at the forefront in the endoscopic management of gastroparesis.

## ENDOSCOPIC DECOMPRESSION OR BYPASS

Enteral nutrition and feeding is sometimes required for

more severe symptoms and can be seen in up to 30% of grade 3 gastroparesis<sup>[57,58]</sup>. Direct percutaneous endoscopic jejunostomy (DPEJ) is a push enteroscopy technique that was first described by Shike *et al*<sup>[59]</sup>, and offers another approach to provide direct postpyloric enteral nutritional support. Percutaneous endoscopic jejunostomy (PEJ) is a safe and effective means to palliate malnutrition in patients with severe gastroparesis<sup>[60,61]</sup>. Maple *et al*<sup>[62]</sup> demonstrated, in the largest cohort study to date, clinical outcomes with DPEJ and included 307 attempts at PEJ placement with a success rate of 68%. While this study included multiple indications for DPEJ placement, gastroparesis was a substantial proportion ( $n = 61$  or 21%). A case series by Toussaint *et al*<sup>[63]</sup>, showed a PEJ technical success rate of 78.6% with no immediate complications reported; however, this was based upon a small sample size of 14 patients total. Based on these data, PEJ should be considered in the algorithm of enteral access for nutritional support before jejunostomy is considered.

The main limitation of DPEJ is technical difficulty as the jejunum is narrow, making it more difficult to advance a needle directly into the lumen<sup>[64]</sup>. This difficulty can be alleviated with balloon-assisted enteroscopy (BAE)<sup>[65]</sup>. Aktas *et al*<sup>[66]</sup> reported the first prospective study in which single-balloon enteroscopy (SBE)-assisted DPEJ was successful in 11 of the 12 procedures (92%). In this prospective case study, SBE was shown to facilitate the identification of an ideal DPEJ insertion site for the placement of a direct percutaneous jejunal feeding tube. While again this study is limited in size, the results were similar to previous small case series using double-balloon enteroscopy (DBE)-assisted DPEJ placement<sup>[65,67]</sup>.

## ALTERNATIVE TO SURGICAL GASTROJEJUNOSTOMY

While surgical gastrojejunostomy is a potential treatment option for patients with gastroparesis, the procedure is associated with substantial morbidity and mortality when patients are in less than ideal clinical condition<sup>[68-71]</sup>. Fritscher-Ravens *et al*<sup>[72,73]</sup> and Binmoeller *et al*<sup>[74]</sup> first described EUS-guided gastrojejunostomy in pigs by using a compression button and lumen-apposing metal stent, respectively. These studies were built upon the success of previous studies - notably Cope *et al*<sup>[75]</sup> creating the first transluminal anastomosis using self-expanding metal stents (SEMS) and Chopita *et al*<sup>[76]</sup> reporting the first clinical trial using fully covered version of the flared stent. While the Fritscher-Ravens *et al*<sup>[72,73]</sup> and Binmoeller *et al*<sup>[74]</sup> studies were performed as possible alternatives to surgical bypass for the palliation of malignant gastric outlet obstruction, more benign conditions such as gastroparesis may potentially benefit from this transluminal therapy.

An additional study performed by Itoi *et al*<sup>[77]</sup>, developed a new enteric tube that allowed for the entrapment of fluid between the double balloons without the need to use tissue-opposing devices such as tilt tags. This maintains distention of the small bowel between the double balloons at the initial FNA needle puncture site. Although simply a pilot study, all stents, with exception of one stent, were successfully deployed without complication. All animals showed normal eating behavior without signs of infection at 1-mo follow-up post procedure. Endoscopic gastric imaging showed patent stents in all pigs. While this study lacks power, the initial findings are impressive. The development of a EUS-guided gastrojejunostomy appears to be promising as a minimally invasive treatment.

Although surgical gastrojejunostomy has been shown to improve gastroparetic symptoms, EUS-guided gastrojejunostomy warrants further investigation owing to unknown long-term stent safety and patency issues<sup>[74]</sup>. Ideally, the stent can be removed after an interval of time, leaving a permanent fistula tract. However, studies are needed to determine the necessary pressure gradient and initial gastrojejunostomy tract diameter in order to maintain long-term fistula patency after stent removal. The minute amount of data available to date, while optimistic and potentially transformative, requires repeat analysis and trials with human study before implementation into the gastroenterologist's everyday arsenal. However, given the technical success of studies above, the future of endoscopic gastrojejunostomy using EUS-guided lumen-apposing metal stents is bright with the potential to diminish the need for invasive surgeries and improve symptoms of gastroparesis refractory to medical management.

## CONCLUSION

In summary, there is a wealth of potential endoscopic approaches that may one day be at the disposal of endoscopists. While many of these procedures and pioneering approaches have the potential to be ground-breaking and radically transform the standard management algorithm of refractory gastroparesis, all of them require more studies to validate, corroborate, and substantiate early optimistic data. The opportunities for less invasive endoscopic treatment of gastroparesis are abundant and inspiring. From further studies to evaluate the true effectiveness of IPBI to the potential for EUS-guided lumen-apposing metal stents for gastrojejunostomy, the role of endoscopic therapies in management of gastroparesis is likely to expand in near future.

## REFERENCES

- Camilleri M. Integrated upper gastrointestinal response to food intake. *Gastroenterology* 2006; **131**: 640-658 [PMID: 16890616 DOI: 10.1053/j.gastro.2006.03.023]
- Parkman HP, Hasler WL, Fisher RS; American Gastroenterological Association. American Gastroenterological Association technical review on the diagnosis and treatment of gastroparesis. *Gastroenterology* 2004; **127**: 1592-1622 [PMID: 15521026]
- Friedenberg FK, Parkman HP. Advances in the management of gastroparesis. *Curr Treat Options Gastroenterol* 2007; **10**: 283-293 [PMID: 17761121]
- Malagelada JR, Rees WD, Mazzotta LJ, Go VL. Gastric motor abnormalities in diabetic and postvagotomy gastroparesis: effect of metoclopramide and bethanechol. *Gastroenterology* 1980; **78**: 286-293 [PMID: 7350052]
- Camilleri M, Brown ML, Malagelada JR. Relationship between impaired gastric emptying and abnormal gastrointestinal motility. *Gastroenterology* 1986; **91**: 94-99 [PMID: 3710086]
- Mearin F, Camilleri M, Malagelada JR. Pyloric dysfunction in diabetics with recurrent nausea and vomiting. *Gastroenterology* 1986; **90**: 1919-1925 [PMID: 3699409]
- Soykan I, Sivri B, Sarosiek I, Kiernan B, McCallum RW. Demography, clinical characteristics, psychological and abuse profiles, treatment, and long-term follow-up of patients with gastroparesis. *Dig Dis Sci* 1998; **43**: 2398-2404 [PMID: 9824125]
- Jung HK, Choung RS, Locke GR, Schleck CD, Zinsmeister AR, Szarka LA, Mullan B, Talley NJ. The incidence, prevalence, and outcomes of patients with gastroparesis in Olmsted County, Minnesota, from 1996 to 2006. *Gastroenterology* 2009; **136**: 1225-1233 [PMID: 19249393 DOI: 10.1053/j.gastro.2008.12.047]
- Abell TL, Camilleri M, Donohoe K, Hasler WL, Lin HC, Maurer AH, McCallum RW, Nowak T, Nusynowitz ML, Parkman HP, Shreve P, Szarka LA, Snape WJ, Ziessman HA; American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. *Am J Gastroenterol* 2008; **103**: 753-763 [PMID: 18028513 DOI: 10.1111/j.1572-0241.2007.01636.x]
- Tougas G, Eaker EY, Abell TL, Abrahamsson H, Boivin M, Chen J, Hocking MP, Quigley EM, Koch KL, Tokayer AZ, Stanghellini V, Chen Y, Huizinga JD, Rydén J, Bourgeois I, McCallum RW. Assessment of gastric emptying using a low fat meal: establishment of international control values. *Am J Gastroenterol* 2000; **95**: 1456-1462 [PMID: 10894578 DOI: 10.1111/j.1572-0241.2000.02076.x]
- McCallum RW, Ricci DA, Rakatansky H, Behar J, Rhodes JB, Salen G, Deren J, Ippoliti A, Olsen HW, Falchuk K. A multicenter placebo-controlled clinical trial of oral metoclopramide in diabetic gastroparesis. *Diabetes Care* 1983; **6**: 463-467 [PMID: 6400707]
- Perkel MS, Moore C, Hersh T, Davidson ED. Metoclopramide therapy in patients with delayed gastric emptying: a randomized, double-blind study. *Dig Dis Sci* 1979; **24**: 662-666 [PMID: 385260]
- Ricci DA, Saltzman MB, Meyer C, Callachan C, McCallum RW. Effect of metoclopramide in diabetic gastroparesis. *J Clin Gastroenterol* 1985; **7**: 25-32 [PMID: 3884697]
- Snape WJ, Battle WM, Schwartz SS, Braunstein SN, Goldstein HA, Alavi A. Metoclopramide to treat gastroparesis due to diabetes mellitus: a double-blind, controlled trial. *Ann Intern Med* 1982; **96**: 444-446 [PMID: 7065559]
- Erbas T, Varoglu E, Erbas B, Tastekin G, Akalin S. Comparison of metoclopramide and erythromycin in the treatment of diabetic gastroparesis. *Diabetes Care* 1993; **16**: 1511-1514 [PMID: 8299441]
- Stevens JE, Jones KL, Rayner CK, Horowitz M. Pathophysiology and pharmacotherapy of gastroparesis: current and future perspectives. *Expert Opin Pharmacother* 2013; **14**: 1171-1186 [PMID: 23663133 DOI: 10.1517/14656566.2013.795948]
- Sturm A, Holtmann G, Goebell H, Gerken G. Prokinetics in patients with gastroparesis: a systematic analysis. *Digestion* 1999; **60**: 422-427 [PMID: 10473966]
- Shin A, Wo JM. Therapeutic applications of ghrelin agonists in the



- treatment of gastroparesis. *Curr Gastroenterol Rep* 2015; **17**: 430 [PMID: 25702264 DOI: 10.1007/s11894-015-0430-8]
- 19 **Wang L**, Mogami S, Karasawa H, Yamada C, Yakabi S, Yakabi K, Hattori T, Taché Y. Preventive effect of rikkunshito on gastric motor function inhibited by L-dopa in rats. *Peptides* 2014; **55**: 136-144 [PMID: 24631952 DOI: 10.1016/j.peptides.2014.02.011]
- 20 **James AN**, Ryan JP, Parkman HP. Inhibitory effects of botulinum toxin on pyloric and antral smooth muscle. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G291-G297 [PMID: 12660140 DOI: 10.1152/ajpgi.00296.2002]
- 21 **Schiavo G**, Shone CC, Rossetto O, Alexander FC, Montecucco C. Botulinum neurotoxin serotype F is a zinc endopeptidase specific for VAMP/syntaxin. *J Biol Chem* 1993; **268**: 11516-11519 [PMID: 8505288]
- 22 **Hou YP**, Zhang YP, Song YF, Zhu CM, Wang YC, Xie GL. Botulinum toxin type A inhibits rat pyloric myoelectrical activity and substance P release in vivo. *Can J Physiol Pharmacol* 2007; **85**: 209-214 [PMID: 17487262 DOI: 10.1139/y07-018]
- 23 **Coleski R**, Anderson MA, Hasler WL. Factors associated with symptom response to pyloric injection of botulinum toxin in a large series of gastroparesis patients. *Dig Dis Sci* 2009; **54**: 2634-2642 [PMID: 19184429 DOI: 10.1007/s10620-008-0660-9]
- 24 **Lacy BE**, Weiser K, Kennedy A. Botulinum toxin and gastrointestinal tract disorders: panacea, placebo, or pathway to the future? *Gastroenterol Hepatol* (N Y) 2008; **4**: 283-295 [PMID: 21960915]
- 25 **Bromer MQ**, Friedenberg F, Miller LS, Fisher RS, Swartz K, Parkman HP. Endoscopic pyloric injection of botulinum toxin A for the treatment of refractory gastroparesis. *Gastrointest Endosc* 2005; **61**: 833-839 [PMID: 15933684]
- 26 **Maksymowych AB**, Reinhard M, Malizio CJ, Goodnough MC, Johnson EA, Simpson LL. Pure botulinum neurotoxin is absorbed from the stomach and small intestine and produces peripheral neuromuscular blockade. *Infect Immun* 1999; **67**: 4708-4712 [PMID: 10456920]
- 27 **Ezzeddine D**, Jit R, Katz N, Gopalswamy N, Bhutani MS. Pyloric injection of botulinum toxin for treatment of diabetic gastroparesis. *Gastrointest Endosc* 2002; **55**: 920-923 [PMID: 12024156]
- 28 **Miller LS**, Szych GA, Kantor SB, Bromer MQ, Knight LC, Maurer AH, Fisher RS, Parkman HP. Treatment of idiopathic gastroparesis with injection of botulinum toxin into the pyloric sphincter muscle. *Am J Gastroenterol* 2002; **97**: 1653-1660 [PMID: 12135014 DOI: 10.1111/j.1572-0241.2002.05823.x]
- 29 **DeSantis ER**, Huang S. Botulinum toxin type A for treatment of refractory gastroparesis. *Am J Health Syst Pharm* 2007; **64**: 2237-2240 [PMID: 17959574 DOI: 10.2146/ajhp060394]
- 30 **Lacy BE**, Crowell MD, Schettler-Duncan A, Mathis C, Pasricha PJ. The treatment of diabetic gastroparesis with botulinum toxin injection of the pylorus. *Diabetes Care* 2004; **27**: 2341-2347 [PMID: 15451898]
- 31 **Reddymasu SC**, Singh S, Sankula R, Lavenbarg TA, Olyae M, McCallum RW. Endoscopic pyloric injection of botulinum toxin-A for the treatment of postvagotomy gastroparesis. *Am J Med Sci* 2009; **337**: 161-164 [PMID: 19174691 DOI: 10.1097/MAJ.0b013e318182ee33]
- 32 **Bai Y**, Xu MJ, Yang X, Xu C, Gao J, Zou DW, Li ZS. A systematic review on intrapyloric botulinum toxin injection for gastroparesis. *Digestion* 2010; **81**: 27-34 [PMID: 20029206 DOI: 10.1159/000235917]
- 33 **Friedenberg FK**, Palit A, Parkman HP, Hanlon A, Nelson DB. Botulinum toxin A for the treatment of delayed gastric emptying. *Am J Gastroenterol* 2008; **103**: 416-423 [PMID: 18070232 DOI: 10.1111/j.1572-0241.2007.01676.x]
- 34 **Arts J**, Holvoet L, Caenepeel P, Bisschops R, Sifrim D, Verbeke K, Janssens J, Tack J. Clinical trial: a randomized-controlled crossover study of intrapyloric injection of botulinum toxin in gastroparesis. *Aliment Pharmacol Ther* 2007; **26**: 1251-1258 [PMID: 17944739 DOI: 10.1111/j.1365-2036.2007.03467.x]
- 35 **Camilleri M**, Parkman HP, Shafi MA, Abell TL, Gerson L; American College of Gastroenterology. Clinical guideline: management of gastroparesis. *Am J Gastroenterol* 2013; **108**: 18-37; quiz 38 [PMID: 23147521 DOI: 10.1038/ajg.2012.373]
- 36 Humanitarian device exemption for Enterra device. *Federal Registry* 2000; **65**: 78495-78496
- 37 **Abell T**, McCallum R, Hocking M, Koch K, Abrahamsson H, Leblanc I, Lindberg G, Konturek J, Nowak T, Quigley EM, Tougas G, Starkebaum W. Gastric electrical stimulation for medically refractory gastroparesis. *Gastroenterology* 2003; **125**: 421-428 [PMID: 12891544]
- 38 **Lin Z**, Forster J, Sarosiek I, McCallum RW. Treatment of gastroparesis with electrical stimulation. *Dig Dis Sci* 2003; **48**: 837-848 [PMID: 12772777]
- 39 **McCallum RW**, Snape W, Brody F, Wo J, Parkman HP, Nowak T. Gastric electrical stimulation with Enterra therapy improves symptoms from diabetic gastroparesis in a prospective study. *Clin Gastroenterol Hepatol* 2010; **8**: 947-954; quiz e116 [PMID: 20538073 DOI: 10.1016/j.cgh.2010.05.020]
- 40 **McCallum RW**, Sarosiek I, Parkman HP, Snape W, Brody F, Wo J, Nowak T. Gastric electrical stimulation with Enterra therapy improves symptoms of idiopathic gastroparesis. *Neurogastroenterol Motil* 2013; **25**: 815-e636 [PMID: 23895180 DOI: 10.1111/nmo.12185]
- 41 **Anand C**, Al-Juburi A, Familoni B, Rashed H, Cutts T, Abidi N, Johnson WD, Minocha A, Abell TL. Gastric electrical stimulation is safe and effective: a long-term study in patients with drug-refractory gastroparesis in three regional centers. *Digestion* 2007; **75**: 83-89 [PMID: 17519527 DOI: 10.1159/000102961]
- 42 **Forster J**, Sarosiek I, Lin Z, Durham S, Denton S, Roeser K, McCallum RW. Further experience with gastric stimulation to treat drug refractory gastroparesis. *Am J Surg* 2003; **186**: 690-695 [PMID: 14672781]
- 43 **Abell T**, Lou J, Tabbaa M, Batista O, Malinowski S, Al-Juburi A. Gastric electrical stimulation for gastroparesis improves nutritional parameters at short, intermediate, and long-term follow-up. *J Parenter Enteral Nutr* 2003; **27**: 277-281 [PMID: 12903891]
- 44 **Chu H**, Lin Z, Zhong L, McCallum RW, Hou X. Treatment of high-frequency gastric electrical stimulation for gastroparesis. *J Gastroenterol Hepatol* 2012; **27**: 1017-1026 [PMID: 22128901 DOI: 10.1111/j.1440-1746.2011.06999.x]
- 45 **National Institute of Health and Care Excellence**. NICE interventional procedures guidance [IPG489]. May 2014. Accessed December 18, 2014. Available from: URL: <https://www.nice.org.uk/guidance/IPG489/chapter/1-recommendations>
- 46 **Abell TL**, Johnson WD, Kedar A, Runnels JM, Thompson J, Weeks ES, Minocha A, Griswold ME. A double-masked, randomized, placebo-controlled trial of temporary endoscopic mucosal gastric electrical stimulation for gastroparesis. *Gastrointest Endosc* 2011; **74**: 496-503.e3 [PMID: 21872708 DOI: 10.1016/j.gie.2011.05.022]
- 47 **Jayanthi NV**, Dexter SP, Sarella AI; Leeds Gastroparesis Multi-Disciplinary Team. Gastric electrical stimulation for treatment of clinically severe gastroparesis. *J Minim Access Surg* 2013; **9**: 163-167 [PMID: 24250062 DOI: 10.4103/0972-9941.118833]
- 48 **Deb S**, Tang SJ, Abell TL, McLawhorn T, Huang WD, Lahr C, To SD, Easter J, Chiao JC. Development of innovative techniques for the endoscopic implantation and securing of a novel, wireless, miniature gastrostimulator (with videos). *Gastrointest Endosc* 2012; **76**: 179-184 [PMID: 22726478 DOI: 10.1016/j.gie.2012.03.177]
- 49 **Deb S**, Tang SJ, Abell TL, Rao S, Huang WD, To SD, Lahr C, Chiao JC. An endoscopic wireless gastrostimulator (with video). *Gastrointest Endosc* 2012; **75**: 411-415, 415.e1 [PMID: 22248609 DOI: 10.1016/j.gie.2011.09.052]
- 50 **Clarke JO**, Sharaiha RZ, Kord Valeshabad A, Lee LA, Kalloo AN, Khashab MA. Through-the-scope transpyloric stent placement improves symptoms and gastric emptying in patients with gastroparesis. *Endoscopy* 2013; **45** Suppl 2 UCTN: E189-E190 [PMID: 23824975 DOI: 10.1055/s-0032-1326400]
- 51 **Saxena P**, Clarke JO, Penas I. Refractory gastroparesis can be successfully managed with transpyloric stent placement and fixation. *Gastroenterology*. Digestive Disease Week; 2014 May 3-6;

- DDW 2014 Chicago, United States. *DDW* 2014; **146** (5 SUPPL. 1): pp S-771
- 52 **Rao SS**, Vemuri S, Harris B, Schulze K. Fundic balloon distension stimulates antral and duodenal motility in man. *Dig Dis Sci* 2002; **47**: 1015-1019 [PMID: 12018896]
  - 53 **Khashab MA**, Stein E, Clarke JO, Saxena P, Kumbhari V, Chander Roland B, Kalloo AN, Stavropoulos S, Pasricha P, Inoue H. Gastric peroral endoscopic myotomy for refractory gastroparesis: first human endoscopic pyloromyotomy (with video). *Gastrointest Endosc* 2013; **78**: 764-768 [PMID: 24120337 DOI: 10.1016/j.gie.2013.07.019]
  - 54 **Pescarus R**, Shlomovitz E, Swanson LL. Per-oral endoscopic myotomy (POEM) for esophageal achalasia. *Curr Gastroenterol Rep* 2014; **16**: 369 [PMID: 24362953 DOI: 10.1007/s11894-013-0369-6]
  - 55 **Pasricha PJ**, Hawari R, Ahmed I, Chen J, Cotton PB, Hawes RH, Kalloo AN, Kantsevoy SV, Gostout CJ. Submucosal endoscopic esophageal myotomy: a novel experimental approach for the treatment of achalasia. *Endoscopy* 2007; **39**: 761-764 [PMID: 17703382 DOI: 10.1055/s-2007-966764]
  - 56 **Shlomovitz E**, Pescarus R, Cassera MA, Sharata AM, Reavis KM, Dunst CM, Swanson LL. Early human experience with peroral endoscopic pyloromyotomy (POP). *Surg Endosc* 2015; **29**: 543-551 [PMID: 25106716 DOI: 10.1007/s00464-014-3720-6]
  - 57 **Abell TL**, Bernstein RK, Cutts T, Farrugia G, Forster J, Hasler WL, McCallum RW, Olden KW, Parkman HP, Parrish CR, Pasricha PJ, Prather CM, Soffer EE, Twillman R, Vinik AI. Treatment of gastroparesis: a multidisciplinary clinical review. *Neurogastroenterol Motil* 2006; **18**: 263-283 [PMID: 16553582 DOI: 10.1111/j.1365-2982.2006.00760.x]
  - 58 **Parkman HP**, Yates K, Hasler WL, Nguyen L, Pasricha PJ, Snape WJ, Farrugia G, Koch KL, Abell TL, McCallum RW, Lee L, Unalp-Arida A, Tonascia J, Hamilton F. Clinical features of idiopathic gastroparesis vary with sex, body mass, symptom onset, delay in gastric emptying, and gastroparesis severity. *Gastroenterology* 2011; **140**: 101-115 [PMID: 20965184 DOI: 10.1053/j.gastro.2010.10.015]
  - 59 **Shike M**, Latkany L, Gerdes H, Bloch AS. Direct percutaneous endoscopic jejunostomies for enteral feeding. *Gastrointest Endosc* 1996; **44**: 536-540 [PMID: 8934158]
  - 60 **Panagiotakis PH**, DiSario JA, Hilden K, Ogara M, Fang JC. DPEJ tube placement prevents aspiration pneumonia in high-risk patients. *Nutr Clin Pract* 2008; **23**: 172-175 [PMID: 18390785 DOI: 10.1177/0884533608314537]
  - 61 **Tang DM**, Friedenberg FK. Gastroparesis: approach, diagnostic evaluation, and management. *Dis Mon* 2011; **57**: 74-101 [PMID: 21329779 DOI: 10.1016/j.disamonth.2010.12.007]
  - 62 **Maple JT**, Petersen BT, Baron TH, Gostout CJ, Wong Kee Song LM, Buttar NS. Direct percutaneous endoscopic jejunostomy: outcomes in 307 consecutive attempts. *Am J Gastroenterol* 2005; **100**: 2681-2688 [PMID: 16393220 DOI: 10.1111/j.1572-0241.2005.00334.x]
  - 63 **Toussaint E**, Van Gossum A, Ballarin A, Le Moine O, Estenne M, Knoop C, Devière J, Arvanitakis M. Percutaneous endoscopic jejunostomy in patients with gastroparesis following lung transplantation: feasibility and clinical outcome. *Endoscopy* 2012; **44**: 772-775 [PMID: 22833022 DOI: 10.1055/s-0032-1309735]
  - 64 **Moran GW**, Fisher NC. Direct Percutaneous Endoscopic Jejunostomy: High Completion Rates with Selective Use of a Long Drainage Access Needle. *Diagn Ther Endosc* 2009; **2009**: 520879 [PMID: 19547660 DOI: 10.1155/2009/520879]
  - 65 **Despott EJ**, Gabe S, Tripoli E, Konieczko K, Fraser C. Enteral access by double-balloon enteroscopy: an alternative method of direct percutaneous endoscopic jejunostomy placement. *Dig Dis Sci* 2011; **56**: 494-498 [PMID: 20585980 DOI: 10.1007/s10620-010-1306-2]
  - 66 **Aktas H**, Mensink PB, Kuipers EJ, van Buuren H. Single-balloon enteroscopy-assisted direct percutaneous endoscopic jejunostomy. *Endoscopy* 2012; **44**: 210-212 [PMID: 22271031 DOI: 10.1055/s-0031-1291442]
  - 67 **Yan KK**, Kelly MI, Samuel DS. Direct endoscopic percutaneous jejunostomy placement with double balloon enteroscopy. *Endoscopy* 2010; **42** Suppl 2: E43 [PMID: 20157882 DOI: 10.1055/s-0029-1214775]
  - 68 **Lesurtel M**, Dehni N, Tiet E, Parc R, Paye F. Palliative surgery for unresectable pancreatic and periampullary cancer: a reappraisal. *J Gastrointest Surg* 2006; **10**: 286-291 [PMID: 16455463 DOI: 10.1016/j.gassur.2005.05.011]
  - 69 **Nuzzo G**, Clemente G, Cadeddu F, Giovannini I. Palliation of unresectable periampullary neoplasms. "surgical" versus "non-surgical" approach. *Hepatogastroenterology* 2004; **51**: 1282-1285 [PMID: 15362733]
  - 70 **Ly J**, O'Grady G, Mittal A, Plank L, Windsor JA. A systematic review of methods to palliate malignant gastric outlet obstruction. *Surg Endosc* 2010; **24**: 290-297 [PMID: 19551436 DOI: 10.1007/s00464-009-0577-1]
  - 71 **Ausania F**, Vallance AE, Manas DM, Prentis JM, Snowden CP, White SA, Charnley RM, French JJ, Jaques BC. Double bypass for inoperable pancreatic malignancy at laparotomy: postoperative complications and long-term outcome. *Ann R Coll Surg Engl* 2012; **94**: 563-568 [PMID: 23131226 DOI: 10.1308/003588412X13373405386934]
  - 72 **Fritscher-Ravens A**, Mosse CA, Mills TN, Mukherjee D, Park PO, Swain P. A through-the-scope device for suturing and tissue approximation under EUS control. *Gastrointest Endosc* 2002; **56**: 737-742 [PMID: 12397289 DOI: 10.1067/mge.2002.129084]
  - 73 **Fritscher-Ravens A**, Mosse CA, Mukherjee D, Mills T, Park PO, Swain CP. Transluminal endosurgery: single lumen access anastomotic device for flexible endoscopy. *Gastrointest Endosc* 2003; **58**: 585-591 [PMID: 14520300]
  - 74 **Binmoeller KF**, Shah JN. Endoscopic ultrasound-guided gastroenterostomy using novel tools designed for transluminal therapy: a porcine study. *Endoscopy* 2012; **44**: 499-503 [PMID: 22531985 DOI: 10.1055/s-0032-1309382]
  - 75 **Cope C**, Clark TW, Ginsberg G, Habecker P. Stent placement of gastroenteric anastomoses formed by magnetic compression. *J Vasc Interv Radiol* 1999; **10**: 1379-1386 [PMID: 10584655]
  - 76 **Chopita N**, Vaillaverde A, Cope C, Bernedo A, Martinez H, Landoni N, Jmelnitzky A, Burgos H. Endoscopic gastroenteric anastomosis using magnets. *Endoscopy* 2005; **37**: 313-317 [PMID: 15824939 DOI: 10.1055/s-2005-861358]
  - 77 **Itoi T**, Itokawa F, Uraoka T, Gotoda T, Horii J, Goto O, Moriyasu F, Moon JH, Kitagawa Y, Yahagi N. Novel EUS-guided gastrojejunostomy technique using a new double-balloon enteric tube and lumen-apposing metal stent (with videos). *Gastrointest Endosc* 2013; **78**: 934-939 [PMID: 24237949 DOI: 10.1016/j.gie.2013.09.025]

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## Minimally invasive treatment of pancreatic pseudocysts

Enver Zerem, Goran Hauser, Svjetlana Loga-Zec, Suad Kunosić, Predrag Jovanović, Dino Crnkić

Enver Zerem, Predrag Jovanović, Department of Gastroenterology, University Clinical Center Tuzla, 75000 Tuzla, Bosnia and Herzegovina

Enver Zerem, Department of Medical Sciences, The Academy of Sciences and Arts of Bosnia and Herzegovina, 71000 Sarajevo, Bosnia and Herzegovina

Goran Hauser, Department of Internal Medicine, Division of Gastroenterology, Clinical Hospital Centre Rijeka, 51000 Rijeka, Croatia

Svjetlana Loga-Zec, Institute of Pharmacology, Medical Faculty, University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina

Suad Kunosić, Department of Physics, Faculty of Natural Sciences and Mathematics, University of Tuzla, 75000 Tuzla, Bosnia and Herzegovina

Dino Crnkić, Faculty of Pharmacology, University of Tuzla, 75000 Tuzla, Bosnia and Herzegovina

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**Correspondence to:** Enver Zerem, MD, PhD, Department of Gastroenterology, University Clinical Center Tuzla, Trnovac bb, 75000 Tuzla, Bosnia and Herzegovina. [zerem@live.com](mailto:zerem@live.com)  
 Telephone: +387-35303300  
 Fax: +387-35266485

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### Abstract

A pancreatic pseudocyst (PPC) is typically a complication of acute and chronic pancreatitis, trauma or pancreatic duct obstruction. The diagnosis of PPC can be made if an acute fluid collection persists for 4 to 6 wk and is enveloped by a distinct wall. Most PPCs regress spontaneously and require no treatment, whereas some may persist and progress until complications occur. The decision whether to treat a patient who has a PPC, as well as when and with what treatment modalities, is a difficult one. PPCs can be treated with a variety of methods: percutaneous catheter drainage (PCD), endoscopic transpapillary or transmural drainage, laparoscopic surgery, or open pseudocystoenterostomy. The recent trend in the management of symptomatic PPC has moved toward less invasive approaches such as endoscopic- and image-guided PCD. The endoscopic approach is suitable because most PPCs lie adjacent to the stomach. The major advantage of the endoscopic approach is that it creates a permanent pseudocysto-gastric track with no spillage of pancreatic enzymes. However, given the drainage problems, the monitoring, catheter manipulation and the analysis of cystic content are very difficult or impossible to perform endoscopically, unlike in the PCD approach. Several conditions must be met to achieve the complete obliteration of the cyst cavity. Pancreatic duct anatomy is an important factor in the prognosis of the treatment outcome, and the recovery of disrupted pancreatic ducts is the main prognostic factor for successful treatment of PPC, regardless of the treatment method used. In this article, we review and evaluate the minimally invasive approaches in the management of PPCs.

**Key words:** Complications; Pseudocyst; Treatment; Drainage; Outcomes

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**Core tip:** Pancreatic pseudocysts (PPCs) are common complications of acute and chronic pancreatitis, pancreatic trauma, and pancreatic duct obstruction. They can be treated with a variety of methods: percutaneous catheter drainage, endoscopic transpapillary or transmural drainage, laparoscopic surgery, or open pseudocystoenterostomy. It is a difficult decision whether to treat a patient with a PPC and if so, with what treatment modalities and when. This article presents and critically evaluates the minimally invasive approaches for the treatment of PPCs.

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## INTRODUCTION

A pancreatic pseudocyst (PPC) is defined as a collection of fluid in the peripancreatic or intra-pancreatic tissues, is surrounded by a well-defined wall and contains essentially no solid material<sup>[1]</sup>. PPCs are usually complications of both acute and chronic pancreatitis, pancreatic trauma, and pancreatic duct (PD) obstruction<sup>[2-6]</sup>.

It is a difficult decision whether to treat a patient who has a PPC, and if so, when and with what treatment modalities. PPCs should initially be managed conservatively because many resolve spontaneously within 4 to 6 wk. Although most PPCs regress spontaneously and require no treatment, some (especially those larger than 6 cm) require treatment to prevent cystic infection, rupture, haemorrhage, and the resultant obstruction of the stomach, small bowel, colon or bile ducts<sup>[7,8]</sup>.

Traditionally, surgical approach was the treatment of choice for symptomatic PPCs. Although surgery is effective, complications can occur in up to 35% of patients, and death from surgery has also been noted<sup>[9]</sup>. The recent trend in the management of symptomatic PPC has been toward less invasive approaches such as endoscopic and image-guided percutaneous catheter drainage (PCD)<sup>[2-13]</sup>.

Several conditions must be met to achieve the complete obliteration of the cyst cavity. PD anatomy is an important factor for the prognosis and the treatment outcomes. When PPC-PD communication is identified, the mean duration of drainage increases to weeks or months, depending on the condition of the PD. The recovery of a disrupted PD is the main prognostic factor for successful treatment of PPC regardless of the treatment method<sup>[2,11,12,14]</sup>. This

review article presents and critically evaluates the minimally invasive approaches for the treatment of PPCs.

## DIAGNOSIS OF PPCS

The distinction between PPC and other similar entities, such as benign and malignant cystic lesions, vascular pathology such as pseudoaneurysms and hematomas, seromas, abscesses, and bilomas, is crucial in the decision to treat a patient who has a PPC, as well as when and by which method. This requires the correlation of often complex and overlapping clinical presentations and laboratory findings with those of imaging studies, such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI)<sup>[15,16]</sup>.

### Clinical presentation and laboratory findings

PPC is typically asymptomatic, and its clinical presentation tends to occur in cases with complications throughout their clinical course. During physical examination, the most common presenting symptoms that might be attributed to the development of symptomatic PPC are persistent abdominal pain and/or an epigastric mass with a persistently raised serum amylase level<sup>[17]</sup>. Clinical presentations of PPC complications are infection, rupture and haemorrhage<sup>[7,17]</sup>. Infection occurs in approximately 10% of cases and is characterized by fever and abdominal pain. The leakage of the content from the PPC into the peritoneum can cause the appearance of pancreatic ascites. However, sudden rupture of the PPC into the peritoneum produces severe peritonitis that is often fatal. Haemorrhage is caused by the erosion of the small vessels that line the cyst wall or the erosion of surrounding major blood vessels. Intracystic bleeding leads to a rapid enlargement of the PPC, which produces pain and shock. Spontaneous rupture of the PPC into the gastrointestinal tract can result in the drainage of its contents into the gastrointestinal tract and the amelioration of symptoms. However, this is often associated with vomiting, hematemesis and melena<sup>[17]</sup>.

Laboratory findings have a limited value in the diagnosis of PPC. Serum amylase and lipase levels are persistently elevated in up to 76% of patients with PPC. When PPC produces a biliary obstruction, the serum bilirubin level is increased<sup>[17]</sup>.

### Imaging evaluation

The diagnosis of a PPC is usually established by imaging studies, whereby a rapid progress in the improvement of diagnostic modalities enables detection with high sensitivity and specificity.

Because transabdominal ultrasonography is a very inexpensive and noninvasive technique, it should be performed as a first step in the diagnosis of PPCs.



US has a diagnostic sensitivity of 75% to 90% in detecting PPCs and the technique is highly dependent on the experience of the examiner. It has a limited role in the assessment of small PPCs (smaller than 10 mm). However, small PPCs are asymptomatic and without clinical significance, usually not requiring any treatment<sup>[2,17]</sup>.

Endoscopic ultrasound (EUS) may typically display a small PPC, being the best method in distinguishing acute fluid collections from pancreatic abscesses and PPCs, with high sensitivity (93% to 100%) and specificity (92% to 98%)<sup>[18,19]</sup>. Besides, EUS can accurately define the proximity of the PPC to the gut lumen and surrounding large blood vessels. Limitations of EUS are its inability to demonstrate large PPCs which extend into peripancreatic areas in their entirety, and display PPCs which are more than 1 cm distant away from the gastric or duodenal wall<sup>[20,21]</sup>.

CT scanning is a standard and precise imaging modality in the setting of PPCs, with 82% to 100% sensitivity and 98% specificity. CT scan is more effective than US in detecting the secondary complications of a PPC, such as infection; hemorrhage, and involvement of adjacent structures<sup>[18,22]</sup>.

Endoscopic retrograde cholangiopancreatography (ERCP) may be useful in patients who require delineation of PD anatomy, helping to devise optimal therapy. Although ERCP provides less information regarding the pancreatic size and surrounding visceral structures than CT and ultrasound, it renders important information on the anatomy of the pancreatic and biliary ductal system<sup>[14]</sup>.

Magnetic resonance imaging (MRI) is a good alternative to CT for detection of PPCs due to its ability to characterize pancreatic and peripancreatic collections as partially or fully fluid in consistency. Magnetic resonance cholangiopancreatography (MRCP) may replace ERCP in the diagnostic evaluation of pancreatic duct. However, the diagnosis of PPC-PD communication is rather difficult because a communication can only be identified by MRCP if a high-intensity fluid tract can be detected between the pseudocyst and the duct<sup>[2,21,23-26]</sup>.

A plain radiograph of the abdomen is rarely helpful in diagnosing PPC. Occasionally, it may demonstrate displacement of the gastric bubble or calcification in the cyst wall. A chest radiograph may show elevations of the diaphragm, pleural effusion, or a mediastinal mass.

#### **Differential diagnosis between pseudocysts and cystic neoplasms**

The differential diagnosis between PPCs and cystic neoplasms may be difficult in patients with a pancreatic fluid collection. Clinical criteria such as prior episodes of acute pancreatitis, and data regarding chronic pancreatitis or a calcified cystic wall less than 1 cm thick, make the diagnosis of PPC more likely. On the contrary, weight loss, a palpable abdominal

mass, the lack of pre-existing pancreatic disease, and multilocular cysts with non-calcified walls thicker than 1 cm, all indicate the likelihood of a cystic malignant tumour. EUS or US-guided diagnostic puncture and sampling of the fluid content and of the PPC wall helps to distinguish cystic malignancies from PPCs<sup>[27-29]</sup>. Research has recently focused on the identification of new biomarkers for the diagnosis of malignant lesions. Important criteria for malignancy are a markedly elevated carcinoembryonic antigen (CEA) value in the cyst fluid (over 192 ng/mL) and increasing viscosity of the cyst content<sup>[18,30]</sup>.

### **INDICATIONS FOR TREATMENT OF PPCS**

The most important question in clinical practice related to acute or chronic PPCs is whether and when they should be treated. A careful preliminary clinical and imaging evaluation of benign pancreatic fluid collections can avoid unnecessary interventions. The majority of the simple PPCs are asymptomatic and do not require interventional treatment. Treatment is indicated if the complications are present or whether intervention is necessary to prevent complications. The indications for interventional procedures in the treatment of PPCs are summarized in Table 1.

Symptoms result from biliary obstruction, the effects of painful or obstructive masses, infection or haemorrhage into the cyst, pancreaticopleural fistula or compression of the surrounding major vessels, and in such cases, interventional treatment is typically indicated. Treatment is also indicated for symptomatic PPCs that cause abdominal distension, nausea and vomiting, pain, or gastrointestinal bleeding (Table 1).

PPCs larger than 4 cm that develop outside the pancreas can be considered independent predictive factors of persistent symptoms because they rarely regress spontaneously and can cause complications<sup>[31]</sup>. Therefore, if they demonstrate either unchanged size and morphology or progression over a period of more than 6 wk of observation, these are relative indications for treatment<sup>[15,31]</sup>. A relative indication for treatment includes PPCs whose wall thickness is between 5 and 10 mm and PPCs caused by the presence of chronic pancreatitis with duct abnormalities or stones in the PD. In these patients, constant irritation promotes inflammation and reduces the rate of spontaneous regression<sup>[18,31,32]</sup>. Whenever a malignant tumour is suspected, surgical treatment is urgently indicated (Table 1)<sup>[33,34]</sup>. When an intervention is required, the best option should be the application of a multidisciplinary approach based on the initial imaging and clinical findings.

### **MINIMALLY INVASIVE APPROACHES TO THE MANAGEMENT OF PPCS**

PPCs as benign fluid collections in the pancreas can

**Table 1 Indications for therapeutic intervention for pancreatic pseudocysts**

Clinical presentations and complications
<i>Local complications</i>
Infection of pancreatic pseudocyst
Hemorrhage into pancreatic pseudocyst
Rupture (can cause pancreatic ascites, shock and peritonitis)
<i>Involving adjacent organs</i>
Gastrointestinal tract:
Esophagus (secondary achalasia, mechanical dysphagia)
Stomach (clinically relevant gastric outlet stenosis, fistula, intramural gastric mass)
Duodenum (clinically relevant duodenal stenosis, fistula)
Colon (clinically relevant colonic stenosis and/or rectal bleeding)
Liver (stenosis of the common bile duct with jaundice due to compression)
Vascular:
Arterial (erosion of gastroduodenal and/or splenic artery)
Venous (thrombosis of portal and/or splenic vein)
Spleen (splenic rupture)
Genitourinary tract (stricture, fistula, ureter obstruction)
Chest (pancreaticopleural fistula, pleural effusion, mediastinal extension)
Skin (subcutaneous fat necrosis)
<i>Symptomatic pancreatic pseudocyst</i>
Abdominal distension
Nausea and vomiting
Pain
Upper gastrointestinal bleeding
<i>Relative indications for intervention in asymptomatic pancreatic pseudocyst</i>
Pseudocyst > 5 cm, unchanged in size and morphology for more than 6 wk <sup>[15]</sup>
Pseudocyst > 4 cm and extrapancreatic complications in patients with chronic alcoholic pancreatitis <sup>[31]</sup>
Cyst wall > 5 mm (mature cyst) <sup>[32]</sup>
Chronic pancreatitis with advanced pancreatic duct changes <sup>[31]</sup>
Suspected cystic pancreatic tumor (requiring surgery) <sup>[33,34]</sup>

mimic cystic neoplasms. Therefore, pretreatment evaluations of pancreatic fluid collections for appropriate therapeutic intervention should be focused on the exclusion of cystic neoplasms that masquerade as pseudocysts<sup>[35]</sup>. The topic of cystic neoplasms of the pancreas is broad, and thus this article focuses primarily on the minimally invasive treatments of benign PPCs. Once a PPC has been diagnosed, it must be determined whether it can be treated conservatively in hopes of a spontaneous resolution, or whether an intervention is necessary to prevent complications. If an intervention is necessary, it must be determined whether surgical, PCD, or endoscopic drainage (ED) is the best approach.

### Conservative management

Based on earlier studies on the clinical course of PPCs, the rate of spontaneous resolution of PPCs has been reported to be from 8% to 70%<sup>[15,17,18]</sup>. This wide range can be attributed to many factors that influence PPCs, including size, chronicity, wall thickness, multiplicity, and aetiology.

The size of the PPC is an important determinant of

spontaneous resolution. The majority of pseudocysts that are over 6 cm in size that persist for over 6 wk have been regarded as unlikely to resolve spontaneously<sup>[17,18,31,36]</sup>. However, some large PPCs (> 6 cm) may undergo spontaneous resolution, which suggests that the size of the PPC alone is not an indication for drainage<sup>[36,37]</sup>.

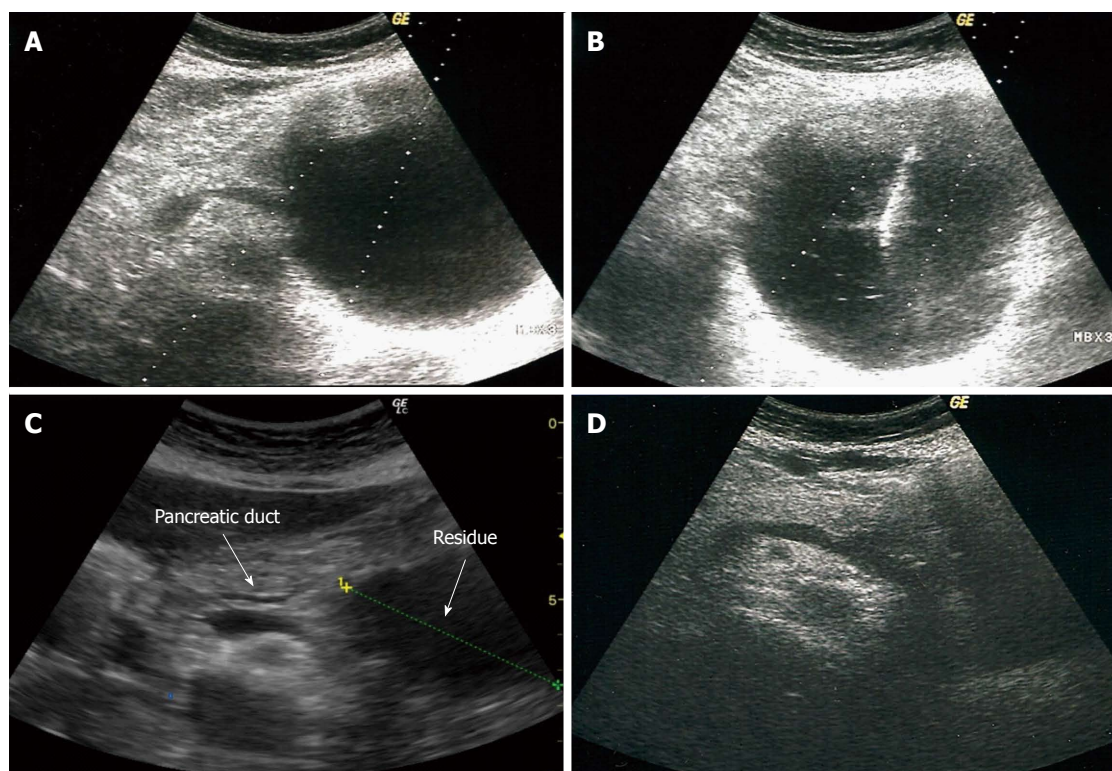
Chronicity adversely affects the healing of PPCs whereby PPCs that persist for 8 to 10 wk are unlikely to resolve spontaneously. Most PPCs that are likely to heal do so within 6 wk, but the resolution may occur after 24 wk or even 28 mo<sup>[17]</sup>. Chronic pancreatitis and pancreatic calcifications are also poor prognostic indicators<sup>[18,38]</sup>.

Other factors that indicate that spontaneous regression is less likely include the presence of multiple cysts<sup>[18,39]</sup>, location in the tail of the pancreas<sup>[37]</sup>, and a wall thickness greater than 1 cm<sup>[40]</sup>. The aetiology may also have some bearing on the outcome. PPCs related to alcohol abuse have a more favourable outcome compared with those of biliary aetiology. However, traumatic PPCs may have a high percentage of spontaneous resolution<sup>[39]</sup>.

The setting of non-interventional conservative management of PPCs is still poorly evaluated. Earlier studies showed that conservative treatment in the hope of spontaneous resolution was not without risks. Several studies have warned against serious, life-threatening complications related to the conservative treatment of PPCs<sup>[41-44]</sup>. However, with improved medical care, the incidence of complications as well as the mortality rate has decreased considerably. Several studies<sup>[36,41,45,46]</sup> have reported that some patients with PPCs can be managed conservatively if the presenting symptoms can be controlled. According to their results, the complication rates with conservative management are low (< 1%).

These results suggest that some patients with PPCs can be managed conservatively and that some pseudocysts can resolve with supportive medical care. Medical care includes the use of intravenous fluids, analgesics and antiemetics to control the presenting symptoms caused by PPC. For patients who can tolerate oral intake, a low fat diet is recommended, whereas for those who cannot tolerate oral nutrition, support can be provided *via* nasoenteral feeding or total parenteral nutrition<sup>[47]</sup>.

Somatostatin (octreotide) has an inhibitory effect on pancreatic exocrine secretion, and it can be used to decrease the pancreatic secretion, which leads to the resolution of PPC. Octreotide has also been used in conjunction with PCD of PPCs, which results in a shorter drainage time. The role of somatostatin in the management of PPCs is not clear because this treatment has not been adequately tested and only a handful of case series have been published<sup>[47-49]</sup>. Prospective controlled trials are necessary to demonstrate its efficacy.



**Figure 1** Appearance on ultrasound of a pancreatic pseudocyst before, during and after treatment. A: Appearance on ultrasound of a PPC in the tail of pancreas before treatment; B: Insertion of a catheter into the PPC; C: Residue of PPC with suspected PPC-PD communication (marked by arrows) immediately after the procedure; D: The appearance of the pancreas several months after the procedure. PPC: Pancreatic pseudocyst; PD: Pancreatic duct.

### Image-guided percutaneous treatment of PPCs

Image-guided percutaneous drainage of PPCs is a well-established and relatively inexpensive drainage method that involves either simple percutaneous aspiration or PCD. It is most commonly performed under ultrasound or CT control, and in some cases, under MRI or fluoroscopic guidance (Figure 1)<sup>[2,4,8,10,17,21,35,47,50-52]</sup>.

Single-step needle aspiration of PPCs is associated with a high recurrence rate (70% or more) and cannot be considered the optimal treatment<sup>[4,8]</sup>. The continuous vacuum drainage system is more effective because it continuously evacuates the cyst content and thereby avoids the lytic action of pancreatic enzymes that may lead to obliteration of the cyst cavity. This approach has achieved high initial drainage success rates (70%-100%) and reduced recurrence rates<sup>[4,8,23,53]</sup>.

Several conditions must be met to achieve the complete obliteration of the cyst cavity. PD disruption is the initial pathologic event that triggers PPC formation, and its anatomy is an important factor in the prognosis of the complete obliteration of the cyst cavity. Therefore, the complete removal of liquid and air, which is necessary to keep the cyst walls in close contact, constitutes the mechanical aspect of obliteration. The recovery of a disrupted PD has been recognized as the main prognostic factor for successful treatment of PPC regardless of the treatment method used<sup>[8,10,14]</sup>. Patients with PPC-PD communication

require a longer duration of drainage, as short-term drainage results in very high recurrence rates. However, some authors consider that the risk of septic complications is potentially increased with prolonged drainage periods<sup>[2,8,14,53-55]</sup>.

Percutaneous techniques are usually performed under local anaesthesia and seem technically feasible in the vast majority of patients with PPCs. Transperitoneal, retroperitoneal, transhepatic, transgastric, and transduodenal approaches are typically used. The access route for drainage depends on the size, location, and the disposition of the surrounding viscera and blood vessels<sup>[2,4,8,23,51]</sup>. Depending on the operator's experience, the tandem trocar technique or the Seldinger technique may be used. If the Seldinger technique is used, the catheter tract should be sequentially dilated over a guidewire. The use of three-dimensional ultrasound and colour Doppler may help to guide the catheters into the cyst cavities and aid in the circumvention of major vascular structures, which increases the safety of the procedure<sup>[2,4,17,21,47,52]</sup>.

After complete evacuation of the cystic content, the catheter should be secured to the skin and connected to a pressure bag for continuous external drainage. Catheter exchange may be performed as indicated. When the PPC has resolved and the drainage output becomes minimal (less than 10 mL/d), the catheter should be removed<sup>[8]</sup>. Percutaneous drainage is a safe and effective method for treatment of PPCs.



Complications include catheter-related secondary infections (9%), bleeding (1%-2%), inadvertent traversing of the pleural space or other viscera (1%-2%), catheter occlusion, cellulitis at the site of entry, and sepsis<sup>[4,55]</sup>. Another limitation of PCD is the development of pancreaticocutaneous fistulae. However, the resulting pancreatic-cutaneous fistula spontaneously resolves with time in 60% to 70% of patients<sup>[4,56]</sup>. Moreover, in some cases, the fistula can be successfully treated by image-guided PCD<sup>[57]</sup>. In the case of superinfection or drainage problems, monitoring, catheter manipulation and analysis of the cystic content can be performed much more easily by PCD than by an endoscopic approach<sup>[8,21,58,59]</sup>.

### Endoscopic drainage of PPCs

ED provides minimally invasive access to the PPC, which may be performed by a trans-papillary or a trans-mural approach. Sometimes a combination of both methods may be necessary to drain a pseudocyst. ED is suitable because most PPCs lie adjacent to the stomach; however, both endoscopic and radiologic skills are required here. The aim of endoscopic treatment is to create a connection between the pseudocyst cavity and the gastrointestinal lumen<sup>[60]</sup>.

Transpapillary/transductal endoscopic drainage is recommended for PPCs that communicate with the main PD or one of its side branches located in the head or the body of the pancreas. A limited number of PPCs may be drained *via* a transpapillary insertion of a stent that bridges the main pancreatic duct or a disrupted side branch. A favourable predictor of successful therapy is a dilated Wirsung duct above a stenotic area underneath the stent<sup>[4,7,61,62]</sup>.

This technique involves pancreatic endoscopic sphincterotomy, balloon dilatation of the commonly detected PD strictures, and insertion of a guidewire through the duct directly into the pseudocyst cavity. Thereafter, a plastic stent of 5 F to 7 F (up to 10 F) in diameter is inserted over the wire<sup>[4,23,63-65]</sup>. The duration of stenting depends on the clinical course of PPC regression<sup>[23,61]</sup>. Stents should be left in place for a longer duration because their removal within 2 mo is associated with a higher incidence of pseudocyst recurrence<sup>[66]</sup>. Some authors have reported on the routine exchange of stents every 6 to 8 wk for as long as the PPCs remained unresolved<sup>[64]</sup>.

Transpapillary drainage appears to be a safe and effective procedure (the immediate success rate is approximately 85%) with low morbidity (6%) and no reported mortality. The best results are obtained when the pseudocyst is older than 6 mo or smaller than 60 mm<sup>[64,67]</sup>. Haemorrhagic complications occurred in less than 1% of patients and pancreatitis occurred in 5%. Stent clogging, which can lead to infection, can be treated with stent changes alone. Broad-spectrum antibiotics are administered in cases of infected PPCs<sup>[4,23,61,64,65]</sup>.

Transmural endoscopic drainage (cystogastrostomy or cystoduodenostomy) is indicated for pseudocysts that do not communicate with the main PD and that are compressed against the digestive tract. Drainage of the cyst fluid by the trans-mural approach is achieved *via* the insertion of a stent between the pseudocyst and the gastric lumen (cystogastrostomy) or between the pseudocyst and the duodenal lumen (cystoduodenostomy). The drainage procedure may be performed either by direct endoscopy as a "semi-blind" procedure if an impression caused by the cyst is present, or by EUS guidance. Technically, cystoduodenostomy should be given preference over cystogastrostomy if both routes are deemed equally feasible. Direct endoscopic drainage can be performed only if the PPC is located next to the gastric or the duodenal lumen. The site of transmural puncture for a direct endoscopic intervention should be determined visually and fluoroscopically by an observed bulge that represents the extrinsic compression of the collection into the gut lumen<sup>[2,68-71]</sup>. Once the bulge is located, its apex can be identified as the optimal needle insertion site. After needle puncture and aspiration of the pseudocyst content (for biochemical and cytological analyses), a guidewire should be inserted along which an incision can be made with either a diathermic coagulation probe or a needle-knife papillotome. Once access has been achieved, a double pigtail catheter can be introduced into the cyst over the wire. The European Society of Gastrointestinal Endoscopy (ESGE) recommends the insertion of at least two double-pigtail plastic stents. Transmural stents should not be retrieved before complete resolution of the PPC as determined by cross-sectional imaging, and not before 2 mo of stenting<sup>[23,65-67,72-74]</sup>.

However, a bulge is often absent with smaller collections, collections with low serum albumin, and collections in or near the pancreatic tail. Therefore, to minimize the risk of complications such as the puncture of adjacent structures, bleeding, and perforation, EUS is increasingly used to perform ED<sup>[2,75]</sup>. Randomized clinical trials of endoscopic transmural drainage with and without EUS guidance showed that EUS-visualization had an advantage over conventional ED<sup>[68,69]</sup>. Even in large bulging pseudocysts, the EUS-guided drainage is superior to the purely endoscopic approach because the puncture of vascular structures and bleeding into the collection can be avoided during and immediately after the procedure by Doppler sonographic visualization<sup>[2,20,76]</sup>. The use of EUS-guided drainage has been reported, especially for PPCs that do not bulge onto the gut wall or PPCs with parietal vessels due to portal hypertension<sup>[4,32,64,77]</sup>. The stent type used for endoscopic drainage is currently a major area of interest. Conventional drainage with plastic stents has its limitations. A covered self-expandable metallic stent is an alternative to conventional drainage with plastic stents because it offers the option of access



to the fistula *via* a larger diameter for drainage, which may increase the final success rate. One problem with covered self expandable metallic stent is dislodgement, so a metallic stent with flared or looped ends at both extremities may be the best option<sup>[78-80]</sup>.

The advantages of the endoscopic approach compared with PCD include internal drainage and avoidance of external fistulae, but limitations include the need for multiple repeated procedures under sedation or anaesthesia; it is also necessary that the location of the PPC be further than 1-1.5 cm from the gut wall<sup>[20,65,67,81]</sup>. Moreover, in the case of superinfection or drainage problems, the monitoring, catheter manipulation and the analysis of cystic content are very difficult by ED<sup>[8,11,21,82]</sup>. A combination of a percutaneous approach with endoscopic transmural drainage can prevent external fistulae and avoid repetitive endoscopic interventions<sup>[83]</sup>.

Some authors advocate the use of a combination of transmural and transpapillary techniques to drain pseudocysts. They have used ERCP in the same endoscopic session to assess and treat any PD leakage; when PD leaks are evident, ERCP is also used for the placement of PD stents to bridge the leak site or stricture. Thus, when the treatment of the cause of the pseudocyst (*i.e.*, the duct leak) is possible by placement of concomitant PD stents, this has been shown to yield better outcomes<sup>[13,61,81,84]</sup>. Additionally, in patients with disconnected duct syndrome, transgastric stent removal results in a lack of a conduit for drainage of pancreatic secretions, which leads to pseudocyst recurrence<sup>[13,85]</sup>.

### Laparoscopic surgery

The classic open surgical approach for the treatment of PPC requires a laparotomy with attendant risks of morbidity and mortality. The development of advanced laparoscopic techniques and technologies offer new modalities for the treatment of PPCs. Laparoscopic surgery is a method in which the lumen of the PPC is anastomosed either to the posterior stomach wall or to the jejunum with a linear endoscopic stapler or with laparoscopic suturing techniques; this provides ongoing internal drainage and decompression of the PPC<sup>[4,7,23,86,87]</sup>.

Laparoscopic drainage of mature PPCs is usually the definitive treatment because it is associated with a low complication rate and a good outcome in the postoperative follow-up period. Currently, most PPCs can be approached and managed by a laparoscopic approach, which is due to the availability of advanced imaging systems and cameras, better haemostatic equipment and excellent suturing skills<sup>[23,88]</sup>. Laparoscopic procedures for PPC include pancreatic pseudocystogastrostomy, pseudocystoduodenostomy, and pseudocystojejunostomy.

Cystogastrostomy is the most commonly used laparoscopic procedure, and it can be performed *via*

the endogastric, transgastric, or exogastric routes. In cases where pseudocysts contain significant debris because of the larger size of the stoma that is created, laparoscopy seems to have a distinct advantage over endoscopic drainage<sup>[4,7,23]</sup>.

Several authors reported that laparoscopic drainage was associated with low morbidity (early postprocedure bleeding and infection), rapid recovery, and recurrence rates comparable to those reported for open surgery. The disadvantage of laparoscopic surgery is that it may not be suitable for patients who are unfit to undergo general anaesthesia or for patients who had undergone extensive previous abdominal surgery. Although laparoscopic management has been reported with encouraging results, long-term follow-up results have yet to show equivalence to those of open surgery. Additionally, randomized controlled trials that compare PCD, laparoscopic and ED techniques for the management of PPCs are required<sup>[7,8,23,89]</sup>.

## CONCLUSION

PPC usually runs asymptotically and its clinical presentation mainly occurs in case of complications during its clinical course. Once a PPC is diagnosed, it must be determined whether it can be treated conservatively with the hope of spontaneous resolution, or if an intervention is necessary to prevent complications. The setting of conservative management of PPCs is still poorly evaluated. Several studies have reported that some patients with PPCs can be managed conservatively with supportive medical care if the presenting symptoms can be controlled.

If intervention is necessary, it must be determined whether surgical treatment, PCD, or ED is the best approach. Much overlap exists in the various treatment options offered by interventional radiologists, gastroenterologists, and surgeons, and often a combined approach is needed. Several conditions must be met to achieve the complete obliteration of the cyst cavity. PD anatomy is an important factor in the results of the treatment. When PPC-PD communication is identified, the mean duration of drainage increases to weeks or months, depending on the condition of the PD. The recovery of disrupted PD is the main prognostic factor for successful treatment of PPC regardless of the treatment method used.

Traditionally, symptomatic PPC has been treated by surgical internal drainage. However, this treatment involves considerable surgical trauma and general anaesthesia.

The recent trend in the management of symptomatic PPC has moved toward less invasive approaches such as ED, image-guided PCD and minimal invasive surgery.

ED of PPCs may be performed by a trans-papillary or a trans-mural approach and is suitable because most PPCs lie adjacent to the stomach. The major

advantage of the endoscopic approach is that it creates a permanent pseudocysto-gastric track with no spillage of pancreatic enzymes, which reduces the risks of formation of pancreatocutaneous fistulas; this is in contrast to PCD. Moreover, PCD that persists too long is not practical, especially for young and professionally active patients. Therefore, some authors suggest that ED should be the preferred modality for PPCs that are located immediately adjacent to the gastric or duodenal wall.

However, with these potential drainage problems (which could appear with both techniques), the monitoring, manipulation or change of stent, and the analysis of cystic content are very difficult or impossible to perform endoscopically, unlike the PCD approach. Moreover, PCD is less aggressive compared with surgical and endoscopic (especially with ERCP) methods, is suitable for the treatment of all PPCs regardless of their location and can be performed without general anaesthesia. Therefore, this treatment option is especially recommended for patients who are unsuitable for more aggressive methods and for those at a high risk for complications of general anaesthesia.

Some authors advocate the use of a combination of transmural and transpapillary techniques to drain pseudocysts. Some have used ERCP in the same endoscopic session to assess and treat any PD leakage, and when PD leaks were evident, ERCP was used for the placement of PD stents to bridge the leak site or stricture. However, because it has not been clearly confirmed that the introduction of stents leads to permanent recovery of PD and permanent cessation of the leakage of pancreatic juice after the stent removal, the use of this intervention is questionable. The reason for this is that it may represent overtreatment in these patients, given the mechanical trauma of the placement and the removal of the PD stent, that the two demanding interventions (ERCP) are performed under conscious sedation, and the cost-benefit effect.

Laparoscopic management has been reported with encouraging results, but long-term follow-up results have yet to show equivalence to open surgery and other minimally invasive methods. The disadvantage of laparoscopic surgery is that it may not be suitable for patients who are unfit to undergo general anaesthesia or for patients with a history of extensive previous abdominal surgery.

Currently, few randomized controlled studies have been performed that compare the various minimally invasive approaches in the management of PPCs. Several groups worldwide have developed new minimally invasive approaches for the treatment of PPC. Applicability of these techniques is highly dependent on the availability of specialized expertise and multidisciplinary teams that are dedicated to the management of pancreatic diseases. This review article is intended to help physicians base their therapeutic decisions about minimally invasive management of PPCs on the current state of therapeutic technology

and published data.

## REFERENCES

- Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS; Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]
- Zerem E**. Treatment of severe acute pancreatitis and its complications. *World J Gastroenterol* 2014; **20**: 13879-13892 [PMID: 25320523 DOI: 10.3748/wjg.v20.i38.13879]
- Johnson MD**, Walsh RM, Henderson JM, Brown N, Ponsky J, Dumot J, Zuccaro G, Vargo J. Surgical versus nonsurgical management of pancreatic pseudocysts. *J Clin Gastroenterol* 2009; **43**: 586-590 [PMID: 19077728 DOI: 10.1097/MCG.0b013e31817440be]
- Bhattacharya D**, Ammori BJ. Minimally invasive approaches to the management of pancreatic pseudocysts: review of the literature. *Surg Laparosc Endosc Percutan Tech* 2003; **13**: 141-148 [PMID: 12819495]
- Giovannini M**, Binmoeller K, Seifert H. Endoscopic ultrasound-guided cystogastrostomy. *Endoscopy* 2003; **35**: 239-245 [PMID: 12584645]
- Zerem E**, Imamović G, Sušić A, Haračić B. Step-up approach to infected necrotising pancreatitis: a 20-year experience of percutaneous drainage in a single centre. *Dig Liver Dis* 2011; **43**: 478-483 [PMID: 21478061 DOI: 10.1016/j.dld.2011.02.020]
- Gumaste VV**, Aron J. Pseudocyst management: endoscopic drainage and other emerging techniques. *J Clin Gastroenterol* 2010; **44**: 326-331 [PMID: 20142757 DOI: 10.1097/MCG.0b013e3181cd9d2f]
- Zerem E**, Imamović G, Omerović S, Ljuca F, Haračić B. Percutaneous treatment for symptomatic pancreatic pseudocysts: Long-term results in a single center. *Eur J Intern Med* 2010; **21**: 393-397 [PMID: 20816592 DOI: 10.1016/j.ejim.2010.06.015]
- Ahn JY**, Seo DW, Eum J, Song TJ, Moon SH, Park do H, Lee SS, Lee SK, Kim MH. Single-Step EUS-Guided Transmural Drainage of Pancreatic Pseudocysts: Analysis of Technical Feasibility, Efficacy, and Safety. *Gut Liver* 2010; **4**: 524-529 [PMID: 21253303 DOI: 10.5009/gnl.2010.4.4.524]
- Zerem E**, Imamović G, Omerović S. What is the optimal treatment for pancreatic pseudocysts? *Scand J Gastroenterol* 2012; **47**: 124-125 [PMID: 21718085 DOI: 10.3109/00365521.2011.599191g]
- Zerem E**, Pavlović-Čalić N, Mavija Z. EUS-guided drainage of debris-containing pancreatic pseudocysts by using combined endoprosthesis and a nasocystic drain. *Gastrointest Endosc* 2014; **79**: 694-695 [PMID: 24630086 DOI: 10.1016/j.gie.2013.10.036]
- Nealon WH**, Walser E. Surgical management of complications associated with percutaneous and/or endoscopic management of pseudocyst of the pancreas. *Ann Surg* 2005; **241**: 948-957; discussion 957-960 [PMID: 15912044 DOI: 10.1097/01.sla.0000164737.86249.81]
- Varadarajulu S**, Bang JY, Sutton BS, Trevino JM, Christein JD, Wilcox CM. Equal efficacy of endoscopic and surgical cystogastrostomy for pancreatic pseudocyst drainage in a randomized trial. *Gastroenterology* 2013; **145**: 583-90.e1 [PMID: 23732774 DOI: 10.1053/j.gastro.2013.05.046]
- Nealon WH**, Walser E. Main pancreatic ductal anatomy can direct choice of modality for treating pancreatic pseudocysts (surgery versus percutaneous drainage). *Ann Surg* 2002; **235**: 751-758 [PMID: 12035030]
- Bradley EL**. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993; **128**: 586-590 [PMID: 8489394 DOI: 10.1001/archsurg.1993.01420170122019]
- Thoeni RF**. The revised Atlanta classification of acute pancreatitis:

- its importance for the radiologist and its effect on treatment. *Radiology* 2012; **262**: 751-764 [PMID: 22357880 DOI: 10.1148/radiol.11110947]
- 17 **Gumaste VV**, Pitchumoni CS. Pancreatic pseudocyst. *Gastroenterologist* 1996; **4**: 33-43 [PMID: 8689144]
- 18 **Lerch MM**, Stier A, Wahnschaffe U, Mayerle J. Pancreatic pseudocysts: observation, endoscopic drainage, or resection? *Dtsch Arztebl Int* 2009; **106**: 614-621 [PMID: 19890418 DOI: 10.3238/arztebl.2009.0614]
- 19 **Lehman GA**. Pseudocysts. *Gastrointest Endosc* 1999; **49**: S81-S84 [PMID: 10049456]
- 20 **Freeman ML**, Werner J, van Santvoort HC, Baron TH, Besselink MG, Windsor JA, Horvath KD, vanSonnenberg E, Bollen TL, Vege SS. Interventions for necrotizing pancreatitis: summary of a multidisciplinary consensus conference. *Pancreas* 2012; **41**: 1176-1194 [PMID: 23086243 DOI: 10.1097/MPA.0b013e318269c660]
- 21 **Maher MM**, Lucey BC, Gervais DA, Mueller PR. Acute pancreatitis: the role of imaging and interventional radiology. *Cardiovasc Intervent Radiol* 2004; **27**: 208-225 [PMID: 15024494 DOI: 10.1007/s00270-003-1907-7]
- 22 **Balthazar EJ**, Freeny PC, vanSonnenberg E. Imaging and intervention in acute pancreatitis. *Radiology* 1994; **193**: 297-306 [PMID: 7972730]
- 23 **Aghdassi A**, Mayerle J, Kraft M, Sielenkämper AW, Heidecke CD, Lerch MM. Diagnosis and treatment of pancreatic pseudocysts in chronic pancreatitis. *Pancreas* 2008; **36**: 105-112 [PMID: 18376299 DOI: 10.1097/MPA.0b013e31815a8887]
- 24 **Arvanitakis M**, Delhaye M, De Maertelaere V, Bali M, Winant C, Coppens E, Jeanmart J, Zalman M, Van Gansbeke D, Devière J, Matos C. Computed tomography and magnetic resonance imaging in the assessment of acute pancreatitis. *Gastroenterology* 2004; **126**: 715-723 [PMID: 14988825 DOI: 10.1053/j.gastro.2003.12.006]
- 25 **Ball CG**, Correa-Gallego C, Howard TJ, Zyromski NJ, House MG, Pitt HA, Nakeeb A, Schmidt CM, Akisik F, Lillemoe KD. Radiation dose from computed tomography in patients with necrotizing pancreatitis: how much is too much? *J Gastrointest Surg* 2010; **14**: 1529-1535 [PMID: 20824381 DOI: 10.1007/s11605-010-1314-8]
- 26 **Pelaez-Luna M**, Vege SS, Petersen BT, Chari ST, Clain JE, Levy MJ, Pearson RK, Topazian MD, Farnell MB, Kendrick ML, Baron TH. Disconnected pancreatic duct syndrome in severe acute pancreatitis: clinical and imaging characteristics and outcomes in a cohort of 31 cases. *Gastrointest Endosc* 2008; **68**: 91-97 [PMID: 18378234 DOI: 10.1016/j.gie.2007.11.041]
- 27 **Dumonceau JM**, Macias-Gomez C. Endoscopic management of complications of chronic pancreatitis. *World J Gastroenterol* 2013; **19**: 7308-7315 [PMID: 24259962 DOI: 10.3748/wjg.v19.i42.7308]
- 28 **Brugge WR**, Lauwers GY, Sahani D, Fernandez-del Castillo C, Warshaw AL. Cystic neoplasms of the pancreas. *N Engl J Med* 2004; **351**: 1218-1226 [PMID: 15371579 DOI: 10.1056/NEJMra031623]
- 29 **van der Waaij LA**, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. *Gastrointest Endosc* 2005; **62**: 383-389 [PMID: 16111956]
- 30 **Linder JD**, Geenen JE, Catalano MF. Cyst fluid analysis obtained by EUS-guided FNA in the evaluation of discrete cystic neoplasms of the pancreas: a prospective single-center experience. *Gastrointest Endosc* 2006; **64**: 697-702 [PMID: 17055859]
- 31 **Gouyon B**, Lévy P, Ruszniewski P, Zins M, Hammel P, Vilgrain V, Sauvanet A, Belghiti J, Bernades P. Predictive factors in the outcome of pseudocysts complicating alcoholic chronic pancreatitis. *Gut* 1997; **41**: 821-825 [PMID: 9462217]
- 32 **Beckingham IJ**, Krige JE, Bornman PC, Terblanche J. Long term outcome of endoscopic drainage of pancreatic pseudocysts. *Am J Gastroenterol* 1999; **94**: 71-74 [PMID: 9934733 DOI: 10.1111/j.1572-0241.1999.00773.x]
- 33 **Lévy P**, Jouannaud V, O'Toole D, Couvelard A, Vullierme MP, Palazzo L, Aubert A, Ponsot P, Sauvanet A, Maire F, Hentic O, Hammel P, Ruszniewski P. Natural history of intraductal papillary mucinous tumors of the pancreas: actuarial risk of malignancy. *Clin Gastroenterol Hepatol* 2006; **4**: 460-468 [PMID: 16616351 DOI: 10.1016/j.cgh.2006.01.018]
- 34 **Ridder GJ**, Maschek H, Klemptner J. Favourable prognosis of cystadeno- over adenocarcinoma of the pancreas after curative resection. *Eur J Surg Oncol* 1996; **22**: 232-236 [PMID: 8654602]
- 35 **Bennett S**, Lorenz JM. The role of imaging-guided percutaneous procedures in the multidisciplinary approach to treatment of pancreatic fluid collections. *Semin Intervent Radiol* 2012; **29**: 314-318 [PMID: 24293805 DOI: 10.1055/s-0032-1330066]
- 36 **Yeo CJ**, Bastidas JA, Lynch-Nyhan A, Fishman EK, Zinner MJ, Cameron JL. The natural history of pancreatic pseudocysts documented by computed tomography. *Surg Gynecol Obstet* 1990; **170**: 411-417 [PMID: 2326721]
- 37 **Maringhini A**, Uomo G, Patti R, Rabitti P, Termini A, Cavallera A, Dardanoni G, Manes G, Ciambra M, Laccetti M, Biffarella P, Pagliaro L. Pseudocysts in acute nonalcoholic pancreatitis: incidence and natural history. *Dig Dis Sci* 1999; **44**: 1669-1673 [PMID: 10492151]
- 38 **Bourliere M**, Sarles H. Pancreatic cysts and pseudocysts associated with acute and chronic pancreatitis. *Dig Dis Sci* 1989; **34**: 343-348 [PMID: 2646086]
- 39 **Aranha GV**, Prinz RA, Esguerra AC, Greenlee HB. The nature and course of cystic pancreatic lesions diagnosed by ultrasound. *Arch Surg* 1983; **118**: 486-488 [PMID: 6830440]
- 40 **Warshaw AL**, Rattner DW. Timing of surgical drainage for pancreatic pseudocyst. Clinical and chemical criteria. *Ann Surg* 1985; **202**: 720-724 [PMID: 4073984]
- 41 **Cheruvu CV**, Clarke MG, Prentice M, Eyre-Brook IA. Conservative treatment as an option in the management of pancreatic pseudocyst. *Ann R Coll Surg Engl* 2003; **85**: 313-316 [PMID: 14594534 DOI: 10.1308/003588403769162413]
- 42 **Morgagni JB**. De sedibuset causis morborum per anatomen indagatis. 4th ed. Paris, 1821: 86-123
- 43 **Jedlica R**. Eine neue Operationsmethode der Pankreaszysten (Pancreatogastrostomie). *Zentralbl Chir* 1923; **50**: 132
- 44 **Han O**. Beitrag zur Behandlung der Pankreasfistein. *Arch Klin Chir* 1928; **143**: 73
- 45 **Vitas GJ**, Sarr MG. Selected management of pancreatic pseudocysts: operative versus expectant management. *Surgery* 1992; **111**: 123-130 [PMID: 1736380]
- 46 **Walt AJ**, Bouwman DL, Weaver DW, Sachs RJ. The impact of technology on the management of pancreatic pseudocyst. Fifth annual Samuel Jason Mixter Lecture. *Arch Surg* 1990; **125**: 759-763 [PMID: 2189377]
- 47 **Habashi S**, Draganov PV. Pancreatic pseudocyst. *World J Gastroenterol* 2009; **15**: 38-47 [PMID: 19115466 DOI: 10.3748/wjg.15.38]
- 48 **Gullo L**, Barbara L. Treatment of pancreatic pseudocysts with octreotide. *Lancet* 1991; **338**: 540-541 [PMID: 1678802]
- 49 **Suga H**, Tsuruta O, Okabe Y, Saitoh F, Noda T, Yoshida H, Ono N, Kinoshita H, Toyonaga A, Sata M. A case of mediastinal pancreatic pseudocyst successfully treated with somatostatin analogue. *Kurume Med J* 2005; **52**: 161-164 [PMID: 16639988]
- 50 **Kariniemi J**, Sequeiros RB, Ojala R, Tervonen O. Feasibility of MR imaging-guided percutaneous drainage of pancreatic fluid collections. *J Vasc Interv Radiol* 2006; **17**: 1321-1326 [PMID: 16923979 DOI: 10.1097/01.RVI.0000231957.91785.63]
- 51 **Zerem E**, Pavlović-Čalić N, Sušić A, Haračić B. Percutaneous management of pancreatic abscesses: long term results in a single center. *Eur J Intern Med* 2011; **22**: e50-e54 [PMID: 21925043 DOI: 10.1016/j.ejim.2011.01.015]
- 52 **Polaków J**, Serwatka W, Dobrzycki S, Ładny JR, Janica J, Puchalski Z. A new diagnostic approach to pancreatic pseudocyst fine-needle puncture: three-dimensional sonography. *J Hepatobiliary Pancreat Surg* 2004; **11**: 159-163 [PMID: 15235887 DOI: 10.1007/s00534-003-0852-9]
- 53 **Spivak H**, Galloway JR, Amerson JR, Fink AS, Branum GD,



- Redvanly RD, Richardson WS, Mauren SJ, Waring JP, Hunter JG. Management of pancreatic pseudocysts. *J Am Coll Surg* 1998; **186**: 507-511 [PMID: 9583690]
- 54 **Adams DB**, Anderson MC. Percutaneous catheter drainage compared with internal drainage in the management of pancreatic pseudocyst. *Ann Surg* 1992; **215**: 571-576; discussion 576-578 [PMID: 1632678]
- 55 **Pitchumoni CS**, Agarwal N. Pancreatic pseudocysts. When and how should drainage be performed? *Gastroenterol Clin North Am* 1999; **28**: 615-639 [PMID: 10503140]
- 56 **Tsiotos GG**, Sarr MG. Management of fluid collections and necrosis in acute pancreatitis. *Curr Gastroenterol Rep* 1999; **1**: 139-144 [PMID: 10980941]
- 57 **Zerem E**, Omerović S. Successful percutaneous drainage with iodine irrigation for pancreatic fistulas and abscesses after necrotizing pancreatitis. *Med Princ Pract* 2012; **21**: 398-400 [PMID: 22398319 DOI: 10.1159/000336594]
- 58 **Neff R**. Pancreatic pseudocysts and fluid collections: percutaneous approaches. *Surg Clin North Am* 2001; **81**: 399-403, xii [PMID: 11392426 DOI: 10.1016/S0039-6109(05)70127-4]
- 59 **Zerem E**, Imamovic G, Omerović S, Imširović B. Randomized controlled trial on sterile fluid collections management in acute pancreatitis: should they be removed? *Surg Endosc* 2009; **23**: 2770-2777 [PMID: 19444515 DOI: 10.1007/s00464-009-0487-2]
- 60 **Baron TH**, Thaggard WG, Morgan DE, Stanley RJ. Endoscopic therapy for organized pancreatic necrosis. *Gastroenterology* 1996; **111**: 755-764 [PMID: 8780582]
- 61 **Barthet M**, Lamblin G, Gasmi M, Vitton V, Desjeux A, Grimaud JC. Clinical usefulness of a treatment algorithm for pancreatic pseudocysts. *Gastrointest Endosc* 2008; **67**: 245-252 [PMID: 18226686 DOI: 10.1016/j.gie.2007.06.014]
- 62 **Godil A**, Chen YK. Endoscopic management of benign pancreatic disease. *Pancreas* 2000; **20**: 1-13 [PMID: 10630377]
- 63 **Vidyarthi G**, Steinberg SE. Endoscopic management of pancreatic pseudocysts. *Surg Clin North Am* 2001; **81**: 405-410, xii [PMID: 11392427 DOI: 10.1016/S0039-6109(05)70128-6]
- 64 **Catalano MF**, Geenen JE, Schmalz MJ, Johnson GK, Dean RS, Hogan WJ. Treatment of pancreatic pseudocysts with ductal communication by transpapillary pancreatic duct endoprosthesis. *Gastrointest Endosc* 1995; **42**: 214-218 [PMID: 7498685]
- 65 **Binmoeller KF**, Seifert H, Walter A, Soehendra N. Transpapillary and transmural drainage of pancreatic pseudocysts. *Gastrointest Endosc* 1995; **42**: 219-224 [PMID: 7498686]
- 66 **Dumonceau JM**, Delhay M, Tringali A, Dominguez-Munoz JE, Poley JW, Arvanitakis M, Costamagna G, Costea F, Devière J, Eisendrath P, Lakhtakia S, Reddy N, Fockens P, Ponchon T, Bruno M. Endoscopic treatment of chronic pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2012; **44**: 784-800 [PMID: 22752888 DOI: 10.1055/s-0032-1309840]
- 67 **Seicean A**, Vultur S. Endoscopic therapy in chronic pancreatitis: current perspectives. *Clin Exp Gastroenterol* 2015; **8**: 1-11 [PMID: 25565876 DOI: 10.2147/CEG.S43096]
- 68 **Varadarajulu S**, Christein JD, Tamhane A, Drelichman ER, Wilcox CM. Prospective randomized trial comparing EUS and EGD for transmural drainage of pancreatic pseudocysts (with videos). *Gastrointest Endosc* 2008; **68**: 1102-1111 [PMID: 18640677 DOI: 10.1016/j.gie.2008.04.028]
- 69 **Park DH**, Lee SS, Moon SH, Choi SY, Jung SW, Seo DW, Lee SK, Kim MH. Endoscopic ultrasound-guided versus conventional transmural drainage for pancreatic pseudocysts: a prospective randomized trial. *Endoscopy* 2009; **41**: 842-848 [PMID: 19798610 DOI: 10.1055/s-0029-1215133]
- 70 **Varadarajulu S**, Bang JY, Phadnis MA, Christein JD, Wilcox CM. Endoscopic transmural drainage of peripancreatic fluid collections: outcomes and predictors of treatment success in 211 consecutive patients. *J Gastrointest Surg* 2011; **15**: 2080-2088 [PMID: 21786063 DOI: 10.1007/s11605-011-1621-8]
- 71 **Seifert H**, Biermer M, Schmitt W, Jürgensen C, Will U, Gerlach R, Kreitmair C, Meining A, Wehrmann T, Rösch T. Transluminal endoscopic necrosectomy after acute pancreatitis: a multicentre study with long-term follow-up (the GEPARD Study). *Gut* 2009; **58**: 1260-1266 [PMID: 19282306 DOI: 10.1136/gut.2008.163733]
- 72 **Chak A**. Endosonographic-guided therapy of pancreatic pseudocysts. *Gastrointest Endosc* 2000; **52**: S23-S27 [PMID: 11115944]
- 73 **Hawes RH**. Endoscopic management of pseudocysts. *Rev Gastroenterol Disord* 2003; **3**: 135-141 [PMID: 14502117]
- 74 **Monkemuller KE**, Kahl S, Malfertheiner P. Endoscopic therapy of chronic pancreatitis. *Dig Dis* 2004; **22**: 280-291 [PMID: 15753611 DOI: 10.1159/000082800]
- 75 **Bang JY**, Varadarajulu S. Endoscopic ultrasound-guided management of pancreatic pseudocysts and walled-off necrosis. *Clin Endosc* 2014; **47**: 429-431 [PMID: 25325003 DOI: 10.5946/ce.2014.47.5.429]
- 76 **Braden B**, Dietrich CF. Endoscopic ultrasonography-guided endoscopic treatment of pancreatic pseudocysts and walled-off necrosis: new technical developments. *World J Gastroenterol* 2014; **20**: 16191-16196 [PMID: 25473173 DOI: 10.3748/wjg.v20.i43.16191]
- 77 **Ng PY**, Rasmussen DN, Vilman P, Hassan H, Gheorman V, Burtea D, Surlin V, Săftoiu A. Endoscopic Ultrasound-guided Drainage of Pancreatic Pseudocysts: Medium-Term Assessment of Outcomes and Complications. *Endosc Ultrasound* 2013; **2**: 199-203 [PMID: 24949396 DOI: 10.4103/2303-9027.121245]
- 78 **Bapaye A**, Itoi T, Kongkam P, Dubale N, Mukai S. New fully covered large-bore wide-flare removable metal stent for drainage of pancreatic fluid collections: Results of a multicenter study. *Dig Endosc* 2015; **27**: 499-504 [PMID: 25545957 DOI: 10.1111/den.12421]
- 79 **Krishnan A**, Ramakrishnan R. EUS-guided endoscopic necrosectomy and temporary cystogastrostomy for infected pancreatic necrosis with self-expanding metallic stents. *Surg Laparosc Endosc Percutan Tech* 2012; **22**: e319-e321 [PMID: 23047418 DOI: 10.1097/SLE.0b013e3182657e03]
- 80 **Tarantino I**, Di Pisa M, Barresi L, Curcio G, Granata A, Traina M. Covered self expandable metallic stent with flared plastic one inside for pancreatic pseudocyst avoiding stent dislodgement. *World J Gastrointest Endosc* 2012; **4**: 148-150 [PMID: 22523616 DOI: 10.4253/wjge.v4.i4.148]
- 81 **Smits ME**, Rauws EA, Tytgat GN, Huibregtse K. The efficacy of endoscopic treatment of pancreatic pseudocysts. *Gastrointest Endosc* 1995; **42**: 202-207 [PMID: 7498683]
- 82 **Zerem E**, Pavlović-Čalić N, Haračić B. Comparative evaluation of outcomes of endoscopic versus percutaneous drainage for symptomatic pancreatic pseudocysts. *Gastrointest Endosc* 2014; **79**: 1028 [PMID: 24856842 DOI: 10.1016/j.gie.2013.12.019]
- 83 **Ross A**, Gluck M, Irani S, Hauptmann E, Fotoohi M, Siegal J, Robinson D, Crane R, Kozarek R. Combined endoscopic and percutaneous drainage of organized pancreatic necrosis. *Gastrointest Endosc* 2010; **71**: 79-84 [PMID: 19863956 DOI: 10.1016/j.gie.2009.06.037]
- 84 **Trevino JM**, Tamhane A, Varadarajulu S. Successful stenting in ductal disruption favorably impacts treatment outcomes in patients undergoing transmural drainage of peripancreatic fluid collections. *J Gastroenterol Hepatol* 2010; **25**: 526-531 [PMID: 20074158 DOI: 10.1111/j.1440-1746.2009.06109.x]
- 85 **Arvanitakis M**, Delhay M, Bali MA, Matos C, De Maertelaer V, Le Moine O, Devière J. Pancreatic-fluid collections: a randomized controlled trial regarding stent removal after endoscopic transmural drainage. *Gastrointest Endosc* 2007; **65**: 609-619 [PMID: 17324413 DOI: 10.1016/j.gie.2006.06.083]
- 86 **Park AE**, Heniford BT. Therapeutic laparoscopy of the pancreas. *Ann Surg* 2002; **236**: 149-158 [PMID: 12170019]
- 87 **Aljarabiah M**, Ammori BJ. Laparoscopic and endoscopic approaches for drainage of pancreatic pseudocysts: a systematic review of published series. *Surg Endosc* 2007; **21**: 1936-1944 [PMID: 17717626 DOI: 10.1007/s00464-007-9515-2]



- 88 **Palanivelu C**, Senthilkumar K, Madhankumar MV, Rajan PS, Shetty AR, Jani K, Rangarajan M, Maheshkumaar GS. Management of pancreatic pseudocyst in the era of laparoscopic surgery--experience from a tertiary centre. *Surg Endosc* 2007; **21**: 2262-2267 [PMID: 17516116 DOI: 10.1007/s00464-007-9365-y]
- 89 **Hamza N**, Ammori BJ. Laparoscopic drainage of pancreatic pseudocysts: a methodological approach. *J Gastrointest Surg* 2010; **14**: 148-155 [PMID: 19789929 DOI: 10.1007/s11605-009-1048-7]

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## Advances in the study of Lynch syndrome in China

Jun-Yu Lu, Jian-Qiu Sheng

Jun-Yu Lu, Jian-Qiu Sheng, Department of Gastroenterology, General Hospital of Beijing Military Region, Beijing 100700, China

Jun-Yu Lu, The Third Military Medical University, Chongqing 400038, China

**Author contributions:** Lu JY and Sheng JQ contributed equally to this work.

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**Correspondence to:** Jian-Qiu Sheng, MD, Professor, Department of Gastroenterology, General Hospital of Beijing Military Region, Nanmencang 5, Dongcheng District, Beijing 100700, China. [jianqiu@263.net](mailto:jianqiu@263.net)  
Telephone: +86-10-66721014  
Fax: +86-10-66721299

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### Abstract

Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer, is an autosomal dominant genetic condition that has a high risk of colon cancer as well as other cancers due to inherited mutations in mismatch repair (MMR) genes. During the last decades, there

have been great advances in research on Chinese Lynch syndrome. This review mainly focuses on the genetic basis, clinicopathologic features, diagnosis, intervention, chemoprevention, and surveillance of Lynch syndrome in China. In addition to frequently altered MMR genes, such as *MLH1*, *MSH2*, *MSH6*, and *MLH3*, other MMR-associated genes, such as those encoding human exonuclease 1, transforming growth factor  $\beta$  receptor 2, and alanine aminopeptidase, metastasis-associated protein 2, adenomatous polyposis coli down-regulated 1, and hepatic and glial cell adhesion molecule have also been implicated in Chinese Lynch syndrome. Most Chinese researchers focused on the clinicopathologic features of Lynch syndrome, and it is noticeable that the most frequent extracolonic tumor in northeast China is lung cancer, which is different from other areas in China. The Chinese diagnostic criteria for Lynch syndrome have been established to identify gene mutation or methylation. With regard to chemoprevention, celecoxib may be effective to prevent polyps relapse in Lynch syndrome carriers. Additionally, a colonoscopy-based surveillance strategy for the prevention and early detection of neoplasms in Lynch-syndrome carriers has been proposed.

**Key words:** Clinicopathologic features; Diagnostic criteria; Genetics; Intervention; Lynch syndrome

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**Core tip:** Lynch syndrome is an autosomal dominant inherited disorder. The estimated number of Lynch syndrome carriers in China is larger than that in any other country worldwide. This review summarized recent advances in studies of Chinese Lynch syndrome.

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## INTRODUCTION

Lynch syndrome is an autosomal dominant inherited disease that is typically characterized by hereditary nonpolyposis colorectal cancer (HNPCC) and has a high risk of other tumors, such as endometrial cancer, ovarian cancer, gastric cancer, intrahepatic cholangiocarcinoma, urological cancer, and skin cancer, which is caused by germline mutation in mismatch repair (MMR) genes. Individuals with unique colon cancer, are categorized as Lynch I. The others, suffering from Lynch syndrome-related tumors, in addition to colorectal cancer (CRC), are considered as Lynch II. HNPCC is the main clinical pattern of Lynch syndrome, and also the most common autosomal dominant hereditary CRC. The two terms, Lynch syndrome and HNPCC, have been used interchangeably, until recent advances in the understanding of the disease led to the term HNPCC falling out of favor. In the population of CRC patients in China, the prevalence of Lynch syndrome meeting the Amsterdam Criteria (AC) I and II, and Japanese Criteria were 0.5%-1.2%, 2.1%-2.9% and 2.4%-2.9%, respectively<sup>[1,2]</sup>. Lynch syndrome accounts for 5.6%-6.4% of all Chinese CRC patients<sup>[3,4]</sup>.

Lynch syndrome is distinctive from sporadic CRC in many aspects, including genetics, clinical features, intervention, and treatment. This article is aimed to review the current knowledge and status of the above aspects in China, by searching on PubMed, CNKI, and VIP databases for relevant studies published between 2004 and 2014.

## GENETICS

It has been demonstrated that germline mutations in MMR genes are the genetic basis of Lynch syndrome, including *MLH1*, *MSH2*, *MSH6*, *MSH3*, *PMS1*, *PMS2*, and *MLH3*. Mutant *MLH1* and *MSH2* were reported to be the most common pathogenic genes in Chinese Lynch syndrome, with the frequency of mutation and loss of expression of *MLH1* higher than that of *MSH2*<sup>[5-7]</sup>. In parts of China, the rates of loss of expression of *MLH1* and *MSH2* in Lynch syndrome patients are 15%-48% and 34%-40%, respectively<sup>[8-10]</sup>. In foreign patients, the mutation rates of those genes varied from 41% to 90%<sup>[11-13]</sup>, whereas the mutation rates of *MLH1* and *MSH2* in Chinese Lynch syndrome patients that met the ACI or ACII criteria were 35% and 14%, respectively<sup>[6]</sup>. A study of 76 Chinese families, who met Bethesda 1, 3, or 4, showed that the mutation rates of these two genes were only 25% and 9%, respectively<sup>[14]</sup>. Endometrial carcinoma (EC) is one of the main Lynch Syndrome-associated extracolonic tumor. An investigation of female EC patients below age 50 in South China showed that the rate of loss expression of MMR proteins was 30%, most of whom had an abnormal expression of *MLH1*<sup>[15]</sup>. For more details, please see Table 1<sup>[16-24]</sup>.

Reports on *MLH3* are scarce, it was reported that

**Table 1 Identification of mutations in mismatch repair genes in China**

Gene	Base variation	Amino acid change	Ref.
<i>MLH1</i>	g.677G>A	Arg226Gln	[7]
	c.107T>A	Ile36Asn	[14]
	IVS2-1G>A	(intron)	[14]
	c.488delG	(intron)	[14]
	c.497T>A	Leu166Och	[14]
	c.498A>C	Leu166Phe	[14]
	c.572G>T	Ser191Ile	[14]
	c.910T>A	Val307Glu	[14]
	c.949C>A	Leu317Met	[14]
	c.1246A>G	Lys416Glu	[14]
	c.1731G>C	-	[14]
	c.1823C>A	Ala608Asp	[14]
	c.1988A>C	Glu663Ala	[14]
	c.2038T>C	Cys680Arg	[14]
	c.2101C>A	Gln701Lys	[14,16-18]
	c.2251_2insAA	-	[14]
	c.1625A>T	-	[18,90]
	c.194G>A	-	[18,19]
	c.199G>A	-	[18,20,21]
	c.649C>T	-	[18,20,21]
	c.1646T>C	-	[18]
	c.1721T>C	-	[18]
	c.1742C>T	-	[18]
	c.1344insG	Glu448Glyfs*30	[27]
	c.157delGAGG	Glu54Alafs*2	[22]
	c.-64G>T	(promoter)	[22]
	c.2157dupT	Val720Cysfs*3	[22]
	c.1731+15delT	-	[23]
	c.503_4insA	(frame shift)	[49]
<i>MSH2</i>	g.610G>T	Gly204X	[7]
	c.1452-1455delAATG	-	[31]
	c.2108C>A	Ser703Tyr	[24]
	c.2583A>G	Q861Q	[10,49]
	c.899_890insAT	(frame shift)	[49]
	IVS7-1G>A	(splice junction)	[49]
	c.-78_-79delGT	(promoter)	[28]
	c.1216_1219dupCGAC	L407fsX417	[29]
	c.23C>T	Thr8Met	[14,66]
	c.1571G>T	Arg524Leu	[14]
	c.1917T>A	His639Gln	[14]
	c.1955C>A	Pro652His	[14]
	c.2047G>A	Gly683Arg	[14]
	c.206-?_792+?del	-	[5]
	c.1077-?_1276+?del	-	[5,83]
	c.1387-?_1510+?del	-	[5]
	c.2211-?_818+?del	-	[5]
	c.2635+?_(*3084)del	-	[5]
	c.2196T>C	-	[24]
	c.2963C>G	-	[24]
<i>MSH6</i>	c.2297delA	His766Leufs*8	[27]
	c.3488A>T	Glu>Val	[30]
<i>PSM2</i>	c.1532C>T	Thr>Met	[37]
<i>MLH3</i>	c.2152C>T	Pro718Scr	[25]
	c.2615C>G	Scr872Stop	[25]
	c.3488G>A	Gly1163Asp	[25]
	c.666G>A	Lys222Lys	[25]
	c.4335C>A	Gln1445Gln	[25]

mutation of *MLH3* in northern Chinese was similar to that in the Western population, but the mutation frequency seemed higher in northern China<sup>[25]</sup>. There is little evidence that *MLH3* mutation prompts the development of Lynch syndrome in northern China

or is an indicator for people at a high risk. Some novel mutation sites in *MLH1*, *MSH2*, and *MSH6* have been found by Chinese researchers during studies of MMR genes<sup>[26-30]</sup>. For example, researchers from the University of Hong Kong found a mutation site (c.1452-1455delAATG) that accounted for 36% of all the germline mutations in *MSH2*<sup>[31]</sup>. Further analysis suggested that this founder mutation may date back between 22 and 103 generations. The identification of this *MSH2* founder mutation has important implications for the design of mutation-detection strategies for the southern Chinese population.

Additionally, epimutation of MMR genes has become to be a hot spot. *MLH1* promoter methylation was mainly found in sporadic colon cancer, so it can be used as a screening biomarker to exclude Lynch syndrome. However the detection rate of this methylation was 13%-22% in Lynch syndrome patients without confirmed germline mutations in *MLH1*, *MSH2*, or *MSH6*<sup>[32-34]</sup>. Carriers of the *MLH1* mutation may show loss of expression of due to the methylation of the functional allele<sup>[35,36]</sup>. Therefore, the diagnosis of Lynch syndrome should not be excluded with only the evidence of *MLH1* promoter methylation, especially for those who were diagnosed with CRC at a young age, and for those with evident family history or other risk factors.

Actually, MMR gene mutation cannot be identified in all patients who meet the diagnostic criteria. Therefore, scientists have begun to explore other genes associated with MMR to identify some novel pathogenic genes of Lynch syndrome. Using gene chips combined with immunohistochemical method, Chinese researchers found and upregulation of genes encoding alanine aminopeptidase and metastasis-associated protein 2, and downregulation of adenomatosis polyposis coli down-regulated 1 and hepatic and glial cell adhesion molecule genes in CRC patients with abnormal expression of MMR genes, compared to those who express MMR genes normally<sup>[37]</sup>. Although germline deletions in *EPCAM* is considered to inactive *MSH2* and therefore result in about 1% Lynch syndrome, there is little literature reporting its effect in Chinese Lynch syndrome.

Schmutte *et al.*<sup>[38]</sup> first discovered that the human exonuclease 1 gene (*EXO1*) can interact with products of *MSH2* and participate in recognition and combination with mismatch sites. After that, some researchers agreed that *EXO1* germline mutation can result in MMR dysfunction and prompt tumor development<sup>[39-41]</sup>. However, others consider that there is no direct relationship between *EXO1* mutation and Lynch syndrome, and that the mutation exists extensively in the normal population. Despite *EXO1* mutation, MMR function can still be available through other mechanisms<sup>[42,43]</sup>.

Transforming growth factor  $\beta$  receptor 2 (TGF $\beta$ R2) is an important conversion factor in the signal transduction system. It has been found that, *TGFBR2*

poly A and G repetitive sequences in microsatellite instability (MSI)-H CRC cells are likely mutated, which accounts for more than 70% in Lynch syndrome patients<sup>[44]</sup>. It is reported that *TGFBR2* expression in most MSI-H colon adenoma and carcinoma is low<sup>[45,46]</sup>. However, mutations within the TGF $\beta$  system can be easily found in many kinds of tumors, and its action in the development of Lynch syndrome and its specific value for diagnosis remain unclear.

In Lynch syndrome, the mutation of an allele of heterozygous MMR genes will lead to MMR dysfunction and then increase mistakes during DNA replication, which result in MSI. MSI is a significant feature of Lynch syndrome. It is found that the positive rate of MSI was 85% in patients with Lynch syndrome, 40% in those with ordinary hereditary CRC, and 10% in sporadic CRC cancer patients<sup>[47]</sup>. Additionally, the positive rates of MSI-H in Lynch syndrome-related adenoma and cancer are significantly higher than in sporadic colorectal adenoma and cancer (64.3% vs 3.1%, 71.4% vs 12.5%, respectively)<sup>[48,49]</sup>.

## CLINICOPATHOLOGIC FEATURES

Lynch syndrome is a form of MSI colon cancer with clinical manifestations that differ from familial adenomatous polyposis and sporadic colon cancer, which are chromosome instability colon cancers. Chinese Lynch syndrome patients possess the following characteristics: (1) the episode age of Lynch syndrome is 10-20 years earlier than that of sporadic colon cancer. The median episode age is 42.5-46.0 years, with a peak age of 40-49 years old, and 75% of patients are diagnosed before age 50<sup>[50]</sup> and 87% before age 60<sup>[51-54]</sup>; (2) men have a higher risk than women with a male to female ratio of 1.3-1.7:1, and the onset age of males is lower than that of females<sup>[52-55]</sup>; (3) vertical transmission: as an autosomal dominant inherited disease, if one of the parents carries pathogenic genes for HNPCC, the chance of transmission to an offspring is 50%, regardless of sex. An investigation of 69 families indicated that 94% of families had more than two generations of vertical transmission<sup>[53]</sup>; (4) proximal colon cancer is more common: international reports showed that 70% of CRC is located at the proximal colon of splenic flexure<sup>[51,56]</sup>, with most studies reporting similar results<sup>[52,53,57-65]</sup>. In addition, 66% of 116 patients with CRC from 34 families had right-sided colon cancer<sup>[52]</sup>, whereas an investigation of 31 Chinese families showed that CRCs were found mainly in left-sided colon and rectum rather than in right-sided colon<sup>[66]</sup>; (5) synchronous and heterochronous multiple primary carcinomas: several investigations showed that the incidence rate of synchronous and heterochronous multiple primary carcinomas in Chinese patients was 10.0%-20.4%<sup>[53,63,67,68]</sup>; and (6) high morbidity of associated extracolonic cancer: some Lynch II patients can develop extracolonic cancer, and gastric cancer was reported as the most common type in China<sup>[61,62,69-72]</sup>.



**Table 2** Frequency of extra colonic cancer related with hereditary nonpolyposis colorectal cancer in China *n* (%)

Ref.	Extra colonic cancer			<i>n</i>	Diagnostic criteria	Hospital location
[73]	Lung 56 (20.07)	Gastric 48 (17.20)	Endometrial 32 (11.47)	279	ACII	Liaoning
[52]	Gastric 13 (41.90)	Glioma 3 (9.68)	Cardiac/retinoblastoma/ovarian 2/2/2 (6.45)	31	ACII, JC	Beijing
[53]	Gastric 18 (28.13)	Endometrial 11 (17.19)	Esophagus 7 (10.94)	64	ACII, JC, BG	Beijing
[63]	Endometrial 9 (19.57)	Brest 7 (15.22)	Lung/Gastric 6/6 (13.04)	46	ACI, ACII, JC	Tianjin
[69]	Gastric 5 (31.25)	Endometrial/Lung/Brest/Bladder 2/2/2/2 (1.63)		16	ACI, ACII	Guangdong
[55]	Gastric 16 (18.60)	Liver 10 (11.63)	Endometrial 8 (9.30)	86	ACI, ACII, JC, BG	Shanghai
[54]	Gastric 8 (25.00)	Endometrial 8 (25.00)	Liver 5 (15.63)	32	ACI, ACII	Shanghai
[70]	Gastric 25 (39.68)	Endometrial 11 (17.46)	Liver 6 (9.52)	63	ACI	Shanghai
[72]	Gastric 9 (37.50)	Esophagus 3 (12.50)	Mouth 2 (8.33)	24	Chinese C	Zhejiang

ACI: Amsterdam criteria I; ACII: Amsterdam criteria II; JC: Japanese criteria; CC: Chinese criteria; BG: Bethesda guideline.

Our group<sup>[52,53]</sup> reported that 20%-23% of all patients with Lynch syndrome experienced extracolonic cancer, of which, 42% had gastric cancer and 18% had endometrial cancer. Others reported that lung cancer was the most common in northeast China<sup>[68,73]</sup> and endometrial cancer was predominant in Tianjin<sup>[63,74]</sup> (Table 2).

## DIAGNOSTIC CRITERIA AND METHODS

The International Collaborate Group on HNPCC established the first unified clinical criteria on Lynch syndrome in Amsterdam, Netherlands in 1990, known as the ACI. However, this criterion only took Lynch I into account, excluding Lynch syndrome-related cancers, and is useless for screening in small families. The ACI was later modified in 1999 as the ACII. During that time, the Japanese Criteria and the Bethesda Guideline and the modified version were issued by the Japanese Society for Cancer of the Colon and Rectum and the National Cancer Institute, respectively. Researchers from Fudan University and Shanghai Cancer Center established the Recommended Fudan Criteria, based on investigations upon the features of Chinese patients: (1) three or more family members, one of whom is a first-degree relative of the other two, with a confirmed diagnosis of HNPCC-related cancers (including CRC, endometrial cancer, gastric cancer, liver cancer, ureter and renal pelvis cancer); (2) two successive affected generations; (3) one or more of the HNPCC-related cancers diagnosed before age 50 years; and (4) exclusion of familial adenomatous polyposis. The difference between these criteria and the ACII lies in the addition of gastric cancer and liver cancer into the category of HNPCC-related cancers. Nevertheless, these criteria are all based on retrospective data, and require several members

with a confirmed diagnosis of HNPCC to determine the Lynch syndrome families. Over the past several decades, the family scale has diminished due to family planning, which makes pedigree analysis, the main method for diagnosis of inherited cancer, increasingly impractical<sup>[75]</sup>. Besides, most of the mutation carriers of MMR genes cannot be identified by these criteria. Therefore, it may be wise to take the advantage of molecular genetic characteristics to screen families with Lynch syndrome.

### MMR gene testing

The test of germline mutation in MMR genes is supposedly the most accurate way to identify families with HNPCC. Once a germline mutation in an MMR gene is identified, regardless of the clinical diagnostic criteria, families with HNPCC can be determined. Methods for detection of MMR gene mutation currently consist of single-strand confirmation polymorphism (SSCP), denaturing high-performance liquid chromatography (DHPLC), multiplex ligation-dependent probe amplification (MLPA), and direct sequencing.

SSCP is appropriate for detection of PCR products  $\leq 500$  bp. The sensitivity of SSCP is comparable to that of DNA sequencing, according to an internal report<sup>[76]</sup>.

DHPLC is also known as temperature-modulated heteroduplex analysis, which is used to detect 200-500 bp PCR products. DHPLC is currently the most sensitive method for the qualitative detection of gene mutations, but its application is still limited to experimental studies<sup>[77]</sup>. Zhang *et al.*<sup>[78]</sup> used DHPLC to detect germline mutations of *MLH1* and *MSH2*, which had been determined by DNA sequencing, and the result indicated that all the known mutations can be identified by DHPLC, and the sensitivity and specificity all reach 100%.

Mutation detection for large fragments of MMR genes in Chinese patients with Lynch syndrome is common<sup>[79]</sup>. We<sup>[5]</sup> investigated Chinese families with Lynch syndrome by MLPA and found that large deletions in *MLH1* and *MSH2* were responsible for approximately 19% of all mutations, and the deletions of *MSH2* were more frequent<sup>[80]</sup>. On the contrary, large deletions of MMR genes have not been identified in sporadic CRC<sup>[81]</sup>.

Theoretically, the sensitivity and specificity of DNA sequencing are all 100%, through which we can figure out the location and types of MMR gene mutations. Although gene test is the most accurate method for diagnosis of Lynch syndrome, its wide application in the clinic is limited by some defects such as long testing time, high cost, and low efficiency.

Mutation tests for *BRAF* in the diagnosis of Lynch syndrome have become increasingly accepted. It has been demonstrated that *BRAF* mutations exist in 15% of CRC cases (most are sporadic). Therefore, if *BRAF* mutations are detected in patients with CRC, then the diagnosis is more likely to be sporadic CRC<sup>[35,82,83]</sup>. It should be noted that *BRAF* mutation is not common in sporadic endometrial cancer, thus, *BRAF* testing is not useful for distinguishing Lynch syndrome-related endometrial cancer from sporadic endometrial cancer<sup>[84]</sup>.

### Immunohistochemistry testing

MMR proteins work in dimers; MSH-2 complexes with MSH-6 or MSH-3, and MLH-1 may complex with PMS-2 or PMS-1. Monomeric MSH-6 and PMS-2 proteins are unstable, thus a germline mutation in *MSH2* typically leads to loss of expression of the proteins MSH-2/MSH-6, and a germline mutation in *MLH1* results in loss of expression of MLH-1/PMS-2. On the contrary, germline mutations in *MSH6* or *PMS2* do not cause loss of expression of MSH-2 or MLH-1<sup>[35]</sup>.

Internal studies indicated that the sensitivity of immunohistochemistry (IHC) testing for MLH-1 and MSH-2 mutations was 79%-95%<sup>[85]</sup>, which was 92% in an international report<sup>[86]</sup>. For most of the clinical testing laboratories, IHC testing is technically easy and convenient. Most loss of gene expression can be detected by IHC, which is cheap and consequently reduces the cost of detection. However, IHC can only determine the loss of expression of MMR proteins, but cannot detect germline mutations. And many other reasons, such as somatic mutation, promoter methylation and oxidative stress, can also lead to loss of expression of MMR proteins.

### MSI assessment

The stability of microsatellites can be evaluated by assessing the stability of microsatellite markers in tumor tissues. In 1997, the National Cancer Institute recommended five microsatellite markers, including BAT25, BAT26, D2S123, D5S346 and D17S250,

among which, the frequency of BAT26 mutation is 95%. CAT25 and BAT26 seemed to be equally effective for screening Lynch syndrome in Chinese population. And the length distribution of CAT25 alleles was more intensive than BAT26, suggesting that CAT25 testing may be more sensitive in large-scale studies<sup>[87]</sup>. We<sup>[88]</sup> found that the positive rates of MSI-H in Lynch syndrome patients who met ACII and Bethesda Guideline 3 criteria in northern China were 85% and 81%, respectively. Xu *et al.*<sup>[89]</sup> reported that the positive rates of MSI-H in patients who met ACI criteria and individuals with highly suspected diagnosis of Lynch syndrome were 94% and 93%, respectively. The sensitivity of MSI testing for the diagnosis of Lynch syndrome was reported as 91%<sup>[90]</sup>. A study from Southern Medical University showed that, MSI (-H and -L) carriers accounted for 85% of CRC patients aged below 40 years in southern China<sup>[91]</sup>. MSI testing can be used in preliminary screening for Lynch syndrome, and can be used in combination with ACII to reduce diagnostic errors<sup>[92]</sup>. However, it is hard to implement MSI testing in every clinical laboratory due to high cost and conditions. MSI cannot be detected in mucinous tumors because of technical challenges such as lack of DNA. Some Lynch syndrome-related cancers resulting from germline mutations in *MSH6* tended to have MSI-L, which may lead to a false-negative result. Additionally, approximately 15% of sporadic CRC patients exhibit MSI<sup>[93]</sup>.

A combination of the above methods could enhance the sensitivity and specificity of diagnosis. It is reported that the sensitivity and specificity of IHC testing for the loss of MLH-1 and MSH-2 expression in Lynch syndrome are 91% and 87%, respectively, and those of MSI assessment of five markers are 100% and 75%, respectively; the sensitivity and specificity are 91% and 93%, respectively, when those two methods are used in combination<sup>[94]</sup>.

### Proteomic analysis

Chinese researchers have used surface enhanced laser desorption/ionization-time of flight-mass spectrometry combined with protein chip to analyze protein components of preoperative serum derived from 20 patients with Lynch syndrome and 25 patients with sporadic CRC<sup>[95]</sup>. Protein profiles were analyzed with Biomarker Wizard and Biomarker Pattern programs (Ciphergen Biosystems, Inc., Fremont, CA, United States). The authors concluded that, under a blind authentication mode, the diagnostic accuracy, sensitivity, total specificity, and positive predictive value were 75.6%, 69.8%, 99.2%, and 100% respectively.

## INTERVENTION OF LYNCH SYNDROME

Nowadays in China, partial colectomy or local resection is usually performed prior to the confirmation of Lynch syndrome, as there are no prospective or retrospective

trials supporting that extended resection provides a survival advantage compared to partial colectomy. Zhou *et al.*<sup>[96]</sup> and Li *et al.*<sup>[97]</sup> reported that after conventional surgical treatment for the first CRC, the five- and ten-year accumulated risks for metachronous primary CRC were estimated at 50% and 52%, respectively. Extended resection could reduce the times of operation for metachronous CRC, but also reduced colonic function and increased the risk for old-age patients at the same time. Therefore, Lynch syndrome patients should be preoperatively identified and carefully considered for correct staging, receive more individualized colonic resection, specific follow-up, and familial screening<sup>[97-99]</sup>. For female Lynch syndrome carriers, resection of the uterus and ovaries could be considered in order to prevent Lynch syndrome-related cancers following a careful discussion of the risks, benefits, and limitations of this procedure<sup>[100]</sup>.

Prophylactic colectomy did not show any survival benefit compared with surveillance for MMR gene mutation carriers without CRC, who should receive genetic counseling and should actively participate in decisions concerning the prophylactic strategies<sup>[101]</sup>.

## CHEMOPREVENTION

It was demonstrated in a clinical study on 1071 patients with Lynch syndrome that four-year administration of aspirin and/or resistant starch has no effect on the incidence of CRC<sup>[102,103]</sup>. However, the extended study also conducted by Burn *et al.*<sup>[104]</sup> showed a trend towards protection with aspirin, but not starch. Researchers found that those who took aspirin for  $\geq 2$  years had an incidence rate of 0.06 per 100 person-years compared with 0.13 per 100 person-years among those who took aspirin  $< 2$  years. Analysis within the placebo group found no significant difference in CRC incidence between those who took aspirin for  $\geq 2$  years (0.14 per 100 person-years) compared with those took aspirin for  $< 2$  years (0.10 per 100 person-years). The authors implied that compliance might play a role on outcomes. Our group<sup>[105]</sup> reported that in 5/6 patients with Lynch syndrome, the polyps completely vanished after nine-month treatment with celecoxib at 400 mg/d, but side effects, such as arrhythmia, stenocardia, and nervous headache, were also observed. When the dose was adjusted to 200 mg/d, polyp recurrence was only observed in two patients two years later, suggesting that celecoxib is a promising drug for the prevention of polyp relapse in Lynch syndrome patients.

## COLONOSCOPY SURVEILLANCE

A study of 140 patients with Lynch syndrome and 2350 patients from suspected Lynch syndrome families and their first-degree relatives indicated that routine colonoscopy and intervention reduced the incidence

of CRC and improved the survival of patients<sup>[106]</sup>. For young patients, suspected patients, and gene mutation carriers, routine colonoscopy and intervention reduced the incidence of colon cancer and advanced adenoma<sup>[106,107]</sup>.

A prospective study suggested that annual colonoscopy can lower the morbidity and mortality of patients with Lynch syndrome to the same levels as mutation-negative relatives<sup>[108]</sup>. For that reason, in 2011, Chinese experts in gastroenterology established a consensus statement upon surveillance and prevention of colon cancers.

## CRC

Colonoscopy repeated at one-to two-year intervals, beginning at age 20-25 years (for *MSH6* and *PMS2* heterozygotes carriers, the risk for colon cancer is lower, colonoscopy screening may be delayed until age 30-35 years<sup>[109,110]</sup>) or at the age ten years lower than the onset age of the youngest colon cancer patient within a family<sup>[108,111]</sup>. Additionally, CT colonography has undergone major advances recent years, and it may exert a significant effect on CRC screening, when colonoscopy is not available<sup>[112,113]</sup>. But this procedure is not sufficiently widespread to become a screening method in China.

## Gastric and duodenal cancers

(1) National Comprehensive Cancer Network guidelines in 2011 suggest that upper gastrointestinal endoscopy should be repeated at two- to three-year intervals, beginning at age 30-35 years, depending on the patient's condition. For individuals with chronic gastritis, atrophic gastritis, and/or intestinal metaplasia, shorter screening intervals are recommended. Of note, it is necessary to evaluate *Helicobacter pylori* infection in the biopsies, and to give appropriate treatments; (2) given that 87% of gastric and duodenal cancers occur after age 45 years, endoscopy beginning at this age may be necessary<sup>[114]</sup>; and (3) a cohort study found that more than 50% of small intestinal tumors of patients with Lynch syndrome were located in duodenum, so screening with upper gastrointestinal endoscopy may be beneficial<sup>[115]</sup>, however, there is currently no evidence to support this.

## Ileal tumors

For ileal tumors, patients should consider undergoing capsule endoscopy once every two to three years, beginning at age 30-35 years. In 2008, a case control study showed that there was no difference in the number of polyps detected between staining endoscopy and carefully repeated colonoscopy<sup>[116]</sup>.

## CONCLUSION

CRC is the third most common malignancy in men and the second in women, with over 1.2 million new cases

and 608700 deaths estimated in 2008<sup>[117]</sup>. Ten to thirty percent of patients with CRC have an obvious genetic predisposition. HNPCC is the main clinical type of Lynch syndrome. China has nearly 20% of the world's total population and is comprised of 56 populations, which is a great advantage for studies of genetic diseases. However, a unified cooperative research organization for Lynch syndrome has not yet been formed. For that reason, nationwide multi-center collaborations are rare and precious. Chinese researchers have been using advanced technologies in the detection, diagnosis, and treatment of Lynch syndrome, and carrying out long-term, large-scale follow-up studies. Our group has been engaged in clinical follow-up for years, and found that estrogen may take part in the regulation of MMR genes<sup>[118-124]</sup>, providing a novel molecular mechanism for Lynch syndrome. However, except for a few in-depth studies, most Chinese researchers are still focusing on the clinicopathologic features and MMR gene mutations. We expect that a unified cooperative research organization for Lynch syndrome will soon be established in order to promote the continuous development of technologies and methods to deepen our understanding of the pathogenesis of Lynch syndrome, to figure out more accurate and convenient diagnostic criteria, to design the best therapy and surveillance protocol, and finally, to reduce the morbidity of Lynch syndrome and increase the life quality of patients.

## REFERENCES

- 1 Zhang YZ, Sheng JQ, Li SR, Wu ZT. [Hereditary predisposition of colorectal cancer and prevalence of hereditary nonpolyposis colorectal cancer in general population of colorectal cancer patients in China]. *Zhonghua Yi Xue Zazhi* 2005; **85**: 2995-3000 [PMID: 16324388]
- 2 Zhang YZ, Wu ZT, Li SR. Clinical pathology and hereditary predisposition in patients with primary colorectal cancer: An analysis of 594 cases. *Shijie Huaren Xiaohua Zazhi* 2004; **12**: 1809-1813 [DOI: 10.3969/j.issn.1009-3079.2004.08.013]
- 3 Jin HY, Liu X, Li VK, Ding Y, Yang B, Geng J, Lai R, Ding S, Ni M, Zhao R. Detection of mismatch repair gene germline mutation carrier among Chinese population with colorectal cancer. *BMC Cancer* 2008; **8**: 44 [PMID: 18257912 DOI: 10.1186/1471-2407-8-44]
- 4 Jin HY, Ding YJ, Liu XF, Yang BL, Lai RS, Ni M, Ge YS. [Screening the hereditary nonpolyposis colorectal cancer by revised Bethesda guideline: a cohort study of 110 cases]. *Zhonghua Yi Xue Zazhi* 2007; **87**: 1445-1447 [PMID: 17785078 DOI: 10.3760/j.issn.0376-2491.2007.21.002]
- 5 Sheng JQ, Fu L, Sun ZQ, Huang JS, Han M, Mu H, Zhang H, Zhang YZ, Zhang MZ, Li AQ, Wu ZT, Han Y, Li SR. Mismatch repair gene mutations in Chinese HNPCC patients. *Cytogenet Genome Res* 2008; **122**: 22-27 [PMID: 18931482 DOI: 10.1159/000151312]
- 6 Ni H. Germ-line mutations in the hMLH1 and hMSH2 gene in the Chinese colorectal cancer (CRC) patients: a systematic analysis. Zhejiang University, 2012. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2012&FileName=1012369659.nh&v=&uid=>
- 7 Liu SR, Wang ZJ, Zhao B, Wan YL, Huang YT. [Clinical features and hMSH2/hMLH1 germline mutation screening of Chinese hereditary nonpolyposis colorectal cancer patients]. *Zhonghua Yi Xue Zazhi* 2004; **84**: 714-717 [PMID: 15200905 DOI: 10.3760/j.issn.0376-2491.2004.09.005]
- 8 Peng Y, Wu ZG, Mao JF, Chen MQ, Dong J. Expression of MMR Proteins in Hereditary Nonpolyposis Colorectal Cancer in Yunnan Region. *Zhongliu Fangzhi Yanjiu* 2011; **38**: 270-273 [DOI: 10.3971/j.issn.1000-8578.2011.03.008]
- 9 Guan SS. Expression and Clinical Significance of mismatch repair genes in Chinese suspected HNPCC. Chinese PLA Medical School, 2012. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2013&FileName=1012432500.nh&v=&uid=>
- 10 Sheng JQ, Zhang H, Ji M, Fu L, Mu H, Zhang MZ, Huang JS, Han M, Li AQ, Wei Z, Sun ZQ, Wu ZT, Xia CH, Li SR. Genetic diagnosis strategy of hereditary non-polyposis colorectal cancer. *World J Gastroenterol* 2009; **15**: 983-989 [PMID: 19248199 DOI: 10.3748/wjg.15.983]
- 11 Wijnen J, Khan PM, Vasen H, Menko F, van der Klift H, van den Broek M, van Leeuwen-Cornelisse I, Nagengast F, Meijers-Heijboer EJ, Lindhout D, Griffioen G, Cats A, Kleibeuker J, Varesco L, Bertario L, Bisgaard ML, Mohr J, Kolodner R, Fodde R. Majority of hMLH1 mutations responsible for hereditary nonpolyposis colorectal cancer cluster at the exonic region 15-16. *Am J Hum Genet* 1996; **58**: 300-307 [PMID: 8571956]
- 12 Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomäki P, de la Chapelle A, Hamilton SR, Vogelstein B, Kinzler KW. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat Med* 1996; **2**: 169-174 [PMID: 8574961 DOI: 10.1038/nm0296-169]
- 13 Kohlmann W, Gruber SB. Lynch Syndrome. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, editors. *Source GeneReviews®* [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015 [PMID: 20301390]
- 14 Fu L, Sheng JQ, Sun ZQ, Han M, Huang JS, Mu H, Han WL, Niu HL, Li AQ, Wu ZT, Li SR. [Mutation of hMLH1 and hMSH2 genes in hereditary nonpolyposis colorectal cancer: analysis of 76 probands]. *Zhonghua Yi Xue Zazhi* 2008; **88**: 1983-1985 [PMID: 19062740]
- 15 Tong J, Wang Y, Yang L, Ding YQ, Zhou J, Yang HJ. Abnormal Expression of MMR Proteins in Low Age Patients with Endometrial Cancer. *Guangdong Yixue Zazhi* 2010; **(8)**: 973-975
- 16 Fan Y, Liu X, Zhang H, Dai J, Zhang X, Zhu M, Gao X, Wang Y. Variations in exon 7 of the MSH2 gene and susceptibility to gastrointestinal cancer in a Chinese population. *Cancer Genet Cytogenet* 2006; **170**: 121-128 [PMID: 17011982]
- 17 Zhang Y, Liu X, Fan Y, Ding J, Xu A, Zhou X, Hu X, Zhu M, Zhang X, Li S, Wu J, Cao H, Li J, Wang Y. Germline mutations and polymorphic variants in MMR, E-cadherin and MYH genes associated with familial gastric cancer in Jiangsu of China. *Int J Cancer* 2006; **119**: 2592-2596 [PMID: 16929514]
- 18 Fan Y, Wang W, Zhu M, Zhou J, Peng J, Xu L, Hua Z, Gao X, Wang Y. Analysis of hMLH1 missense mutations in East Asian patients with suspected hereditary nonpolyposis colorectal cancer. *Clin Cancer Res* 2007; **13**: 7515-7521 [PMID: 18094436]
- 19 Cai Q, Sun MH, Fu G, Ding CW, Mo SJ, Cai SJ, Ren SX, Min DL, Xu XL, Zhu WP, Zhang TM, Shi DR. [Mutation analysis of hMSH2 and hMLH1 genes in Chinese hereditary nonpolyposis colorectal cancer families]. *Zhonghua Bing Li Xue Zazhi* 2003; **32**: 323-328 [PMID: 14514376]
- 20 Yuan Y, Huang YQ, Cai SR, Song YM, Zheng S, Zhang SZ. Genetic characterization of Chinese hereditary non-polyposis colorectal cancer by DHPLC and multiplex PCR. *Jpn J Clin Oncol* 2004; **34**: 660-666 [PMID: 15613555]
- 21 Huang YQ, Yuan Y, Wang YP, Zhu M, Zhang SZ, Zheng S. [Mutation detection of mismatch repair genes in hereditary nonpolyposis colorectal cancer by denaturing high-performance liquid chromatography]. *Zhonghua Wai Ke Zazhi* 2005; **43**: 317-320 [PMID: 15842942]
- 22 Wei WQ. Mutation detection and analysis of Chinese hereditary



- nonpolyposis colorectal cancer (HNPCC). Fudan University, 2011. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2012&FileName=1011197483.nh&v=&uid=>
- 23 **Wang Y.** Gene detection and analysis of hereditary nonpolyposis colorectal cancer. Southern Medical University, 2008. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2009&FileName=2009024039.nh&v=&uid=>
- 24 **Jin HY, Yan HL, Song LH, Cui L, Ding YJ, Sun SH.** The Identification and functional analysis of a novel germ-line mutation in hMSH2 from a Chinese hereditary nonpolyposis colorectal cancer family. *Dier Junyi Daxue Xuebao* 2005; **26**: 888-891
- 25 **Zhang H.** The Clinical Characterization of HNPCC Families from Northern Chinese Population and the Role of hMLH3 and hEXO1 Germline Mutation in HNPCC. Third Military Medical University, 2005. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2006&FileName=2005148016.nh&v=&uid=>
- 26 **Fan Y, Chen J, Wang W, Wu P, Zhi W, Xue B, Zhang W, Wang Y.** Influence of eight unclassified missense variants of the MLH1 gene on Lynch syndrome susceptibility. *Biochem Genet* 2012; **50**: 84-93 [PMID: 21952876 DOI: 10.1007/s10528-011-9467-z]
- 27 **Hou S.** The study of mismatch repair genes mutation in two Lynch syndrome families. Central South University, 2012. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2013&FileName=1012478354.nh&v=&uid=>
- 28 **Yan H, Jin H, Xue G, Mei Q, Ding F, Hao L, Sun SH.** Germline hMSH2 promoter mutation in a Chinese HNPCC kindred: evidence for dual role of LOH. *Clin Genet* 2007; **72**: 556-561 [PMID: 17894833 DOI: 10.1111/j.1399-0004.2007.00911.x]
- 29 **Zheng D, Li T, Liu X, Hu W, Chen H, Yang Y.** A novel MSH2 mutation in a Chinese family with hereditary non-polyposis colorectal cancer. *Int J Colorectal Dis* 2007; **22**: 875-879 [PMID: 17333219 DOI: 10.1007/s00384-006-0253-z]
- 30 **Yan SY, Zhou XY, Du X, Zhang TM, Lu YM, Cai SJ, Xu XL, Yu BH, Zhou HH, Shi DR.** Three novel missense germline mutations in different exons of MSH6 gene in Chinese hereditary nonpolyposis colorectal cancer families. *World J Gastroenterol* 2007; **13**: 5021-5024 [PMID: 17854147]
- 31 **Chan TL, Chan YW, Ho JW, Chan C, Chan AS, Chan E, Lam PW, Tse CW, Lee KC, Lau CW, Gwi E, Leung SY, Yuen ST.** MSH2 c.1452-1455delAATG is a founder mutation and an important cause of hereditary nonpolyposis colorectal cancer in the southern Chinese population. *Am J Hum Genet* 2004; **74**: 1035-1042 [PMID: 15042510 DOI: 10.1086/383591]
- 32 **Ji M.** Study of methylation of mismatch repair gene MLH1 in hereditary nonpolyposis colorectal cancer. *Weichangbingxue He Ganzangbingxue Zazhi* 2008; **17**: 291-293
- 33 **Xu XL.** Epigenetics of colorectal cancer: multiple gene promoter methylation profile and its clinicopathological significance. Fudan University, 2004. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2005&FileName=2004134988.nh&v=&uid=>
- 34 **Zhou HH, Yan SY, Zhou XY, Du X, Zhang TM, Cai X, Lu YM, Cai SJ, Shi DR.** MLH1 promoter germline-methylation in selected probands of Chinese hereditary non-polyposis colorectal cancer families. *World J Gastroenterol* 2008; **14**: 7329-7334 [PMID: 19109866 DOI: 10.3748/wjg.14.7329]
- 35 **Bellizzi AM, Frankel WL.** Colorectal cancer due to deficiency in DNA mismatch repair function: a review. *Adv Anat Pathol* 2009; **16**: 405-417 [PMID: 19851131 DOI: 10.1097/PAP.0b013e3181bb6bdc]
- 36 **Niessen RC, Hofstra RM, Westers H, Ligtenberg MJ, Kooi K, Jager PO, de Groote ML, Dijkhuizen T, Olderde-Berends MJ, Hollema H, Kleibeuker JH, Sijmons RH.** Germline hypermethylation of MLH1 and EPCAM deletions are a frequent cause of Lynch syndrome. *Genes Chromosomes Cancer* 2009; **48**: 737-744 [PMID: 19455606 DOI: 10.1002/gcc.20678]
- 37 **Sheng X, Zhou HH, Zhou XY, Du X, Zhang TM, Cai SJ, Sheng WQ, Shi DR.** Germline mutation analysis of hPMS2 gene in Chinese families with hereditary nonpolyposis colorectal cancer. *World J Gastroenterol* 2010; **16**: 3847-3852 [PMID: 20698049 DOI: 10.3748/wjg.v16.i30.3847]
- 38 **Schmutte C, Marinescu RC, Sadoff MM, Guerrette S, Overhauser J, Fishel R.** Human exonuclease I interacts with the mismatch repair protein hMSH2. *Cancer Res* 1998; **58**: 4537-4542 [PMID: 9788596]
- 39 **Knudsen NØ, Nielsen FC, Vinther L, Bertelsen R, Holten-Andersen S, Liberti SE, Hofstra R, Kooi K, Rasmussen LJ.** Nuclear localization of human DNA mismatch repair protein exonuclease 1 (hEXO1). *Nucleic Acids Res* 2007; **35**: 2609-2619 [PMID: 17426132 DOI: 10.1093/nar/gkl1166]
- 40 **Wu Y, Berends MJ, Post JG, Mensink RG, Verlind E, Van Der Sluis T, Kempinga C, Sijmons RH, van der Zee AG, Hollema H, Kleibeuker JH, Buys CH, Hofstra RM.** Germline mutations of EXO1 gene in patients with hereditary nonpolyposis colorectal cancer (HNPCC) and atypical HNPCC forms. *Gastroenterology* 2001; **120**: 1580-1587 [PMID: 11375940 DOI: 10.1053/gast.2001.25117]
- 41 **Sun X, Zheng L, Shen B.** Functional alterations of human exonuclease 1 mutants identified in atypical hereditary nonpolyposis colorectal cancer syndrome. *Cancer Res* 2002; **62**: 6026-6030 [PMID: 12414623]
- 42 **Smith CE, Mendillo ML, Bowen N, Hombauer H, Campbell CS, Desai A, Putnam CD, Kolodner RD.** Dominant mutations in *S. cerevisiae* PMS1 identify the Mlh1-Pms1 endonuclease active site and an exonuclease 1-independent mismatch repair pathway. *PLoS Genet* 2013; **9**: e1003869 [PMID: 24204293 DOI: 10.1371/journal.pgen.1003869]
- 43 **Zhang H, Sheng JQ, Li SR, Han Y, Zhang YZ, Shen Y, Huang JS, Chen JG, Li AQ.** Mutation of hEXO1 gene in patients with hereditary nonpolyposis colorectal cancer. *Weichangbingxue He Ganzangbingxue Zazhi* 2006; **15**: 95-97
- 44 **Garre P, Pérez-Segura P, Diaz-Rubio E, Caldés T, de la Hoya M.** Reassessing the TARB2 mutation rate in hereditary nonpolyposis colorectal cancer. *Nat Genet* 2010; **42**: 817-818; author reply 818 [PMID: 20877318 DOI: 10.1038/ng1010-817]
- 45 **Geng HG, Sheng JQ, Han WL, Zhang YH, Li AQ, Huang JS, Han M, Sun ZQ, Mu H, Wang ZH, Wu ZT, Li SR.** The relationship between microsatellite instability and the expression of TGFβRII in HNPCC adenocarcinomas and adenomas. *Weichangbingxue He Ganzangbingxue Zazhi* 2008; **17**: 572-575
- 46 **Gu GL, Wei XM, Wang SL, Ren L, Hu YY, Li DC.** Expression of hMSH2, hMLH1, transforming growth factor β receptor type II, matrix metalloproteinase-7, tissue inhibitor of metalloproteinase-2 and their correlations with the biological behaviors of hereditary nonpolyposis colorectal cancer. *Shijie Huaren Xiaohua Zazhi* 2007; **15**: 1103-1109
- 47 **Liu WZ, Jin F, Zhang ZH, Wang SB.** Role of detection of microsatellite instability in Chinese with hereditary nonpolyposis colorectal cancer or ordinary hereditary colorectal cancer. *World J Gastroenterol* 2006; **12**: 4745-4749 [PMID: 16937450]
- 48 **Geng HG, Sheng JQ, Zhang YH, Huang JS, Han M, Mu H, Sun ZQ, Wang ZH, Li AQ, Wu ZT, Li SR.** Microsatellite Genotyping of Adenoma and Adenocarcinoma in Patients with Hereditary Nonpolyposis Colorectal Cancer. *Weichangbingxue* 2008; **13**: 140-144
- 49 **Sheng JQ, Chan TL, Chan YW, Huang JS, Chen JG, Zhang MZ, Guo XL, Mu H, Chan AS, Li SR, Yuen ST, Leung SY.** Microsatellite instability and novel mismatch repair gene mutations in northern Chinese population with hereditary non-polyposis colorectal cancer. *Chin J Dig Dis* 2006; **7**: 197-205 [PMID: 17054581 DOI: 10.1111/j.1443-9573.2006.00269.x]
- 50 **Luo DC, Cai Q, Sun MH, Ni YZ, Tao CW, Chen ZJ, Shi DR.** [Clinical analysis and molecular genetic study of hereditary nonpolyposis colorectal cancer kindreds]. *Zhonghua Wai Ke Zazhi* 2004; **42**: 158-162 [PMID: 15062061]
- 51 **Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, Cavalieri RJ, Boland CR.** Genetics, natural history, tumor

- spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993; **104**: 1535-1549 [PMID: 8482467]
- 52 **Sheng J**, Shen Z, Fan C. [Clinical phenotypes of hereditary nonpolyposis colorectal cancer in Chinese population]. *Zhonghua Yi Xue Zazhi* 2002; **82**: 1371-1374 [PMID: 12509915]
  - 53 **Zhang H**, Wang J, Sheng JQ, Zhang YZ, Li SR. The Clinical Characterization of Hereditary Nonpolyposis Colorectal Cancer Families from Chinese Population. *Weichangbingxue He Ganzangbingxue Zazhi* 2005; **14**: 186-189
  - 54 **Yan SY**, Zhou XY, Cai SJ, Yu BH, Luo DC, Du X, Shi DR. Analysis of clinicopathological features in patients with Chinese hereditary nonpolyposis colorectal kindreds. *Zhonghua Xiaohua Zazhi* 2007; **27**: 813-816 [DOI: 10.3760/j.issn:0254-1432.2007.12.006]
  - 55 **Cai SJ**, Cai Q, Sun MH, Xu Y, Mo SJ, Xu XL, Cai H, Wang YN, Shi YQ, Shi DR. Clinicopathological study of hereditary nonpolyposis colorectal cancer families in China. *Zhonghua Xiaohua Zazhi* 2004; **24**: 26-29 [DOI: 10.3760/j.issn:0254-1432.2004.02.008]
  - 56 **Boland CR**, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; **138**: 2073-2087.e3 [PMID: 20420947]
  - 57 **Zhang YZ**, Sheng JQ, Li SR, Zhang H. Clinical phenotype and prevalence of hereditary nonpolyposis colorectal cancer syndrome in Chinese population. *World J Gastroenterol* 2005; **11**: 1481-1488 [PMID: 15770724]
  - 58 **Wang SL**, Gu GL, Wei XM. Clinicopathological features of hereditary nonpolyposis colorectal cancer: analysis of 20 genealogies in 81 cases. *Zhonghua Puwaike Shoushuxue Zazhi* 2008; **2**: 86-90 [DOI: 10.3969/j.issn.1674-3946.2008.01.018]
  - 59 **Wang D**, Xue YW, Zhou XJ, Qiao F, Zhang Y, Li H, Zhao YS. Hereditary non-polyposis colorectal cancer syndrome: an analysis of 13 pedigrees. *Shijie Huaren Xiaohua Zazhi* 2005; **13**: 30-33 [DOI: 10.3969/j.issn.1009-3079.2005.02.007]
  - 60 **Xu YC**, Xu AG. Clinical analysis of hereditary nonpolyposis colorectal cancer in 6 kindreds. *Xiandai Xiaohua and Jieru Zhenliao* 2007; **12**: 13-16 [DOI: 10.3969/j.issn.1672-2159.2007.01.004]
  - 61 **Zhang YZ**, Zhang ZF, Zhang F, Ren YM, Gao HF, Zhang H, Feng Y, Gao YY, Chen XP. Common features of clinical phenotype and extracolonic malignant tumor spectrum of hereditary nonpolyposis colorectal cancer in Chinese population. *Weichangbingxue He Ganzangbingxue Zazhi* 2011; **20**: 513-518 [DOI: 10.3969/j.issn.1006-5709.2011.06.009]
  - 62 **Xu Y**, Cai SJ, Mo SJ, Guan ZQ, Sun MH, Cai Q, Shi DR. Characteristics of hereditary nonpolyposis colorectal cancer among Chinese patients. *Zhonghua Xiaohua Zazhi* 2002; **22**: 25-27 [DOI: 10.3760/j.issn:0254-1432.2002.03.009]
  - 63 **Wang J**, Luo MH, Zhang ZX, Zhang PD, Ma DW, Suo RZ, Zhao LZ, Jiang XL, Jiang T. Genealogical Analysis of the Hereditary Non-polyposis Colorectal Cancer. *Shiyong Aizheng Zazhi* 2004; **19**: 381-384 [DOI: 10.3969/j.issn.1001-5930.2004.04.016]
  - 64 **Ding YS**. The screening of HNPCC families and the analysis of expression of hMLH1 and hMSH2 protein in HNPCC families. Shihezi University, 2011. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2012&FileName=1011407186.nh&v=&uid=>
  - 65 **Liu WZ**, Wang YD, Liu ZL, Wang BS. Detection of hMLH1/hMSH2 expression for identifying patients with hereditary nonpolyposis colorectal carcinoma. *Zhongguo Aizheng Zazhi* 2009; **19**: 662-666 [DOI: 10.3969/j.issn.1007-3639.2009.09.003]
  - 66 **Wang XL**, Yuan Y, Zhang SZ, Cai SR, Huang YQ, Xu ZF, Zheng S. [Clinical and genetic characterization of Chinese hereditary nonpolyposis colorectal cancer families]. *Zhonghua Putong Waike Zazhi* 2006; **21**: 419-422 [DOI: 10.3760/j.issn:1007-631X.2006.06.012]
  - 67 **Jin HY**, Cui L, Ding YJ, Yan YT, Meng RG, Liu F, Zhang XC, Yu DH. [Analysis of the clinicopathological features of Chinese hereditary nonpolyposis colorectal cancer]. *Zhonghua Wei Chang Wai Ke Zazhi* 2005; **8**: 316-318 [PMID: 16167250 DOI: 10.3760/cma.j.issn.1671-0274.2005.04.013]
  - 68 **Li XX**, Guo J, Ma SP, Zhang JR, Lin T, Yu WH, Xu L, Shi Y, Wang SB. Demographic and Clinical fetures of hereditary nonpolyposis colorectal cancer. *Zhonghua Putong Waike Zazhi* 2005; **20**: 146-148 [DOI: 10.3760/j.issn: 1007-631X.2005.03.004]
  - 69 **Zhang CH**, He YL, Zhan WH, Cai SR, Huang MJ, Wang JP, Chen CQ. Hereditary nonpolyposis colorectal cancer: an analysis of 11 kindreds. *Zhongguo Shiyong Waike Zazhi* 2005; **25**: 222-224 [DOI: 10.3321/j.issn:1005-2208.2005.04.014]
  - 70 **Xu Y**, Deng W, Cai SJ, Mo SJ, Sun MH, Cai GX, Lian P, Guan ZQ, Shi DR. Analysis of life time risk of different cancer in Patients with hereditary nonpolyposis colorectal cancer. *Aizheng Yanjiu He Linchuang* 2005; **17**: 15-18 [DOI: 10.3760/cma.j.issn.1006-9801.2005.05.003]
  - 71 **Cao WM**, Yuan Y, Song YM, Cai SR, Zhang SZ. [Clinical features and mutation analysis of a poly-(A)8 tract in M3 cholinergic receptor gene in Chinese HNPCC families]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2004; **33**: 399-402 [PMID: 15476321 DOI: 10.3785/j.issn.1008-9292.2004.05.006]
  - 72 **Yuan Y**, Cao WM, Cai SR, Zhang SZ. [Clinical phenotype of Chinese hereditary nonpolyposis colorectal cancer (HNPCC) families]. *Zhonghua Zhong Liu Zazhi* 2006; **28**: 36-38 [PMID: 16737618 DOI: 10.3760/j.issn:0253-3766.2006.01.010]
  - 73 **Li XX**, Tang YX, Sun GP, Li X, Zhao M, Yan YF, Meng J. Analysis of Features of Extracolonic Carcinoma Spectrum in Northeast Chinese with Hereditary Nonpolyposis Colorectal Cancer. *Zhongguo Puwai He Jichu Yanjiu* 2012; **19**: 288-291
  - 74 **Wang Y**, Xue F, Broadus RR, Tao X, Xie SS, Zhu Y. Clinicopathological features in endometrial carcinoma associated with Lynch syndrome in China. *Int J Gynecol Cancer* 2009; **19**: 651-656 [PMID: 19509565 DOI: 10.1111/IGC.0b013e3181a12fb9]
  - 75 **Wang YJ**, Wang N, Wang Y. Diagnosis of Chinese Hereditary Tumors following with Family Planning for 30 Years. *Yixue Yanjiu Zazhi* 2011; **40**: 2-4 [DOI: 10.3969/j.issn.1673-548X.2011.10.002]
  - 76 **Cui L**, Jin HY, Cheng HY, Yan YD, Meng RG, Yu DH. Genetic detection of Chinese hereditary nonpolyposis colorectal cancer. *World J Gastroenterol* 2004; **10**: 209-213 [PMID: 14716824]
  - 77 **Zhang CH**, He YL, Wang FJ, Song W, Yuan XY, Yang DJ, Chen CQ, Cai SR, Zhan WH. Detection of hMSH2 and hMLH1 mutations in Chinese hereditary non-polyposis colorectal cancer kindreds. *World J Gastroenterol* 2008; **14**: 298-302 [PMID: 18186571]
  - 78 **Zhang YZ**, Sheng JQ, Geng HG, Han Y, Li SR. Screening of Minor Mutation of hMLH1 and hMSH2 Based on Denatured HPLC. *Xiandai Shengwu Yixue Jinzhan* 2008; **8**: 2315-2320
  - 79 **Yan SY**. Study on the gemline mutation of hMSH6 gene,large genomic deletions of mismatch repair genes and the methylation of the hHLH1 promoter in hereditary nonpolyposis colorectal cancer families. Fudan University, 2008. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CDFD&dbName=CDFD2009&FileName=2009017846.nh&v=&uid=>
  - 80 **Zhang H**, Sheng JQ, Geng HG, Han Y, Li SR, Li AQ. [Detection of large intragenic mismatch repair genes deletions in Chinese hereditary nonpolyposis colorectal cancer families with multiplex ligation-dependent probe amplification technique]. *Zhongguo Yi Xue Ke Xue Yuan Xuebao* 2006; **28**: 837-839 [PMID: 17260478]
  - 81 **Zhu M**, Liu XR, Huang YQ, Yuan Y, Li JT, Zhang XM, Zhang YY, Wang YP. The analysis for identifying large DNA fragment aberrations of MSH2 and MLH1 genes from familial colorectal cancer in China. *Zhonghua Yi Xue Yi Chuan Xue Zazhi* 2005; **22**: 603-606 [PMID: 16331552]
  - 82 **Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group**. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med* 2009; **11**: 35-41 [PMID: 19125126 DOI: 10.1097/GIM.0b013e31818fa2ff]
  - 83 **Bouzourene H**, Hutter P, Losi L, Martin P, Benhattar J. Selection of patients with germline MLH1 mutated Lynch syndrome by determination of MLH1 methylation and BRAF mutation. *Fam*

- Cancer* 2010; **9**: 167-172 [PMID: 19949877 DOI: 10.1007/s10689-009-9302-4]
- 84 **Kawaguchi M**, Yanokura M, Banno K, Kobayashi Y, Kuwabara Y, Kobayashi M, Nomura H, Hirasawa A, Susumu N, Aoki D. Analysis of a correlation between the BRAF V600E mutation and abnormal DNA mismatch repair in patients with sporadic endometrial cancer. *Int J Oncol* 2009; **34**: 1541-1547 [PMID: 19424571]
- 85 **Wang J**, Luo MH, Zhang ZX, Zhang PD, Jiang XL, Ma DW, Suo RZ, Zhao LZ, Qi QH. Clinical and molecular analysis of hereditary non-polyposis colorectal cancer in Chinese colorectal cancer patients. *World J Gastroenterol* 2007; **13**: 1612-1617 [PMID: 17461458 DOI: 10.3748/wjg.v13.i10.1612]
- 86 **Shia J**. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. *J Mol Diagn* 2008; **10**: 293-300 [PMID: 18556767 DOI: 10.2353/jmoldx.2008.080031]
- 87 **Zhang GC**. The Role of CAT-25 in screening of HNPCC. Huazhong University of Science and Technology, 2010. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2012&FileName=1011277622.nh&v=&uid=>
- 88 **Sheng JQ**, Tian SL, Lv Y, Chen XY, Li SR. Study of microsatellite instability in hereditary nonpolyposis colorectal cancer. *Weichangbingxue He Ganzangbingxue Zazhi* 2004; **13**: 537-539 [DOI: 10.3969/j.issn.1006-5709.2004.05.026]
- 89 **Xu Y**, Cai SJ, Sun MH, Mo SJ, Shi DR. Evaluation of microsatellite instability for detection of Chinese hereditary nonpolyposis colorectal cancer patients. *Zhongguo Aizheng Zazhi* 2006; **16**: 128-131 [DOI: 10.3969/j.issn.1007-3639.2006.02.012]
- 90 **Yan HL**, Hao LQ, Jin HY, Xing QH, Xue G, Mei Q, He J, He L, Sun SH. Clinical features and mismatch repair genes analyses of Chinese suspected hereditary non-polyposis colorectal cancer: a cost-effective screening strategy proposal. *Cancer Sci* 2008; **99**: 770-780 [PMID: 18307539 DOI: 10.1111/j.1349-7006.2008.00737.x]
- 91 **Yang L**, Ding YQ, Li GX, Xu J, Wang Y, Zhou J, Yang HJ, Zhang JH. The application of the detection of microsatellite instability in the screening of young patients with HNPCC. *Guangdong Yixue* 2007; **28**: 219-221 [DOI: 10.3969/j.issn.1001-9448.2007.02.020]
- 92 **Sheng JQ**, Geng HG, Han M, Huang JS, Mu H, Han WL, Chen JG, Niu HL, Li AQ, Zhang YH, Wu ZT, Li SR. Discussion of novel method of primary screening with microsatellite in HNPCC Patients. Chinese Medical Association seventh national conference on Digestive Diseases, 2007. Jinan, Shandong, China. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CPFD&dbName=CPFD2008&FileName=ZHYX200705006988&v=&uid=>
- 93 **Xiao XY**, Zhou XY, Sun MH, Yan G, Du X. [Microsatellite instability of sporadic colorectal carcinomas and its clinicopathological significance]. *Zhonghua Zhong Liu Zazhi* 2006; **28**: 289-293 [PMID: 16875631 DOI: 10.3760/j.issn.0253-3766.2006.04.013]
- 94 **Jin HY**, Cui L, Meng RG, Liu F, Yan YD, Ding YJ, Yao H, Fu CG, Yu DH. [The role of the immunohistochemistry for hMLH1 and hMSH2 with detection of microsatellite instability to identify the kindreds with hereditary nonpolyposis colorectal cancer]. *Zhonghua Wai Ke Zazhi* 2003; **41**: 809-811 [PMID: 14703452 DOI: 10.3760/j.issn.0529-5815.2003.11.003]
- 95 **Wei W**, Shang XJ, Lv BZ. Serum proteomic analysis of hereditary nonpolyposis colorectal cancer. *Shijie Huaren Xiaohua Zazhi* 2011; **19**: 1417-1421
- 96 **Zhou X**, Zhou JN, Xu FP, Shang JQ. Surgical treatment for colorectal carcinoma in hereditary nonpolyposis colorectal cancer (HNPCC) kindreds. *Jiezhichang Gangmen Watke Zazhi* 2007; **13**: 228-230 [DOI: 10.3969/j.issn.1674-0491.2007.04.009]
- 97 **Li Z**, Wang GC, Han GS, Li JD. Analysis of Curative Effect on Patient with Hereditary Nonpolyposis Colorectal Cancer Underwent Two Types of Colectomy. *Zhongguo Zhongliu Linchuang* 2004; **31**: 38-39 [DOI: 10.3969/j.issn.1000-8179.2004.19.012]
- 98 **Rodriguez-Bigas MA**, Möeslein G. Surgical treatment of hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome). *Fam Cancer* 2013; **12**: 295-300 [PMID: 23508345 DOI: 10.1007/s10689-013-9626-y]
- 99 **Baiocchi GL**, Portolani N, Vermi W, Baronchelli C, Gheza F, Zogno C, Scaglia A, Marchina E, Tiberio GA, Giulini SM. Lynch syndrome from a surgeon perspective: retrospective study of clinical impact of mismatch repair protein expression analysis in colorectal cancer patients less than 50 years old. *BMC Surg* 2014; **14**: 9 [PMID: 24533633 DOI: 10.1186/1471-2482-14-9]
- 100 **Lynch HT**, Casey MJ. Prophylactic surgery prevents endometrial and ovarian cancer in Lynch syndrome. *Nat Clin Pract Oncol* 2007; **4**: 672-673 [PMID: 17971803]
- 101 **Celentano V**, Luglio G, Antonelli G, Tarquini R, Bucci L. Prophylactic surgery in Lynch syndrome. *Tech Coloproctol* 2011; **15**: 129-134 [PMID: 21287222 DOI: 10.1007/s10151-010-0666-0]
- 102 **Burn J**, Bishop DT, Mecklin JP, Macrae F, Möeslein G, Olschwang S, Bisgaard ML, Ramesar R, Eccles D, Maher ER, Bertario L, Jarvinen HJ, Lindblom A, Evans DG, Lubinski J, Morrison PJ, Ho JW, Vasen HF, Side L, Thomas HJ, Scott RJ, Dunlop M, Barker G, Elliott F, Jass JR, Fodde R, Lynch HT, Mathers JC. Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. *N Engl J Med* 2008; **359**: 2567-2578 [PMID: 19073976 DOI: 10.1056/NEJMoa0801297]
- 103 **Burn J**, Mathers J, Bishop DT. Lynch syndrome: history, causes, diagnosis, treatment and prevention (CAPP2 trial). *Dig Dis* 2012; **30** Suppl 2: 39-47 [PMID: 23207931 DOI: 10.1159/000341892]
- 104 **Burn J**, Mathers JC, Bishop DT. Chemoprevention in Lynch syndrome. *Fam Cancer* 2013; **12**: 707-718 [PMID: 23880960 DOI: 10.1007/s10689-013-9650-y]
- 105 **Sheng JQ**, Li SR, Yang XY, Zhang YH, Su H, Yu DL, Yan W, Geng HG. [Clinical management of adenomatous polyposis in patients with hereditary non-polyposis colorectal cancer and familial adenomatous polyposis]. *Zhonghua Yi Xue Zazhi* 2006; **86**: 526-529 [PMID: 16681880 DOI: 10.3760/j.issn.0376-2491.2006.08.006]
- 106 **Sheng JQ**, Li AQ, Han M, Huang JS, Mu H, Han WL, Chen JG, Niu HL, Geng HG, Yu DL, Han Y, Li SR. The significance of monitoring and intervention and treatment with colonoscope in HNPCC patients and their relatives. Chinese Medical Association seventh national conference on Digestive Diseases, 2007. Jinan, Shandong, China. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CPFD&dbName=CPFD2008&FileName=ZHYX200705006988&v=&uid=>
- 107 **Fu L**, Sheng JQ, Li XO, Jin P, Mu H, Han M, Huang JS, Sun ZQ, Li AQ, Wu ZT, Li SR. Mismatch repair gene mutation analysis and colonoscopy surveillance in Chinese Lynch syndrome families. *Cell Oncol (Dordr)* 2013; **36**: 225-231 [PMID: 23640085 DOI: 10.1007/s13402-013-0130-z]
- 108 **Järvinen HJ**, Renkonen-Sinisalo L, Aktán-Collán K, Peltomäki P, Aaltonen LA, Mecklin JP. Ten years after mutation testing for Lynch syndrome: cancer incidence and outcome in mutation-positive and mutation-negative family members. *J Clin Oncol* 2009; **27**: 4793-4797 [PMID: 19720893 DOI: 10.1200/JCO.2009.23.7784]
- 109 **Senter L**, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, Lindblom A, Lagerstedt K, Thibodeau SN, Lindor NM, Young J, Winship I, Dowty JG, White DM, Hopper JL, Baglietto L, Jenkins MA, de la Chapelle A. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology* 2008; **135**: 419-428 [PMID: 18602922 DOI: 10.1053/j.gastro.2008.04.026]
- 110 **Baglietto L**, Lindor NM, Dowty JG, White DM, Wagner A, Gomez Garcia EB, Vriends AH, Cartwright NR, Barnettson RA, Farrington SM, Tenesa A, Hampel H, Buchanan D, Arnold S, Young J, Walsh MD, Jass J, Macrae F, Antill Y, Winship IM, Giles GG, Goldblatt J, Parry S, Suthers G, Leggett B, Butz M, Aronson M, Poynter JN, Baron JA, Le Marchand L, Haile R, Gallinger S, Hopper JL, Potter J, de la Chapelle A, Vasen HF, Dunlop MG, Thibodeau SN, Jenkins MA. Risks of Lynch syndrome cancers for MSH6 mutation



- carriers. *J Natl Cancer Inst* 2010; **102**: 193-201 [PMID: 20028993 DOI: 10.1093/jnci/djp473]
- 111 **Engel C**, Rahner N, Schulmann K, Holinski-Feder E, Goecke TO, Schackert HK, Kloor M, Steinke V, Vogelsang H, Mösllein G, Görgens H, Dechant S, von Knebel Doeberitz M, Rüschoff J, Friedrichs N, Büttner R, Loeffler M, Propping P, Schmiegeler W. Efficacy of annual colonoscopic surveillance in individuals with hereditary nonpolyposis colorectal cancer. *Clin Gastroenterol Hepatol* 2010; **8**: 174-182 [PMID: 19835992 DOI: 10.1016/j.cgh.2009.10.003]
  - 112 **Baiocchi GL**, Mazza G, Baronchelli C, Marchina E, Tiberio GA, Grazioli L, Portolani N, Giulini SM. Right colon cancer missed by virtual colonoscopy in HNPCC patient. *J Gastrointest Cancer* 2012; **43**: 518-520 [PMID: 21987022 DOI: 10.1007/s12029-011-9331-8]
  - 113 **Spada C**, Stoker J, Alarcon O, Barbaro F, Bellini D, Bretthauer M, De Haan MC, Dumonceau JM, Ferlitsch M, Halligan S, Helbren E, Hellstrom M, Kuipers EJ, Lefere P, Mang T, Neri E, Petruzzello L, Plumb A, Regge D, Taylor SA, Hassan C, Laghi A. Clinical indications for computed tomographic colonography: European Society of Gastrointestinal Endoscopy (ESGE) and European Society of Gastrointestinal and Abdominal Radiology (ESGAR) Guideline. *Endoscopy* 2014; **46**: 897-915 [PMID: 25268304 DOI: 10.1055/s-0034-1378092]
  - 114 **Capelle LG**, Van Grieken NC, Lingsma HF, Steyerberg EW, Klokman WJ, Bruno MJ, Vasen HF, Kuipers EJ. Risk and epidemiological time trends of gastric cancer in Lynch syndrome carriers in the Netherlands. *Gastroenterology* 2010; **138**: 487-492 [PMID: 19900449 DOI: 10.1053/j.gastro.2009.10.051]
  - 115 **Schulmann K**, Brasch FE, Kunstmann E, Engel C, Pagenstecher C, Vogelsang H, Krüger S, Vogel T, Knaebel HP, Rüschoff J, Hahn SA, Knebel-Doeberitz MV, Moeslein G, Meltzer SJ, Schackert HK, Tympner C, Mangold E, Schmiegeler W. HNPCC-associated small bowel cancer: clinical and molecular characteristics. *Gastroenterology* 2005; **128**: 590-599 [PMID: 15765394 DOI: 10.1053/j.gastro.2004.12.051]
  - 116 **Stoffel EM**, Turgeon DK, Stockwell DH, Zhao L, Normolle DP, Tuck MK, Bresalier RS, Marcon NE, Baron JA, Ruffin MT, Brenner DE, Syngal S. Missed adenomas during colonoscopic surveillance in individuals with Lynch Syndrome (hereditary nonpolyposis colorectal cancer). *Cancer Prev Res (Phila)* 2008; **1**: 470-475 [PMID: 19138994 DOI: 10.1158/1940-6207.CAPR-08-0098]
  - 117 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
  - 118 **Jin P**. The correlation between serum estrogen level and the expression of mismatch repair genes in colonic epithelial cells of healthy individuals. The Third Military Medical University, 2010. Available from: URL: <http://www.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbName=CMFD2011&FileName=2010250425.nh&v=&uid=>
  - 119 **Lu XJ**, Yu DL, Wang JX, Pan XL, Jin P, Li SR, Sheng JQ. [Effect of estrogen on mismatch repair gene expression in colon cancer cells]. *Xi Bao Yu Fen Zi Mian Yi Xue Zazhi* 2011; **27**: 754-756 [PMID: 21722527]
  - 120 **Lu JY**, Jin P, Gao W, Lu XJ, Wang YT, Sheng JQ. Construction and identification of luciferase reporter gene vector containing hMLH1 promoter. *Jichu Yixue He Linchuang* 2012; **32**: 338-341
  - 121 **He YQ**, Sheng JQ, Ling XL, Fu L, Jin P, Yen L, Rao J. Estradiol regulates miR-135b and mismatch repair gene expressions via estrogen receptor- $\beta$  in colorectal cells. *Exp Mol Med* 2012; **44**: 723-732 [PMID: 23143558 DOI: 10.3858/em.2012.44.12.079]
  - 122 **Jin P**, Lu XJ, Sheng JQ, Fu L, Meng XM, Wang X, Shi TP, Li SR, Rao J. Estrogen stimulates the expression of mismatch repair gene hMLH1 in colonic epithelial cells. *Cancer Prev Res (Phila)* 2010; **3**: 910-916 [PMID: 20663978 DOI: 10.1158/1940-6207.CAPR-09-0228]
  - 123 **Gao W**, Jin P, Lu XJ, Lu JY, Wang YT, Sheng JQ. Knocking down mismatch repair gene hMLH1 may influence microsatellite stability in human embryonic kidney cell line 293t. *Jichu Yixue He Linchuang* 2012; **32**: 346-349
  - 124 **Wang YT**, Jin P, Lu JY, Su XM, Gao W, Lu XJ, Sheng JQ. The role of mismatch repair gene hMLH1 in estrogen-induced apoptosis of colon cancer cell HCT116. *Jichu Yixue He Linchuang* 2012; **32**: 342-345

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## Basic Study

# Induction of endoplasmic reticulum-derived oxidative stress by an occult infection related S surface antigen variant

In-Kyung Lee, Seoung-Ae Lee, Hong Kim, You-Sub Won, Bum-Joon Kim

In-Kyung Lee, Seoung-Ae Lee, Hong Kim, You-Sub Won, Bum-Joon Kim, Department of Microbiology and Immunology, Liver Research Institute, Cancer Research Institute and SNUMRC, College of Medicine, Seoul National University, Seoul 110-799, South Korea

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**Correspondence to:** Bum-Joon Kim, Professor, Department of Microbiology and Immunology, Liver Research Institute, Cancer Research Institute and SNUMRC, College of Medicine, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 110-799, South Korea. [kbumjoon@snu.ac.kr](mailto:kbumjoon@snu.ac.kr)  
Telephone: +82-2-7408316  
Fax: +82-2-7430881

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## Abstract

**AIM:** To investigate the mechanism of endoplasmic reticulum (ER) stress induction by an occult infection related hepatitis B virus S surface antigen (HBsAg) variant.

**METHODS:** We used an HBsAg variant with lower secretion capacity, which was a KD variant from a Korean subject who was occultly infected with the genotype C. We compared the expression profiles of ER stress-related proteins between HuH-7 cells transfected with HBsAg plasmids of a wild-type and a KD variant using Western blot.

**RESULTS:** Confocal microscopy indicated that the KD variant had higher levels of co-localization with ER than the wild-type HBsAg. The KD variant up-regulated ER stress-related proteins and induced reactive oxygen species (ROS) compared to the wild-type *via* an increase in calcium. The KD variant also down-regulated anti-oxidant proteins (HO-1, catalase and SOD) compared to the wild-type, which indicates positive amplification loops of the ER-ROS axis. The KD variant also induced apoptotic cell death *via* the up-regulation of caspase proteins (caspase 6, 9 and 12). Furthermore, the KD variant induced a higher level of nitric oxide than wild-type HBsAg *via* the up-regulation of the iNOS protein.

**CONCLUSION:** Our data indicate that occult infection related HBsAg variants can lead to ER-derived oxidative stress and liver cell death in HuH-7 cells.

**Key words:** Endoplasmic reticulum oxidative stress; Hepatitis B virus; KD variant; Colocalization; Reactive

oxidative species; Apoptotic cell death

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**Core tip:** The molecular mechanisms underlying the relationships between occult hepatitis B virus infection and liver disease progression remain a mystery. The present study demonstrated that the HBsAg variant KD, which exhibits a secretion defective phenotype, universally induced endoplasmic reticulum (ER) stress pathways in hepatocytes. This induction of ER stress may evoke ER stress-mediated biological actions that induce liver damaging processes, including ROS production, nitric oxide production, and apoptosis induction. In conclusion, occult infection related to hepatitis B virus S surface antigen variants may play a very pivotal role in the progression of liver diseases primarily *via* ER-derived oxidative stress and apoptosis in hepatocytes.

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## INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem, and more than 350 million people are chronic carriers of the virus<sup>[1]</sup>. South Korea is an endemic area of HBV infection, and the Korean National Health and Nutrition Survey of 2007 listed the prevalence of hepatitis B virus S surface antigen (HBsAg) as 4.2% in men and 3.1% in women<sup>[2]</sup>. Moreover, an extraordinary prevalence of genotype C2 was reported in this area<sup>[3]</sup>. This genotype is more virulent than genotype B<sup>[4]</sup>, and it may contribute to the distribution of characteristic HBV mutation patterns that are related to the progression of liver diseases<sup>[5-15]</sup>.

HBV surface open reading frames (ORFs) encode 3 types of proteins that share C terminals, large (L), middle (M), and small (S) surface antigen. The HBsAg is expressed at high levels, and it can be secreted independently of L and M envelope proteins. The L and S envelope proteins are needed for virion secretion, but the M protein is dispensable<sup>[16]</sup>. Overexpression of the L protein blocks HBsAg secretion<sup>[17]</sup>. Proper stoichiometry between L and S envelope proteins is important for the secretion of HBsAg and virions.

Occult HBV infection is defined as an infection state that is negative for HBsAg serology, but the presence of HBsAg DNA is demonstrated using polymerase chain reaction (PCR). Generally, HBV infection is diagnosed using serological detection of the

circulating HBsAg<sup>[18,19]</sup>. Occult HBV infection is highly prevalent, particularly in HBV endemic areas, and it is significantly related to severe forms of liver diseases, such as cirrhosis or hepatocellular carcinoma (HCC)<sup>[20]</sup>. However, the exact molecular mechanisms underlying the relationship between occult infection and severe liver diseases are not known.

The endoplasmic reticulum (ER) performs multiple important functions that are essential to cell survival and normal cellular function, including Ca<sup>2+</sup> storage, post-translational modification, and the folding and assembly of newly synthesized secretory proteins. Various disturbances can cause an accumulation of unfolded/misfolded proteins in the ER, which triggers an evolutionarily conserved response termed the unfolded/misfolded protein response (UPR)<sup>[21,22]</sup>. Viral infection may also trigger the UPR because of an overloading of the ER, and viral infection is one of the ancient evolutionary pressures that links ER stress to cell suicide to avoid viral replication and spreading<sup>[23,24]</sup>. Several mutations in S ORFs lead to ER stress in hepatocytes through the accumulation of HBV virions because of failure of the appropriate production of 3 proteins in the S ORF, which may contribute to hepatocarcinogenesis and liver damage. However, most studies of HBV-induced ER stress focused on a specific deletion type of large surface proteins (LHBs)<sup>[25,26]</sup>. HBsAg has the most potent secretory capacity of the HBV encoding proteins. Therefore, it is reasonable that unfolded proteins as a result of an LHB mutation may increase the ER stress response. Nevertheless, to the best of our knowledge, ER stress mechanisms that focus on HBsAg variants of occult infection (occult HBsAg) are rarely introduced<sup>[27]</sup>.

Recently, we reported that a variety of novel HBsAg variants that are absent or rarely encountered in other areas were observed in Korean subjects with occult genotype C infections<sup>[13]</sup>. Notably, we found that some occult HBsAg exhibited deficiencies in HBsAg secretion<sup>[15]</sup>. We hypothesized that these HBsAg could induce strong ER stress in hepatocytes *via* UPR activation. Therefore, the current study elucidated the molecular mechanisms to provide a positive link between occult infection and liver disease progression. The current study focused on ER stress-linked pathways that are induced by occult HBsAg. The present study used a HBsAg KD variant from Korean subjects with occult infections of HBV genotype C, which was previously reported to exhibit very low levels of HBsAg secretion but a higher level of accumulated intracellular HBsAg compared to the wild-type HBsAg<sup>[15]</sup>.

## MATERIALS AND METHODS

### **Samples and drugs used in this study**

The serum for the HBsAg variant (KD) related to occult infection and the control HBsAg (wild-type or

NOR) were acquired from a 54-year-old Korean occult subject showing HBsAg seronegativity and a Korean chronic patient, respectively<sup>[15]</sup>. The institutional review board of the Seoul National University Hospital approved this study (1202-051-398). Salubrinal (Santa cruz Biotech, CA, United States), thapsigargin (Sigma-Aldrich, MO, United States), and N-acetyl-cysteine (NAC; Sigma-Aldrich, MO, United States) were used as an ER stress inhibitor, an ER stress inducer, and an inhibitor of ROS, respectively.

### Plasmid construction of sub-genomic HBV DNA from subjects

Previously reported<sup>[15]</sup> plasmids encoding two types of HBsAg (NOR and KD variant) were used in this study. Briefly, the nested PCR method was used for HBsAg DNA amplification. First-round PCR was performed using the PreS2-Del-F2 and HB2R primers (PreS2-Del-F2: 5'-GGG TCA CCA TAT TCT TGG G-3'; HB2R: 5'-CAT ACT TTC CAA TCA ATA GG-3'), which target the large surface region of HBsAg. Second-round amplification was performed using the Cystein-S-F1 and Cystein-S-R1 primers (Cystein-S-F1: 5'-ATG GAG ARC ACM ACA TCA GGA TTC C-3'; Cystein-S-R1: 5'-TYA AAT GTA TAC CCA AAG ACA MAA G-3'), which amplify the small surface region. The amplified 681-bp products of the NOR and KD variant were cloned into the Topo TA cloning vector (Invitrogen, Massachusetts, United States) according to the manufacturer's protocol. The inserted target region in the TA vector was digested using *EcoRI* (Takara, Shiga, Japan), and the target regions were finally re-cloned into the pIRES2-EGFP vector (Clontech, CA, United States).

### HBsAg ELISA

The secretion capacity of the occult infection-related KD variant and NOR were compared using ELISA for HBsAg in the supernatant and lysed pellet using the commercial Bioelisa HBsAg Colour ELISA Kit (BIOKIT, Barcelona, Spain) according to the manufacturer's protocol. Additionally, the pCMV- $\beta$ -gal vector containing  $\beta$ -galactosidase was co-transfected and analyzed according to the recommendation of the  $\beta$ -Galactosidase Enzyme Assay System kit (Promega, WI, United States) to normalize the HBsAg ELISA in cloned HBsAg.

### Cell culture and transfection

The human hepatoma cell line HuH-7 was used for *in vitro* cell culture studies. Cells were maintained at 37 °C in an atmosphere of 5% CO<sub>2</sub> in RPMI-1640 (GibcoBRL, NY, United States) supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, and 100 U/mL penicillin/streptomycin (Gibco BRL). Cells were plated at  $4 \times 10^5$  cells in six-well plates one day before transfection. Lipofectamine 2000 (Invitrogen, CA, United States) was used to transfect the three plasmid DNAs [void pIRES2 vector (MOCK), NOR, and

KD] using opti-MEM (Gibco BRL) for starvation and incubation.

### Western blot

Proteins were quantified using a Qubit fluorometer (Invitrogen), and Western blotting was performed. Isolated proteins (50  $\mu$ g) were loaded and separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lysate proteins were transferred to nitrocellulose membranes. Each membrane was incubated for 1 h at room temperature in phosphate-buffered saline (PBS) containing 5% skim milk powder (blocking solution) prior to incubation with a primary antibody (1:1000 dilution, 1 h) and a secondary antibody (1:2000 dilution, 30 min). A commercial kit was used to obtain the nuclear fraction (Thermo, IL, United States). Protein analyses involved the following antibodies: activating transcription factor 6 (ATF6), phosphorylated pancreatic ER eIF2 $\alpha$  kinase (pPERK), phosphorylated insulin-response element 1, X-box binding protein 1 (XBP1), phosphorylated eukaryotic initiation factor- $\alpha$ , heme oxygenase 1 (HO-1), CHOP, glucose-regulated protein, 78-kDa (GRP78), JNK, phosphorylated JNK, Bcl-2, Bax, caspase 12, caspase 9, caspase 6 (all from Cell Signaling, MA, United States), inducible nitric oxide synthase (iNOS; BD, NJ, United States), catalase (RD Systems, MN, United States), Cu/ZnSOD (BioDesign, NY, United States), and manganese superoxide dismutase (MnSOD) (Stressgen, Victoria, BC, Canada). Immunoreactive signals were detected using a WEST-one™ Western Blot Detection System (iNtRON, Kyungkido, Republic of Korea) and LAS-3000 (Fujifilm, Tokyo, Japan).

### Calcium measurements

Rhod-2 AM (Invitrogen, Carlsbad, United States) was used as an indicator of intracellular calcium concentration. Cells ( $5 \times 10^4$ ) were seeded in six-well plates, incubated overnight, and transiently transfected. Cells were incubated for 24 h, and Rhod-2 AM was applied at a final concentration of 4  $\mu$ mol/L in PBS containing 0.1 g/L CaCl<sub>2</sub> for 30 min. Intracellular calcium levels were analyzed using fluorescence-activated cell sorting (FACS) in a FACSCan II apparatus (BD, NJ, United States).

### Measurement of intracellular ROS

Dichlorodihydrofluorescein diacetate (DCF-DA; Molecular Probes, OR, United States) and dihydrogen-rhodamine123 (DHR123; Calbiochem, CA, United States) were used to detect intracellular ROS levels. Cells ( $4 \times 10^5$ ) were seeded in six-well plates, and incubated for 24 h. Cells were transiently transfected with occult HBsAg DNA using Lipofectamine 2000 (Invitrogen, Carlsbad, United States) and incubated at 37 °C in 5% CO<sub>2</sub> overnight. The day after transfection, cells were treated with ROS lysis buffer. Supernatants

(100  $\mu$ L) were transferred to 96-well plates and treated with DCF-DA or DHR123 at a final concentration of 10  $\mu$ mol/L for 30 min. Supernatants were analyzed using an LS 55 luminescence spectrometer (Perkin-Elmer, MA, United States) and a FACSCalibur apparatus (BD, NJ, United States).

#### **Nitric oxide ELISA**

Samples were prepared after transient transfection and analyzed in triplicate to determine nitric oxide (NO) levels (Assay Designs, MI, United States). The quantitative determination of NO production was determined using the Griess reaction. NO levels were assessed spectrophotometrically at 540 nm.

#### **DNA fragmentation assay**

Cells ( $5 \times 10^4$ ) were plated in 96-well plates for 24 h. Cells were transfected with plasmid DNA (MOCK, NOR, or KD) using opti-MEM medium for 24 h. Supernatants were recovered, and DNA fragmentation was quantified using the Cell Death Detection ELISA<sup>PLUS</sup> kit (Roche, Mannheim, Germany). For each assay, 20  $\mu$ L of supernatant was added to each well containing 80  $\mu$ L of immunoreagent. Mixtures were gently shaken for 2 h, and each well was rinsed three times with incubation buffer. The buffer after the final rinse was carefully removed, and the substrate solution was pipetted into each well. Each plate was incubated until the color development was sufficient for analysis. Color development was terminated by the addition of a stop solution to each well, and the absorbance was measured at an optical density of 405 nm.

#### **Immunofluorescence assay for colocalization of HBsAg and ER using confocal microscopy**

Immunofluorescence double-staining assays were performed using confocal microscopy to co-localize HBsAg in ER in KD variants and NOR in the HuH-7 cell line. HuH-7 cell lines were transiently transfected with one empty vector (pIRES2-EGFP-MOCK) or two different small surface protein expression vectors (pIRES2-EGFP-NOR and KD). Cells were harvested 2 d post-transfection, fixed with 4% paraformaldehyde, permeabilized using 10% fetal bovine serum-0.1% Triton X-100 in PBS for 1 h at room temperature, and stained for HBsAg (green) and the ER marker calnexin (red). DAPI stained the nucleus a blue color. Cells were incubated with a polyclonal Ab against HBsAg (Abcam, Cambridge, MA, United States) and a monoclonal Ab against the ER marker calnexin (Santa Cruz, CA, United States) to analyze the colocalization of HBsAg and the ER. Goat anti-rabbit-Alexa Fluor 488 (Invitrogen, Carlsbad, United States) and goat anti-mouse-Alexa Fluor 594 (Invitrogen, Carlsbad, United States) were used as secondary antibodies for the experiments. Cell nuclei were counterstained with 10  $\mu$ g/mL of DAPI (Sigma-Aldrich, MO, United States). The results were visualized under a Confocal A1

(Nikon, Japan) confocal microscope. Comparisons of colocalization between HBsAg and ER in the cytoplasm were estimated using colocalization coefficients according to the lasso ROI selection. Statistical comparisons were performed using one-way ANOVA, and the average coefficients of ten images examined in a double-blind manner are shown.

#### **Ethics statement**

This retrospective study was reviewed and approved by the Institutional Review Board of Seoul National University Hospital (IRB Grant No. C-0803-013-237), and the patients' medical records were anonymized and de-identified prior to analyses.

## **RESULTS**

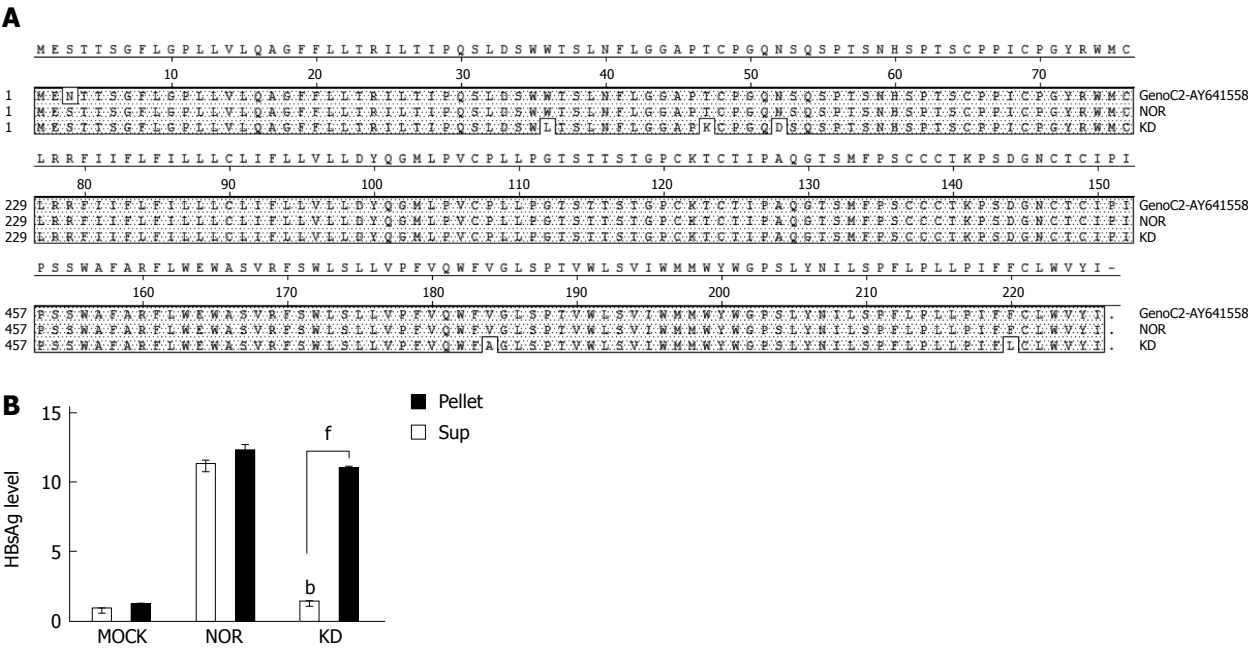
### **Comparison of ER colocalization coefficients between the KD variant and wild-type HBsAg in HuH-7 transfected cells**

Sequencing analyses of cloned plasmids revealed no mutations in wild-type HBsAg from a chronic carrier (NOR). However, a total of five mutations (W36L, T47K, N52D, V184A, and F220L) were mainly concentrated in the N-terminal region in the KD variant, but no mutations were found in the major hydrophilic region (MHR), as previously reported<sup>[15]</sup> (Figure 1A). Comparisons of the 2 cloned HBsAg plasmids (wild-type (NOR) and KD variant) in the pellets and supernatants of HuH-7 cells using ELISA revealed that the KD variant had deficiencies in secretion capacity similar to the negative control compared to the wild-type (Figure 1B). These results suggest a higher level of ER accumulation of the KD variant compared to the wild-type. Colocalization coefficients of the ER and HBsAg between wild-type and KD variant were compared 2 d after transfection of HBsAg plasmids into HuH-7 cells to address this issue. HBsAg of the KD variant showed significantly higher colocalization coefficients with the ER marker calnexin compared to the wild-type (Figure 2A and B).

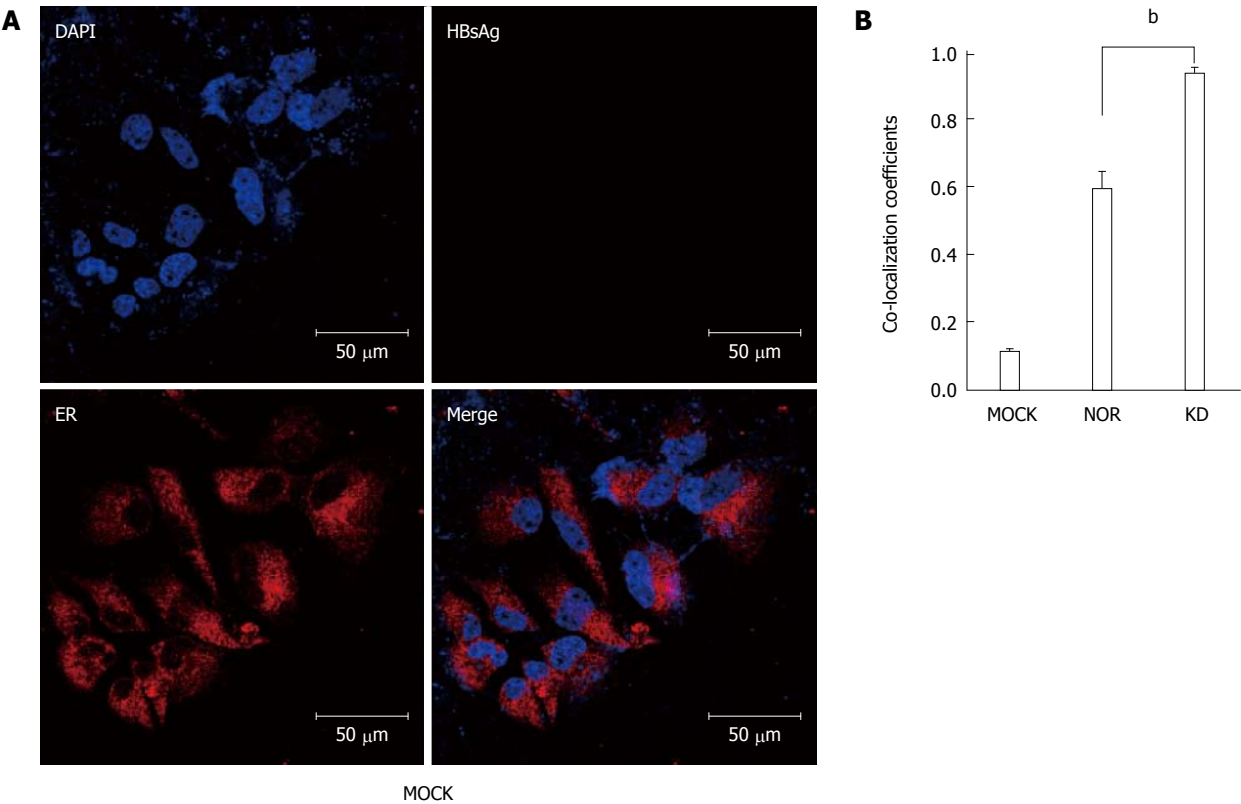
### **KD variant leads to ER stress and increases the intracellular $Ca^{2+}$ concentration**

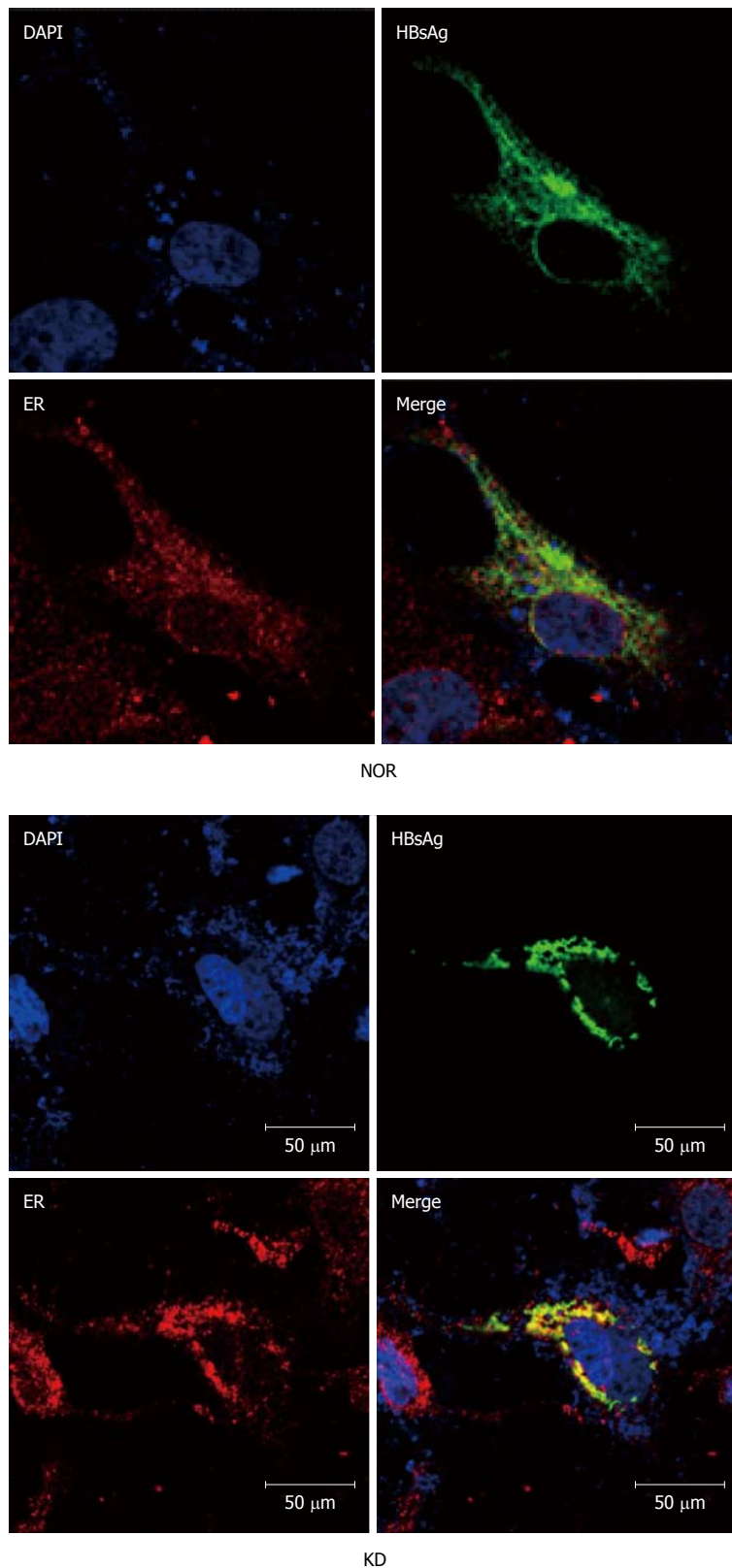
We compared the expression of ER stress-related proteins between the KD variant and wild-type to address issue of whether the compromising of normal HBsAg secretion function in the KD variant leads to ER stress response. The KD variant up-regulated the expression of the seven main ER stress-related proteins, namely, IRE1, ATF6, PERK, eIF2, XBP1, CHOP, and GRP 78, in the absence (Figure 3A) or presence (Figure 3B) of the ER stress inducer TG compared to MOCK and NOR. These results indicate that the reduced secretion of HBsAg spontaneously induced ER stress in the absence of ER stress inducers. The ER stress inhibitor salubrinal was used after the transient transfection of HuH-7 cell lines to further investigate



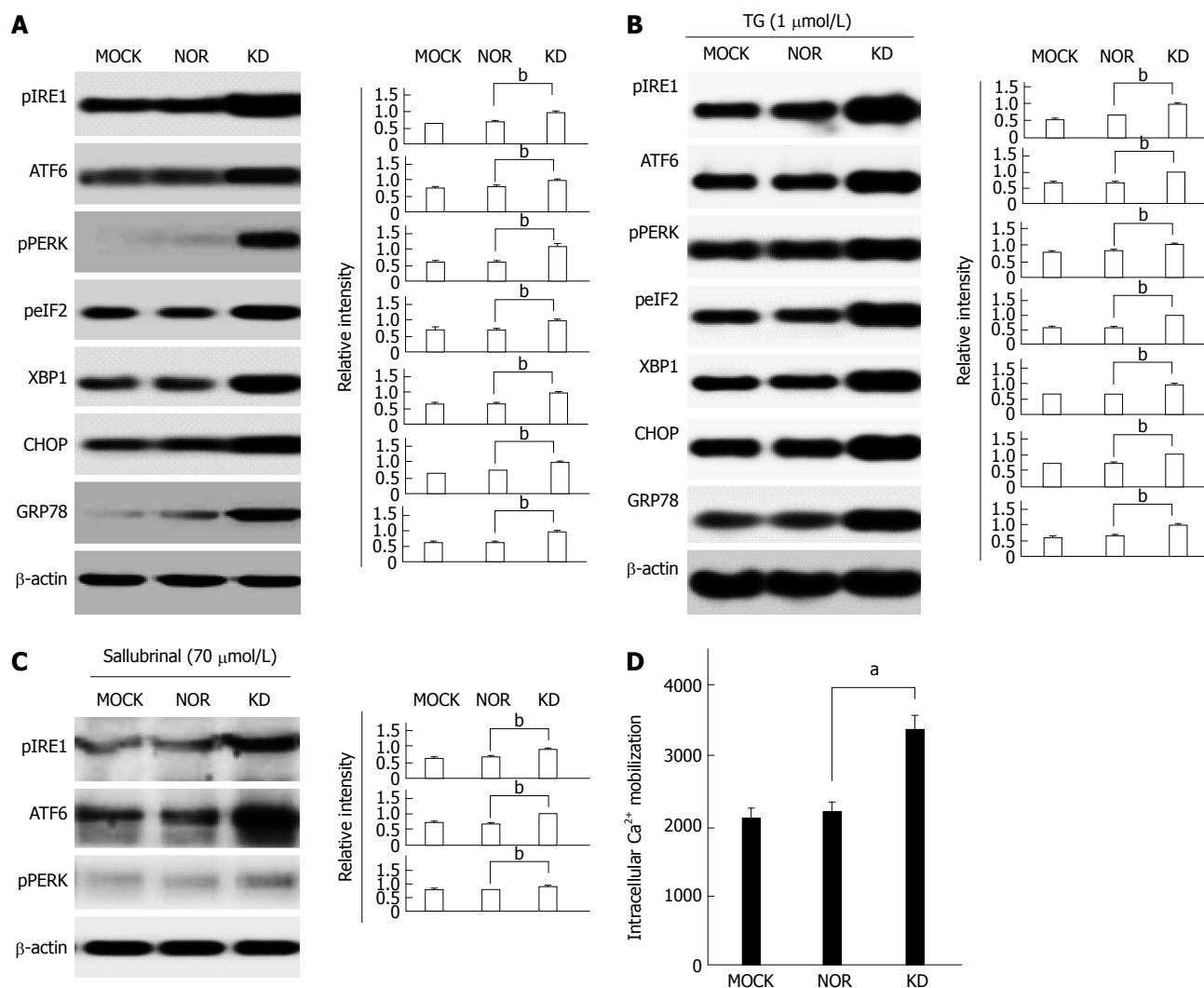


**Figure 1** Mutation patterns of hepatitis B virus S surface antigen (KD) related to occult infection and a wild-type hepatitis B virus S surface antigen (NOR) and comparisons of secretory and intracellular S surface antigen levels using hepatitis B virus S surface antigen ELISAs. Sequence variations were compared between wild-type (NOR) and KD variant (W36L, T47K, N52D, V184A, and F220L) HBsAg using the MegAlign software program (DNASTAR, Wisconsin, United States) (A). Secretory and intracellular HBsAg levels after transient transfection of plasmids (MOCK, NOR, and KD) into HuH-7 cells were measured using the HBsAg ELISA assay (B). All data are expressed as the mean  $\pm$  SD within a group. <sup>b</sup> $P < 0.01$  vs NOR supernatant; <sup>f</sup> $P < 0.01$  vs supernatant and pellet.





**Figure 2** Comparisons of co-localization signals of endoplasmic reticulum and a hepatitis B virus S surface antigen variant (KD) and wild-type hepatitis B virus S surface antigen (NOR) using confocal microscopy. **A:** Colocalization of the KD HBsAg variant (KD) and wild-type HBsAg (Nor) in the ER was visualized using confocal microscopy (Confocal A1, Nikon, Japan) after immunofluorescence double-staining assays. HuH-7 cells were transiently transfected with an empty vector (pIRES2-EGFP-Mock) or two different small hepatitis B virus S surface antigen (HBsAg) protein expression vectors (pIRES2-EGFP-Wild-type, pIRES2-EGFP-KD). Cells were harvested 2 d post-transfection, fixed with 4% paraformaldehyde, and stained for HBsAg (green) and the ER marker calnexin (red). The blue color of the nucleus is DAPI staining. Scale bars represent 10, 20, and 50 μm; **B:** Colocalizations of the HBsAg and ER in the cytoplasm were compared to each other using co-localization coefficients according to the lasso ROI selection. Statistical comparisons were performed using one-way ANOVA. The average of the coefficients of ten images examined in a double-blinded manner is shown. ( $^bP < 0.01$  vs control).



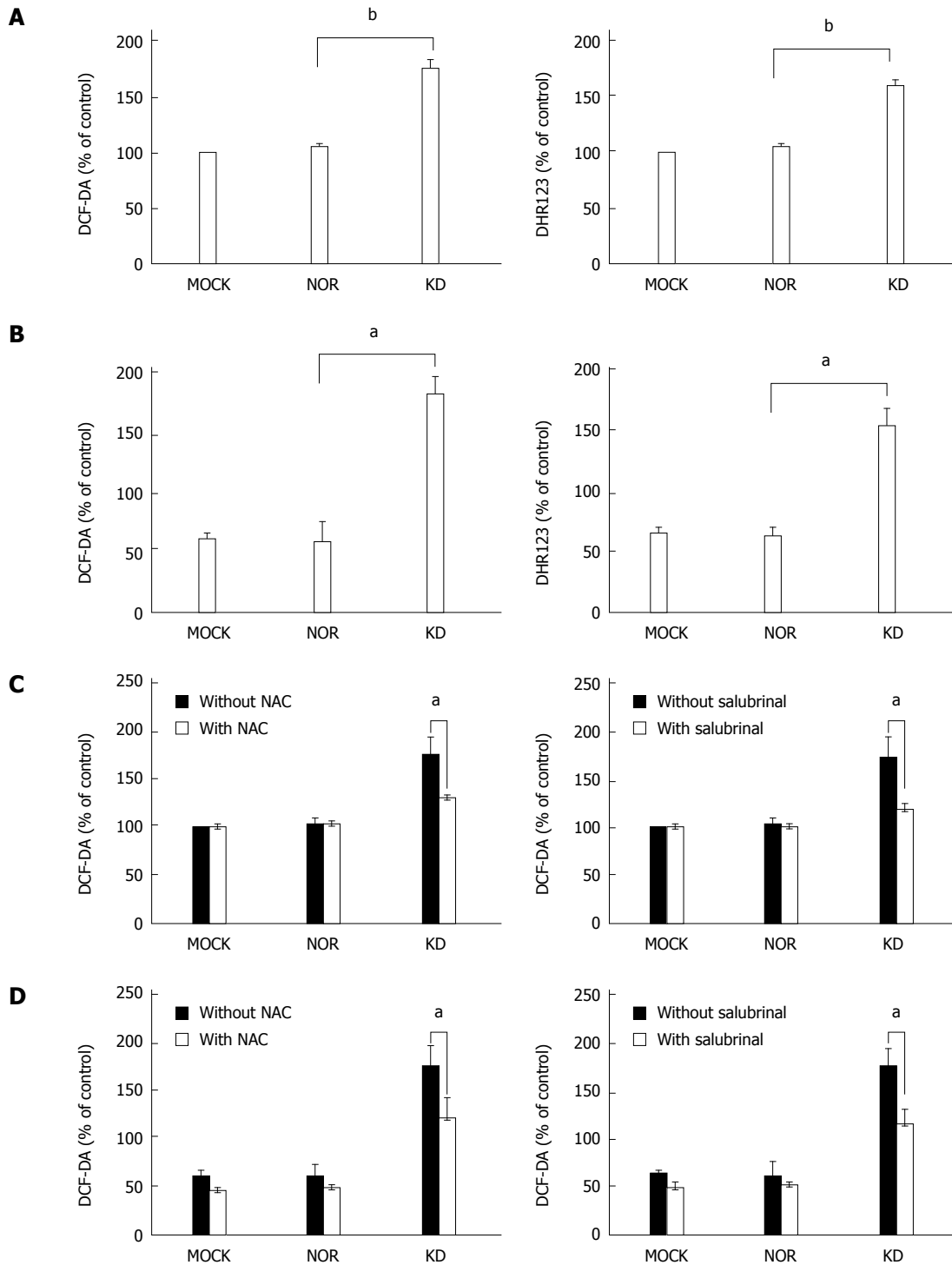
**Figure 3** Induction of endoplasmic reticulum stress and intracellular calcium levels by an occult infection related hepatitis B virus S surface antigen variant (KD). After transient transfection of plasmids (MOCK, NOR, and KD) into HuH-7 cells, up-regulation of the seven main endoplasmic reticulum (ER) stress-related proteins, namely, IRE1, ATF6, PERK, eIF2, XBP1, CHOP, and GRP 78, was confirmed using Western blot in the absence (A) and presence (B) of 1  $\mu$ mol/L thapsigargin (TG); C: Effect of the ER stress inhibitor salubrinal (70  $\mu$ mol/L) on HBsAg variant-induced activation of ER stress-related proteins (IRE1, ATF6, and PERK). D: After transient transfection,  $\text{Ca}^{2+}$  was measured using Rhod-2-AM. The mean values are presented in the graphic (all data were confirmed using green fluorescent protein and Western blot to determine transfection efficiency). The relative intensity was determined, and all data are expressed as the mean  $\pm$  SD within a group. Data from the three experiments were compared using Tukey's multiple post-hoc test ( $^aP < 0.05$ ,  $^bP < 0.01$  vs control).

the effects of the KD variant on ER stress. The data show that ER stress transducers, which induce effects of IRE1, ATF6, and PERK, were greatly reduced in salubrinal-treated cells compared to cells without salubrinal treatment (Figure 3C). The up-regulation of ER stress pathways induces the release of  $\text{Ca}^{2+}$  from the ER lumen to the cytoplasm<sup>[26]</sup>. We monitored  $\text{Ca}^{2+}$  mobilization using the fluorescent membrane permeable fluorochrome Rhod2-AM to investigate this issue. The FACS data show that the KD variant induced an increase in intracellular  $\text{Ca}^{2+}$  concentrations, which contrasts MOCK and NOR cells (Figure 3D).

#### **KD variant leads to ROS generation via ER stress pathways**

Released  $\text{Ca}^{2+}$  may induce ROS generation via mitochondrial membrane perturbation<sup>[27]</sup>. Therefore,

an ROS assay was performed in transiently transfected HuH-7 cells with or without salubrinal treatment to investigate whether the KD variant induces ROS generation through ER stress pathways. Cells were loaded with DHR123 and DCF-DA to assess the levels of mitochondrial and intracellular ROS, respectively. Two different methods for ROS measurement, ELISA (Figure 4A) and FACS analysis (Figure 4B), were used in this study. The data show that the KD variant induced a higher level of ROS generation in mitochondria and cytoplasm compared to MOCK and NOR cells (Figure 4A and B). Salubrinal treatment reduced these inducing effects (Figure 4C and D), which suggests that the KD variant induces ROS generation in an ER stress-dependent manner<sup>[28]</sup>. The HBsAg variant may also play a key role in increased ROS generation within hepatocytes. Figure 4C and D shows that the

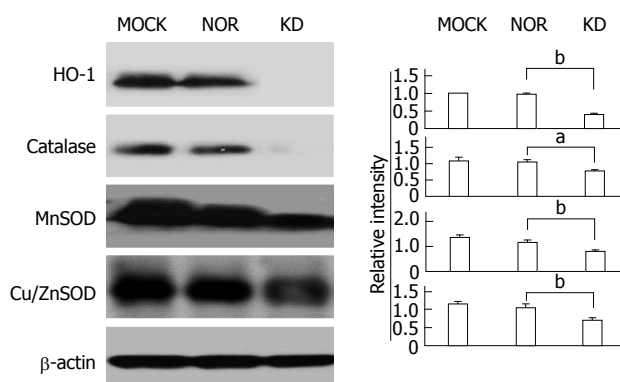


**Figure 4** Reactive oxygen species generation by an occult infection related hepatitis B virus S surface antigen variant, KD. Final concentrations (25  $\mu\text{mol/L}$ ) of DCF-DA and DHR123 were incubated for 20 min after transient transfection of plasmids (MOCK, NOR, and KD) into HuH-7 cells to assess mitochondrial and intracellular ROS levels. Results were determined using luminescence spectrometry (A) or FACS (B). The mean values of the experiments are presented in the graphic. The effects of the ROS inhibitor NAC (40 mmol/L) and the ER stress inhibitor salubrinal (70  $\mu\text{mol/L}$ ) on ROS generation were evaluated using DCF-DA and luminescence spectrometry (C) or FACS (D). All experiments were performed in triplicate. The relative intensity was determined, and all data are expressed as the means  $\pm$  SD within a group. Data from the three experiments were compared using Tukey's multiple post-hoc test (<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control).

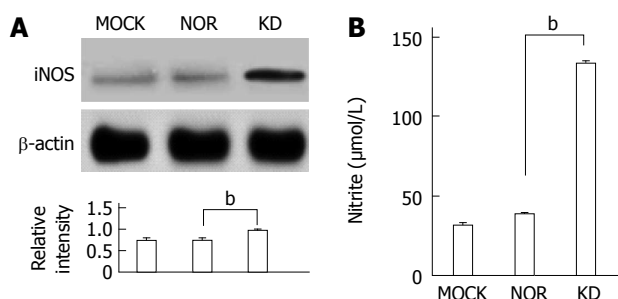
ROS inhibitor N-acetyl-cysteine (NAC) reduced ROS induction by the KD variant to a level similar to MOCK

and NOR, which verifies the validity of the above experiment.





**Figure 5 Down-regulation of anti-oxidant proteins by an occult infection related to an S surface antigen variant (KD).** After transient transfection of plasmids (MOCK, NOR, and KD) into HuH-7 cells, the down-regulation of four anti-oxidant proteins, MnSOD, CuZnSOD, HO-1, and catalase in the KD variant was confirmed using Western blot. The results of the KD variant contrasted MOCK and NOR. The relative intensity values were determined, and all data are expressed as the mean  $\pm$  SD within a group. The three experiments were compared using Tukey's multiple post-hoc test ( $^aP < 0.05$ ,  $^bP < 0.01$  vs three experiments).



**Figure 6 Nitric oxide increase via up-regulation of iNOS expression by a hepatitis B virus S surface antigen variants (KD).** A: After transient transfection of plasmids (MOCK, NOR, and KD) into HuH-7 cells, up-regulation of iNOS expression was observed in the KD variant compared to the MOCK and NOR. The relative intensity was determined, and all data are expressed as the mean  $\pm$  SD within a group. The three experiments were compared using Tukey's multiple post-hoc test ( $^aP < 0.01$  vs three experiments); B: After transient transfection, NO levels were assessed using a nitric oxide (NO) ELISA (Assay Designs, MI, United States). NO levels in the KD HBsAg variant increased significantly compared to the MOCK and NOR. The relative intensity was determined, and all data are expressed as the mean  $\pm$  SD within a group. The three experiments were compared using Tukey's multiple post-hoc test ( $^aP < 0.01$  vs three experiments).

#### **KD variant down-regulates anti-oxidant proteins and lead to NO increase via up-regulation of iNOS expression**

The balance between oxidants and anti-oxidants is a major concern in hepatocyte damage<sup>[29]</sup>. The expression of antioxidant-related enzymes after plasmid transfection was measured to analyze the effect of the KD variant on the expression of antioxidant proteins (Figure 5). Generally, the expression levels of anti-oxidant ROS scavenger proteins, such as SOD (MnSOD and CuZn SOD), HO-1, and catalase, were significantly down-regulated by the KD variant, which leads to a positive amplification loop of the ER stress-ROS axis. Experiments using iNOS protein expression and a

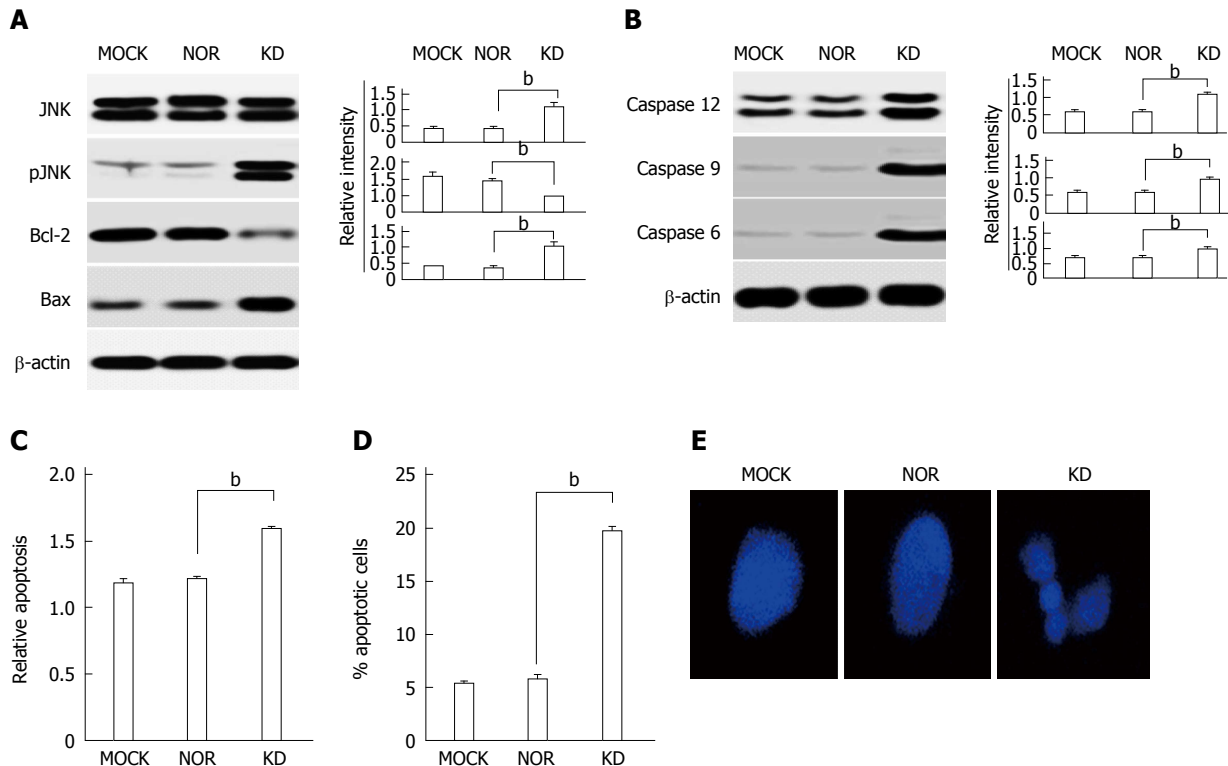
commercially available nitrite assay were performed to investigate the effects of the KD variant on NO levels. The KD variant significantly up-regulated iNOS (Figure 6A) and increased nitrite production compared to MOCK and NOR (Figure 6B); these findings suggest that the KD variant may play a pivotal role in NO production in hepatocytes *via* up-regulation of the iNOS protein.

#### **KD variant induces apoptosis in hepatocytes**

Apoptosis induction in hepatocytes by stress signals, such as ER stress, is closely related to the progression of liver diseases<sup>[30]</sup>. We investigated the effects of the KD variant on the expression of apoptosis-related genes to examine whether the KD variant induces apoptosis in hepatocytes. First, the data showed that phosphorylation of the JNK protein, which plays a key role in apoptosis induction because of ER stress, was up-regulated in the KD variant, but not MOCK or NOR. The up-regulation of the pro-apoptotic protein, Bax, and the down-regulation of the anti-apoptotic protein, Bcl-2, which followed JNK activation, were also observed in the KD variant (Figure 7A). Caspases 3, 9, and 12, which directly mediate apoptotic cell death, were also up-regulated in the KD variant but not MOCK or NOR (Figure 7B). Additionally, we conducted cell death ELISAs to investigate the effect of the KD variant on apoptosis by *in vitro* determination of cytoplasmic histone-associated DNA fragments. Highly elevated apoptosis levels were found in the KD variant compared to MOCK and NOR (Figure 7C). Additionally, PI staining using FACS was performed for apoptosis detection, and increased levels were observed in the KD variant (Figure 7D). Finally, DNA fragmentation associated with apoptosis was assessed for morphological identification. We found enhanced DNA fragmentation as a hallmark of apoptosis in the KD variant (Figure 7E). These results collectively indicate that occult infection related HBsAg variant (KD) induces apoptosis in hepatocytes *via* the ER stress-JNK activation axis.

## **DISCUSSION**

HBsAg is the strongest secretory HBV protein. Therefore, it is reasonably expected that HBsAg variants with deficient secretory capacity could induce ER stress in liver cells *via* the UPR, which could lead to liver cell damage<sup>[22,31]</sup>. This process provides a plausible link between occult infection and the progression of liver diseases. Therefore, the present study investigated the above hypothesis by examining whether our KD variant showing a secretion defect could induce ER stress pathways and evoke ER stress-mediated biological actions that are associated with the progression of liver cell diseases. Our data indicated that the KD variant activated ER-related genes and induced ER stress, which mediated the induction of ROS, NO, and apoptosis in similar manners. These results suggest that the KD variant may affect an



**Figure 7 Apoptosis induction by an occult infection related hepatitis B virus S surface antigen variant (KD).** A: After transient transfection of plasmids (MOCK, NOR, and KD) into HuH-7 cells, up-regulation of p-JNK and the pro-apoptotic proteins Bax and down-regulation of the anti-apoptotic protein Bcl-2 were observed in the KD HBsAg variant compared to the MOCK and NOR. These results were confirmed using Western blot; B: After transfection, up-regulation of apoptosis-related caspase proteins (caspases 6, 9, and 12) was observed in the KD HBsAg variant compared to the MOCK and NOR. Apoptosis induction in the KD variant was demonstrated using a DNA Fragmentation Assay and the Cell Death Detection ELISA<sup>PLUS</sup> kit (Roche, Mannheim, Germany); C: PI staining using FACS. Morphological identification (D) of DNA fragmentation (E). The relative intensity values were determined, and all data are expressed as the mean  $\pm$  SD within a group. The three experiments were compared using Tukey's multiple post-hoc test ( $^*P < 0.01$  vs three experiments).

upstream signal of the ER stress pathway.

This study investigated possible links between a KD variant and the progression of liver disease and focused on three biological actions that could lead to liver damage, including ER stress and ROS production, NO production, and apoptosis induction. First, our data showed that the KD variant induced ER stress and ROS production *via* intracellular calcium increases in hepatocytes (Figures 3 and 4). Generally, a relatively mild level of ROS induction is advantageous in cell signaling, and it induced anti-oxidant genes that enabled elevated ROS levels to remain at normal levels in this case<sup>[32]</sup>. Increased ROS levels in hepatocytes due to the hepatitis C virus (HCV) core proteins induce the expression of anti-oxidant genes, which may maintain ROS homeostasis. This process may otherwise induce hepatocyte apoptosis or HCC<sup>[33]</sup>. In contrast to the HCV core protein, our KD variant down-regulated the anti-oxidant proteins HO-1, MnSOD, Cu/Zn SOD, and catalase (Figure 5), which suggests a positive amplification of the ER stress-ROS production axis. The differences between these two cases may be due to differences in the intensity of inducing ER stress or ROS production. Perhaps the excessive ER stress and ROS level induced by the KD variant were beyond the threshold necessary to maintain normal

cell function, which facilitated cell death by increasing cytosolic ROS levels *via* the abrogation of anti-oxidant gene function. The excessive ER stress and ROS level also provide possible links between occult HBsAg variants and apoptosis.

Second, the KD variant increased NO production in hepatocytes in an iNOS expression-dependent manner. Recently, the role of NO-mediated oxidative stress was well established in chronic viral hepatitis induced by HBV and HCV<sup>[34,35]</sup>. Therefore, the iNOS-mediated NO synthesis that was observed by the KD variant in the present study (Figure 6) was likely induced by ER stress-ROS mediated inflammation. The elevated NO production by the HBsAg variant may be responsible for the generation of chronic hepatitis and the progression of liver diseases *via* peroxynitrite, which is a potential oxidant that is produced by the reduction of superoxide anion, a species of ROS, with NO<sup>[36]</sup>.

Third, apoptosis plays a central role in liver diseases<sup>[37]</sup>. The apoptosis of hepatocytes, which are the major component of liver cells, is directly related to the failure of liver functions. Additionally, the regeneration of liver cells after chronic apoptosis in combination with the accumulation of mutated DNA because of ER stress-ROS production may induce hepatocarcinogenesis. Figure 7 shows that the KD variant induced apoptosis

in hepatocytes *via* at least three potential pathways, which are more inter-connected than distinct. First, apoptosis may be mediated by CHOP activation *via* the major ER stress transducer PERK-EIF2 $\alpha$  axis (Figure 3A). Second, apoptosis may be mediated by intracellular calcium increases *via* ER stress (Figure 3D). Finally, apoptosis may also be mediated by ER stress-mediated JNK activation, which induces anti-apoptotic BCL-2 down-regulation and pro-apoptotic Bax-2 activation (Figure 7A). The synergistic combination of these three pathways may lead to apoptosis in hepatocytes in a caspase-dependent manner (Figure 7B).

In conclusion, the KD variant that is related to occult infections may play a pivotal role in the progression of liver diseases, such as chronic hepatitis, liver fibrosis, cirrhosis, and HCC, during the natural course of HBV infections primarily *via* ER stress and ROS production. The possible down-regulation of HBV replication because of ROS and NO production by HBsAg variants may also provide a likely explanation for the relationship between occult HBV infection and ER stress-mediated liver diseases. The present study provides two primary observations. First, the results of this study emphasized the role of HBsAg among other HBV products in the pathogenesis of liver diseases. Second, the data obtained in this study strongly support a hypothesis that one resource of occult HBV infections, at least in the Korean population, may be chronic patients with advanced liver diseases. Therefore, horizontal transfer of occult HBV variants may occur between occult HBV patients, chronic HBV patients, and otherwise healthy people.

## COMMENTS

### Background

Occult hepatitis B virus (HBV) infection is highly prevalent, particularly in HBV endemic areas, and it is significantly related to severe forms of liver diseases, such as cirrhosis and hepatocellular carcinoma. However, the exact molecular mechanisms underlying occult infections that are related to severe liver diseases are not known.

### Research frontiers

The data indicated that an occult infection related to the S surface antigen (HBsAg) variant KD with lower secretion capacity led to endoplasmic reticulum (ER)-derived oxidative stress and liver cell death in HuH-7 cells. These results provide novel insight into relationships between occult infection and liver disease progression.

### Innovations and breakthroughs

This report is the first study to demonstrate that an occult infection related to an HBsAg variant may have a pivotal function in liver disease progression *via* ER stress-related pathways.

### Applications

An occult infection related to the HBsAg variant KD, which was introduced in this study, could be effectively used for further investigation of ER stress pathways.

### Terminology

Occult HBV infection is defined as an infection state that is negative for HBsAg serology, but viral genome persistence is observed in infected individuals. The ER has multiple important functions that are essential to cell survival and normal cellular functions, including Ca<sup>2+</sup> storage, post-translational modification, and the folding and assembly of newly synthesized secretory proteins.

## Peer-review

The authors examined the underlying mechanisms of the relationship between occult HBV infection and liver disease progression and focused on the induction of ER stress because of occult infection related to an HBsAg variant. The results revealed that an occult infection related to the HBsAg variant KD with lower secretion capacity enhanced ER-derived oxidative stress and liver cell death in HuH-7 cells compared to wild-type HBsAg. These results provide novel insight into the relationships between occult infection and liver disease progression.

## REFERENCES

- 1 Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; **337**: 1733-1745 [PMID: 9392700 DOI: 10.1056/Nejm199712113372406]
- 2 KCDC Trend in Seroprevalence of Hepatitis B surface Antigen (HBsAg) in Korea Report of National Health and Nutrition Survey. 2013. Available from: URL: <http://www.cdc.go.kr>
- 3 Kim H, Jee YM, Song BC, Shin JW, Yang SH, Mun HS, Kim HJ, Oh EJ, Yoon JH, Kim YJ, Lee HS, Hwang ES, Cha CY, Kook YH, Kim BJ. Molecular epidemiology of hepatitis B virus (HBV) genotypes and serotypes in patients with chronic HBV infection in Korea. *Intervirology* 2007; **50**: 52-57 [PMID: 17164558 DOI: 10.1159/000096313]
- 4 Orito E, Mizokami M, Sakugawa H, Michitaka K, Ishikawa K, Ichida T, Okanoue T, Yotsuyanagi H, Iino S. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. *Hepatology* 2001; **33**: 218-223 [PMID: 11124839 DOI: 10.1053/jhep.2001.20532]
- 5 Song BC, Kim SH, Kim H, Ying YH, Kim HJ, Kim YJ, Yoon JH, Lee HS, Cha CY, Kook YH, Kim BJ. Prevalence of naturally occurring surface antigen variants of hepatitis B virus in Korean patients infected chronically. *J Med Virol* 2005; **76**: 194-202 [PMID: 15834881 DOI: 10.1002/Jmv.20354]
- 6 Kim HJ, Park JH, Jee Y, Lee SA, Kim H, Song BC, Yang S, Lee M, Yoon JH, Kim YJ, Lee HS, Hwang ES, Kook YH, Kim BJ. Hepatitis B virus X mutations occurring naturally associated with clinical severity of liver disease among Korean patients with chronic genotype C infection. *J Med Virol* 2008; **80**: 1337-1343 [DOI: 10.1002/Jmv.21219]
- 7 Mun HS, Lee SA, Jee Y, Kim H, Park JH, Song BC, Yoon JH, Kim YJ, Lee HS, Hyun JW, Hwang ES, Kook YH, Kim BJ. The prevalence of hepatitis B virus preS deletions occurring naturally in Korean patients infected chronically with genotype C. *J Med Virol* 2008; **80**: 1189-1194 [PMID: 18461612 DOI: 10.1002/Jmv.21208]
- 8 Mun HS, Lee SA, Kim H, Hwang ES, Kook YH, Kim BJ. Novel F141L pre-S2 mutation in hepatitis B virus increases the risk of hepatocellular carcinoma in patients with chronic genotype C infections. *J Virol* 2011; **85**: 123-132 [PMID: 20962085 DOI: 10.1128/Jvi.01524-10]
- 9 Lee SA, Mun HS, Kim H, Lee HK, Kim BJ, Hwang ES, Kook YH, Kim BJ. Naturally occurring hepatitis B virus X deletions and insertions among Korean chronic patients. *J Med Virol* 2011; **83**: 65-70 [PMID: 21108340 DOI: 10.1002/Jmv.21938]
- 10 Lee SA, Cho YK, Lee KH, Hwang ES, Kook YH, Kim BJ. Gender disparity in distribution of the major hydrophilic region variants of hepatitis B virus genotype C according to hepatitis B e antigen serostatus. *J Med Virol* 2011; **83**: 405-411 [PMID: 21264860 DOI: 10.1002/Jmv.21988]
- 11 Lee SA, Kim K, Kim H, Kim BJ. Nucleotide change of codon 182 in the surface gene of hepatitis B virus genotype C leading to truncated surface protein is associated with progression of liver diseases. *J Hepatol* 2012; **56**: 63-69 [PMID: 21827734 DOI: 10.1016/j.jhep.2011.06.028]
- 12 Kim DW, Lee SA, Hwang ES, Kook YH, Kim BJ. Naturally occurring precore/core region mutations of hepatitis B virus genotype C related to hepatocellular carcinoma. *PLoS One* 2012; **7**: e47372 [PMID: 23071796 DOI: 10.1371/journal.pone.0047372]
- 13 Kim H, Lee SA, Kim DW, Lee SH, Kim BJ. Naturally occurring mutations in large surface genes related to occult infection of

- hepatitis B virus genotype C. *PLoS One* 2013; **8**: e54486 [PMID: 23349904 DOI: 10.1371/journal.pone.0054486]
- 14 **Lee SA**, Kim KJ, Kim DW, Kim BJ. Male-specific W4P/R mutation in the pre-S1 region of hepatitis B virus, increasing the risk of progression of liver diseases in chronic patients. *J Clin Microbiol* 2013; **51**: 3928-3936 [PMID: 24025913 DOI: 10.1128/JCM.01505-13]
  - 15 **Kim H**, Lee SA, Won YS, Le HJ, Kim BJ. Occult infection related hepatitis B surface antigen variants showing lowered secretion capacity. *World J Gastroenterol* 2014; **21**: 1794-1803 [PMID: 25684944 DOI: 10.3748/wjg.v21.i6.1794]
  - 16 **Bruss V**, Ganem D. The role of envelope proteins in hepatitis B virus assembly. *Proc Natl Acad Sci USA* 1991; **88**: 1059-1063 [PMID: 1992457 DOI: 10.1073/pnas.88.3.1059]
  - 17 **Molnar-Kimber KL**, Jarocki-Witek V, Dheer SK, Vernon SK, Conley AJ, Davis AR, Hung PP. Distinctive properties of the hepatitis B virus envelope proteins. *J Virol* 1988; **62**: 407-416 [PMID: 3336067]
  - 18 **Liang TJ**, Baruch Y, Ben-Porath E, Enat R, Bassan L, Brown NV, Rimon N, Blum HE, Wands JR. Hepatitis B virus infection in patients with idiopathic liver disease. *Hepatology* 1991; **13**: 1044-1051 [PMID: 2050320 DOI: 10.1016/0270-9139(91)92470-S]
  - 19 **Koike K**, Kobayashi M, Gondo M, Hayashi I, Osuga T, Takada S. Hepatitis B virus DNA is frequently found in liver biopsy samples from hepatitis C virus-infected chronic hepatitis patients. *J Med Virol* 1998; **54**: 249-255 [PMID: 9557290 DOI: 10.1002/(Sici)1096-9071(199804)54]
  - 20 **Bánhegyi G**, Baumeister P, Benedetti A, Dong D, Fu Y, Lee AS, Li J, Mao C, Margittai E, Ni M, Paschen W, Piccirella S, Senesi S, Sitia R, Wang M, Yang W. Endoplasmic reticulum stress. *Ann N Y Acad Sci* 2007; **1113**: 58-71 [PMID: 17483206 DOI: 10.1196/annals.1391.007]
  - 21 **Schröder M**, Kaufman RJ. ER stress and the unfolded protein response. *Mutat Res* 2005; **569**: 29-63 [PMID: 15603751 DOI: 10.1016/j.mrfmmm.2004.06.056]
  - 22 **Ji C**, Kaplowitz N. ER stress: can the liver cope? *J Hepatol* 2006; **45**: 321-333 [PMID: 16797772 DOI: 10.1016/j.jhep.2006.06.004]
  - 23 **Lin JH**, Walter P, Yen TS. Endoplasmic reticulum stress in disease pathogenesis. *Annu Rev Pathol* 2008; **3**: 399-425 [PMID: 18039139 DOI: 10.1146/annurev.pathmechdis.3.121806.151434]
  - 24 **Wang HC**, Huang W, Lai MD, Su IJ. Hepatitis B virus pre-S mutants, endoplasmic reticulum stress and hepatocarcinogenesis. *Cancer Sci* 2006; **97**: 683-688 [PMID: 16863502 DOI: 10.1111/j.1349-7006.2006.00235.x]
  - 25 **Hsieh YH**, Su IJ, Wang HC, Chang WW, Lei HY, Lai MD, Chang WT, Huang W. Pre-S mutant surface antigens in chronic hepatitis B virus infection induce oxidative stress and DNA damage. *Carcinogenesis* 2004; **25**: 2023-2032 [PMID: 15180947 DOI: 10.1093/carcin/bgh207]
  - 26 **Chua PK**, Wang RYL, Lin MH, Masuda T, Suk FM, Shih C. Reduced secretion of virions and hepatitis B virus (HBV) surface antigen of a naturally occurring HBV variant correlates with the accumulation of the small S envelope protein in the endoplasmic reticulum and Golgi apparatus. *J Virol* 2005; **79**: 13483-13496 [DOI: 10.1128/Jvi.79.21.13483-13496.2005]
  - 27 **Görlach A**, Klappa P, Kietzmann T. The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. *Antioxid Redox Signal* 2006; **8**: 1391-1418 [PMID: 16986999 DOI: 10.1089/ars.2006.8.1391]
  - 28 **Yang H**, Westland C, Xiong S, Delaney WE. In vitro antiviral susceptibility of full-length clinical hepatitis B virus isolates cloned with a novel expression vector. *Antiviral Res* 2004; **61**: 27-36 [PMID: 14670591 DOI: 10.1016/j.antiviral.2003.07.003]
  - 29 **Alisi A**, Piemonte F, Pastore A, Panera N, Passarelli C, Tozzi G, Petrini S, Pietrobattista A, Bottazzo GF, Nobili V. Glutathionylation of p65NF-kappaB correlates with proliferating/apoptotic hepatoma cells exposed to pro- and anti-oxidants. *Int J Mol Med* 2009; **24**: 319-326 [PMID: 19639223 DOI: 10.3892/ijmm.00000235]
  - 30 **Hara Y**, Hino K, Okuda M, Furutani T, Hidaka I, Yamaguchi Y, Korenaga M, Weinman SA, Lemon SM, Okita K. Hepatitis C virus core protein inhibits deoxycholic acid-mediated apoptosis despite generating mitochondrial reactive oxygen species. *J Gastroenterol* 2006; **41**: 257-268 [DOI: 10.1007/s00535-005-1738-1]
  - 31 **Xu Z**, Jensen G, Yen TS. Activation of hepatitis B virus S promoter by the viral large surface protein via induction of stress in the endoplasmic reticulum. *J Virol* 1997; **71**: 7387-7392 [PMID: 9311817]
  - 32 **Trachootham D**, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov* 2009; **8**: 579-591 [PMID: 19478820 DOI: 10.1038/nrd2803]
  - 33 **Mahmood S**, Kawanaka M, Kamei A, Izumi A, Nakata K, Niiyama G, Ikeda H, Hanano S, Suehiro M, Togawa K, Yamada G. Immunohistochemical evaluation of oxidative stress markers in chronic hepatitis C. *Antioxid Redox Signal* 2004; **6**: 19-24 [PMID: 14713333 DOI: 10.1089/152308604771978318]
  - 34 **García-Monzón C**, Majano PL, Zubia I, Sanz P, Apolinario A, Moreno-Otero R. Intrahepatic accumulation of nitrotyrosine in chronic viral hepatitis is associated with histological severity of liver disease. *J Hepatol* 2000; **32**: 331-338 [PMID: 10707875 DOI: 10.1016/S0168-8278(00)80080-X]
  - 35 **Kandemir O**, Polat A, Kaya A. Inducible nitric oxide synthase expression in chronic viral hepatitis and its relation with histological severity of disease. *J Viral Hepat* 2002; **9**: 419-423 [PMID: 12431203 DOI: 10.1046/j.1365-2893.2002.00382.X]
  - 36 **Oishi P**, Grobe A, Benavidez E, Ovadia B, Harmon C, Ross GA, Hendricks-Munoz K, Xu J, Black SM, Fineman JR. Inhaled nitric oxide induced NOS inhibition and rebound pulmonary hypertension: a role for superoxide and peroxynitrite in the intact lamb. *Am J Physiol Lung Cell Mol Physiol* 2006; **290**: L359-L366 [PMID: 16258003 DOI: 10.1152/ajplung.00019.2005]
  - 37 **Fabregat I**, Roncero C, Fernández M. Survival and apoptosis: a dysregulated balance in liver cancer. *Liver Int* 2007; **27**: 155-162 [PMID: 17311609 DOI: 10.1111/j.1478-3231.2006.01409.x]

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## Basic Study

# Downregulation of microRNA-382 is associated with poor outcome of esophageal squamous cell carcinoma

Bo Qi, Jian-Guo Lu, Wen-Jian Yao, Ting-Min Chang, Xiu-Guang Qin, Ying-Hua Ji, Tian-Yun Wang, Shang-Guo Liu, Han-Chen Li, Yu-Zhen Liu, Bao-Sheng Zhao

Bo Qi, Jian-Guo Lu, Wen-Jian Yao, Xiu-Guang Qin, Shang-Guo Liu, Han-Chen Li, Yu-Zhen Liu, Bao-Sheng Zhao, Department of Thoracic Surgery, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan Province, China

Ting-Min Chang, Department of Gastroenterology, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan Province, China

Ying-Hua Ji, Department of Oncology, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan Province, China

Tian-Yun Wang, Department of Molecular Biology, Xinxiang Medical University, Xinxiang 453003, Henan Province, China

**Author contributions:** Zhao BS contributed to conception and design; Qi B designed methods; Lu JG, Yao WJ, Chang TM, Qin XG and Ji YH performed the majority of the experiments; Wang TY carried out the laboratory experiments; Liu SG analyzed the data; Liu YZ interpreted the results and wrote the paper; and Li HC contributed to revision for intellectual content.

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Correspondence to: Bao-Sheng Zhao, MD, Professor, Chief, Department of Thoracic Surgery, The First Affiliated Hospital of Xinxiang Medical University, 88 Jiangkang Road, Weihui 453100, Henan Province, China. [zhaobsn@126.com](mailto:zhaobsn@126.com)

Telephone: +86-373-4404340

Fax: +86-373-4402573

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## Abstract

**AIM:** To study the potential prognostic role of microRNA-382 (miR-382) in esophageal squamous cell carcinoma (ESCC).

**METHODS:** Forty six patients were divided into 2 groups according to postoperative survival time: the poor outcome group (28 patients), who showed early metastasis but no recurrence, and died within 1 year after surgery, 12 patients of the group received postoperative chemotherapy treatment that was given after early metastasis happening; the good outcome group (18 patients), who had no clinical metastasis and recurrence, and survived 5 years or more after surgery, all patients did not receive any postoperative treatment. Total RNA was extracted from the patients' formalin-fixed and paraffin-embedded esophageal cancer tissues. miR-382 level was evaluated using high-throughput real-time quantitative polymerase chain reaction analysis. The correlation between miR-382 level and clinicopathologic features was analyzed through COX regression model, and Kaplan-Meier analysis was used to analyze the relationship between

miR-382 level and patient survival time.

**RESULTS:** miR-382 was differentially expressed in the two groups. Overall the average miR-382 level in the ESCC patients with good outcome was  $9.8 \pm 3.8$ , while miR-382 level in the ESCC patients with poor outcome was  $3.0 \pm 0.8$ . The differences of miR-382 levels between two groups were significant ( $P < 0.05$ ). Kaplan-Meier analysis results showed that miR-382 expression level generally had a significant reverse-correlation with ESCC patient survival time ( $P < 0.001$ ), in which the patients with higher expressions of miR-382 had a longer survival time either among individuals with the same tumor stage or among the overall patients.

**CONCLUSION:** miR-382 levels are reverse-correlated with ESCC poor outcomes, suggesting that miR-382 could be a potential predictive biomarker for both prognosis and treatment of ESCC.

**Key words:** Esophageal squamous cell carcinoma; miR-382; Metastasis; Outcome; Prognosis

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**Core tip:** Esophageal squamous cell carcinoma (ESCC) patients often have significantly different outcomes due to early metastasis happening or not, although the patients are at the same pathological stage and receive the similar surgical therapy. Exploring novel biomarkers related with ESCC metastasis is required for monitoring the progression of the disease, and predicting the outcome of the patient after clinical intervention. Current research addressed a potential prognostic role of microRNA-382 in ESCC.

Qi B, Lu JG, Yao WJ, Chang TM, Qin XG, Ji YH, Wang TY, Liu SG, Li HC, Liu YZ, Zhao BS. Downregulation of microRNA-382 is associated with poor outcome of esophageal squamous cell carcinoma. *World J Gastroenterol* 2015; 21(22): 6884-6891 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6884.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6884>

## INTRODUCTION

Esophageal cancer (EC) is one of the most common gastrointestinal cancers. Up to 300 thousand people worldwide die from this disease each year<sup>[1]</sup>. More than 50% of the global incidence of EC is in China<sup>[2]</sup>. Histologically, EC is divided into two main types: squamous cell carcinoma and adenocarcinoma. Esophageal squamous cell carcinoma (ESCC) is the major histological type of EC in China. The global 5-year survival rate of EC is only about 15% with most patients dying within one year of diagnosis<sup>[3]</sup>.

This is mainly based on EC's highly invasive characteristic, which often leads to early metastasis and reduces treatment efficacy<sup>[4,5]</sup>. Although Tumor Node Metastasis (TNM) staging system is still a major criteria for EC prognosis, our clinical observations show that ESCC patients receiving similar therapy (e.g., surgical therapy by the same surgeon) at the same pathological stage had significantly different outcomes. Moreover, metastasis in the early postoperative stage also occurred in early-stage ESCC patients. These observations suggested the hypothesis that metastasis-related molecules may be heterogeneously present in ESCC individuals. Therefore, exploring novel biomarkers related to ESCC metastasis is required for monitoring the progression of the disease, and predicting the prognosis of the patient after clinical intervention.

MicroRNA (miRNA) is a small non-coding RNA with 22-25 nucleotides in length, and controls gene expression via the regulation of translation efficiency and mRNA stability by binding to the complementary site in 3'-untranslated region (UTR) of the mRNA<sup>[6,7]</sup>. miRNAs are abundantly expressed and play an essential role in the regulation of a large number of biological processes, including cancer<sup>[8,9]</sup>. The expression patterns and biological functions of miRNAs in ESCC have been investigated in recent years<sup>[10-12]</sup>.

Human miR-382 (has-mir-382, MIMAT0000737, 5'-GAAGUUGUUCGUGGUGGAUUCG-3') resides in a miRNA cluster in the imprinted DLK1-DIO3 region on the 14q32 locus which hosts one of the largest miRNA clusters in the genome. Many of these miRNAs are differentially expressed in several pathologic processes and various cancers. Recent studies reported that miR-382 was decreased or increased in several types of human malignancy<sup>[13,14]</sup>, suggesting that the role of miR-382 contributing to tumor development and metastasis is tissue specific.

Using high-throughput real-time quantitative polymerase chain reaction, we initially evaluated the expression profiles of 754 miRNAs in paraffin-embedded tumor specimens from two ESCC patients with TNM IIa stage. These patients had different outcomes although they both received same postoperative treatment (chemotherapy twice)<sup>[15]</sup>. Our results showed that the levels of multiple miRNAs were significantly different between the two patients. One of the findings was that the ESCC patient who had neck lymph node metastasis which occurred four months after surgery and died 1 year after surgery had significantly lower miR-382 level compared to another patient who did not have metastasis and survived five years after surgery.

The present study was to further validate a potential role for miR-382 as an ESCC prognostic biomarker. For this purpose, miR-382 levels were examined from 46 ESCC patients with different outcomes; afterwards the relationship between miR-382 level and clinicopathological characteristics of

the patients was assessed. We found that expression of miR-382 was weaker in the specimens from the patients with poor outcome compared to those with good outcome ( $P < 0.05$ ) and miR-382 level was inversely correlated with ESCC patient survival time ( $P < 0.001$ ).

## MATERIALS AND METHODS

### *Patients and esophageal cancer tissue collection*

Our aim was to examine miR-382 levels in cancer tissues from ESCC patients who were at the same TNM stage but had different outcomes. Forty-six patients with ESCC diagnosed by histopathological examination between 2006 and 2009 were enrolled in this study. All patients' clinicopathological information had been recorded and the specimens had been collected before this work started. Additionally, all patients had not received any radiotherapy or chemotherapy prior to the surgical procedure performed in the Department of Thoracic Surgery, the First Affiliated Hospital of Xinxiang Medical University. TNM classifications after surgery were made according to International Union Against Cancer (UICC) staging criteria for esophageal cancer, sixth edition (2002). The 46 patients were divided into 2 groups according to postoperative survival time: the poor outcome group, who showed early metastasis but no recurrence, and died within 1 year after surgery; the good outcome group, who had no clinical metastasis and recurrence, and survived 5 years or more after surgery. This study did not include the patients surviving between 1-5 years after surgery. In addition, postoperative survival time of all stage IV patients undergoing surgical procedure in our department was less than 5 years when we started this work. Thus, patients with stage IV disease were excluded from this study. The postoperative follow-up was a standardized process that included routine computed tomography scan and upper gastrointestinal endoscopy. All patients ( $n = 18$ ) with good outcome did not receive any postoperative treatment, and 12 out of 28 patients with poor outcome received postoperative chemotherapy treatment that was given after early metastasis happening.

### *Ethics*

The present study was conducted in accordance with the declaration of Helsinki, and approved by the Institutional Review Board for Human Research of the First Affiliated Hospital of Xinxiang Medical University. Written informed consent form was obtained from all patients.

### *Tissue sample collection and RNA extraction*

Formalin-fixed and paraffin-embedded esophageal cancer tissues from the patients were used for RNA extraction. Using a microtome, 10- $\mu$ m slices of tissue from each patient were captured and placed into 1.5

mL microcentrifuge tubes. Paracancerous normal esophageal mucous membranes (8 cm distant to the verge of the tumor tissue) from 4 ESCC patients were taken as controls. Total RNA (including miRNAs) was extracted using TRI Reagent (Applied Biosystems, Foster City, United States) according to manufacturer's instructions. The RNA yield was determined using a UV spectrophotometer, and then stored at  $-80^{\circ}\text{C}$  for further processing.

### *Reverse transcription reaction*

Total RNA samples were reverse-transcribed using Taqman MicroRNA reverse transcription kit in combination with Megaplex reverse transcription (RT) primer Human pool set v3.0 (Applied Biosystems, Foster City, United States). Briefly, 3  $\mu$ L of total RNA was supplemented with Megaplex RT primer mix ( $\times 10$ ), RT buffer ( $\times 10$ ), Multiscribe reverse transcriptase (50 U/ $\mu$ L), dNTPs with dTTP (100 mmol/L),  $\text{MgCl}_2$  (25 mmol/L), and RNase inhibitor (20 U/ $\mu$ L) in a total reaction volume of 8  $\mu$ L. RT reaction was performed for 40 cycles of  $16^{\circ}\text{C}$  for 2 min,  $42^{\circ}\text{C}$  for 1 min and  $50^{\circ}\text{C}$  for 1 s, followed by a final reverse transcriptase inactivation at  $85^{\circ}\text{C}$  for 5 min. cDNA samples were kept at  $-80^{\circ}\text{C}$  until PCR analysis.

### *Pre-amplification of cDNA*

2.5  $\mu$ L of cDNA samples was pre-amplified using Applied Biosystems' Taqman preamp master mix ( $\times 2$ ) and Megaplex preamp primers ( $\times 5$ ) in a 25  $\mu$ L PCR. Megaplex™ PreAmp Primers (Applied Biosystems, Foster City, United States) that contained forward primers specific to miR-382 were used. The pre-amplification cycling conditions were as follows:  $95^{\circ}\text{C}$  for 10 min,  $55^{\circ}\text{C}$  for 2 min and  $75^{\circ}\text{C}$  for 2 min, followed by 12 cycles of  $95^{\circ}\text{C}$  for 15 s and  $60^{\circ}\text{C}$  for 4 min.

### *Real-time quantitative polymerase chain reaction analysis*

qPCR was performed through 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, United States). Pre-amplified cDNA samples were diluted with low-EDTA (0.1 mmol/L) TE buffer (1:50) and qPCR reaction included TaqMan® 2X Universal PCR Master Mix (No AmpErase® UNG) 10  $\mu$ L, TaqMan® MicroRNA Assays, 20X TaqMan® Assay 1  $\mu$ L, PreAmp Product 1  $\mu$ L and nuclease free water 8  $\mu$ L. qPCR cycling conditions were as follows:  $95^{\circ}\text{C}$  for 10 min, followed by 40 cycles of  $97^{\circ}\text{C}$  for 30 s and  $59.7^{\circ}\text{C}$  for 1 min. The small rRNA U6 (Assay ID: 001093) was used as an endogenous control in RT-qPCR. We run PCR in triplicate for each sample and all samples' Ct values are less 30, which is in a reasonable range. The relative quantitative method was used. Gene expression was quantitated based on the following formula:  $F = 2^{-\Delta\Delta\text{Ct}}$  where  $\Delta\Delta\text{Ct} = (\text{mean Ct of has-mir-382 in the test sample} - \text{mean Ct of the housekeeping gene in the test sample}) - (\text{mean Ct of has-mir-382 in the control sample} - \text{mean Ct of the housekeeping gene in the control sample})$ .

**Table 1** Characteristics of patients with esophageal squamous cell carcinoma

Characteristic	n (%)
All patients	46 (100)
Sex	
Male	23 (50.0)
Female	23 (50.0)
Age	
< 60	25 (54.3)
≥ 60	21 (45.7)
Size of tumor	
≤ 5 cm	27 (58.7)
> 5 cm	19 (41.3)
Site of tumor	
Upper thoracic	4 (8.7)
Middle thoracic	35 (76.1)
Lower thoracic	7 (15.2)
Differentiation	
Good	11 (23.9)
Moderate	19 (41.3)
Poor	16 (34.8)
Depth of invasion	
Tis, T1	8 (17.4)
T2	6 (13.0)
T3	32 (69.6)
Stage (TNM)	
0- I	7 (15.2)
II a- II b	21 (45.7)
III	18 (39.1)
Lymph node metastasis	
Negative	27 (58.7)
Positive	19 (41.3)
Metastasis	
Early metastasis	28 (60.9)
Non metastasis	18 (39.1)

Ct of the housekeeping gene in the control sample). A high *F*-value indicates a relatively high expression of miR-382.

### Statistical analysis

Two-tailed student *t* test was used to determine the expression levels of miR-382, and results were expressed as mean ± SE. *P* < 0.05 was considered statistically significant. The  $\chi^2$  test was used to determine the relationship between miR-382 expression level and clinicopathological features, and Kaplan-Meier was used to analyze the relationship between miRNA expression and survival time. COX regression model was used to analyze the influence of the related factor on the survival time of patients with ESCC. SPSS 17.0 software (SPSS Inc., Chicago, United States) was used for data analysis.

## RESULTS

### Demographic and clinicopathological characteristics

The study included 46 patients with ESCC. The patients were followed up for 3 to 84 mo, and were subsequently divided into two groups - good outcome and poor outcome. The group with good outcome

included 18 (39.1%) patients who showed neither clinical metastasis nor recurrence, and survived 5 years or more after surgery; the group with poor outcome included 28 (60.9%) patients who showed early metastasis but no recurrence, and died within 1 year after surgery.

Demographic variables of the patients are listed in Table 1. In this study, there were 23 (50%) males and 23 (50%) females with ages ranging from 45 to 71 years (median, 59 years). The tumor size in 19 (41.3%) cases was more than 5 cm and in 27 (58.7%) cases was less than 5 cm. Regarding the histological differentiation, 11 (23.9%) patients had well differentiated ESCC, 19 (41.3%) patients had moderately differentiated ESCC, and 16 (34.8%) had poorly differentiated ESCC. Lymph node metastasis occurred in 19 (41.3%) patients, and did not occur in 27 (58.7%) cases. 7 (15.2%) cases were at stage 0 and I (1 case at stage 0 and 6 cases at stage I), 21 (45.7%) cases were at stage II (II a and II b), and 18 (39.1%) cases were at stage III. This study did not include the patients at stage IV and the patients living between 1-5 years.

### Differential expression of miR-382 in ESCC patients and their significance in prognosis

RT-qPCR was performed to examine the expression level of miR-382. qPCR amplification plot with  $\Delta$ Ct values of miR-382 is shown as Figure 1. All Ct values from each sample were less than 30 (data not shown). miR-382 levels shown in Figure 2 were differentially expressed in ESCC patients. Overall the miR-382 level (Figure 2A) in the patients with good outcome was  $9.8 \pm 3.8$  (mean ± SE), while miR-382 level in the patients with poor outcome was  $3.0 \pm 0.8$  (mean ± SE). Therefore, miR-382 average level from all ESCC patients with poor outcome was lower than that from all ESCC patients with good outcome. The differences of miR-382 levels between two groups were significant (*P* < 0.05). Furthermore, in each TNM stage shown in Figure 2B-D, miR-382 levels were decreased in the patients with poor outcome when compared with the patients with good outcome.

### miR-382 expression was associated with ESCC patient survival time

Kaplan-Meier analysis results showed that miR-382 expression level generally had a significant reverse-correlation with ESCC patient survival time (Figure 3A, *P* < 0.001). Similarly, at individual stage as showed in Figure 3B-D, expression levels of miR-382 were reversely correlated with patient postoperative survival time. Cox single factor related risk analysis results showed that patient TNM stage (*P* = 0.023), tumor size (*P* = 0.006), postoperative time (*P* = 0.010), miR-382 level (*P* = 0.007), and patient survival time had a significant correlation.



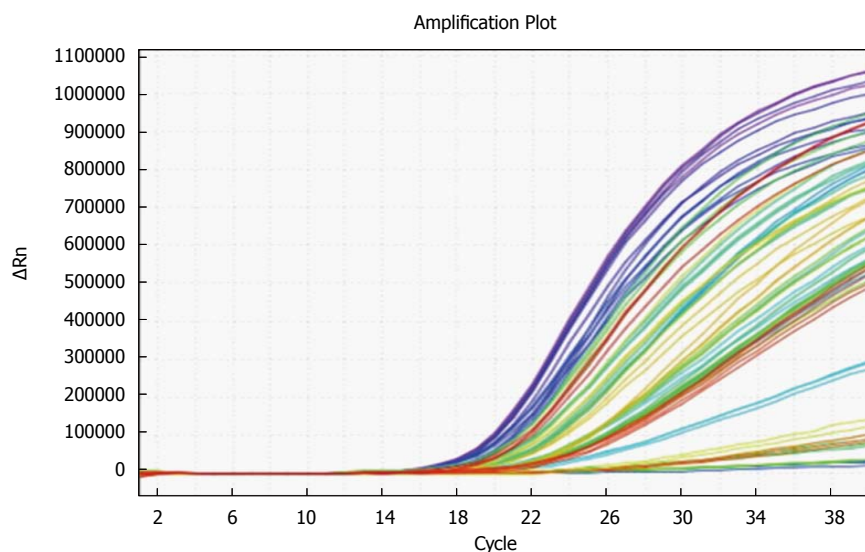


Figure 1 Amplification plot with  $\Delta C_t$  values of miR-382 in all patients.

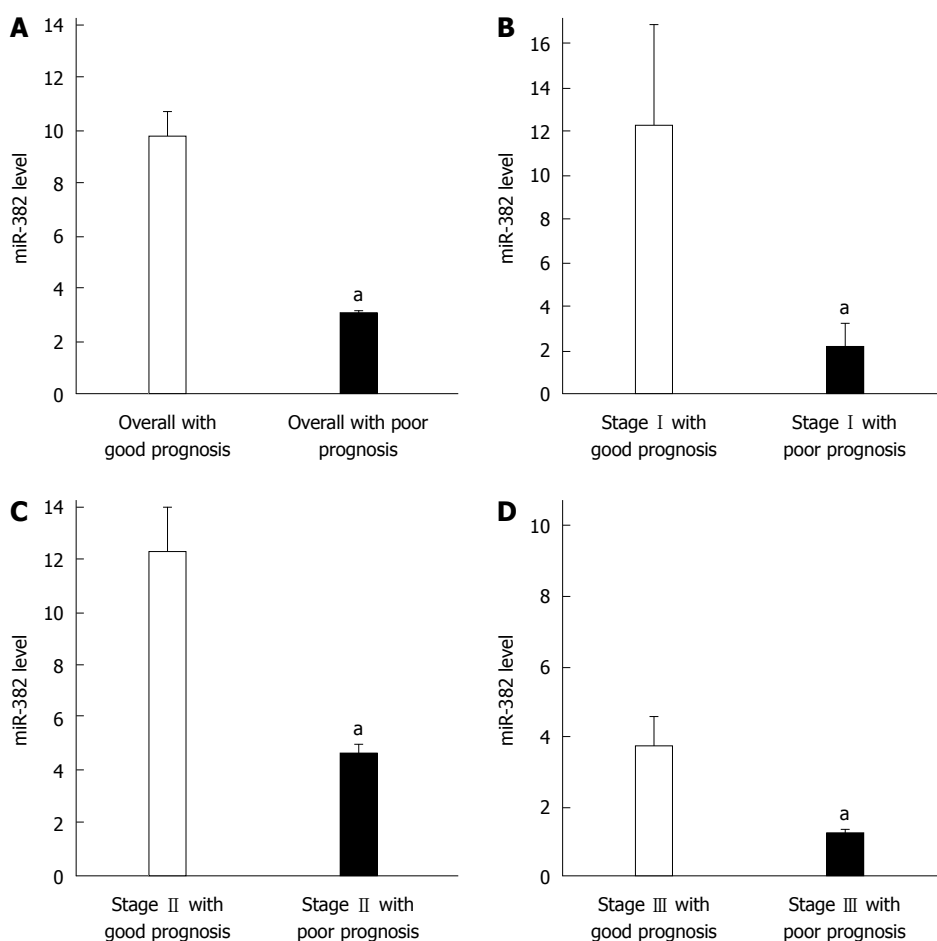
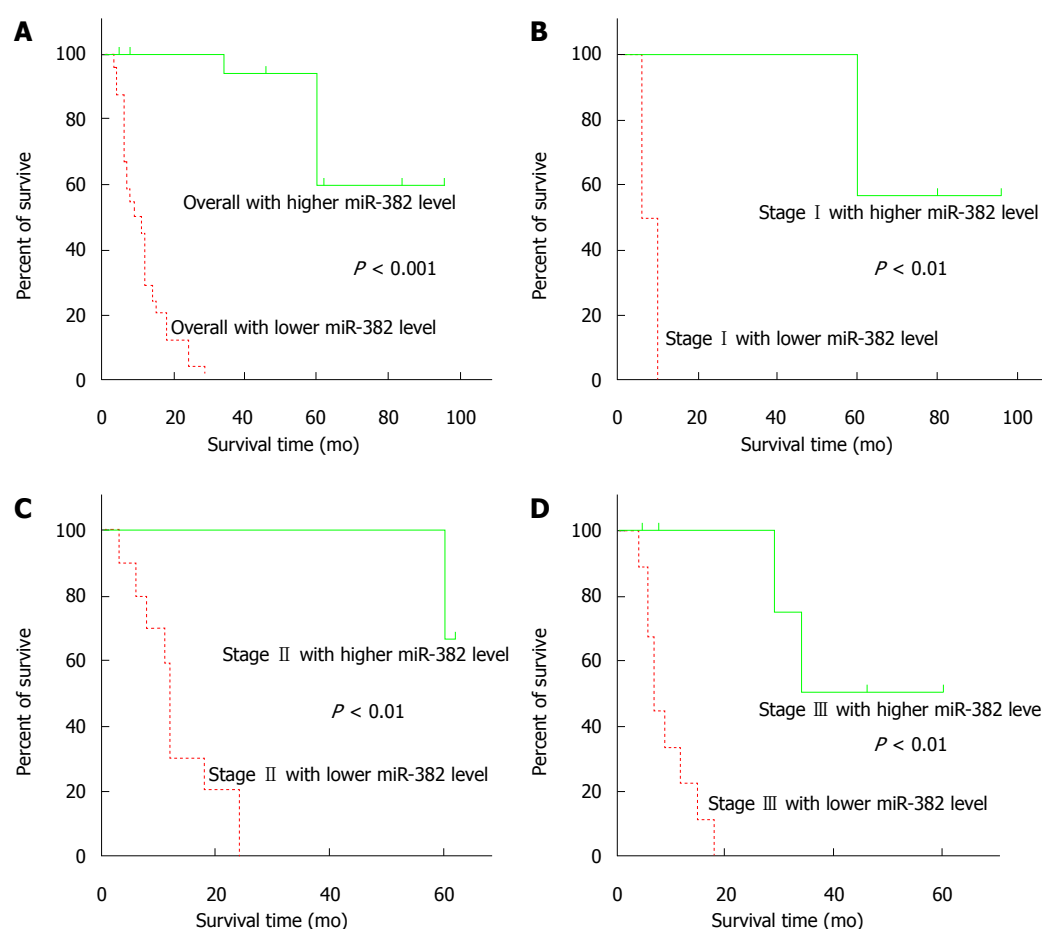


Figure 2 miR-382 expression levels were associated with outcomes of esophageal squamous cell carcinoma patients. A: The esophageal squamous cell carcinoma (ESCC) patients with good outcome generally exhibited higher miR-382 expression than those with poor outcome ( $^aP < 0.05$ , good outcome vs poor outcome); B: Stage I ESCC patients with good outcome exhibited higher levels of miR-382 than those with poor prognosis ( $^aP < 0.05$ , good outcome vs poor outcome); C: Stage II ESCC with good outcome exhibited higher levels of miR-382 than those with poor outcome ( $^aP < 0.05$ , good outcome vs poor outcome); D: Stage III ESCC with good outcome exhibited higher levels of miR-382 than those with poor outcome ( $^aP < 0.05$ , good outcome vs poor outcome).



**Figure 3** miR-382 expression levels were reverse-correlated with esophageal squamous cell carcinoma patient survival times. A: The esophageal squamous cell carcinoma (ESCC) with higher miR-382 expression generally exhibited longer survival times, compared to those with lower expression levels ( $P < 0.001$ , higher vs lower miR-382 level); B: Stage I ESCC patients with higher levels of miR-382 exhibited longer survival times, compared to those with lower expression levels ( $P < 0.01$ , higher vs lower miR-382 level); C: Stage II ESCC patients with higher levels of miR-382 exhibited longer survival times, compared to those with lower expression levels ( $P < 0.01$ , higher vs lower miR-382 level); D: Stage III ESCC patients with higher levels of miR-382 exhibited longer survival times, compared to those with lower expression levels ( $P < 0.01$ , higher vs lower miR-382 level).

## DISCUSSION

In the current study, we investigated the prognostic impact of miR-382 level in 46 patients with ESCC. We discovered that miR-382 was differentially expressed in cancer specimens from ESCC patients with different outcomes, although the patients were at the same pathological stage and received similar surgical treatment. We showed that miR-382 expression was significantly decreased in specimens from ESCC patients with poor outcomes compared to the patients with good outcomes. Moreover, our clinical results revealed that miR-382 level was inversely correlated with ESCC patient survival time, and significantly linked to the patients' outcomes. These data suggest a potential role for miR-382 as a biomarker for identifying patients who will experience an unfavorable clinical outcome.

ESCC is a common malignancy<sup>[2]</sup>. Accurate judgments of the clinical stage and prognosis of ESCC are important bases for clinicians to take a rational approach to the treatment of this disease. Although

TNM staging is traditionally considered the single most important factor to guide treatment decisions and prognosis for ESCC, clinical observations showed that same TNM stage ESCC patients who received the same surgical treatment by the same surgeon had different outcomes, indicating that ESCC metastasis susceptibility is somehow individually specific. Other traditional examinations based on medical equipment such as CT, X-ray barium meal fluoroscopy, gastroscopy, and B-ultrasonography, can be utilized to discover cancer metastases, whereas all these medical examinations are unable to predict distinct metastasis susceptibility. New research methods in molecular techniques have facilitated the biomarker discoveries of cancer metastasis susceptibility<sup>[16]</sup>. The current clinical biomarkers for ESCC prognosis are not ideal, as there remains a lack of reliable biomarkers that can specifically distinguish between ESCC patients who are susceptible to metastasis and those who are not.

Numerous studies have established miRNAs as broad and powerful regulators of protein expression in physiology and diseases<sup>[17]</sup>. Correlation analyses

between miRNAs and cancers have shown miRNA as potential prognostic biomarkers in variant cancers<sup>[18-20]</sup>. miRNAs that are upregulated in cancers are proposed to be oncogenes, whereas those that are downregulated are considered tumor suppressors<sup>[21]</sup>. Some miRNAs, such as has-miR-335, has-miR-181d, has-miR-25, has-miR-7, and has-miR-495, have been reported to directly participate in the initiation and development of ESCC<sup>[22-24]</sup>.

The relationship between miR-382 levels and prognoses has been examined in several types of human malignancy. Decreased miR-382 levels were associated with poor survival in osteosarcoma patients<sup>[14]</sup>, indicating that the role of miR-382 in osteosarcoma as a tumor suppressor. Conversely increased miR-382 levels were found in acute myeloid leukemia tumor tissue<sup>[15]</sup>, indicating that miR-382 plays the role of an oncogene in this disease. Consequently the role of miR-382 in tumor development and metastasis is a heterogeneous one for which the physical, cellular and molecular determinants adapt and react throughout the progression of the disease in a cell-driven and tissue-driven manner.

Our study revealed that the average level of miR-382 was significantly lower in specimens from ESCC patients who showed a poor outcome than those in the patients who showed a good outcome, and miR-382 level was reverse-correlated with patient's survival time and linked to a poor outcome. Metastasis had occurred in all ESCC patients with poor outcome but not in the patients with good outcome in our study. Thus, our results indicate that miR-382 is involved in the ESCC metastasis process and is a potential biomarker for ESCC patients who are individual susceptible to metastasis. This could at least partially explain why the ESCC patients at similar clinicopathological stages and receiving similar surgical treatment had completely different outcomes.

The mechanism by which miR-382 affects ESCC behavior is not yet clear. One study reported that miR-382 as a tumor suppressor in osteosarcoma negatively regulated the expression of KLF12 and HIPK3 by directly targeting their 3' UTR sequences to inhibit tumor cell growth *in vivo* and *in vitro*<sup>[25]</sup>. The downstream signals of miRNA contributing to tumor development and metastasis are tissue heterogeneous. Thus our future work will be aimed at determining the specific downstream molecules by which miR-382 affects ESCC behavior<sup>[26]</sup>.

The specimens we used in this study were formalin-fixed and paraffin-embedded esophageal cancer tissues. The expression level of miR-382 in the cancer tissue is dependent on the individual ESCC patient situation rather than the specimen type used for examination. Hence, our results should be applicable to other specimen types such as fresh surgical specimen and forceps biopsies obtained during upper gastrointestinal endoscopy.

In this study, the level of miR-382 we mentioned

just means the relative quantification when comparing the results from two groups. The total number of patients assessed in our study was relatively small, especially for the number of individual pathological stage patients. Accordingly, it is difficult to determine the cutoff level of miR-382 as a biomarker for clinical utility from our current results. A large size cohort study must therefore be an objective of future projects to determine the miR-382 cutoff level, which can be used not only for predicting ESCC outcomes but also for being a supplemental criterion to TNM staging or postoperative treatment decision.

In conclusion, it is the first study to show that miR-382 was downregulated in ESCC patients with early metastasis, and that miR-382 levels were significantly reverse-correlated with ESCC patient outcomes. Therefore, miR-382 could be a potential predictive biomarker for both outcome prognosis and treatment of ESCC. Further studies are needed to define the detailed mechanisms and determine the cutoff level of miR-382.

## COMMENTS

### Background

Different outcomes often happen to esophageal squamous cell carcinoma (ESCC) patients with the same pathological stage and receiving similar therapy due to early metastasis happening or not. It is important to explore reliable biomarkers that can specifically distinguish between ESCC patients who are susceptible to metastasis and those who are not. microRNAs (miRNAs) as broad and powerful regulators of protein expression are potential prognostic biomarkers of cancers. microRNA-382 (miR-382) has been shown to relate with tumor development and metastasis in variant cancers.

### Research frontiers

It was reported that miR-382 was reduced and associated with poor survival in osteosarcoma patients. Conversely increased miR-382 levels were found in acute myeloid leukemia tumor tissue. Consequently the role of miR-382 in tumor development and metastasis is a heterogeneous one in a cell-driven and tissue-driven manner.

### Innovations and breakthroughs

This study revealed that miR-382 was downregulated in ESCC patients with early metastasis comparing with the patients without metastasis. This is the first study to show that miR-382 levels were significantly reverse-correlated with ESCC patient outcomes. Therefore, the results indicate that miR-382 is involved in ESCC metastasis process and is a potential biomarker for ESCC patients who are individual susceptible to metastasis.

### Applications

By understanding the differential expression of miR-382 in the specimen of ESCC patient with or without early metastasis, and its relationship with clinicopathological characteristics and patient outcomes, this study may provide a future strategy in the development of a novel biomarker for both metastasis predictor and treatment of ESCC.

### Terminology

Metastasis is a complex process that involves the spread of a tumor or cancer from its original site to other places in the body. miRNA is a small non-coding RNA with about 22 nucleotides in length, and controls gene expression via the regulation of translation efficiency and mRNA stability. miRNAs have been shown as potential prognostic biomarkers in variant cancers. miR-382 is one of these, the role of miR-382 to tumor development and metastasis is tissue specific.

### Peer-review

This is an interested topic aiming to advance management for this difficult disease. The authors first provide the evidence of the divergent expression of microRNA-382 in tumor tissues from ESCC patients with different outcomes,

which indicate that it is associated with prognosis and may develop a novel biomarker for both diagnosis and treatment of ESCC.

## REFERENCES

- 1 Sun X, Chen W, Chen Z, Wen D, Zhao D, He Y. Population-based case-control study on risk factors for esophageal cancer in five high-risk areas in China. *Asian Pac J Cancer Prev* 2010; **11**: 1631-1636 [PMID: 21338208]
- 2 Mao WM, Zheng WH, Ling ZQ. Epidemiologic risk factors for esophageal cancer development. *Asian Pac J Cancer Prev* 2011; **12**: 2461-2466 [PMID: 22320939]
- 3 Siegel R, Naishadham D, Jemal A. Cancer statistics for Hispanics/Latinos, 2012. *CA Cancer J Clin* 2012; **62**: 283-298 [PMID: 22987332 DOI: 10.3322/caac.21153]
- 4 Zhu ZJ, Hu Y, Zhao YF, Chen XZ, Chen LQ, Chen YT. Early recurrence and death after esophagectomy in patients with esophageal squamous cell carcinoma. *Ann Thorac Surg* 2011; **91**: 1502-1508 [PMID: 21354552 DOI: 10.1016/j.athoracsur.2011.01.007]
- 5 Griffin SM, Burt AD, Jennings NA. Lymph node metastasis in early esophageal adenocarcinoma. *Ann Surg* 2011; **254**: 731-76; discussion 731-76; [PMID: 21997815 DOI: 10.1097/SLA.0b013e318236048b]
- 6 Zamore PD, Haley B. Ribo-gnome: the big world of small RNAs. *Science* 2005; **309**: 1519-1524 [PMID: 16141061]
- 7 Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. microRNAs in cancer management. *Lancet Oncol* 2012; **13**: e249-e258 [PMID: 22652233 DOI: 10.1016/S1470-2045(12)70073-6]
- 8 Bushati N, Cohen SM. microRNA functions. *Annu Rev Cell Dev Biol* 2007; **23**: 175-205 [PMID: 17506695]
- 9 Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002; **99**: 15524-15529 [PMID: 12434020]
- 10 Zhou ZQ, Cao WH, Xie JJ, Lin J, Shen ZY, Zhang QY, Shen JH, Xu LY, Li EM. Expression and prognostic significance of THBS1, Cyr61 and CTGF in esophageal squamous cell carcinoma. *BMC Cancer* 2009; **9**: 291 [PMID: 19698122 DOI: 10.1186/1471-2407-9-291]
- 11 He B, Pan Y, Cho WC, Xu Y, Gu L, Nie Z, Chen L, Song G, Gao T, Li R, Wang S. The association between four genetic variants in microRNAs (rs11614913, rs2910164, rs3746444, rs2292832) and cancer risk: evidence from published studies. *PLoS One* 2012; **7**: e49032 [PMID: 23155448 DOI: 10.1371/journal.pone.0049032]
- 12 Yang M, Liu R, Sheng J, Liao J, Wang Y, Pan E, Guo W, Pu Y, Yin L. Differential expression profiles of microRNAs as potential biomarkers for the early diagnosis of esophageal squamous cell carcinoma. *Oncol Rep* 2013; **29**: 169-176 [PMID: 23124769 DOI: 10.3892/or.2012.2105]
- 13 Sarver AL, Thayanithy V, Scott MC, Cleton-Jansen AM, Hogendoorn PC, Modiano JF, Subramanian S. MicroRNAs at the human 14q32 locus have prognostic significance in osteosarcoma. *Orphanet J Rare Dis* 2013; **8**: 7 [PMID: 23311495 DOI: 10.1186/1750-1172-8-7]
- 14 Li Z, Lu J, Sun M, Mi S, Zhang H, Luo RT, Chen P, Wang Y, Yan M, Qian Z, Neilly MB, Jin J, Zhang Y, Bohlander SK, Zhang DE, Larson RA, Le Beau MM, Thirman MJ, Golub TR, Rowley JD, Chen J. Distinct microRNA expression profiles in acute myeloid leukemia with common translocations. *Proc Natl Acad Sci USA* 2008; **105**: 15535-15540 [PMID: 18832181 DOI: 10.1073/pnas.0808266105]
- 15 Zhao BS, Liu SG, Wang TY, Ji YH, Qi B, Tao YP, Li HC, Wu XN. Screening of microRNA in patients with esophageal cancer at same tumor node metastasis stage with different prognoses. *Asian Pac J Cancer Prev* 2013; **14**: 139-143 [PMID: 23534712]
- 16 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438]
- 17 Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. *Curr Genomics* 2010; **11**: 537-561 [PMID: 21532838 DOI: 10.2174/138920210793175895]
- 18 Gomes AQ, Nolasco S, Soares H. Non-coding RNAs: multi-tasking molecules in the cell. *Int J Mol Sci* 2013; **14**: 16010-16039 [PMID: 23912238 DOI: 10.3390/ijms140816010]
- 19 Liang M. MicroRNA: a new entrance to the broad paradigm of systems molecular medicine. *Physiol Genomics* 2009; **38**: 113-115 [PMID: 19470802 DOI: 10.1152/physiolgenomics.00080.2009]
- 20 Lin RJ, Xiao DW, Liao LD, Chen T, Xie ZF, Huang WZ, Wang WS, Jiang TF, Wu BL, Li EM, Xu LY. MiR-142-3p as a potential prognostic biomarker for esophageal squamous cell carcinoma. *J Surg Oncol* 2012; **105**: 175-182 [PMID: 21882196 DOI: 10.1002/jso.22066]
- 21 Guo Y, Chen Z, Zhang L, Zhou F, Shi S, Feng X, Li B, Meng X, Ma X, Luo M, Shao K, Li N, Qiu B, Mitchelson K, Cheng J, He J. Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma. *Cancer Res* 2008; **68**: 26-33 [PMID: 18172293 DOI: 10.1158/0008-5472.CAN-06-4418]
- 22 Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Dev Biol* 2007; **302**: 1-12 [PMID: 16989803]
- 23 Mathé EA, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, Braun R, Reimers M, Kumamoto K, Hughes D, Altorki NK, Casson AG, Liu CG, Wang XW, Yanaiharu N, Hagiwara N, Dannenberg AJ, Miyashita M, Croce CM, Harris CC. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clin Cancer Res* 2009; **15**: 6192-6200 [PMID: 19789312 DOI: 10.1158/1078-0432]
- 24 Feber A, Xi L, Luketich JD, Pennathur A, Landreneau RJ, Wu M, Swanson SJ, Godfrey TE, Litle VR. MicroRNA expression profiles of esophageal cancer. *J Thorac Cardiovasc Surg* 2008; **135**: 255-60; discussion 260 [PMID: 18242245 DOI: 10.1016/j.jtcvs.2007.08.055]
- 25 Kong KL, Kwong DL, Chan TH, Law SY, Chen L, Li Y, Qin YR, Guan XY. MicroRNA-375 inhibits tumour growth and metastasis in oesophageal squamous cell carcinoma through repressing insulin-like growth factor 1 receptor. *Gut* 2012; **61**: 33-42 [PMID: 21813472 DOI: 10.1136/gutjnl-2011-300178]
- 26 Xu M, Jin H, Xu CX, Sun B, Mao Z, Bi WZ, Wang Y. miR-382 inhibits tumor growth and enhance chemosensitivity in osteosarcoma. *Oncotarget* 2014; **5**: 9472-9483 [PMID: 25344865]

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## Case Control Study

# Nonalcoholic fatty liver disease, spleen and psoriasis: New aspects of low-grade chronic inflammation

Nicola Balato, Maddalena Napolitano, Fabio Ayala, Cataldo Patruno, Matteo Megna, Giovanni Tarantino

Nicola Balato, Maddalena Napolitano, Fabio Ayala, Cataldo Patruno, Matteo Megna, Department of Dermatology, Federico II University of Naples, Naples 80131, Italy  
Giovanni Tarantino, Department of Clinical Medicine and Surgery, Medical School, Federico II University of Naples, Naples 80131, Italy  
Giovanni Tarantino, National Cancer Institute "Pascale Foundation," IRCS, Mercogliano (AV) 83013, Italy

**Author contributions:** Balato N, Tarantino G, and Ayala F designed the research; Tarantino G, Megna M, and Napolitano M performed the research; Tarantino G and Napolitano M analyzed the data; and Napolitano M, Patruno C, and Tarantino G wrote the paper.

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**Correspondence to:** Giovanni Tarantino, MD, Department of Clinical Medicine and Surgery, Medical School, Federico II University of Naples, 5 Via Sergio Pansini, Naples 80131, Italy. [giovanni.tarantino5@me.com](mailto:giovanni.tarantino5@me.com)  
Telephone: +39-81-7462024  
Fax: +39-81- 5466152

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## Abstract

**AIM:** To investigate spleen status in psoriasis and its

relationship with hepatic steatosis, Psoriasis Area and Severity Index, and insulin resistance.

**METHODS:** Seventy-nine psoriatic patients who were not suffering from any chronic inflammatory disease were retrospectively selected for inclusion in this study, and their complete medical records were accessed. An age- and sex-matched group of 80 non-psoriatic, obese patients was included as a control. The following relevant data were collected: age, sex, weight, height, body mass index, waist circumference, blood pressure, insulin resistance status, age at psoriasis onset, and severity of psoriasis. Abdominal ultrasonography was performed to determine spleen longitudinal diameter (SLD), and hepatic steatosis grade.

**RESULTS:** The SLD of control obese patients was greater than that of psoriatic subjects ( $P = 0.013$ ), but body mass index predicted the size of the spleen in psoriatic patients ( $P < 0.001$ ). The SLD of psoriatic patients with normal weight was significantly reduced with respect to the overweight/obese psoriatic patients ( $P = 0.002$ ). A multiple regression analysis revealed that body mass index was a unique predictor of the spleen size ( $P < 0.001$ ). Finally, the disease duration predicted the spleen size in psoriatic subjects ( $P = 0.038$ ).

**CONCLUSION:** This study shows a correlation between the SLD and the duration of psoriasis.

**Key words:** Hepatic steatosis; Inflammation; Nonalcoholic fatty liver disease; Psoriasis; Spleen size

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**Core tip:** The specific role of the spleen in psoriatics could help in more comprehensively understanding the inflammatory mechanism underlying this illness; psoriasis would be the most superficial manifestation of

a chronic inflammatory process involving various organs and systems. The increased diameter of the spleen found in psoriatic patients with long-term illness may be the expression of the immune system's response to the state of chronic inflammation.

Balato N, Napolitano M, Ayala F, Patruno C, Megna M, Tarantino G. Nonalcoholic fatty liver disease, spleen and psoriasis: New aspects of low-grade chronic inflammation. *World J Gastroenterol* 2015; 21(22): 6892-6897 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6892.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6892>

## INTRODUCTION

Psoriasis is a chronic, relapsing, inflammatory skin disease that affects about 2% of the Caucasian population, causing a significant impairment of quality of life, particularly if it is diffuse and recalcitrant to treatments<sup>[1-3]</sup>. Much attention has been drawn towards upgrading psoriasis from a skin condition to a systemic disease, as serum biomarkers for inflammation [interleukin (IL)-1 $\beta$ , IL-6, IL-10, C-reactive protein, intracellular adhesion molecule-1, E-selectin, and tumour necrosis factor- $\alpha$ ] are raised. Psoriatic patients could therefore have a higher risk of developing systemic comorbidities, including psoriatic arthritis, inflammatory bowel diseases (Crohn's disease and ulcerative colitis), or cardio-metabolic disorders (such as myocardial infarction hypertension, obesity, diabetes, dyslipidemia, fatty liver disease, and hyperuricemia)<sup>[4-7]</sup>. Recently, hospital-based observational studies suggested that patients with psoriasis are 1.5- to 3-fold more likely to have nonalcoholic fatty liver disease (NAFLD). This increased risk of NAFLD and subsequent risk of liver damage was explained by an increased prevalence of NAFLD risk factors such as obesity, diabetes mellitus, and alcohol consumption among patients with psoriasis<sup>[8]</sup>. Moreover, among the various pathways that contribute to the development of hepatic steatosis (HS), circulating concentrations of inflammatory cytokines are considered to be the most important factor in causing and maintaining insulin resistance (IR). Low-grade chronic inflammation, of which IL-6 is the main involved cytokine, is fundamental in the progression of NAFLD toward higher-risk cirrhotic states, *via* nonalcoholic steatohepatitis (NASH)<sup>[9]</sup>. NASH is a progressive liver disease characterized by Kupffer cell dysfunction, which contributes to its pathogenesis. Noteworthy, the reticular-endothelial system also plays a key role in the spleen. Indeed, Tsushima *et al.*<sup>[10]</sup> found an association between NAFLD and enlarged spleen volume measured by computed tomography. Moreover, as it is now known, obesity and IR are strongly associated with systemic markers of inflammation<sup>[11]</sup>. Studies attempting to find a noninvasive method that could likely assess the presence of NASH and using

histology as a gold standard to diagnose NAFLD, revealed that the NASH subjects had a higher spleen longitudinal diameter (SLD) and significantly higher IL-6 and vascular endothelial growth factor concentrations than healthy controls and patients with fatty liver<sup>[12]</sup>. The aim of this retrospective study was to establish if psoriatic patients presented a larger spleen volume, as an index of low-grade chronic inflammation, and to what extent HS was present. Further the relationships between Psoriasis Area and Severity Index (PASI), the most used score to evaluate the clinical severity, and anthropometric data, IR, grade of HS, and spleen volume were analyzed.

## MATERIALS AND METHODS

Seventy-nine consecutive psoriatic patients who attended the Dermatology Clinic of the University of Naples Federico II between July 2012 and October 2013, and who were not suffering from any chronic inflammatory diseases, viral or bacterial infections, or cancer were retrospectively included in this study, and their complete medical records were accessed. A similar group of 80 obese patients with body mass index (BMI) > 30 attending the outpatient obesity clinic, without psoriasis, and were well matched for age (max tolerance: three years of difference) and sex was compared as a control. The source population for cases and controls was the same.

Patients aged  $\geq 18$  years with moderate or severe plaque psoriasis were included in the study. Psoriasis was diagnosed according to clinical criteria. Severity was assessed according to PASI, body surface area (BSA) measurement, and static Physician's Global Assessment. Disease severity was classified as  $\geq 10$  according to the PASI. Disease severity was scored as moderate when PASI was 10-20 and severe when PASI score was > 20. Chronic plaque psoriasis was considered localized or disseminated when it covered less or more than 10% of the BSA, respectively. No patient suffering from psoriasis was on beta-blockers, lithium, or anti-malarials.

Patients receiving any systemic treatment for psoriasis including acitretin, ciclosporin, methotrexate, phototherapy, or biologics for  $\geq 6$  mo before enrollment were not included in the study. After signed informed consent was obtained, all subjects were visited by a dermatologist who registered demographic, biometric, and other relevant data on a case-report form. Relevant data collected included age, sex, weight, height, BMI, waist circumference, blood pressure, smoking habit, age at psoriasis onset, type and severity of psoriasis, concomitant medications, and abdominal US. Obesity was determined by measuring BMI and was corrected for abdominal adiposity. Measurements of height and body weight were taken by a trained research staff for each patient. Height was measured to the nearest 1 mm and weight was measured to the nearest 0.1 kg. The BMI was calculated as weight in

kg divided by height in  $m^2$  and was categorized into three groups: normal weight was defined as  $BMI < 25 \text{ kg/m}^2$ ; overweight was defined as  $25 \text{ kg/m}^2 < BMI < 29.99 \text{ kg/m}^2$ ; obesity was defined as  $BMI > 30 \text{ kg/m}^2$ . Visceral obesity was identified by measuring waist circumference at the midpoint between the lower border of the rib cage and the iliac crest. Hip circumference was measured around the widest part of the buttocks, with the tape parallel to the floor, and the waist to hip ratio was calculated. IR status was determined by the homeostatic metabolic assessment (HOMA), which was assessed by the formula: fasting insulin ( $\mu\text{U/mL}$ )  $\times$  fasting glucose ( $\text{mg/dL}$ )/405. Moreover, as the repeated HOMA measurements presented high within-person variability in obese patients, HOMA values were averaged on the basis of at least five determinations to avoid misclassification. Patients with psoriatic arthritis, inflammatory bowel diseases, rheumatoid arthritis, or other autoimmune disease such as lupus erythematosus and primary biliary cirrhosis were excluded.

### Ultrasound evaluation

Ultrasonographic measurements were performed using an Esaote system (Genoa, Italy). SLD as an index of low-grade chronic inflammation was chosen to evaluate spleen volume and was carried out by postero-lateral scanning<sup>[12]</sup>. Maximum length (the optically greatest overall longitudinal dimension obtained from one of the two poles) and cranio-caudal length (the optically maximal transversal dimension intercepting one of the two poles) were measured; the resulting values were then averaged, as the two measurements do not always coincide. The classification of HS (commonly defined as "bright liver") was based on the following scale of hyper-echogenicity at ultrasound (US): grade 0 = absent, grade 1 = light, grade 2 = moderate, grade 3 = severe, pointing out the difference between the densities of the liver and the right kidney<sup>[13]</sup>. Technically, echo intensity can be influenced by many factors, particularly by gain intensity. To avoid confounding factors that could modify echo intensity and thus bias comparisons, mean brightness levels of both liver and right kidney cortex were obtained on the same longitudinal sonographic plane.

### Statistical analysis

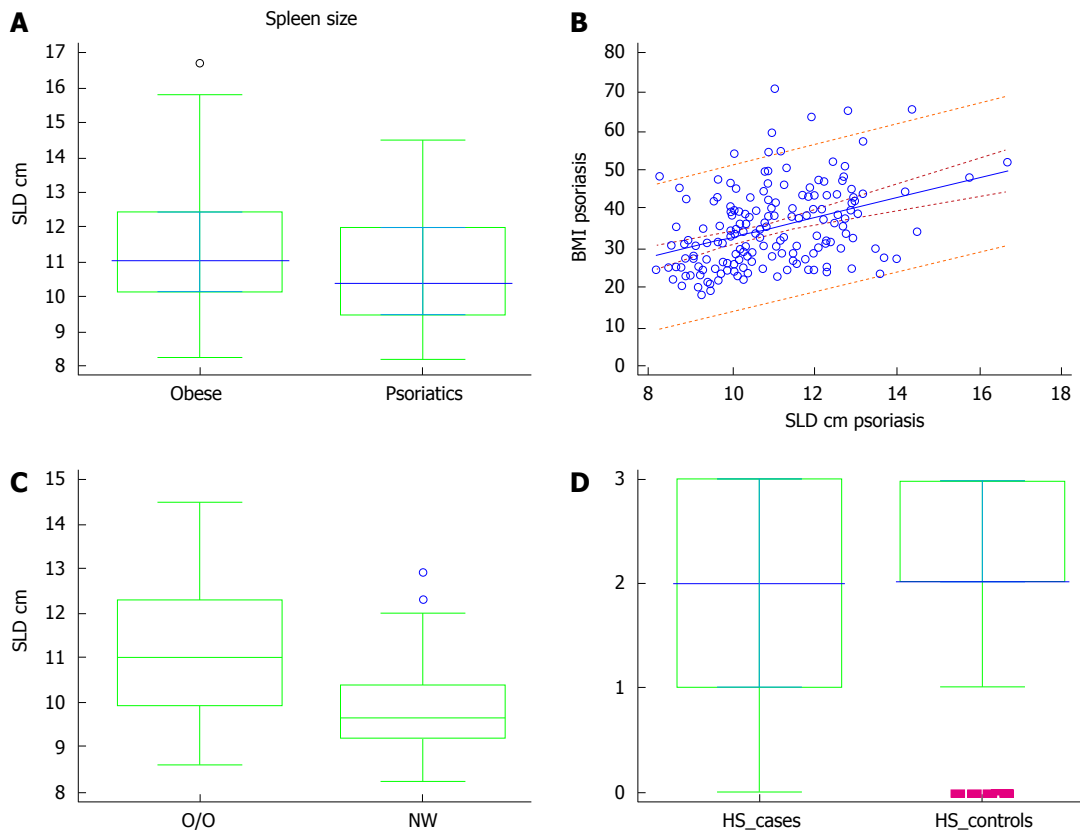
Alanine and aspartate aminotransferase level ratio, SLD, and HOMA were not normally distributed when analyzed by the Shapiro-Wilk (S-W) test ( $P < 0.05$ ), and were expressed as median (interquartile range). Age and PASI were derived from a normally distributed population, and were articulated as mean  $\pm$  SD. Grade of HS was an ordinal variable and analyzed by a nonparametric method. Appropriate tests for matched case-control studies included the paired  $t$  test or the nonparametric Wilcoxon test. McNemar's test for frequencies was used instead of the independent  $t$  and  $\chi^2$  tests because the former take into account the

dependent nature of the case and control subjects<sup>[14]</sup>. When dealing with subgroup analyses, *i.e.*, psoriatic patient with normal weight or overweight/obese, the Man-Whitney  $U$  test was used. For univariate analysis, to assess the independent effect of a quantitative variable on the prediction of another one, the linear regression analysis (least squares) was used, evaluating the coefficient with its standard error and the  $t$  (t-stat). A  $t > 1.96$  with a significance  $< 0.05$  indicates that the independent variable is a significant predictor of the dependent variable within and beyond the sample. To evaluate the association between spleen size and the grade of HS in psoriatics, the Spearman's coefficient of rank correlation ( $\rho$ ) was calculated. To establish what the best combination of independent variables would be to predict the dependent variable, a multiple regression (enter method) was adopted. BMI was used as dependent variable, while the independent ones were BMI, HOMA, and PASI. To avoid multi-collinearity, *i.e.*, situations in which the predictors are correlated with each other to some degree, the variance inflation factor and tolerance were set at  $> 10$  and  $< 0.1$ , respectively. Similarly, to get the sense of which variables contribute more or less to the regression equation, the magnitude of standardized coefficient beta ( $\beta$ ) was calculated.

## RESULTS

Seventy-nine patients with psoriasis (51 male and 28 female; age:  $46.55 \pm 15.56$  years, range: 18-76 years), and 80 age- and sex-matched controls (52 male and 28 female; age:  $44.3 \pm 12.87$  years, range: 19-73 years) participated in the study. No statistically significant difference was noted in age between the groups. The mean duration of psoriasis was 17.9 years (range: 1-63 years). PASI score ranged from 6.4 to 59.0 ( $14.75 \pm 12.78$ ), and 55/79 (69.62%) had moderate to severe psoriasis (PASI  $> 10$ ). BSA ranged from 2% to 85%, ( $15.23\% \pm 11.09\%$ ), while 43/79 (54.43%) patients had involved BSA  $> 10\%$ . SLD of obese patients was greater than that of psoriasis group [ $11.1$  ( $10.2$ - $12.4$ ) vs  $10.4$  ( $9.4$ - $11.9$ ),  $P = 0.013$ ] (Figure 1A). BMI predicted the size of the spleen, evaluated as SLD in psoriatic patients (coefficient: 2.56,  $t = 5.57$ ;  $P < 0.001$ ) (Figure 1B). The SLD of psoriatic patients with normal weight was significantly reduced when compared to the SLD of the overweight/obese psoriatic patients [ $9.6$  ( $9.2$ - $10.4$ ) vs  $11$  ( $9.9$ - $12.3$ ),  $P < 0.01$ ] (Figure 1C). There was no difference in frequency of HS presence in the two groups; 65/79 psoriatic patients and 73/80 obese subjects showed HS (McNemar test;  $P = 0.070$ ). The median grade of HS was significantly different between patients and obese controls [ $2.0$  ( $1.0$ - $3.0$ ) vs  $2.0$  ( $2.0$ - $3.0$ ),  $P = 0.006$ ] (Figure 1D).

Homa IR predicted the severity of psoriasis evaluated by the means of PASI (coefficient: 0.051,  $t = 2.35$ ;  $P = 0.020$ ) (Figure 2A). The grade of HS was predicted by PASI (coefficient 0.05,  $t = 2.9$ ;  $P = 0.005$ ) (Figure



**Figure 1** Spleen size, body mass index, and hepatic steatosis. A: Spleen longitudinal diameter (SLD) in the psoriatic patients and obese controls; B: Prediction of body mass index (BMI) on SLD of patients with psoriasis (dotted lines near the regression line are the 95% confidence curves, the far ones are the 95% prediction curves); C: SLD in overweight/obese psoriatic patients (O/O) and normal weight (NW) psoriatic patients; D: Hepatic steatosis (HS) grade in patients (cases) and controls: grade 0 = absent; 1 = mild; 2 = moderate; 3 = severe. Boxes in A, C and D indicate the interquartile ranges and the transverse lines represent the median.

2B). The spleen size strongly predicted the grade of HS (coefficient: 0.47,  $t = 3.6$ ;  $P < 0.001$ ) (Figure 2C). However, SLD did not predict PASI (coefficient: 0.03,  $t = 54$ ). A multiple regression analysis including the anthropometric and disease severity measures showed that BMI was the unique predictor of spleen size ( $\beta = 0.39$ ;  $P < 0.001$ ). Finally, when evaluating the impact of disease age in psoriatic patients, the duration of psoriasis (counted as years of disease) predicted the SLD (coefficient: 1.8,  $t = 2.11$ ;  $P = 0.038$ ) (Figure 2D). Interestingly, the values of transaminases of this population fell in the upper normal range: alanine transaminase, 40 (34-46) U/L; aspartate transaminase, 34 (30-43) U/L. Finally, the association between spleen size and the severity of HS was significant ( $\rho = 0.415$ ;  $P < 0.001$ ).

## DISCUSSION

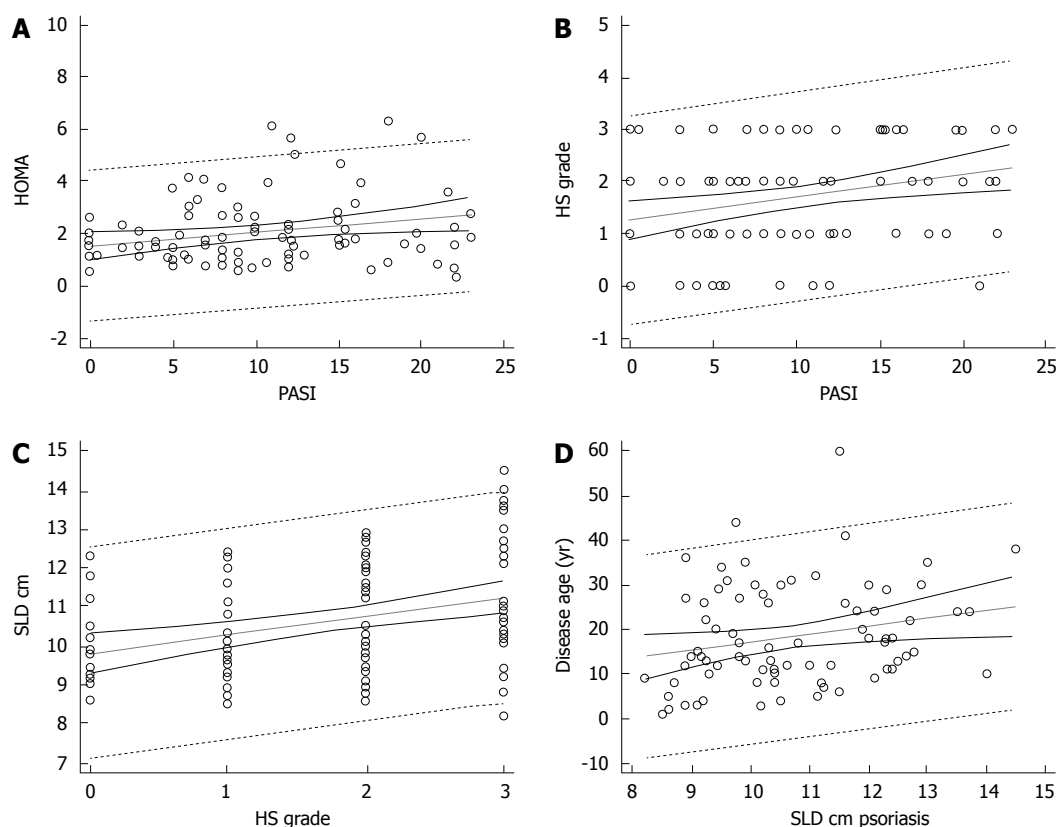
The key findings of this research can be summarized as follows. Firstly, robust links were found among HS, further expression of the metabolic syndrome, and PASI, as well as between IR and PASI. Secondly, a strict relationship between disease duration and spleen size was observed. However, few studies published during the last decade investigated the link between spleen and psoriasis. On the other hand, many studies

worldwide have shown that people with psoriasis have comorbidities such as diabetes, hypertension, and lipid abnormalities<sup>[15-17]</sup>.

With regard to possible mechanisms to explain these findings, we hypothesize that this association to the psoriatic march, the process by which inflammatory mediators released in the course of the psoriatic autoimmune reaction, causes IR, which is correlated to an increased prevalence of metabolic syndrome<sup>[18]</sup>. Earlier studies have shown that IR is common in patients with psoriasis and that the PASI is the major determinant of IR<sup>[19]</sup>. The data presented here confirm this association. These observations support the concept of synergistic effects from the chronic state of inflammation caused by obesity and the chronic systemic Th1 lymphocyte-mediated inflammation characteristic of psoriasis, which has recently been put forward by Hamminga *et al.*<sup>[20]</sup>. In the literature, there is no data about the importance of the spleen as an indicator of systemic inflammation in patients with psoriasis. Although the data reported here do not reveal a correlation between PASI and SLD, there is a correlation between the duration of the disease and the SLD.

The spleen is the largest lymphoid organ in the body and plays an important role in host immune function and blood filtration *via* the removal and destruction of aged or damaged erythrocytes and other blood





**Figure 2** Insulin resistance, hepatic steatosis, spleen size, severity of psoriasis, and disease age of psoriatic patients. A: Prediction of insulin resistance on severity of psoriasis; B: Prediction of hepatic steatosis (HS) on severity of psoriasis; C: Prediction of SLD on severity of psoriasis; D: Prediction of disease age of psoriatic patients on SLD. Dotted lines near the regression line are the 95% confidence curves, the far ones are the 95% prediction curves. HOMA: Homeostatic metabolic assessment; PASI: Psoriasis area and severity index; SLD: Spleen longitudinal diameter.

cells<sup>[21]</sup>. Splenic gene expression of proinflammatory cytokines, such as tumor necrosis factor- $\alpha$  and IL-6, is decreased in the setting of obesity<sup>[22]</sup>. In contrast, IL-10, which is synthesized within multiple organs, including the spleen, is a potent anti-inflammatory cytokine that inhibits the synthesis of proinflammatory cytokines. Large amounts of IL-10 are produced from activated B-cells that mature in the marginal zone of the spleen. Recent studies suggest that IL-10-producing B-cells play a regulatory role in suppressing harmful immune responses<sup>[23]</sup>. Gotoh *et al.*<sup>[24]</sup> have supported the hypothesis that obesity suppresses the splenic synthesis of the anti-inflammatory cytokine, IL-10, thereby resulting in chronic inflammation. IL-10 may contribute to disease susceptibility in psoriasis, and it has been reported that IL-10 deficiency is a feature of psoriasis<sup>[25]</sup>. The increased diameter of the spleen found in our psoriatic patients with long-term illness may be the expression of the immune system's response to the state of chronic inflammation. It should be emphasized that the increased spleen volume represents a clinical finding, not a comorbidity, such as obesity, linked to the low-grade chronic inflammatory status.

The results of this study show a clear link between psoriasis and HS as well as the spleen. The specific role of the spleen in psoriatics could help us to more comprehensively understand the inflammatory

mechanism underlying this illness: psoriasis would be the most superficial manifestation of a chronic inflammatory process involving various organs and systems. Evaluation of the SLD could help to ameliorate the approach of psoriasis, helping clinicians to identify psoriatic patients who need early attention *via* modification of their lifestyle and alimentary habits. However, further studies are needed to better understand the potential involvement of the spleen in psoriasis inflammatory context.

### Limitations

A limitation of the present study is the lack of liver biopsies to better define the HS, even though the US determination of moderate- to high-grade HS is quite reliable<sup>[26]</sup>. Furthermore, detection of the levels of serum inflammatory markers, clues of both psoriasis and NAFLD, would have strengthened the impact of these results. However, there is a large body of evidence that confirms the main role of C-reactive protein and IL-6 as main mechanisms of psoriasis and NAFLD, evidenced by high serum levels of this acute-phase reactant and cytokine, respectively<sup>[11,12]</sup>.

## COMMENTS

### Background

Psoriasis is a chronic, relapsing, inflammatory skin disease causing a

significant impairment of quality of life, particularly if it is diffuse and recalcitrant to treatments.

### Research frontiers

Much attention has been drawn towards upgrading psoriasis from a skin condition to a systemic disease, as serum biomarkers for inflammation [interleukin (IL)-1 $\beta$ , IL-6, IL-10, C-reactive protein, intracellular adhesion molecule-1, E-selectin, and tumor necrosis factor- $\alpha$ ] are raised.

### Innovations and breakthroughs

Summarizing the advances from other research, this study shows a clear link between psoriasis and the spleen, showing a correlation between the duration of the disease and spleen size.

### Applications

The actual application values of this research lie in a better comprehension of psoriasis mechanisms, especially those related to hepatic steatosis, strictly linked to spleen involvement.

### Peer-review

There are studies showing a higher prevalence of metabolic syndrome in patients with psoriasis. In this paper, the authors wish to say that spleen longitudinal diameter of obese patients is greater in psoriatic patient and normal in non-obese individuals. This is an interesting study reflecting the role of body mass index in psoriasis.

## REFERENCES

- 1 Langley RG, Krueger GG, Griffiths CE. Psoriasis: epidemiology, clinical features, and quality of life. *Ann Rheum Dis* 2005; **64** Suppl 2: ii18-ii23; discussion ii18-ii23; [PMID: 15708928 DOI: 10.1136/ard.2004.033217]
- 2 Kupetsky EA, Keller M. Psoriasis vulgaris: an evidence-based guide for primary care. *J Am Board Fam Med* 2012; **26**: 787-801 [PMID: 24204077 DOI: 10.3122/jabfm.2013.06.130055]
- 3 Patruno C, Ayala F, Megna M, Napolitano M, Balato N. Patient-physician relationship in patients with psoriasis. *Indian J Dermatol Venereol Leprol* 2013; **78**: 228 [PMID: 22421670 DOI: 10.4103/0378-6323.93657]
- 4 Dowlatshahi EA, van der Voort EA, Arends LR, Nijsten T. Markers of systemic inflammation in psoriasis: a systematic review and meta-analysis. *Br J Dermatol* 2013; **169**: 266-282 [PMID: 23550658 DOI: 10.1111/bjd.12355]
- 5 Balato A, Di Caprio R, Canta L, Mattii M, Lembo S, Raimondo A, Schiattarella M, Balato N, Ayala F. IL-33 is regulated by TNF- $\alpha$  in normal and psoriatic skin. *Arch Dermatol Res* 2014; **306**: 299-304 [PMID: 24522896 DOI: 10.1007/s00403-014-1447-9]
- 6 Ghazizadeh R, Shimizu H, Tosa M, Ghazizadeh M. Pathogenic mechanisms shared between psoriasis and cardiovascular disease. *Int J Med Sci* 2010; **7**: 284-289 [PMID: 20827428]
- 7 Davidovici BB, Sattar N, Prinz J, Puig L, Emery P, Barker JN, van de Kerkhof P, Stähle M, Nestle FO, Girolomoni G, Krueger JG. Psoriasis and systemic inflammatory diseases: potential mechanistic links between skin disease and co-morbid conditions. *J Invest Dermatol* 2010; **130**: 1785-1796 [PMID: 20445552 DOI: 10.1038/jid.2010.103]
- 8 van der Voort EA, Koehler EM, Dowlatshahi EA, Hofman A, Stricker BH, Janssen HL, Schouten JN, Nijsten T. Psoriasis is independently associated with nonalcoholic fatty liver disease in patients 55 years old or older: Results from a population-based study. *J Am Acad Dermatol* 2014; **70**: 517-524 [PMID: 24373781 DOI: 10.1016/j.jaad.2013.10.044]
- 9 Tarantino G, Savastano S, Colao A. Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance. *World J Gastroenterol* 2010; **16**: 4773-4783 [PMID: 20939105 DOI: 10.3748/wjg.v16.i38.4773]
- 10 Tsushima Y, Endo K. Spleen enlargement in patients with nonalcoholic fatty liver: correlation between degree of fatty infiltration in liver and size of spleen. *Dig Dis Sci* 2000; **45**: 196-200 [PMID: 10695635]
- 11 Tarantino G, Colicchio P, Conca P, Finelli C, Di Minno MN, Tarantino M, Capone D, Pasanisi F. Young adult obese subjects with and without insulin resistance: what is the role of chronic inflammation and how to weigh it non-invasively? *J Inflamm (Lond)* 2009; **6**: 6 [PMID: 19291292 DOI: 10.1186/1476-9255-6-6]
- 12 Tarantino G, Conca P, Pasanisi F, Ariello M, Mastrolia M, Arena A, Tarantino M, Scopacasa F, Vecchione R. Could inflammatory markers help diagnose nonalcoholic steatohepatitis? *Eur J Gastroenterol Hepatol* 2009; **21**: 504-511 [PMID: 19318968 DOI: 10.1097/MEG.0b013e32832829b40]
- 13 Webb M, Yeshua H, Zelber-Sagi S, Santo E, Brazowski E, Halpern Z, Oren R. Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. *AJR Am J Roentgenol* 2009; **192**: 909-914 [PMID: 19304694 DOI: 10.2214/AJR.07.4016]
- 14 Breslow NE, Day NE. Classical Methods of Analysis of Matched Data. In: Breslow NE, Day NE, editors. Statistical methods in cancer research. Volume 1-The analysis of case-control studies. Lyon, France: International Agency for Research on Cancer, 1980: 162-192
- 15 Christophers E. Comorbidities in psoriasis. *J Eur Acad Dermatol Venereol* 2006; **20**: 52-55
- 16 Balato N, Balato A, Gallo L, Napolitano M, Patruno C, Ayala F. Psoriasis and osteoporosis: data from a Southern Italian population. *Arch Osteoporos* 2012; **7**: 321-323 [PMID: 23203734]
- 17 Miller IM, Ellervik C, Yazdanyar S, Jemec GB. Meta-analysis of psoriasis, cardiovascular disease, and associated risk factors. *J Am Acad Dermatol* 2013; **69**: 1014-1024 [PMID: 24238156 DOI: 10.1016/j.jaad.2013.06.053]
- 18 Boehncke WH, Boehncke S, Schön MP. Managing comorbid disease in patients with psoriasis. *BMJ* 2010; **340**: b5666 [PMID: 20080817 DOI: 10.1136/bmj.b5666]
- 19 Savastano S, Balato N, Gaudiello F, Di Somma C, Brancato V, Colao A, Ayala F, Tarantino G. Insulin-like growth factor-I, psoriasis, and inflammation: a ménage à trois? *European J of Inflammation* 2011; **9**: 277-284
- 20 Hamminga EA, van der Lely AJ, Neumann HA, Thio HB. Chronic inflammation in psoriasis and obesity: implications for therapy. *Med Hypotheses* 2006; **67**: 768-773 [PMID: 16781085 DOI: 10.1016/j.mehy.2005.11.050]
- 21 Dameshek W. Hypersplenism. *Bull N Y Acad Med* 1955; **31**: 113-136 [PMID: 13230762]
- 22 Lamas O, Martínez JA, Martí A. Decreased splenic mRNA expression levels of TNF- $\alpha$  and IL-6 in diet-induced obese animals. *J Physiol Biochem* 2004; **60**: 279-283 [PMID: 15957247]
- 23 Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol* 2004; **22**: 929-979 [PMID: 15032600 DOI: 10.1146/annurev.immunol.22.012703.104622]
- 24 Gotoh K, Inoue M, Masaki T, Chiba S, Shimasaki T, Ando H, Fujiwara K, Katsuragi I, Kakuma T, Seike M, Sakata T, Yoshimatsu H. A novel anti-inflammatory role for spleen-derived interleukin-10 in obesity-induced inflammation in white adipose tissue and liver. *Diabetes* 2012; **61**: 1994-2003 [PMID: 22648387 DOI: 10.2337/db11-1688]
- 25 Asadullah K, Sterry W, Stephanek K, Jasulaitis D, Leupold M, Audring H, Volk HD, Döcke WD. IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach. *J Clin Invest* 1998; **101**: 783-794 [PMID: 9466973 DOI: 10.1172/JCI1476]
- 26 Palmentieri B, de Sio I, La Mura V, Masarone M, Vecchione R, Bruno S, Torella R, Persico M. The role of bright liver echo pattern on ultrasound B-mode examination in the diagnosis of liver steatosis. *Dig Liver Dis* 2006; **38**: 485-489 [PMID: 16716779]

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## Case Control Study

# Association of colorectal cancer susceptibility variants with esophageal cancer in a Chinese population

Ting-Ting Geng, Xiao-Jie Xun, Sen Li, Tian Feng, Li-Ping Wang, Tian-Bo Jin, Peng Hou

Ting-Ting Geng, Li-Ping Wang, Peng Hou, Department of Endocrinology, the First Affiliated Hospital of Xi'an Jiaotong University School of Medicine, Xi'an 710061, Shaanxi Province, China

Ting-Ting Geng, Xiao-Jie Xun, Tian Feng, Tian-Bo Jin, National Engineering Research Center for Miniaturized Detection Systems, Xi'an 710069, Shaanxi Province, China

Xiao-Jie Xun, Tian-Bo Jin, School of Life Sciences, Northwest University, Xi'an 710069, Shaanxi Province, China

Sen Li, School of Life Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZB, United Kingdom

**Author contributions:** Geng TT and Xun XJ contributed equally to this work; Geng TT, Xun XJ, Jin TB and Hou P designed the research; Geng TT, Xun XJ and Li S performed the research; Feng T and Wang LP contributed new reagents/analytic tools and analyzed the data; Xun XJ wrote the paper; all authors approved the final version for publication.

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Correspondence to: Peng Hou, PhD, Professor, Department

of Endocrinology, the First Affiliated Hospital of Xi'an Jiaotong University School of Medicine, The Wild Goose Pagoda West Road No. 277, Xi'an 710061, Shaanxi Province, China. penghou1@163.com  
Telephone: +86-29-88305769  
Fax: +86-29-88305769

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## Abstract

**AIM:** To investigate the association between colorectal cancer (CRC) genetic susceptibility variants and esophageal cancer in a Chinese Han population.

**METHODS:** A case-control study was conducted including 360 esophageal cancer patients and 310 healthy controls. Thirty-one single-nucleotide polymorphisms (SNPs) associated with CRC risk from previous genome-wide association studies were analyzed. SNPs were genotyped using Sequenom Mass-ARRAY technology, and genotypic frequencies in controls were tested for departure from Hardy-Weinberg equilibrium using a Fisher's exact test. The allelic frequencies were compared between cases and controls using a  $\chi^2$  test. Associations between the SNPs and the risk of esophageal cancer were tested using various genetic models (codominant, dominant, recessive, overdominant, and additive). ORs and 95% CIs were calculated by unconditional logistic regression with adjustments for age and sex.

**RESULTS:** The minor alleles of rs1321311 and rs4444235 were associated with a 1.53-fold (95%CI:

1.15-2.06;  $P = 0.004$ ) and 1.28-fold (95%CI: 1.03-1.60;  $P = 0.028$ ) increased risk of esophageal cancer in the allelic model analysis, respectively. In the genetic model analysis, the C/C genotype of rs3802842 was associated with a reduced risk of esophageal cancer in the codominant model (OR = 0.52, 95%CI: 0.31-0.88;  $P = 0.033$ ) and recessive model (OR = 0.55, 95%CI: 0.34-0.87;  $P = 0.010$ ). The rs4939827 C/T-T/T genotype was associated with a 0.67-fold (95%CI: 0.46-0.98;  $P = 0.038$ ) decreased esophageal cancer risk under the dominant model. In addition, rs6687758, rs1321311, and rs4444235 were associated with an increased risk. In particular, the T/T genotype of rs1321311 was associated with an 8.06-fold (95%CI: 1.96-33.07;  $P = 0.004$ ) increased risk in the codominant model.

**CONCLUSION:** These results provide evidence that known genetic variants associated with CRC risk confer risk for esophageal cancer, and may bring risk for other digestive system tumors.

**Key words:** Colorectal cancer; Esophageal cancer; Single-nucleotide polymorphism; Susceptibility

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**Core tip:** This case-control study investigates the association between colorectal cancer susceptibility variants (single-nucleotide polymorphisms) and esophageal cancer in a Chinese Han population. The minor alleles of rs1321311 and rs4444235 were associated with a 1.53-fold and 1.28-fold increased risk of esophageal cancer in allelic model analysis, respectively. In the genetic model analysis, rs3802842 and rs4939827 were associated with a decreased esophageal cancer risk, whereas rs6687758 was associated with an increased risk. These results provide evidence that known genetic variants associated with colorectal cancer risk may also confer risk for esophageal cancer.

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## INTRODUCTION

Esophageal cancer, classified as adenocarcinoma or squamous cell carcinoma, is one of the top ten most malignant and deadly cancers worldwide, for which China has among the highest rates of incidence and mortality<sup>[1]</sup>. Highly advanced cancers of the esophagus have poor prognostic outcomes<sup>[2,3]</sup>. Of the two forms

of esophageal cancer, squamous cell carcinoma is the most common, and prognosis highly correlates with disease stage and advancement.

Epidemiologic studies indicate that tobacco smoking, alcohol intake, nutritional deficiencies, and dietary carcinogen exposure contribute to the etiology of esophageal cancer<sup>[4,5]</sup>. However, only a small proportion of individuals exposed to these factors actually develop esophageal cancer, suggesting that genetic factors also play a vital role in susceptibility. It has been reported that susceptibility to esophageal cancer is not dependent on a single gene and is affected by population differences<sup>[6,7]</sup>.

Colorectal cancer (CRC) is the most common malignant tumor of the digestive tract, and the second most common of all gastrointestinal tumors<sup>[8]</sup>. Recent studies have identified haplotype-tagging single-nucleotide polymorphisms (SNPs) that are associated with an increased colorectal cancer risk in the general population<sup>[9-12]</sup>.

Previous genetic polymorphism studies in the Chinese population were focused solely on SNPs associated with esophageal cancer risk in genome-wide association studies (GWAS)<sup>[13-15]</sup>. The purpose of the present study was to identify digestive system tumor common susceptibility loci. To achieve this, 31 high-frequency SNPs associated with CRC risk in the Chinese population were evaluated with respect to esophageal cancer risk.

## MATERIALS AND METHODS

### Study participants

All participants were Chinese Han that were seen between January 2011 and February 2014 at the First Affiliated Hospital of the Medical College of Xi'an Jiaotong University. None of the study participants received neoadjuvant therapy or had previous histories of other cancers, chemotherapy, or radiotherapy. Participants were chosen without restrictions of age, sex, or disease stage. None of the healthy control subjects had any chronic or severe endocrine, metabolic or nutritional diseases. A total of 360 esophageal cancer cases and 310 controls were included in the study. Esophageal cancer was newly diagnosed according to the criteria established by the International Union Against Cancer tumor-node-metastasis classification system (7<sup>th</sup> ed)<sup>[16]</sup>.

### Clinical data and demographic information

We used a standard epidemiologic questionnaire and in-person interviews to collect personal data, including residential region, age, sex, education status, and family history of cancer. The case information was collected through consultation with treating physicians or from medical chart review. All of the participants signed an informed consent agreement. The Human Research Committee for Approval of Research Involving



**Table 1** Characteristics of cases and controls in this study  
*n* (%)

Variable	Cases ( <i>n</i> = 360)	Controls ( <i>n</i> = 310)	<i>P</i> value
Sex			< 0.001
Male	288 (62.0)	197 (36.5)	
Female	72 (20.0)	113 (36.5)	
Age, yr (mean ± SD)	60.7 ± 8.9	49.4 ± 7.9	< 0.001

Human Subjects, The First Affiliated Hospital of the Medical College of Xi'an Jiaotong University approved the use of human tissue in this study.

### Selection of SNPs and methods of genotyping

Thirty-one SNPs from 17 genes were chosen for analysis in this study. These SNPs were chosen from CRC GWAS<sup>[9-12]</sup>. Minor allele frequencies of all SNPs were > 5% in the HapMap of the Chinese Han Beijing population.

DNA was extracted from whole-blood samples using GoldMag-Mini Whole Blood Genomic DNA Purification Kits (GoldMag Co., Ltd., Hainan City, China), and quantified with a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, United States). The multiplexed SNP MassEXTENDED assay was designed using Sequenom MassARRAY Assay Design 3.0 Software<sup>[17]</sup> (Sequenom Inc., San Diego, CA, United States). Genotyping was performed with the MassARRAY RS1000 system (Sequenom) using the standard protocol recommended by the manufacturer. Data management and analysis were performed using Sequenom Typer 4.0 Software<sup>[17,18]</sup>.

### Statistical analysis

Data were analyzed using SPSS version 18.0 statistical software (SPSS Inc., Chicago, IL, United States) and Excel (Microsoft Corp., Redmond, WA, United States). The lower frequency alleles were coded as the minor allele. A Fisher's exact test was used to assess the variation in each SNP frequency from the Hardy-Weinberg equilibrium in the control subjects. Differences in SNP genotype distribution between cases and controls were compared by the  $\chi^2$  test. ORS<sup>[19]</sup> and 95% CIs were determined using unconditional logistic regression analysis with adjustments for age and sex. All two-sided *P* values < 0.05 were considered statistically significant.

Associations between SNPs and risk of esophageal cancer were tested in genetic models using SNP Stats software (<http://bioinfo.iconcologia.net>). For the additive model, individuals were assigned a 0, 1, or 2, representing the number of risk alleles they possessed for that SNP. For the dominant model, individuals were coded as 1 if they carried at least one risk allele and 0 otherwise; for the recessive model, individuals were coded as 1 if they were homozygous for the risk allele, and 0 otherwise. Akaike's Information Criterion

and Bayesian Information Criterion were applied to estimate the best-fit model for each SNP.

The statistical methods of this study were reviewed by Tianfeng from the National Engineering Research Center for Miniaturized Detection Systems.

## RESULTS

There were significant differences in age and sex distribution between the case and control groups (*P* < 0.01) (Table 1).

Table 2 summarizes the major allelic frequencies of the SNPs among the individuals in the case and control groups. Three SNPs (rs10774214, rs2423279, and rs4925386) were excluded for significant deviation from Hardy-Weinberg equilibrium (*P* < 0.05); the other SNPs in the control group were similar to those of the HapMap Asian population (<http://hapmap.ncbi.nlm.nih.gov/>). A  $\chi^2$  analysis revealed that rs1321311 and rs4444235 were significantly associated with a 1.53-fold and 1.28-fold increased esophageal cancer risk, respectively (*P* < 0.05 for both).

In the genetic model analyses, the minor T allele of rs1321311 was associated with an increased risk of esophageal cancer based on analysis using the codominant and recessive models (*P* < 0.01 for both; Table 3). The minor C allele of rs4444235 was also significantly associated with an increased cancer risk in codominant and dominant models (*P* < 0.05 for both). The G/G genotype of rs6687758 was associated with a 2.54-fold increased risk in the recessive model (*P* < 0.05). In contrast, the minor C allele of rs3802842 was associated with a 0.52-fold and 0.55-fold reduced risk of esophageal cancer as revealed by the codominant and recessive models, respectively (*P* < 0.05 for both). Additionally, the dominant model showed that the rs4939827 SNP was significantly associated with an 0.67-fold decreased esophageal cancer risk (*P* < 0.05).

## DISCUSSION

This study identifies three SNPs (rs1321311, rs4444235, and rs6687758) associated with an increased risk of esophageal cancer. The SNP rs1321311, located near *CDKN1A* at 6p21, has previously been associated with an increased risk of CRC<sup>[20,21]</sup>. This association was not strongly modified by sex, body mass index, alcohol, smoking, aspirin or various dietary factors<sup>[20]</sup>. The SNP rs4444235, which is located 9.4 kb upstream of the gene encoding bone morphogenetic protein 4 (*BMP4*), was previously associated with CRC and gastric cancer risk<sup>[22,23]</sup>. Although the CT genotype showed a protective effect against gastric cancer<sup>[23]</sup>, it was also associated with an increased CRC risk<sup>[24]</sup>. This SNP has been proposed to act as a cis-regulator of *BMP4* and thus confer a risk for CRC<sup>[25,26]</sup>. Based on the results of the present study, the CT genotype is also associated with an increased risk of esophageal cancer. The rs6687758 SNP, which has been shown

**Table 2** Allele frequencies in cases and controls and odds ratio estimates for esophageal cancer

SNP	Gene(s)	Locus	Alleles (A <sup>1</sup> /B)	Major allelic frequency		HWE <i>P</i> value	OR	95%CI	<i>P</i> value
				Case	Control				
rs1912453	<i>C1orf110</i>	1q23.3	C/T	0.404	0.392	0.812	1.054	0.846-1.314	0.639
rs10911251	<i>LAMC1</i>	1q25.3	C/A	0.487	0.494	0.256	0.976	0.787-1.211	0.826
rs6687758		1q41	G/A	0.243	0.222	0.191	1.127	0.873-1.455	0.358
rs11903757		2q32.3	C/T	0.039	0.044	1.000	0.889	0.518-1.525	0.668
rs10936599	<i>ARPM1</i>	3q26.2	C/T	0.480	0.453	0.909	1.116	0.899-1.384	0.320
rs13130787		4q22.2	C/T	0.307	0.315	0.239	0.965	0.765-1.217	0.765
rs367615		5q21.3	T/C	0.447	0.431	0.908	1.070	0.861-1.328	0.542
rs647161		5q31.1	A/C	0.296	0.274	0.198	1.113	0.877-1.413	0.377
rs1321311		6p21.2	T/G	0.200	0.140	0.234	1.534	1.145-2.055	0.004
rs2057314	<i>DCBLD1</i>	6q22.1	C/T	0.483	0.455	0.302	1.120	0.901-1.391	0.308
rs9365723	<i>SYNJ2</i>	6q25.3	G/A	0.411	0.373	0.224	1.169	0.935-1.462	0.171
rs7758229	<i>SLC22A3</i>	6q25.3	T/G	0.229	0.268	1.000	0.811	0.632-1.042	0.101
rs39453		7p15.3	C/T	0.304	0.339	0.528	0.851	0.676-1.073	0.172
rs10505477	<i>POU5F1B</i>	8q24.21	T/C	0.431	0.435	0.205	0.983	0.791-1.221	0.874
rs6983267			G/T	0.425	0.437	0.490	0.950	0.765-1.181	0.645
rs7014346			A/G	0.288	0.323	0.438	0.848	0.672-1.071	0.167
rs10114408		9q22.32	T/A	0.160	0.177	0.175	0.881	0.662-1.174	0.388
rs1665650	<i>HSPA12A</i>	10q25.3	A/G	0.304	0.306	0.595	0.991	0.784-1.251	0.937
rs3824999	<i>POLD3</i>	11q13.4	C/A	0.370	0.379	0.542	0.961	0.768-1.202	0.726
rs3802842	<i>C11orf93</i>	11q23.1	C/A	0.421	0.451	0.065	0.885	0.712-1.100	0.272
rs10774214	<i>CCND2</i>	12p13.32	T/C	0.320	0.318	0.026 <sup>2</sup>	1.011	0.803-1.274	0.923
rs3217901			G/A	0.482	0.498	0.113	0.936	0.755-1.161	0.548
rs59336	<i>TBX3</i>	12q24.21	T/A	0.417	0.379	1.000	1.171	0.939-1.461	0.160
rs7315438			T/C	0.361	0.342	0.528	1.085	0.866-1.360	0.478
rs4444235	<i>BMP4</i>	14q22.2	C/T	0.497	0.435	0.730	1.281	1.027-1.599	0.028
rs4779584	<i>SCG5</i>	15q13.3	C/T	0.216	0.190	0.716	1.175	0.899-1.537	0.237
rs9929218	<i>CDH1</i>	16q22.1	A/G	0.163	0.163	1.000	0.997	0.745-1.334	0.984
rs4939827	<i>SMAD7</i>	18q21.1	T/C	0.204	0.248	0.227	0.776	0.600-1.004	0.053
rs961253		20p12.3	A/C	0.107	0.102	0.537	1.062	0.747-1.510	0.737
rs2423279			C/T	0.359	0.344	0.033 <sup>2</sup>	1.069	0.853-1.340	0.563
rs4925386	<i>LAMA5</i>	20q13.33	T/C	0.251	0.253	0.010 <sup>2</sup>	0.987	0.770-1.265	0.916

<sup>1</sup>Minor allele; <sup>2</sup>Site with HWE *P* ≤ 0.05 excluded. HWE: Hardy-Weinberg equilibrium; SNP: Single-nucleotide polymorphism.

**Table 3** Logistic regression analysis of the association between the single-nucleotide polymorphisms and esophageal cancer risk *n* (%)

SNP	Model	Genotype	Cases	Controls	OR <sup>1</sup> (95%CI)	<i>P</i> value	AIC	BIC
rs1321311	Codominant	G/G	226 (64.2)	226 (72.9)	1	0.004	670.6	693.0
		G/T	111 (31.5)	81 (26.1)	1.27 (0.84-1.93)			
		T/T	15 (4.3)	3 (1.0)	8.06 (1.96-33.07)			
	Dominant	G/G	226 (64.2)	226 (72.9)	1	0.066	676.1	694.1
	Recessive	G/T-T/T	126 (35.8)	84 (27.1)	1.46 (0.97-2.19)			
		G/G-G/T	337 (95.7)	307 (99.0)	1	0.002	669.9	687.8
rs4444235	Codominant	T/T	76 (23.5)	100 (32.4)	1	0.046	651.8	674.0
		C/T	174 (53.7)	149 (48.2)	1.69 (1.08-2.65)			
		C/C	74 (22.8)	60 (19.4)	1.75 (1.01-3.02)			
	Dominant	T/T	76 (23.5)	100 (32.4)	1	0.013	649.8	667.6
	Recessive	C/T-C/C	248 (76.5)	209 (67.6)	1.71 (1.12-2.62)			
		T/T-C/T	250 (77.2)	249 (80.6)	1	0.350	655.0	672.8
rs6687758	Codominant	C/C	74 (22.8)	60 (19.4)	1.25 (0.79-1.98)			
		A/A	212 (59.2)	183 (59.2)	1	0.066	679.0	701.5
		G/A	118 (33.0)	115 (37.2)	0.86 (0.58-1.28)			
	Dominant	G/G	28 (7.8)	11 (3.6)	2.40 (1.01-5.74)			
		A/A	212 (59.2)	183 (59.2)	1	0.950	682.4	700.4
	Recessive	G/A-G/G	146 (40.8)	126 (40.8)	0.99 (0.68-1.44)			
		A/A-G/A	330 (92.2)	298 (96.4)	1	0.027	677.5	695.5
		G/G	28 (7.8)	11 (3.6)	2.54 (1.08-6.00)			

rs3802842	Codominant	A/A	119 (33.4)	101 (32.8)	1	0.033	681.0	703.5
		C/A	174 (48.9)	136 (44.2)	0.91 (0.60-1.40)			
		C/C	63 (17.7)	71 (23.1)	0.52 (0.31-0.88)			
	Dominant	A/A	119 (33.4)	101 (32.8)	1	0.190	684.1	702.1
		C/A-C/C	237 (66.6)	207 (67.2)	0.77 (0.52-1.14)			
	rs4939827	Recessive	A/A-C/A	293 (82.3)	237 (77.0)	1	0.010	679.2
C/C			63 (17.7)	71 (23.1)	0.55 (0.34-0.87)			
Codominant		C/C	228 (63.3)	179 (57.7)	1	0.110	687.0	709.5
		C/T	117 (32.5)	108 (34.8)	0.68 (0.46-1.02)			
		T/T	15 (4.2)	23 (7.4)	0.58 (0.26-1.33)			
Dominant		C/C	228 (63.3)	179 (57.7)	1	0.038	685.1	703.2
	C/T-T/T	132 (36.7)	131 (42.3)	0.67 (0.46-0.98)				
	Recessive	C/C-C/T	345 (95.8)	287 (92.6)	1	0.330	688.5	706.5
		T/T	15 (4.2)	23 (7.4)	0.67 (0.30-1.50)			

<sup>1</sup>Adjusted by sex and age. AIC: Akaike's information criterion; BIC: Bayesian information criterion; SNP: Single-nucleotide polymorphism.

to be associated with an increased CRC risk<sup>[20]</sup>, also increased the risk for esophageal cancer. However, the functional consequence of this polymorphism remains unknown.

This study also identifies two SNPs, rs3802842 and rs4939827, associated with a decreased risk for esophageal cancer. Although these SNPs have been associated with CRC risk, the results are inconsistent<sup>[27,28]</sup>. It is possible that these results reflect different variant alleles in the populations studied, given that the minor allele frequency was different. The rs4939827 SNP likely influences cancer *via* inhibition of SMAD7<sup>[28,29]</sup>, a component of the transforming growth factor- $\beta$  signalling pathway that regulates growth and apoptosis and plays an important role in cancer initiation and progression<sup>[30-33]</sup>.

Despite the adequate statistical power of the current study, some limitations should be considered. First, the sample size of our study was relatively small. Second, the association between genetic polymorphism and clinicopathologic type (adenocarcinoma or squamous cell carcinoma) was not evaluated. This could be an important factor, as rs4444235 and rs4939827 were shown to be over-represented in CRC-free patients with adenomas<sup>[32,33]</sup>.

In conclusion, this association study investigated 31 SNPs identified from CRC GWAS as genetic susceptibility factors for esophageal cancer in a Chinese population. The replication of genetic associations from CRC to esophageal cancer highlights the utility of case-control studies to confirm novel associations characterized in large GWAS of digestive system diseases. Our study provides the first reported data of a possible association between the SNPs rs1321311, rs4444235, rs6687758, rs3802842, and rs4939827 and esophageal cancer risk. However, further investigations are needed to confirm these associations in other populations.

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sample and data collection for this study.

## COMMENTS

### Background

Esophageal cancer and colorectal cancer (CRC) are the most common malignant tumors of the digestive tract, and are among the top ten most malignant and deadly cancers worldwide. China is among the countries with the highest incidence and mortality of esophageal cancer. Previous studies implicate the role of genetic factors in the susceptibility to these cancers. However, previous genome-wide association studies (GWAS) in the Chinese population have focused solely on single-nucleotide polymorphisms (SNPs) in esophageal cancer.

### Research frontiers

Epidemiologic studies have revealed that tobacco smoking, alcohol intake, nutritional deficiencies, and dietary carcinogen exposure may contribute to the etiology of esophageal cancer. However, only a small proportion of exposed individuals actually develop esophageal cancer, suggesting that genetic factors also play a vital role in susceptibility.

### Innovations and breakthroughs

This study aimed to identify common digestive system tumor susceptibility loci in a Chinese population. Thirty-one SNPs previously identified from CRC GWAS were selected to assess their association with risk for esophageal cancer. Five of these SNPs were identified as associated with both CRC and esophageal cancer risk, highlighting the utility of case-control studies to confirm novel associations characterized in large GWAS. This study provides the first reported data of a possible association between the SNPs rs1321311, rs4444235, rs6687758, rs3802842, and rs4939827 and esophageal cancer risk.

### Applications

This study sheds new light on the study of susceptibility variants in digestive system tumors. The results suggest that genetic variation (rs1321311, rs4444235, rs6687758, rs3802842, and rs4939827) influences susceptibility to esophageal cancer, and may have a clinical impact in the future.

### Peer-review

The article provides a novel result for esophageal cancer genetic risk factors, which has significance for clinical application.

## REFERENCES

- 1 **Hoeijmakers JH.** Genome maintenance mechanisms for preventing cancer. *Nature* 2001; **411**: 366-374 [PMID: 11357144 DOI: 10.1038/35077232]
- 2 **Metzger R,** Warnecke-Eberz U, Alakus H, Kütting F, Brabender J, Vallböhmer D, Grimminger PP, Mönig SP, Drebber U, Hölscher AH, Bollschweiler E. Neoadjuvant radiochemotherapy in adenocarcinoma of the esophagus: ERCC1 gene polymorphisms for prediction of response and prognosis. *J Gastrointest Surg* 2012; **16**: 26-34; discussion 34 [PMID: 21956434 DOI: 10.1007/s11605-011-1700-x]
- 3 **Enzinger PC,** Mayer RJ. Esophageal cancer. *N Engl J Med* 2003;

- 349: 2241-2252 [PMID: 14657432 DOI: 10.1056/NEJMra035010]
- 4 **Coleman HG**, Bhat S, Johnston BT, McManus D, Gavin AT, Murray LJ. Tobacco smoking increases the risk of high-grade dysplasia and cancer among patients with Barrett's esophagus. *Gastroenterology* 2012; **142**: 233-240 [PMID: 22062359 DOI: 10.1053/j.gastro.2011.10.034]
- 5 **Lin Y**, Totsuka Y, He Y, Kikuchi S, Qiao Y, Ueda J, Wei W, Inoue M, Tanaka H. Epidemiology of esophageal cancer in Japan and China. *J Epidemiol* 2013; **23**: 233-242 [PMID: 23629646 DOI: 10.2188/jea.JE20120162]
- 6 **Gu H**, Ding G, Zhang W, Liu C, Chen Y, Chen S, Jiang P. Replication study of PLCE1 and C20orf54 polymorphism and risk of esophageal cancer in a Chinese population. *Mol Biol Rep* 2012; **39**: 9105-9111 [PMID: 22744421 DOI: 10.1007/s11033-012-1782-x]
- 7 **Umar M**, Upadhyay R, Khurana R, Kumar S, Ghoshal UC, Mittal B. Role of p53 and p73 genes polymorphisms in susceptibility to esophageal cancer: a case control study in a northern Indian population. *Mol Biol Rep* 2012; **39**: 1153-1162 [PMID: 21573788 DOI: 10.1007/s11033-011-0844-9]
- 8 **Schoen RE**, Pinsky PF, Weissfeld JL, Yokochi LA, Church T, Laiyemo AO, Bresalier R, Andriole GL, Buys SS, Crawford ED, Fouad MN, Isaacs C, Johnson CC, Reding DJ, O'Brien B, Carrick DM, Wright P, Riley TL, Purdue MP, Izmirlian G, Kramer BS, Miller AB, Gohagan JK, Prorok PC, Berg CD. Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy. *N Engl J Med* 2012; **366**: 2345-2357 [PMID: 22612596 DOI: 10.1056/NEJMoal114635]
- 9 **Hu N**, Wang C, Hu Y, Yang HH, Giffen C, Tang ZZ, Han XY, Goldstein AM, Emmert-Buck MR, Buetow KH, Taylor PR, Lee MP. Genome-wide association study in esophageal cancer using GeneChip mapping 10K array. *Cancer Res* 2005; **65**: 2542-2546 [PMID: 15805246 DOI: 10.1158/0008-5472.can-04-3247]
- 10 **Wang AH**, Liu Y, Wang B, He YX, Fang YX, Yan YP. Epidemiological studies of esophageal cancer in the era of genome-wide association studies. *World J Gastrointest Pathophysiol* 2014; **5**: 335-343 [PMID: 25133033 DOI: 10.4291/wjgp.v5.i3.335]
- 11 **Le Marchand L**. Genome-wide association studies and colorectal cancer. *Surg Oncol Clin N Am* 2009; **18**: 663-668 [PMID: 19793573 DOI: 10.1016/j.soc.2009.07.004]
- 12 **He J**, Wilkens LR, Stram DO, Kolonel LN, Henderson BE, Wu AH, Le Marchand L, Haiman CA. Generalizability and epidemiologic characterization of eleven colorectal cancer GWAS hits in multiple populations. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 70-81 [PMID: 21071539 DOI: 10.1158/1055-9965]
- 13 **Yang CX**, Matsuo K, Ito H, Shinoda M, Hatooka S, Hirose K, Wakai K, Saito T, Suzuki T, Maeda T, Tajima K. Gene-environment interactions between alcohol drinking and the MTHFR C677T polymorphism impact on esophageal cancer risk: results of a case-control study in Japan. *Carcinogenesis* 2005; **26**: 1285-1290 [PMID: 15790587 DOI: 10.1093/carcin/bgi076]
- 14 **Yang CX**, Wang HY, Wang ZM, Du HZ, Tao DM, Mu XY, Chen HG, Lei Y, Matsuo K, Tajima K. Risk factors for esophageal cancer: a case-control study in South-western China. *Asian Pac J Cancer Prev* 2005; **6**: 48-53 [PMID: 15780032]
- 15 **Yang SJ**, Wang HY, Li XQ, Du HZ, Zheng CJ, Chen HG, Mu XY, Yang CX. Genetic polymorphisms of ADH2 and ALDH2 association with esophageal cancer risk in southwest China. *World J Gastroenterol* 2007; **13**: 5760-5764 [PMID: 17963305 DOI: 10.3748/wjg.v13.i43.5760]
- 16 **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474 [PMID: 20180029 DOI: 10.1245/s10434-010-0985-4]
- 17 **Gabriel S**, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet* 2009; **Chapter 2**: Unit 2.12 [PMID: 19170031 DOI: 10.1002/0471142905.hg0212s60]
- 18 **Thomas RK**, Baker AC, Debiassi RM, Winckler W, Laframboise T, Lin WM, Wang M, Feng W, Zander T, MacConaill L, Lee JC, Nicoletti R, Hatton C, Goyette M, Girard L, Majmudar K, Ziaugra L, Wong KK, Gabriel S, Beroukhir R, Peyton M, Barretina J, Dutt A, Emery C, Greulich H, Shah K, Sasaki H, Gazdar A, Minna J, Armstrong SA, Mellinghoff IK, Hodi FS, Dranoff G, Mischel PS, Cloughesy TF, Nelson SF, Liao LM, Mertz K, Rubin MA, Moch H, Loda M, Catalona W, Fletcher J, Signoretti S, Kaye F, Anderson KC, Demetri GD, Dummer R, Wagner S, Herlyn M, Sellers WR, Meyerson M, Garraway LA. High-throughput oncogene mutation profiling in human cancer. *Nat Genet* 2007; **39**: 347-351 [PMID: 17293865]
- 19 **Pesch B**, Casjens S, Stricker I, Westerwick D, Taeger D, Rabstein S, Wiethage T, Tannapfel A, Brünning T, Johnen G. NOTCH1, HIF1A and other cancer-related proteins in lung tissue from uranium miners--variation by occupational exposure and subtype of lung cancer. *PLoS One* 2012; **7**: e45305 [PMID: 23028920 DOI: 10.1371/journal.pone.0045305]
- 20 **Kantor ED**, Hutter CM, Minnier J, Berndt SI, Brenner H, Caan BJ, Campbell PT, Carlson CS, Casey G, Chan AT, Chang-Claude J, Chanock SJ, Cotterchio M, Du M, Duggan D, Fuchs CS, Giovannucci EL, Gong J, Harrison TA, Hayes RB, Henderson BE, Hoffmeister M, Hopper JL, Jenkins MA, Jiao S, Kolonel LN, Le Marchand L, Lemire M, Ma J, Newcomb PA, Ochs-Balcom HM, Pflugeisen BM, Potter JD, Rudolph A, Schoen RE, Seminara D, Slaterry ML, Stelling DL, Thomas F, Thornquist M, Ulrich CM, Warnick GS, Zanke BW, Peters U, Hsu L, White E. Gene-environment interaction involving recently identified colorectal cancer susceptibility loci. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 1824-1833 [PMID: 24994789 DOI: 10.1158/1055-9965.epi-14-0062]
- 21 **Dunlop MG**, Dobbins SE, Farrington SM, Jones AM, Palles C, Whiffin N, Tenesa A, Spain S, Broderick P, Ooi LY, Domingo E, Smillie C, Henrion M, Frampton M, Martin L, Grimes G, Gorman M, Semple C, Ma YP, Barclay E, Prendergast J, Cazier JB, Olver B, Penegar S, Lubbe S, Chander I, Carvajal-Carmona LG, Ballereau S, Lloyd A, Vijayakrishnan J, Zgaga L, Rudan I, Theodoratou E, Starr JM, Deary I, Kirac I, Kovacevic D, Aaltonen LA, Renkonen-Sinisalo L, Mecklin JP, Matsuda K, Nakamura Y, Okada Y, Gallinger S, Duggan DJ, Conti D, Newcomb P, Hopper J, Jenkins MA, Schumacher F, Casey G, Easton D, Shah M, Pharoah P, Lindblom A, Liu T, Smith CG, West H, Cheadle JP, Midgley R, Kerr DJ, Campbell H, Tomlinson IP, Houlston RS. Common variation near CDKN1A, POLD3 and SHROOM2 influences colorectal cancer risk. *Nat Genet* 2012; **44**: 770-776 [PMID: 22634755 DOI: 10.1038/ng.2293]
- 22 **Zhou CP**, Pan HZ, Li FX, Hu NY, Li M, Yang XX. Association analysis of colorectal cancer susceptibility variants with gastric cancer in a Chinese Han population. *Genet Mol Res* 2014; **13**: 3673-3680 [PMID: 24854447 DOI: 10.4238/2014.May.9.10]
- 23 **Li FX**, Yang XX, Hu NY, Du HY, Ma Q, Li M. Single-nucleotide polymorphism associations for colorectal cancer in southern Chinese population. *Chin J Cancer Res* 2012; **24**: 29-35 [PMID: 23359760 DOI: 10.1007/s11670-012-0029-7]
- 24 **Liu L**, Su Q, Li L, Lin X, Gan Y, Chen S. The common variant rs444235 near BMP4 confers genetic susceptibility of colorectal cancer: an updated meta-analysis based on a comprehensive statistical strategy. *PLoS One* 2014; **9**: e100133 [PMID: 24932582 DOI: 10.1371/journal.pone.0100133]
- 25 **Houlston RS**, Webb E, Broderick P, Pittman AM, Di Bernardo MC, Lubbe S, Chandler I, Vijayakrishnan J, Sullivan K, Penegar S, Carvajal-Carmona L, Howarth K, Jaeger E, Spain SL, Walther A, Barclay E, Martin L, Gorman M, Domingo E, Teixeira AS, Kerr D, Cazier JB, Niittymäki I, Tuupanen S, Karhu A, Aaltonen LA, Tomlinson IP, Farrington SM, Tenesa A, Prendergast JG, Barnetson RA, Cetnarskyj R, Porteous ME, Pharoah PD, Koessler T, Hampe J, Buch S, Schafmayer C, Tepel J, Schreiber S, Völzke H, Chang-Claude J, Hoffmeister M, Brenner H, Zanke BW, Montpetit A, Hudson TJ, Gallinger S, Campbell H, Dunlop MG. Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. *Nat Genet* 2008; **40**: 1426-1435 [PMID: 19011631 DOI: 10.1038/ng.262]



- 26 **Lubbe SJ**, Pittman AM, Olver B, Lloyd A, Vijayakrishnan J, Naranjo S, Dobbins S, Broderick P, Gómez-Skarmeta JL, Houlston RS. The 14q22.2 colorectal cancer variant rs4444235 shows cis-acting regulation of BMP4. *Oncogene* 2012; **31**: 3777-3784 [PMID: 22158048 DOI: 10.1038/ncr.2011.564]
- 27 **Broderick P**, Carvajal-Carmona L, Pittman AM, Webb E, Howarth K, Rowan A, Lubbe S, Spain S, Sullivan K, Fielding S, Jaeger E, Vijayakrishnan J, Kemp Z, Gorman M, Chandler I, Papaemmanuil E, Penegar S, Wood W, Sellick G, Qureshi M, Teixeira A, Domingo E, Barclay E, Martin L, Sieber O, Kerr D, Gray R, Peto J, Cazier JB, Tomlinson I, Houlston RS. A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. *Nat Genet* 2007; **39**: 1315-1317 [PMID: 17934461 DOI: 10.1038/ng.2007.18]
- 28 **Tenesa A**, Farrington SM, Prendergast JG, Porteous ME, Walker M, Haq N, Barnetson RA, Theodoratou E, Cetnarskyj R, Cartwright N, Semple C, Clark AJ, Reid FJ, Smith LA, Kavoussanakis K, Koessler T, Pharoah PD, Buch S, Schafmayer C, Tepel J, Schreiber S, Völzke H, Schmidt CO, Hampe J, Chang-Claude J, Hoffmeister M, Brenner H, Wilkerson S, Canzian F, Capella G, Moreno V, Deary IJ, Starr JM, Tomlinson IP, Kemp Z, Howarth K, Carvajal-Carmona L, Webb E, Broderick P, Vijayakrishnan J, Houlston RS, Rennert G, Ballinger D, Rozek L, Gruber SB, Matsuda K, Kidokoro T, Nakamura Y, Zanke BW, Greenwood CM, Rangrej J, Kustra R, Montpetit A, Hudson TJ, Gallinger S, Campbell H, Dunlop MG. Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. *Nat Genet* 2008; **40**: 631-637 [PMID: 18372901 DOI: 10.1038/ng.133]
- 29 **Loh YH**, Mitrou PN, Wood A, Luben RN, McTaggart A, Khaw KT, Rodwell SA. SMAD7 and MGMT genotype variants and cancer incidence in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *Cancer Epidemiol* 2011; **35**: 369-374 [PMID: 21075068 DOI: 10.1016/j.canep.2010.09.011]
- 30 **Edlund S**, Bu S, Schuster N, Aspenström P, Heuchel R, Heldin NE, ten Dijke P, Heldin CH, Landström M. Transforming growth factor-beta1 (TGF-beta)-induced apoptosis of prostate cancer cells involves Smad7-dependent activation of p38 by TGF-beta-activated kinase 1 and mitogen-activated protein kinase kinase 3. *Mol Biol Cell* 2003; **14**: 529-544 [PMID: 12589052]
- 31 **Halder SK**, Beauchamp RD, Datta PK. Smad7 induces tumorigenicity by blocking TGF-beta-induced growth inhibition and apoptosis. *Exp Cell Res* 2005; **307**: 231-246 [PMID: 15922743 DOI: 10.1016/j.yexcr.2005.03.009]
- 32 **Aragón E**, Goerner N, Xi Q, Gomes T, Gao S, Massagué J, Macias MJ. Structural basis for the versatile interactions of Smad7 with regulator WW domains in TGF- $\beta$  Pathways. *Structure* 2012; **20**: 1726-1736 [PMID: 22921829 DOI: 10.1016/j.str.2012.07.014]
- 33 **Carvajal-Carmona LG**, Zauber AG, Jones AM, Howarth K, Wang J, Cheng T, Riddell R, Lanis A, Morton D, Bertagnolli MM, Tomlinson I. Much of the genetic risk of colorectal cancer is likely to be mediated through susceptibility to adenomas. *Gastroenterology* 2013; **144**: 53-55 [PMID: 22999960 DOI: 10.1053/j.gastro.2012.09.016]

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## Retrospective Cohort Study

# Clinicopathologic and molecular features associated with patient age in gastric cancer

Ji Yeon Seo, Eun Hyo Jin, Hyun Jin Jo, Hyuk Yoon, Cheol Min Shin, Young Soo Park, Nayoung Kim, Hyun Chae Jung, Dong Ho Lee

Ji Yeon Seo, Department of Internal Medicine and Healthcare Research Institute, Healthcare System Gangnam Center, Seoul National University Hospital, Seoul 135-984, South Korea  
Eun Hyo Jin, Hyun Chae Jung, Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul 110-744, South Korea  
Hyun Jin Jo, Hyuk Yoon, Cheol Min Shin, Young Soo Park, Nayoung Kim, Dong Ho Lee, Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do 463-707, South Korea

**Author contributions:** Seo JY and Lee DH designed the research; Seo JY and Jin EH performed the research; Seo JY, Jo HJ, Yoon H and Shin CM analyzed and interpreted data; Seo JY and Lee DH wrote the paper; Seo JY, Park YS, Kim N, Jung HC and Lee DH critically reviewed the manuscript.

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**Informed consent:** Informed consent was exempted, which was approved by the Ethical Committee at the Seoul National University Bundang Hospital.

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Correspondence to: Dong Ho Lee, MD, PhD, Department of Internal Medicine, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam-si, Gyeonggi-do 463-707, South Korea. [dhlee@yaho.co.kr](mailto:dhlee@yaho.co.kr)  
Telephone: +82-31-7877006  
Fax: +82-31-7874051

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## Abstract

**AIM:** To compare characteristics and prognosis of gastric cancer based on age.

**METHODS:** A retrospective study was conducted on clinical and molecular data from patients ( $n = 1658$ ) with confirmed cases of gastric cancer in Seoul National University Bundang Hospital (Seoul, South Korea) from 2003 to 2010 after exclusion of patients diagnosed with lymphoma, gastrointestinal stromal tumor, and metastatic cancer in the stomach. DNA was isolated from tumor and adjacent normal tissue, and a set of five markers was amplified by polymerase chain reaction to assess microsatellite instability (MSI). MSI was categorized as high, low, or stable if  $\geq 2$ , 1, or 0 markers, respectively, had changed. Immunohistochemistry was performed on tissue sections to detect levels of expression of p53, human epidermal growth factor receptor (HER)-2, and epidermal growth factor receptor. Statistical analysis of clinical and molecular data was performed to assess prognosis based on the stratification of patients by age ( $\leq 45$  and  $> 45$  years).

**RESULTS:** Among the 1658 gastric cancer patients, the number of patients with an age  $\leq 45$  years was 202 (12.2%;  $38.9 \pm 0.4$  years) and the number of patients  $> 45$  years was 1456 (87.8%;  $64.1 \pm 0.3$  years). Analyses revealed that females were predominant in

the younger group ( $P < 0.001$ ). Gastric cancers in the younger patients exhibited more aggressive features and were at a more advanced stage than those in older patients. Precancerous lesions, such as atrophic gastritis and intestinal metaplasia, were observed less frequently in the older than in the younger group ( $P < 0.001$ ). Molecular characteristics, including overexpression of p53 ( $P < 0.001$ ), overexpression of HER-2 ( $P = 0.006$ ), and MSI ( $P = 0.006$ ), were less frequent in gastric cancer of younger patients. Cancer related mortality was higher in younger patients ( $P = 0.048$ ), but this difference was not significant after adjusting for the stage of cancer.

**CONCLUSION:** Gastric cancer is distinguishable between younger and older patients based on both clinicopathologic and molecular features, but stage is the most important predictor of prognosis.

**Key words:** Age; Gastric cancer; Microsatellite instability; Molecular pathology; Prognosis; Stage

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**Core tip:** Whether gastric cancer exhibits distinguishable characteristics based on age remains controversial. In this original article, results are presented that highlight differences in clinical characteristics, pathology, and molecular features of younger and older gastric cancer patients. In particular, the pathologic degree of precancerous lesions associated with each group illuminated potential differences in the pathogenesis of the disease. Although gastric cancer in younger patients presented with more aggressive features, the primary factor in predicting the prognosis of patients with the disease was the stage of the cancer, and not the age of the patient.

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## INTRODUCTION

Gastric cancer is the fourth most commonly diagnosed cancer and the second most common cause of cancer related death worldwide<sup>[1,2]</sup>. Due to successful screening, the detection of early gastric cancer and the cure rate of gastric cancer have increased annually<sup>[1,2]</sup>. Regardless of this effort however, mortality of gastric cancer remains high, particularly in East Asian countries.

In general, the peak incidence for gastric cancer is in patients aged 65-74 years<sup>[3]</sup>, with only approximately 3%-10% of gastric cancers overall occurring in pa-

tients younger than 40 years<sup>[4]</sup>. Some case series have focused specifically on younger patients and have reported the cases to be highly advanced gastric cancers (AGC) with poor prognoses<sup>[5]</sup>. Intriguingly, these gastric cancers were found to be more common in women, frequently diffusely spread in the stomach, more poorly differentiated, and more advanced in stage than gastric cancers from older patients<sup>[6,7]</sup>. These findings remain controversial<sup>[6,8]</sup>, particularly with regard to patient survival, but have raised the possibility of a disease course that is potentially distinct from that in older patients. Some studies report a better prognosis in younger patients<sup>[9,10]</sup>, while others have found poorer prognoses in young gastric cancer patients relative to older patients<sup>[4,11]</sup>. Still others have demonstrated no significant differences at all in survival between the two age groups<sup>[6-8]</sup>.

While the majority of reports have focused only on the clinical or pathologic features of gastric cancer in younger patients<sup>[4,6-11]</sup>, molecular characteristics that may distinguish tumors between the two age groups have not been well described. Therefore, this study aimed to determine whether differences in clinicopathologic and molecular characteristics exist in gastric cancer based on age.

## MATERIALS AND METHODS

### Ethics statement

This study protocol was approved by the Ethical Committee at the Seoul National University Bundang Hospital (SNUBH; IRB number: B-1403/244-116), and conformed to the provisions of the Declaration of Helsinki. Informed consent was exempted by the committee.

### Study population

A retrospective cohort study was conducted to identify differences between young (age  $\leq 45$  years) and older (age  $> 45$  years) patients with gastric cancer. The study was performed on patients who had been diagnosed with gastric cancer in SNUBH from June 2003 to December 2010. Exclusion criteria were the following: (1) patients  $< 20$  years of age; (2) patients diagnosed with gastric mucosa-associated lymphoid tissue lymphoma, gastrointestinal stromal tumor, or other metastatic cancer located in the stomach; (3) patients for whom a pathologic diagnosis of gastric cancer was not confirmed; (4) patients who did not undergo a stage workup of gastric cancer; (5) patients who were lost in follow-up from SNUBH after diagnosis; or (6) patients who were not initially diagnosed with gastric cancer during the search period.

### Data collection and pathologic examination

Demographic factors of patients, characteristics of gastric cancer, pathology, stage, molecular features, *Helicobacter pylori* (*H. pylori*) status, treatment, recurrence, and mortality were reviewed from electro-

nic medical records. Location of the primary tumor was assigned to the proximal, middle, or distal third of the stomach. Gastric cancer that extended into more than two of the three sections was defined as diffusely located<sup>[8]</sup>. The type of early gastric cancer followed Paris classification (I to III)<sup>[12]</sup>, and the type of AGC followed Borrmann classification (I to IV)<sup>[13]</sup>. Gastric cancer was staged according to the 7<sup>th</sup> edition of the American Joint Committee on Cancer TNM staging system<sup>[14]</sup>.

The pathology of gastric cancers was categorized as intestinal, diffuse, or mixed by Lauren's classification<sup>[15]</sup>. The degree of *H. pylori* infection, neutrophil infiltration, mononuclear cell infiltration, atrophic gastritis, and intestinal metaplasia was scored as 0 (absent), 1 (mild), 2 (moderate), or 3 (marked) for statistical analysis, according to the Updated Sydney System<sup>[16]</sup>.

### Immunohistochemistry

Paraffin-embedded sections (4  $\mu$ m) were deparaffinized and incubated with monoclonal antibodies against p53, human epidermal growth factor receptor (HER)-2, and epidermal growth factor receptor (EGFR). Detection of primary antibodies and amplification of signal was performed with the streptavidin-biotin method as previously described<sup>[17,18]</sup>. Staining was recorded as positive or negative expression<sup>[17,18]</sup>. Overexpression of p53 in > 10% of tumor cells, which generally reflects an underlying mutation in the p53 gene, was as considered positive<sup>[19]</sup>. Scoring for HER-2 protein expression was performed as previously reported: 0, membrane staining of less than 10% of tumor cells; 1+, faint partial membrane staining in > 10% of tumor cells; 2+, weak to moderate staining of whole membranes in > 10% of tumor cells; and 3+, strong staining of whole membranes in > 10% of tumor cells. Scores of 2+ and 3+ were classified as HER-2 overexpression<sup>[18]</sup>. A similar scoring method was applied to immunohistochemistry (IHC) staining for the EGFR protein, with scores of 2+ and 3+ classified as overexpression<sup>[18]</sup>.

### Microsatellite instability analysis

Tumor and normal DNAs were extracted from paraffin-embedded tissue. Five markers (BAT-25, BAT-26, D2S123, D5S346, and D17S250) were used following the guidelines of the International Workshop of the National Cancer Institute. Marker sequences from tumor and matched normal DNAs were amplified with polymerase chain reaction and compared. Tumors with two or more novel markers were classified as microsatellite instability (MSI)-high, whereas tumors with one marker shift were classified as MSI-low. Microsatellite stability was defined as when all markers were identical in tumor and normal DNAs<sup>[20]</sup>.

### Evaluation of outcomes

The primary and secondary outcomes that were compared in this study were mortality and recurrence.

Cause of death was categorized as one of the following three scenarios: (1) gastric cancer-related death or mortality due to the progression of gastric cancer; (2) treatment-related death, including severe complications due to surgery or infection after chemotherapy; or (3) other causes not directly related to gastric cancer. Time to recurrence was estimated for those who were cured after endoscopic or surgical resection of gastric cancer.

### Statistical analysis

Values are expressed as the mean  $\pm$  SD for continuous variables and as frequencies (percent) for categorical variables. A Fisher's exact test,  $\chi^2$  analysis, and a Student's *t*-test were used for analyzing characteristics of gastric cancer. Independent risk factors for mortality were analyzed with univariate and multivariate analyses using the Cox proportional hazards model. Variables with  $P < 0.05$  in univariate analyses were included in multivariate analyses. Overall survival and recurrence-free survival was estimated using the Kaplan-Meier method and the log-rank test. A  $P \leq 0.05$  was considered statistically significant. All statistical analyses were performed with SPSS, version 18.0 (SPSS Inc., Chicago, IL, United States).

## RESULTS

### Baseline characteristics

Patients ( $n = 2416$ ) with a diagnosis of gastric cancer were identified from electronic records from June 2003 to December 2010 at SNUBH. The following patients ( $n = 758$ ) were excluded from further analysis: < 20 years of age ( $n = 1$ ); diagnosis of gastric mucosa-associated lymphoid tissue lymphoma ( $n = 87$ ), gastrointestinal stromal tumor ( $n = 18$ ), metastatic cancer in the stomach ( $n = 3$ ), pathologic diagnosis of gastric cancer was not confirmed ( $n = 11$ ), did not undergo a staging workup ( $n = 32$ ), lost in follow-up ( $n = 89$ ), and diagnosed with gastric cancer and receiving treatment ( $n = 517$ ) (Figure 1). The remaining patients ( $n = 1658$ ) were analyzed.

The number of younger patients ( $\leq 45$  years) was 202 (12.2%), and the number of older patients ( $> 45$  years) was 1456 (87.8%). The mean age of diagnosis was  $61.0 \pm 0.3$  years for all patients,  $38.9 \pm 0.4$  years for younger patients, and  $64.1 \pm 0.3$  years for older patients. A summary of the baseline characteristics for all patients is presented in Table 1. Analyses revealed that the number of female patients was predominant in the younger group ( $P < 0.001$ ). The majority of younger patients requested medical examination because of symptoms (56.9%). In contrast, gastric cancer was detected in about half of the older patients as a result of screening (49.7%). Older patients had more comorbid diseases ( $P < 0.001$ ). Gastric cancer in younger patients was more frequently diffusely spread in the stomach ( $P < 0.001$ ) with more incidences of Borrmann type IV AGC ( $P < 0.001$ ). There were no



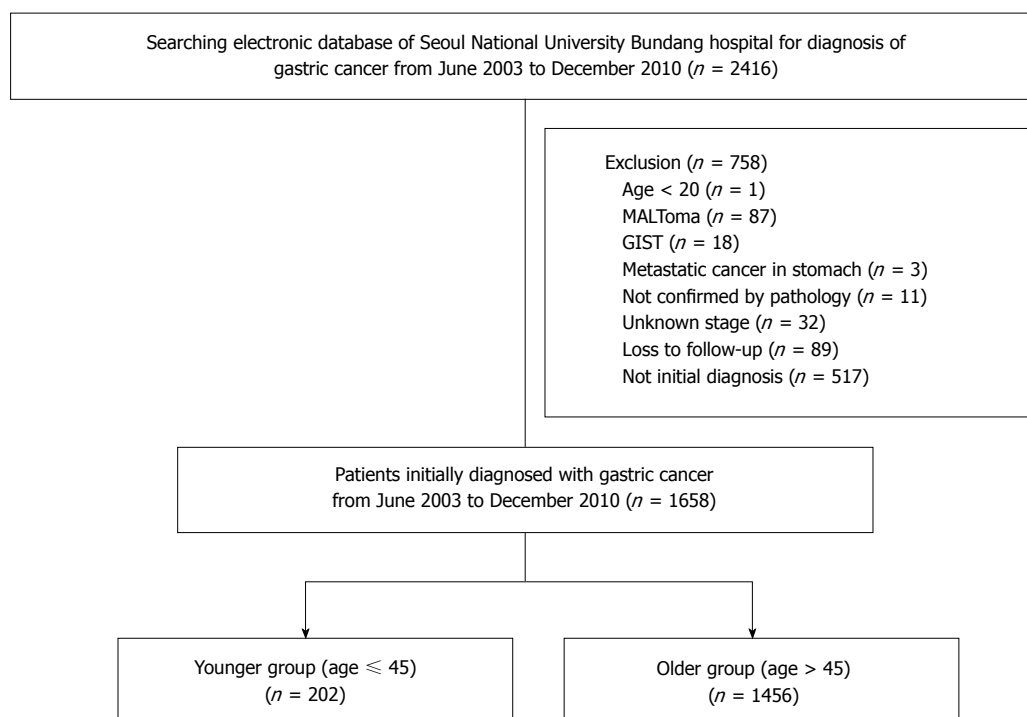


Figure 1 Flow chart for selection criteria of patients.

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

Characteristic	Age ≤ 45 yr ( <i>n</i> = 202)	Age > 45 yr ( <i>n</i> = 1456)	<i>P</i> value
Gender			< 0.001
Male	111 (55.0)	1023 (70.3)	
Female	91 (45.0)	433 (29.7)	
Reason for medical checkup			< 0.001
Screening	70 (34.7)	724 (49.7)	
Symptoms <sup>1</sup>	115 (56.9)	613 (42.1)	
Bleeding/anemia	13 (6.4)	105 (7.2)	
Weight loss	4 (2.0)	14 (1.0)	
Comorbidity			< 0.001
No	188 (93.1)	1200 (82.4)	
Yes	14 (6.9)	256 (17.6)	
Size, cm	4.0 ± 0.3	3.7 ± 0.1	0.083
CEA	1.8 ± 0.2	4.5 ± 1.1	0.631
CA 19-9	38.3 ± 13.0	30.7 ± 6.5	0.090
Synchronous gastric cancer			0.226
No	193 (95.5)	1414 (97.1)	
Yes	9 (4.5)	42 (2.9)	
Location			< 0.001
Proximal	20 (9.9)	132 (9.1)	
Middle	88 (43.6)	433 (29.7)	
Distal	59 (29.2)	753 (51.7)	
Diffuse	35 (17.3)	138 (9.5)	
EGC type	116 (57.4)	889 (61.1)	0.084
I	1 (0.5)	50 (3.4)	
II	113 (55.9)	820 (56.3)	
III	2 (1.0)	19 (1.3)	
AGC type (Borrmann)	86 (42.6)	567 (38.9)	< 0.001
I	0 (0)	22 (1.5)	
II	6 (3.0)	118 (8.1)	
III	47 (23.3)	334 (22.9)	
IV	31 (15.3)	71 (4.9)	

<sup>1</sup>Symptoms included abdominal discomfort, abdominal pain, soreness, indigestion, anorexia, nausea, or vomiting. AGC: Advanced gastric cancer; CA 19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; EGC: Early gastric cancer.

differences in the size of the tumor, or expression of carcinoembryonic antigen (CEA) or carbohydrate antigen 19-9 (CA 19-9) based on age.

### Pathology and stage of gastric cancer

The pathology and the stages of gastric cancer for all patients are presented in Table 2. Statistical analyses revealed that more tumors in younger patients were diagnosed as diffuse based on Lauren's classification ( $P < 0.001$ ) (Table 2). Depth of invasion, frequency of distant metastasis, and final stage were all higher in the younger patient group ( $P = 0.001$ ). The pathologic features of venous ( $P = 0.024$ ) and perineural invasion ( $P = 0.043$ ) were also more frequently observed in gastric cancers from younger patients. In contrast, baseline adenoma occurred less often in these patients ( $P < 0.001$ ).

### Treatment and clinical outcomes of gastric cancer

Treatment for gastric cancer varied among patients of the study. For example, not all patients underwent curative resection. The various treatment strategies utilized are presented in Table 3. A significantly higher percentage of younger patients received palliative resection for AGC ( $P = 0.018$ ), N3 dissection ( $P = 0.017$ ), and chemotherapy ( $P < 0.001$ ) compared to older patients.

The clinical outcomes are summarized in Table 4. The mean time for follow-up was  $35.4 \pm 23.7$  mo. The mean time to recurrence was  $17.8 \pm 4.1$  in younger patients, and  $16.9 \pm 1.3$  mo in older patients. The recurrence rate for cured patients was similar in both age groups, with peritoneal metastasis as the most common site of recurrence. Furthermore,

**Table 2 Pathology and stage of gastric cancer *n* (%)**

Variable	Age ≤ 45 yr ( <i>n</i> = 202)	Age > 45 yr ( <i>n</i> = 1456)	<i>P</i> value
Pathology (Lauren)			< 0.001
Intestinal	39 (19.3)	870 (59.8)	
Diffuse	157 (77.7)	526 (36.1)	
Mixed	6 (3.0)	60 (4.1)	
Depth of invasion			0.001
T1	117 (57.9)	876 (60.2)	
T2	10 (5.0)	143 (9.8)	
T3	15 (7.4)	186 (12.8)	
T4	33 (16.3)	146 (10.0)	
Lymph node metastasis			0.052
N0	122 (60.4)	962 (66.1)	
N1	8 (4.0)	127 (8.7)	
N2	15 (7.4)	74 (5.1)	
N3	25 (12.4)	155 (10.6)	
Distant metastasis			< 0.001
M0	153 (75.7)	1267 (87.0)	
M1	49 (24.3)	189 (13.0)	
Stage			< 0.001
I	122 (60.4)	954 (65.5)	
II	10 (5.0)	149 (10.2)	
III	21 (10.4)	166 (11.4)	
IV	49 (24.3)	187 (12.8)	
Lymphatic invasion			0.612
No	121 (59.9)	902 (62.0)	
Yes	50 (24.8)	408 (28.0)	
Venous invasion			0.024
No	149 (73.8)	1207 (82.9)	
Yes	22 (10.9)	102 (7.0)	
Perineural invasion			0.043
No	125 (61.9)	1042 (71.6)	
Yes	46 (22.8)	264 (18.1)	
Baseline adenoma			< 0.001
No	165 (81.7)	1101 (75.6)	
Yes	4 (2.0)	195 (13.4)	

gastric cancer-related death was the most common cause of death in both groups. Mortality occurred in 26 (12.9%) and 159 (10.9%) of younger and older patients, respectively. Cumulative probabilities of overall mortality were not different between the two age groups (Figure 2). The cumulative rate of gastric cancer-related death was significantly higher in the younger age group ( $P = 0.048$ ) (Figure 3). However, when adjusted for the stage, gastric cancer-related death was not significantly different between the two age groups ( $P = 0.191$ ). The cumulative rate of treatment-related death was not different between the two age groups.

#### Molecular pathology and *H. pylori* status

The results of molecular pathology are shown in Table 5. Gastric cancer in the younger age group had significantly less positive staining for p53 ( $P < 0.001$ ), HER-2 overexpression ( $P = 0.006$ ), and MSI ( $P = 0.006$ ). EGFR protein expression, however, did not differ between the two groups ( $P = 0.899$ ).

The status of *H. pylori* and related changes of the stomach are presented in Table 6. The level of *H. pylori* in pathologic specimens was higher in younger patients

**Table 3 Treatment of gastric cancer *n* (%)**

Variable	Age ≤ 45 yr ( <i>n</i> = 202)	Age > 45 yr ( <i>n</i> = 1456)	<i>P</i> value
Endoscopic resection	9 (4.5)	192 (13.2)	0.516
EMR	6 (3.0)	104 (7.1)	
ESD	3 (1.5)	88 (6.0)	
Operation	172 (85.1)	1160 (79.7)	0.018
Curative resection	157 (77.7)	1108 (76.1)	
Palliative resection	15 (7.4)	52 (3.6)	
LN dissection			0.017
Not performed	9 (4.5)	24 (1.6)	
N1	62 (30.7)	404 (27.7)	
N2	95 (47.0)	714 (49.0)	
N3	6 (3.0)	18 (1.2)	
Radiation			0.101
No	197 (97.5)	1440 (98.9)	
Yes	5 (2.5)	16 (1.1)	
Chemotherapy			< 0.001
No	132 (65.3)	1194 (82.1)	
Yes	70 (34.7)	261 (17.9)	

EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection; LN: Lymph node.

**Table 4 Mortality and recurrence of gastric cancer *n* (%)**

Variable	Age ≤ 45 yr ( <i>n</i> = 202)	Age > 45 yr ( <i>n</i> = 1456)
Mean follow-up duration, mo	36.9 ± 25.5	35.2 ± 23.5
Mean time to recurrence, mo <sup>1</sup>	17.8 ± 4.1	16.9 ± 1.3
Cured patients	162 (80.2)	1246 (85.6)
Recurrence	17 (10.5)	127 (10.2)
Mortality		
Survival	116 (57.4)	838 (57.6)
Death	26 (12.9)	159 (10.9)
Loss to follow-up	60 (29.7)	459 (31.5)
Cause of death		
Gastric cancer-related death	21 (10.4)	87 (6.0)
Treatment-related death	2 (1.0)	13 (0.9)
Other causes <sup>2</sup>	0 (0)	21 (1.4)
Not available	3 (1.5)	38 (2.6)

<sup>1</sup>Calculated only for cured patients; <sup>2</sup>Other causes of death included malignancy other than gastric cancer, infection not related to treatment of gastric cancer, myocardial infarction, hepatic failure, and trauma.

( $P = 0.012$ ). The pathologic degrees of atrophic gastritis and intestinal metaplasia were, however, significantly higher in older patients ( $P < 0.001$ ).

#### Predictors of overall survival

Risk factors for overall mortality in gastric cancer patients were analyzed using a Cox proportional hazards model (Table 7). In univariate analyses, the following were significant risk factors for mortality: non-curative resection, elevated CEA, elevated CA 19-9, larger size of gastric cancer, diffuse pathology, higher T, N, or M stage, final stage, and lymphatic, venous, and perineural invasion (all  $P < 0.001$ ). Multivariate analyses demonstrated that only M stage (adjusted HR = 6.70; 95%CI: 1.58-24.49;  $P = 0.010$ ) and final stage (adjusted HR = 10.78; 95%CI: 2.69-43.22;  $P = 0.001$ )

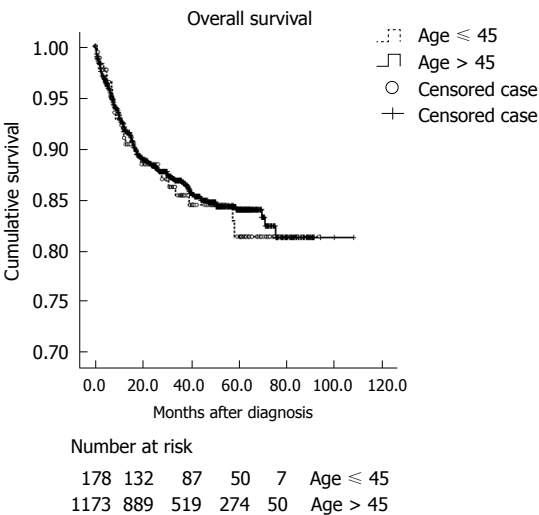


Figure 2 Cumulative probabilities of overall survival based on age (log-rank test,  $P = 0.780$ ).

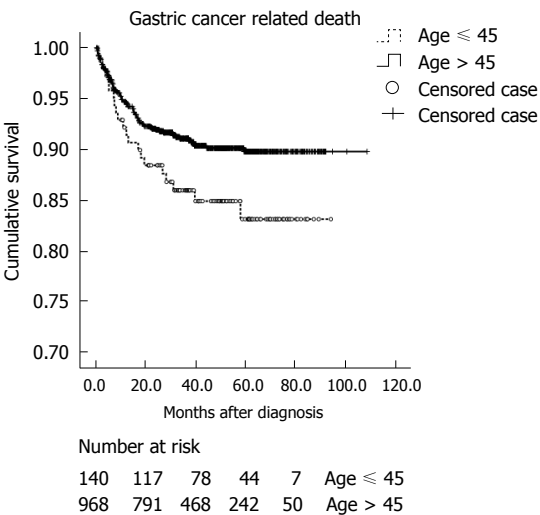


Figure 3 Cumulative probabilities of cancer-related death based on age (log-rank test,  $P = 0.048$ ).

Table 5 Molecular pathology of gastric cancer <i>n</i> (%)				
Variable	Age ≤ 45 yr ( <i>n</i> = 202)	Age > 45 yr ( <i>n</i> = 1456)	<i>P</i> value	
p53			< 0.001	
Negative	123 (60.9)	681 (46.8)		
Positive	44 (21.8)	560 (38.5)		
MSI			0.006	
Stable	85 (42.1)	566 (38.9)		
MSI-L	4 (2.0)	43 (3.0)		
MSI-H	1 (0.5)	79 (5.4)		
HER-2 status			0.006	
Negative	78 (38.6)	528 (36.3)		
Positive	6 (3.0)	125 (8.6)		
EGFR status			0.899	
Negative	94 (46.5)	674 (46.3)		
Positive	12 (5.9)	99 (6.8)		

EGFR: Epidermal growth factor receptor; HER-2: Human epidermal growth factor receptor-2; MSI: Microsatellite instability; MSI-H: MSI-high; MSI-L: MSI-low.

were independent risk factors for mortality.

## DISCUSSION

This retrospective study of 1658 gastric cancer patients indicates that clinicopathologic features, such as being female, Borrmann type IV AGC, and diffuse type pathology, were more commonly associated with the younger group of patients. Gastric cancers from patients ≤ 45 years of age exhibited more advanced stages than from patients > 45 years, but younger patients received more aggressive treatment such as palliative resection and chemotherapy. These findings are in agreement with previous studies<sup>[6-8,11]</sup>.

One of the most intriguing implications of the results is that the pathogenesis of gastric cancer may differ between age groups. *H. pylori* infection is most commonly acquired in children<sup>[21]</sup>, and with increasing age, the stomach changes stepwise

Table 6 <i>Helicobacter pylori</i> and associated changes of gastric cancer				
Variable	Age ≤ 45 yr ( <i>n</i> = 202)	Age > 45 yr ( <i>n</i> = 1456)	<i>P</i> value	
<i>Helicobacter pylori</i> grade	0.8 ± 1.0	0.6 ± 0.9	0.012	
Neutrophil infiltration	1.4 ± 0.9	1.4 ± 0.9	0.866	
Mononuclear cell infiltration	1.9 ± 0.5	1.9 ± 0.5	0.679	
Atrophic gastritis	0.5 ± 0.7	1.0 ± 0.9	< 0.001	
Intestinal metaplasia	0.5 ± 0.7	1.0 ± 0.8	< 0.001	

Scored according to the Updated Sydney System: 0 (absent), 1 (mild), 2 (moderate), and 3 (marked).

from atrophic gastritis, intestinal metaplasia, p53 alteration, and dysplasia, to intestinal-type gastric adenocarcinoma; this transition is known as Correa's cascade<sup>[22]</sup>. Therefore, the presence of higher degrees of atrophic gastritis and intestinal metaplasia, and increased incidence of p53 overexpression, adenoma, and intestinal-type gastric cancer observed in older patients of this cohort largely corroborates this model. Higher grade *H. pylori* infection in the absence of precancerous changes in younger patients from the cohort, however, does not support this model. In the majority of cases from this cohort, the grade of *H. pylori* infection was evaluated from resected cancer specimens, which were primarily located in the distal third of the stomach of older patients (51.7%). As atrophic gastritis and intestinal metaplasia were more common in older patients, the degree of *H. pylori* infection as determined from pathologic specimens could be underestimated<sup>[23]</sup>. In fact, positivity of *H. pylori* determined from serology, pathology, and the rapid urease test did not differ between age groups (64.7% vs 62.4% in younger and older patients, respectively). The results of the current study indicate that gastric cancer in older patients tends to progress through a series of sequential changes starting with

**Table 7** Univariate and multivariate analyses of predictors for mortality

Parameter	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age ( $\leq 45$ / $> 45$ yr)	1.10 (0.72-1.66)	0.664		
Gender (male/female)	0.87 (0.63-1.19)	0.384		
Curative resection (no/yes)	6.38 (4.33-9.40)	$< 0.001$		
CEA	1.00 (1.00-1.00)	$< 0.001$		
CA19-9	1.00 (1.00-1.00)	$< 0.001$		
Size, cm	1.24 (1.20-1.28)	$< 0.001$		
Pathology				
Intestinal	1.00 (reference)			
Diffuse	2.77 (2.03-3.78)	$< 0.001$		
Mixed	1.95 (0.89-4.26)	0.095		
T stage (III, IV <i>vs</i> I, II)	26.07 (16.42-41.40)	$< 0.001$		
N stage (N+ <i>vs</i> N-)	21.29 (12.81-35.36)	$< 0.001$		
M stage (M+ <i>vs</i> M-)	42.17 (30.75-57.84)	$< 0.001$	6.70 (1.58-24.49)	0.010
Stage (III, IV <i>vs</i> I, II)	46.39 (30.75-69.98)	$< 0.001$	10.78 (2.69-43.22)	0.001
Lymphatic invasion (yes/no)	11.84 (7.45-18.83)	$< 0.001$		
Venous invasion (yes/no)	14.75 (9.95-21.87)	$< 0.001$		
Perineural invasion (yes/no)	14.34 (9.42-251.85)	$< 0.001$		
Atrophic gastritis	1.06 (0.77-1.45)	0.736		
Intestinal metaplasia	1.00 (0.76-1.30)	0.977		
p53 (positive/negative)	1.45 (0.99-2.12)	0.058		
MSI (MSI-L <i>vs</i> stable)	1.30 (0.53-3.21)	0.565		
MSI (MSI-H <i>vs</i> stable)	0.82 (0.53-1.76)	0.602		
HER-2 (positive/negative)	0.57 (0.26-1.25)	0.158		
EGFR (positive/negative)	0.66 (0.27-1.65)	0.377		

CA 19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; EGFR: Epidermal growth factor receptor; HER-2: Human epidermal growth factor receptor-2; MSI: Microsatellite instability; MSI-H: MSI-high; MSI-L: MSI-low.

*H. pylori* infection and leading to intestinal-type gastric cancer. In younger patients, however, a gastric cancer of a diffuse pathology was more prevalent. To the best of our knowledge, this study is the first to reveal potential age-associated biologic differences in gastric cancer and its development.

The results also highlight important differences in the molecular pathology of gastric cancer from the two groups. First, a higher incidence of MSI was detected in tumors from older patients. These results agree with a previous report that demonstrated an increased frequency of MSI specifically in gastric cancers with an antral location, intestinal pathology, and lower incidence of lymph node metastasis<sup>[17]</sup>. Second, while overexpression of HER-2 has been reported to predict poor prognosis in gastric cancer patients<sup>[24,25]</sup>, an association between HER-2 and age has not yet been identified. The results of the current study indicate that overexpression of HER-2 in gastric cancer is in fact more common in older patients.

Interestingly, while younger patients exhibited a more advanced stage of gastric cancer, the overall mortality rate did not differ between the two age groups in this cohort. Younger patients did have a higher cumulative rate of cancer-related death compared to the older age group. However, this difference between the two age groups was not statistically significant once the data was adjusted for the stage of gastric cancer. Thus, these findings suggest that survival is not associated with age, which is in agreement with previous studies<sup>[6-8]</sup>. At the same

time, treatment differences do exist between the two groups. For example, in spite of the higher stage of gastric cancer, younger patients received more palliative resections than older patients. Because it is possible that palliative gastrectomy could improve overall survival<sup>[26,27]</sup>, aggressive treatment in younger patients might have extended their overall survival. However, despite potential advantage in treatment strategies in younger patients, gastric cancer-related death did not differ between the two groups when adjusted by stage. Further support for this conclusion is gained from the results that only stage and distant metastasis could predict mortality, whereas age was not found to be an independent risk factor in a Cox proportional hazards model. Therefore, other factors, such as the diffuse pathology or size, might more strongly influence overall survival.

Several limitations are inherent in this study, primarily because of the retrospective design. First, molecular pathology was not performed on all tumors. Results for MSI are particularly inconsistent, as the method described here has been applied to tumor samples starting only in the year 2007. Second, fluorescence *in situ* hybridization (FISH) is required to validate IHC scores of 2+ for the accurate diagnosis of the overexpression of HER-2<sup>[28]</sup>. As FISH was not performed on tumor sections from most patients, overexpression of HER-2 was determined based on IHC results alone. Third, the influence of chemotherapeutic treatment or specific protocol was not evaluated. A greater proportion of younger patients received



chemotherapy than older patients, and furthermore, different regimens could affect survival.

Nevertheless, our study presents several novel findings. To the best of our knowledge, this study is the first to identify differences based on age in the molecular pathology and *H. pylori*-associated precancerous changes of gastric cancer. Therefore, a novel concept on the basis of these results is that the disease pathogenesis differs between the two groups. However, additional studies are necessary to validate the role of *H. pylori* in disease progression, as well as the accompanying molecular changes in gastric cancer, of younger patients.

In conclusion, gastric cancer in younger and older patients differed in clinical characteristics, pathology, and molecular pathology. Although gastric cancer in younger patients often presented with more aggressive features, the primary factor in predicting prognosis was the stage of the gastric cancer and not the age of the patient.

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## COMMENTS

### Background

Clinicopathologic features differ between young and old patients with gastric cancer. Young patients predominantly are female, and their cancers display a more poorly differentiated pathology and advanced stage compared to older patients.

### Research frontiers

Molecular differences and *Helicobacter pylori* (*H. pylori*)-associated changes, such as atrophic gastritis and intestinal metaplasia, have not yet been elucidated based on age. Whether prognosis is related to age also remains controversial. Therefore, this study aimed to illuminate differences in clinicopathologic, molecular, and biologic characteristics of gastric cancer associated with age.

### Innovations and breakthroughs

Although gastric cancers in younger patients displayed more aggressive features than in older patients, cancer-related mortality did not differ between the two age groups after adjustment for the stage of cancer. Gastric cancer in younger patients was less frequently associated with atrophic gastritis, intestinal metaplasia, overexpression of p53, and microsatellite instability than in older patients.

### Applications

The significant differences in the pathologic degree of precancerous lesions and in molecular pathology indicated a distinct pathogenesis of the disease associated with age. The results are consistent with the model that gastric cancer in older patients tends to follow a dynamic series of sequential changes initiated by *H. pylori* infection and leading to intestinal-type gastric cancer. As diffuse-type gastric cancer predominated in younger patients, molecular changes due to factors other than *H. pylori* infection may be more important in pathogenesis in patients  $\leq 45$  years of age. Based on these results, prevention or tailored therapy based on age could be considered in the future.

### Terminology

Gastric cancer patients were stratified as younger or older according to the age of 45 years.

### Peer-review

This is a very interesting article that discusses a little population studied in

gastric cancer where was observed an increase in the disease especially in the West. A population of a considerable volume center was analyzed, complementing clinical aspects with molecular variables and even preneoplastic lesions.

## REFERENCES

- 1 Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]
- 2 Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1893-1907 [PMID: 20647400 DOI: 10.1158/1055-9965.EPI-10-0437]
- 3 Wu CW, Tsay SH, Hsieh MC, Lo SS, Lui WY, P'eng FK. Clinicopathological significance of intestinal and diffuse types of gastric carcinoma in Taiwan Chinese. *J Gastroenterol Hepatol* 1996; **11**: 1083-1088 [PMID: 8985835]
- 4 Theuer CP, de Virgilio C, Keese G, French S, Arnell T, Tolmos J, Klein S, Powers W, Oh T, Stabile BE. Gastric adenocarcinoma in patients 40 years of age or younger. *Am J Surg* 1996; **172**: 473-46; discussion 473-46; [PMID: 8942547 DOI: 10.1016/S0002-9610(96)00223-1]
- 5 Kath R, Fiehler J, Schneider CP, Höffken K. Gastric cancer in very young adults: apropos four patients and a review of the literature. *J Cancer Res Clin Oncol* 2000; **126**: 233-237 [PMID: 10782897]
- 6 Park JC, Lee YC, Kim JH, Kim YJ, Lee SK, Hyung WJ, Noh SH, Kim CB. Clinicopathological aspects and prognostic value with respect to age: an analysis of 3,362 consecutive gastric cancer patients. *J Surg Oncol* 2009; **99**: 395-401 [PMID: 19347884 DOI: 10.1002/jso.21281]
- 7 Hsieh FJ, Wang YC, Hsu JT, Liu KH, Yeh CN. Clinicopathological features and prognostic factors of gastric cancer patients aged 40 years or younger. *J Surg Oncol* 2012; **105**: 304-309 [PMID: 22116742 DOI: 10.1002/jso.22084]
- 8 Kulig J, Popiela T, Kolodziejczyk P, Sierzega M, Jedrys J, Szczepanik AM. Clinicopathological profile and long-term outcome in young adults with gastric cancer: multicenter evaluation of 214 patients. *Langenbecks Arch Surg* 2008; **393**: 37-43 [PMID: 17618451 DOI: 10.1007/s00423-007-0208-z]
- 9 Moreira H, Pinto-de-Sousa J, Carneiro F, Cardoso de Oliveira M, Pimenta A. Early onset gastric cancer no longer presents as an advanced disease with ominous prognosis. *Dig Surg* 2009; **26**: 215-221 [PMID: 19468231 DOI: 10.1159/000219331]
- 10 Eguchi T, Takahashi Y, Yamagata M, Kasahara M, Fujii M. Gastric cancer in young patients. *J Am Coll Surg* 1999; **188**: 22-26 [PMID: 9915238]
- 11 Smith BR, Stabile BE. Extreme aggressiveness and lethality of gastric adenocarcinoma in the very young. *Arch Surg* 2009; **144**: 506-510 [PMID: 19528381]
- 12 The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest Endosc* 2003; **58**: S3-43 [PMID: 14652541]
- 13 Kajitani T. The general rules for the gastric cancer study in surgery and pathology. Part I. Clinical classification. *Jpn J Surg* 1981; **11**: 127-139 [PMID: 7300058]
- 14 Washington K. 7th edition of the AJCC cancer staging manual: stomach. *Ann Surg Oncol* 2010; **17**: 3077-3079 [PMID: 20882416 DOI: 10.1245/s10434-010-1362-z]
- 15 Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histological classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675]
- 16 Stolte M, Meining A. The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. *Can J Gastroenterol* 2001; **15**: 591-598 [PMID: 11573102]
- 17 Wu MS, Lee CW, Shun CT, Wang HP, Lee WJ, Chang MC, Sheu JC, Lin JT. Distinct clinicopathologic and genetic profiles in sporadic gastric cancer with different mutator phenotypes. *Genes Chromosomes Cancer* 2000; **27**: 403-411 [PMID: 10719371]

- 18 **Choi JS**, Kim MA, Lee HE, Lee HS, Kim WH. Mucinous gastric carcinomas: clinicopathologic and molecular analyses. *Cancer* 2009; **115**: 3581-3590 [PMID: 19479974 DOI: 10.1002/cncr.24422]
- 19 **Seo YH**, Joo YE, Choi SK, Rew JS, Park CS, Kim SJ. Prognostic significance of p21 and p53 expression in gastric cancer. *Korean J Intern Med* 2003; **18**: 98-103 [PMID: 12872447]
- 20 **Kim JY**, Shin NR, Kim A, Lee HJ, Park WY, Kim JY, Lee CH, Huh GY, Park do Y. Microsatellite instability status in gastric cancer: a reappraisal of its clinical significance and relationship with mucin phenotypes. *Korean J Pathol* 2013; **47**: 28-35 [PMID: 23483099 DOI: 10.4132/KoreanJPathol.2013.47.1.28]
- 21 **Pounder RE**, Ng D. The prevalence of Helicobacter pylori infection in different countries. *Aliment Pharmacol Ther* 1995; **9** Suppl 2: 33-39 [PMID: 8547526]
- 22 **Correa P**, Haenszel W, Cuello C, Tannenbaum S, Archer M. A model for gastric cancer epidemiology. *Lancet* 1975; **2**: 58-60 [PMID: 49653]
- 23 **El-Zimaity HMT O**, Kim JG, Akamatsu T, Güler IE, Simjee AE, Graham DY. Geographic differences in the distribution of intestinal metaplasia in duodenal ulcer patients. *Am J Gastroenterol* 2001; **96**: 666-672 [PMID: 11280531 DOI: 10.1111/j.1572-0241.2001.03601.x]
- 24 **Chen C**, Yang JM, Hu TT, Xu TJ, Yan G, Hu SL, Wei W, Xu WP. Prognostic role of human epidermal growth factor receptor in gastric cancer: a systematic review and meta-analysis. *Arch Med Res* 2013; **44**: 380-389 [PMID: 23871709 DOI: 10.1016/j.arcmed.2013.07.001]
- 25 **Terashima M**, Kitada K, Ochiai A, Ichikawa W, Kurahashi I, Sakuramoto S, Katai H, Sano T, Imamura H, Sasako M. Impact of expression of human epidermal growth factor receptors EGFR and ERBB2 on survival in stage II/III gastric cancer. *Clin Cancer Res* 2012; **18**: 5992-6000 [PMID: 22977193 DOI: 10.1158/1078-0432.CCR-12-1318]
- 26 **Lasithiotakis K**, Antoniou SA, Antoniou GA, Kaklamanos I, Zoras O. Gastrectomy for stage IV gastric cancer. a systematic review and meta-analysis. *Anticancer Res* 2014; **34**: 2079-2085 [PMID: 24778009]
- 27 **Sun J**, Song Y, Wang Z, Chen X, Gao P, Xu Y, Zhou B, Xu H. Clinical significance of palliative gastrectomy on the survival of patients with incurable advanced gastric cancer: a systematic review and meta-analysis. *BMC Cancer* 2013; **13**: 577 [PMID: 24304886 DOI: 10.1186/1471-2407-13-577]
- 28 **Hofmann M**, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, Ochiai A, Rüschoff J, Henkel T. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 2008; **52**: 797-805 [PMID: 18422971 DOI: 10.1111/j.1365-2559.2008.03028.x]

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## Retrospective Study

# Medical training fails to prepare providers to care for patients with chronic hepatitis B infection

Stephanie D Chao, Bing-Mei Wang, Ellen T Chang, Li Ma, Samuel K So

Stephanie D Chao, Department of Surgery, Stanford University School of Medicine, Asian Liver Center at Stanford University, School of Medicine, Palo Alto, CA 94304, United States  
 Stephanie D Chao, Bing-Mei Wang, Ellen T Chang, Li Ma, Samuel K So, Asian Liver Center at Stanford University, Stanford University School of Medicine, Asian Liver Center at Stanford University School of Medicine, Palo Alto, CA 94304, United States

Ellen T Chang, Division of Epidemiology, Department of Health Research and Policy, Stanford University School of Medicine, Stanford, CA 94305, United States

**Author contributions:** Chao SD and So SK designed the research; Chao SD and Wang BM performed the research; Chao SD, Wang BM and Chang ET analyzed the data; Chao SD, Wang BM and Ma L wrote the paper; all authors have read and approved the final version to be published.

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**Correspondence to:** Stephanie D Chao, MD, Assistant Professor, Department of Surgery, Stanford University School of Medicine, Asian Liver Center at Stanford University, School of Medicine, 780 Welch Road, CJ 130, Palo Alto, CA 94304, United States. [stephanie.chao@stanford.edu](mailto:stephanie.chao@stanford.edu)  
 Telephone: +1-650-5668861  
 Fax: +1-650-5668863

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## Abstract

**AIM:** To investigate physicians' knowledge including chronic hepatitis B (CHB) diagnosis, screening, and management in various stages of their training.

**METHODS:** A voluntary 20-question survey was administered in Santa Clara County, CA where Asian and Pacific Islanders (API) account for a third of the population. Among the 219 physician participants, there were 63 interns, 60 second-year residents, 26 chief residents and 70 attending physicians. The survey asked questions regarding respondents' demographics, general hepatitis B virus knowledge questions (*i.e.*, transmission, prevalence, diagnostic testing, prevention, and treatment options), as well as, self-reported practice behavior and confidence in knowledge.

**RESULTS:** Knowledge about screening and managing patients with CHB was poor: only 24% identified the correct tests to screen for CHB, 13% knew the next steps for patients testing positive for CHB, 18% knew the high prevalence rate among API, and 31% knew how to screen for liver cancer. Wald chi-square analysis determined the effect of training level on knowledge; in all cases except for knowledge of liver cancer screening ( $P = 0.0032$ ), knowledge did not significantly increase with length in residency training or completion of residency.

**CONCLUSION:** Even in a high-risk region, both medical school and residency training have not adequately

prepared physicians in the screening and management of CHB.

**Key words:** Liver cancer; Hepatitis B; Asian Pacific Islander; Liver disease; Health disparity

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**Core tip:** Chronic hepatitis B (CHB) affects 1.25 million Americans and CHB disproportionately impacting the Asian population. The Centers for Disease Control and Prevention recommends routine preventive screening for high-risk populations. However, our study demonstrates that our system of medical training may not adequately train providers how to screen high-risk patients, who to screen for CHB, or how to manage patients who test positive for CHB. Physician knowledge is poor overall and does not improve during medical training. Prompt attention is needed to reduce the burden of chronic liver disease and liver cancer in the high prevalence Asian and Pacific Islander Population.

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## INTRODUCTION

Chronic hepatitis B infection (CHB) remains a global epidemic resulting in significant morbidity and mortality. An estimated 240-360 million people worldwide are chronically infected with hepatitis B virus (HBV), resulting in nearly 800000 deaths annually from CHB-related liver disease<sup>[1-3]</sup>. A vaccine that is safe, effective, and cost-saving has been available for over 30 years. However, global disease burden remains high and CHB continues to be the leading cause of liver cancer and chronic liver disease globally. In the United States, the major risk factor for chronic hepatitis B is foreign born in an endemic country where many became chronically infected at an early age. An estimated 1.3 million foreign born persons have CHB, and Asian and Pacific Islander Americans (API) are disproportionately affected<sup>[4]</sup>. There is a greater than 50-fold difference in CHB prevalence between the API and White non-Hispanic population<sup>[5]</sup>. The Institute of Medicine report estimates that two-thirds are not aware of their infection<sup>[6]</sup>. Without receiving care and treatment, 15%-25% will die as a result of disease progression. The Centers for Disease Control and Prevention (CDC) and the United States Preventive Services Task Force (USPSTF) recommended HBV screening of all individuals born in countries with HBV

prevalence  $\geq 2\%$ <sup>[7,8]</sup>. Our group has demonstrated that HBV screening of API, treatment of the infected and ring vaccination of household contact is a cost-effective intervention<sup>[9]</sup>.

Although early detection of CHB offers the best opportunity for prevention and treatment<sup>[7,8]</sup>, screening rates among API remain extremely low<sup>[10]</sup>. It is incumbent on physicians to recognize the importance of routine screening and how to screen this high prevalence population. Yet physicians' knowledge remains deficient and adherence to screening guidelines is inadequate. Even among API physicians serving predominantly API patient populations, 50% did not routinely screen API patients for HBV infection and most reported less than half of their API patients had been immunized against HBV<sup>[10]</sup>. While many studies have demonstrated the inadequacy of HBV knowledge among established providers<sup>[10-12]</sup>, no studies have assessed attainment of HBV knowledge as trainees progress through medical school and residency training.

In this study we examine the incremental gains in knowledge regarding CHB screening and management by surveying physicians at all levels of training from recent medical school graduates to attending physicians. We hypothesize that as providers progress through the medical education system: (1) HBV knowledge would improve; and (2) providers would report better adherence to screening, diagnosis, referral and treatment guidelines as delineated by the CDC and the American Association for the Study of Liver Diseases (AASLD)<sup>[10]</sup>.

## MATERIALS AND METHODS

### Recruitment

Between June and October 2009, physicians were recruited from a tertiary care academic center, as well as, a local continuing medical education conference both located in Santa Clara County, CA. API account for nearly 35% of the population of Santa Clara County. Providers were eligible if their area of practice was in one of five medical specialties: internal medicine, family medicine, pediatrics, obstetrics and gynecology, and surgery. Subjects were recruited from the following physician cohort by training level: incoming interns, outgoing interns, outgoing residents, and attending physicians. The study protocol was approved by the Stanford University Institutional Review Board and all subjects provided informed signed consent. Participants were offered a nominal incentive for study participation. Participants were also offered the option of receiving additional educational materials regarding CHB after survey participation.

### Survey instrument

A voluntary written survey that comprised of 20 questions was administered in-person or *via* a web-based survey platform. Survey responses were anony-



**Table 1** Distribution of demographic and professional characteristics among respondents *n* (%)

Characteristic	Value <sup>1</sup>
Age (yr)	
24-27	65 (30)
28-31	68 (32)
32-39	43 (20)
40+	39 (18)
Median	
Sex	
Male	99 (47)
Female	113 (53)
Racial/ethnic background	
White, non-Hispanic	78 (38)
Chinese or Taiwanese	22 (11)
South Asian	21 (10)
Other or unspecified Asian or Pacific Islander	43 (21)
Black or African American	17 (8)
Hispanic or Latino	13 (6)
Other or mixed	14 (7)
Level of training	
Incoming intern	63 (29)
Outgoing intern	60 (27)
Senior resident	26 (12)
Attending physician	70 (32)
Specialty	
Internal medicine	88 (40)
Family medicine	8 (4)
Pediatrics	42 (19)
Obstetrics and gynecology	10 (5)
General surgery	22 (10)
Emergency medicine	10 (5)
Psychiatry	8 (4)
Other	31 (14)
Practice setting	
Academic practice	170 (78)
Private practice	28 (13)
Other or multiple	19 (9)

<sup>1</sup>Summed frequencies > 100 are due to spreadsheet rounding. Missing data are excluded.

mous and assigned random identifiers. The survey asked questions regarding respondents' demographics, general HBV knowledge questions (*i.e.*, transmission, prevalence, diagnostic testing, prevention, and treatment options), as well as, self-reported practice behavior and confidence in knowledge. Confidence questions were based on a five-point Likert Scale. The survey also contained internal controls to establish baseline medical knowledge and practices (*i.e.*, regarding breast cancer and colon cancer screening, smoking cessation).

### Statistical analysis

All data were de-identified and pooled for analysis. Our objectives were to describe knowledge and management characteristics of surveyed physicians and compare these characteristics across different levels of clinical training. Pooled descriptive statistics regarding HBV knowledge and management practices were calculated for each cohort: incoming interns, outgoing interns, outgoing residents, and attending

physicians. Individual subjects were assigned knowledge scores based on total number of correct responses for knowledge questions.

Associations between clinical training level and specific HBV knowledge (*i.e.*, a correct response to each HBV-related knowledge question) were estimated as relative risks (odds ratios) using multivariable logistic regression models adjusted for continuous age. Additional characteristics such as gender, race/ethnicity, and clinical specialty were also included in multivariable models if they were significantly associated with the outcome. Internal control questions regarding counseling about smoking cessation and colon and breast cancer screening were used as indicators of positive general preventive care practices ("Good Screeners") and similarly included in multivariable models. Statistical significance was defined as a two-sided *P*-value  $\leq 0.05$ . Associations between clinical training level and total HBV knowledge were estimated using multivariable linear regression models adjusted for age and additional physician characteristics that were significantly associated with overall HBV knowledge. Analyses were conducted using SAS version 9.2.

## RESULTS

### Demographics

This study recruited a total of 219 physician participants. Participants had a median age of thirty years. The distribution of specialties was 40% internal medicine (*n* = 88), 4% family medicine (*n* = 8), 19% pediatrics (*n* = 42), 5% obstetrics and gynecology (*n* = 10), 10% general surgery (*n* = 22), and 23% other (emergency medicine, psychiatry, *etc.*). Of the respondents, 29% were new interns (*n* = 63), 27% were outgoing interns (*n* = 60), 12% were outgoing residents (graduating senior residents) (*n* = 26), and 32% were attending physicians (*n* = 70). Of attending physicians surveyed, 31 reported an academic practice setting vs 39 who reported a non-academic practice (Table 1).

### HBV knowledge questions

Only 24% of physicians surveyed could identify the appropriate serologic test to screen for chronic hepatitis B and only 41% could identify all the modes of HBV transmission. Although most correctly answered HBV infection was vaccine preventable (99%), only about half of physicians would vaccinate children and household contacts of those with CHB and only 61% reported being confident about who should be vaccinated against HBV. Less than half (49%) of respondents were able to identify API as having a higher relative CHB prevalence rate. Furthermore, only 13% of physicians knew the proper next steps to refer patients who tested positive for hepatitis B. Over half of the physicians (56%) did not know they are

**Table 2** Correct responses to hepatitis B knowledge assessment

	Number	Percent	Percent <sup>1</sup>
Through which of the following can HBV be transmitted?	89	41%	47%
Which blood test(s) would you order to screen for chronic hepatitis B infection?	52	24%	20%
Which blood test(s) would you order to confirm immunity to hepatitis B after vaccination?	153	70%	73%
Which of the following viral hepatitis infections can be prevented by immunization?	160	73%	77%
According to California law, who is/are required to report new hepatitis B diagnoses?	31	14%	11%
A patient has been told by a previous physician that he is a healthy hepatitis B carrier. What are the appropriate next steps, if any?	28	13%	16%
Which of the following patient groups has the highest prevalence of chronic hepatitis B?	137	63%	64%
Asians > Caucasians, African Americans, and Hispanics/Latinos (CORRECT RESPONSE)	107	49%	
Which of the following is most likely to result in chronic infection with hepatitis B?	105	48%	52%
Which of the following conditions can be caused by chronic infection with hepatitis B?	173	79%	83%
What are the symptoms of most patients with chronic hepatitis B?	138	63%	69%
Without proper monitoring, what is the chance of dying from chronic hepatitis B?	35	26%	18%
Is there a cure for chronic hepatitis B?	194	89%	91%
Is there a treatment for chronic hepatitis B?	191	87%	89%
According to the American Association for the Study of Liver Diseases (AASLD) guidelines, which of the following are appropriate screening tests for liver cancer?	68	31%	34%

<sup>1</sup>Adjusted for positive control measuring baseline cancer screening knowledge and self-reported counseling practices.

**Table 3** Physician self-reported practices *n* (%)

Question	Value
Do you routinely screen asymptomatic patients for chronic hepatitis B in your practice?	
Yes	52 (24)
Would you screen for chronic hepatitis B in an HIV-positive individual?	
Yes <sup>1</sup>	208 (95)
No	3 (1)
Don't know	8 (4)
Would you screen for chronic hepatitis B in a 40-yr-old with a history of hemophilia?	
Yes <sup>1</sup>	167 (76)
No	39 (18)
Don't know	13 (6)
Would you screen for chronic hepatitis B in a healthy 28-yr-old man born in China?	
Yes <sup>1</sup>	156 (71)
No	57 (26)
Don't know	6 (3)
Would you screen for chronic hepatitis B in a Caucasian woman who travels frequently to Central America?	
Yes	71 (32)
No <sup>1</sup>	133 (61)
Don't know	15 (7)
Would you screen for chronic hepatitis B in a cafeteria food server during a pre-employment physical?	
Yes	86 (39)
No <sup>1</sup>	121 (55)
Don't know	12 (5)

<sup>1</sup>Indicates correct response.

mandated by California law to report seropositive test results to county public health departments (Table 2).

While most physicians recognized that CHB can result in severe liver disease (79%), just 26% recognized the high mortality rate associated with CHB. Only 31% could identify the proper screening tests for liver cancer and 61% of physicians did not know when a CHB patient should be referred to a specialist (Table 2). Eighty percent did not feel well

prepared by their medical training to care for patients with CHB. Only 24% of providers routinely screen asymptomatic individuals for CHB (Table 3).

### Provider confidence

This lack of knowledge is reflected in providers' self-reported confidence level in managing patients with CHB. Table 4 illustrates the physician confidence level in providing care of CHB patients. Only 31% reported feeling confident about knowing who to screen for CHB, as signified by answering "agree" or "strongly agree" to the survey question. Although 61% felt comfortable knowing who to vaccinate against HBV, only 38% reported knowing when it would be appropriate to refer a CHB patient to a specialist and only 20% felt well trained by residency to care for CHB patients.

### Comparisons of physicians' knowledge

Table 5 compares the likelihood of a correct response across training levels for select knowledge questions. In all questions assessing CHB knowledge, except for knowledge of liver cancer screening ( $P = 0.0032$ ), there was no significant effect of years of medical training on likelihood of answering correctly.

Table 6 compares the overall knowledge between the cohorts. In order to compare knowledge between cohorts, each subject's knowledge was assessed based on the number of questions answered correctly (out of a possible total score of 24). The mean knowledge score was 11.6/24 among incoming interns, 11.5/24 among outgoing interns, 12.2/24 among outgoing residents and 10.6/24 among attending physicians. The overall average was 11.3/24, or 47% correct. Overall, senior residents scored marginally higher than other cohorts, including attending physicians (+1.7 difference in knowledge score when compared with incoming interns,  $P = 0.01$ ).

**Table 4** Distribution of responses to questions about confidence in hepatitis B knowledge *n* (%)

Question	Response	Value
I am confident that I know who should be screened for chronic hepatitis B	Strongly disagree	11 (5)
	Disagree	60 (27)
	Neutral	78 (36)
	Agree	59 (27)
	Strongly agree	8 (4)
	Did not reply	3 (1)
I am confident that I know who should be vaccinated for chronic hepatitis B	Strongly disagree	7 (3)
	Disagree	23 (11)
	Neutral	52 (24)
	Agree	104 (47)
	Strongly agree	31 (14)
	Did not reply	3 (1)
I am confident that I know when a patient with chronic hepatitis B should be referred to a specialist	Strongly disagree	5 (2)
	Disagree	57 (26)
	Neutral	72 (33)
	Agree	67 (31)
	Strongly agree	16 (7)
	Did not reply	2 (1)
	Very poorly	8 (4)
	Not well	65 (30)
	Neutral	101 (46)
	Fairly well	41 (19)
	Very well	2 (1)
	Did not reply	2 (1)

Questions that assessed specific aspects of CHB management (*i.e.*, screening, vaccination) were grouped together in Table 6 to assess if cohorts had more knowledge in a particular aspect of CHB management. We used the generalized least squares method to compare total knowledge score, screening knowledge score, and vaccination knowledge score. Total hepatitis B knowledge score between incoming interns and outgoing residents showed marginal improvement ( $P = 0.01$ ), however no difference was observed between incoming interns and attending physicians. Additionally, no difference was noted among cohorts specifically pertaining to screening knowledge or vaccination knowledge (Table 6). In fact, older age was inversely correlated with knowledge score ( $P = 0.003$ ).

There was a trend towards significance ( $RR = 1.7$ ,  $P = 0.09$ ) in the association between provider ethnicity and recognition of increased prevalence of CHB among API. Self-identified API providers were more likely to respond correctly to relative prevalence of CHB infection (API vs all other ethnic groups).

#### **Correlation between physician knowledge and self-reported confidence levels**

Based on physician confidence levels reported in Table 4, sub-group analyses were performed to

assess for correlation between confidence level and actual knowledge. In sub-group analysis of those who reported confidence in knowing which populations to screen ("agree", "strongly agree"), confidence was not associated with correct responses for the proper screening test. Confidence in vaccination knowledge did not correlate with correct responses for the proper test to demonstrate serologic evidence of immunity. Confidence in screening knowledge and being a "Good Screener" was not associated with proper identification of hepatitis B surface antigen (HBsAg) as the most appropriate CHB screening test, nor was it associated with routinely screening asymptomatic individuals. Confidence in knowing who should be vaccinated HBV vaccination knowledge was not associated with identification of the proper test to confirm immunity after vaccination.

Confidence in knowledge about referral for chronic hepatitis B was associated with correct response to proper patient referral upon HBsAg seropositivity, ( $RR = 6.0$ ,  $P = 0.0001$ , Table 5). Confidence in screening knowledge and vaccination were also associated with a correct response, but not after adjusting for confidence in referral knowledge. The model adjusting for referral knowledge has the best "fit" among the models adjusting for any of the three confidence measures, indicating that referral confidence is associated with screening and vaccination confidence and also independently associated with correct response. Liver cancer screening knowledge improved with age and further training. However, "Good Screeners" did not demonstrate better knowledge for liver cancer screening.

## **DISCUSSION**

Chronic infection with hepatitis B and its sequelae continue to be major public health problems that all physicians will likely encounter and need to manage at some point in their practice. Early diagnosis through screening can lead to the prevention of devastating consequences like liver cancer and liver failure. The under-diagnosis, under-referral, and inadequate treatment of chronic hepatitis B are well-documented, especially in the API population<sup>[10-24]</sup>. Barriers to hepatitis B care in API can be distilled into three meaningful and interacting elements: resource-related, patient-related, and provider-related<sup>[25]</sup>. Resource-related barriers include lower per capita income, inadequate health care coverage, and underutilization of health services<sup>[15,25,26]</sup>. Patient-related barriers are associated with poor hepatitis B knowledge, low-English fluency, and the culture, beliefs, and attitudes surrounding hepatitis B and related care<sup>[22,27-29]</sup>. Provider-related barriers also revolve around physician knowledge, attitudes, and beliefs about hepatitis B and liver cancer in the API population<sup>[11]</sup>.

Our study identifies physician-related barriers,

**Table 5** Associations of training level and other characteristics with correct (compared with incorrect) responses to questions about hepatitis B knowledge and clinical practice

Question	Characteristic	Relative risk <sup>1</sup>	(95%CI)	P value
Which blood test(s) would you order to screen for chronic hepatitis B infection? <sup>2</sup>	Incoming intern	1.0	(Reference)	0.0001
	Outgoing intern	1.2	(0.5-3.1)	
	Senior resident	2.1	(0.7-6.1)	
	Attending	2.0	(0.7-5.7)	
Do you routinely screen asymptomatic patients for chronic hepatitis B in your practice? (yes vs no)	Incoming intern	1.0	(Reference)	
	Outgoing intern	1.1	(0.4-3.1)	
	Senior resident	1.5	(0.5-5.2)	
	Attending	1.5	(0.4-5.3)	
According to California law, who is/are required to report new hepatitis B diagnoses? <sup>2</sup>	Incoming intern	1.0	(Reference)	
	Outgoing intern	0.7	(0.3-2.1)	
	Senior resident	0.2	(0.02-1.6)	
	Attending	0.6	(0.1-2.4)	
A patient has been told by a previous physician that he is a healthy hepatitis B carrier. What are the appropriate next steps, if any? <sup>2</sup>	Incoming intern	1.0	(Reference)	
	Outgoing intern	1.8	(0.5-6.7)	
	Senior resident	1.4	(0.2-8.7)	
	Attending	0.9	(0.1-7.8)	
Not confident in knowledge about referral for chronic hepatitis B <sup>3</sup>		1.0	(Reference)	
Confident in knowledge about referral for chronic hepatitis B <sup>3</sup>		6.0	(2.4-15.0)	
What is the relative prevalence of chronic hepatitis B in Caucasians, African Americans, Asians, and Hispanics/Latinos? <sup>2</sup>	Incoming intern	1.0	(Reference)	
	Outgoing intern	0.8	(0.4-1.7)	
	Senior resident	1.8	(0.7-4.6)	
	Attending	1.2	(0.5-2.9)	
Which of the following is most likely to result in chronic infection with hepatitis B? <sup>2</sup>	Incoming intern	1.0	(Reference)	0.080
	Outgoing intern	2.0	(1.0-4.2)	
	Senior resident	3.3	(1.2-9.0)	
	Attending	1.9	(0.7-5.0)	
Without proper monitoring, what is the chance of dying from chronic hepatitis B? <sup>2</sup>	Incoming intern	1.0	(Reference)	
	Outgoing intern	0.8	(0.3-1.9)	
	Senior resident	0.3	(0.1-1.5)	
	Attending	0.6	(0.2-2.3)	
According to the American Association for the Study of Liver Diseases guidelines, which of the following are appropriate screening tests for liver cancer? <sup>2</sup>	Incoming intern	1.0	(Reference)	
	Outgoing intern	3.6	(1.5-8.7)	
	Senior resident	5.6	(1.9-16.4)	
	Attending	4.8	(1.6-14.9)	0.005
	Age (5-yr increase)	0.7	(0.5-0.9)	0.005

<sup>1</sup>All estimates adjusted for age (continuous years); <sup>2</sup>Questions included in calculation of total hepatitis B knowledge score; <sup>3</sup>"Confident": respondent "strongly agrees" or "agrees" that he or she is confident about knowledge; "not confident": respondent "disagrees," "strongly disagrees," or is neutral.

which include gaps in physician knowledge and attitudes which can impede appropriate screening and prevention. Only a minority of physicians we surveyed were able to correctly identify the proper screening tests for CHB infection. Moreover, even fewer physicians were able to identify the appropriate next steps in management once a diagnosis of CHB infection was made. Most physicians expressed a lack of confidence in screening and caring for patients with CHB infection. In our study, although most physicians recognized that the API population carried the highest burden of disease, the majority greatly underestimated the disease prevalence among API. Just 18% recognized that 1 in 10 API are chronically infected with HBV. When presented with clinical

case scenarios, our data show that providers were more likely to screen HIV-positive individuals (95% of respondents) and patients with hemophilia (76% of respondents) for CHB than a health 28-year-old man born in China (71% of respondents). Only 32% of providers surveyed reported feeling confident in knowing who to screen for CHB. These data suggest that providers may not be aware of racial, ethnic, or cultural differences in CHB risk, or may not be aware of the CDC screening guidelines<sup>[7,30,31]</sup>. Upadhyaya *et al.*<sup>[31]</sup> similarly reported that although providers were aware of the high prevalence of CHB in API, this awareness is not associated with higher rates of screening and detection<sup>[30]</sup>. In a national survey of racial and ethnic minorities in the US, persons at high risk of CHB (API



**Table 6** Differences in total hepatitis B knowledge scores by cohort

Cohort	Difference in score <sup>1</sup>	(95%CI)	P value
Total hepatitis b knowledge score			
Incoming intern	0.0	(Reference)	
Outgoing intern	0.1	(-0.9-1.2)	
Senior resident <sup>2</sup>	1.7	(0.4-3.0)	0.010
Attending Physician	0.9	(-0.4-2.2)	
Age (5-yr increase) <sup>2</sup>	-0.4	[-0.6-(-0.1)]	0.003
Responded incorrectly to other cancer screening questions and smoking cessation practices	0.0	(Reference)	
Responded correctly to other cancer screening questions and smoking cessation practices <sup>2</sup>	1.0	(0.2-1.8)	0.010
Age (5-yr increase)	-0.4	[-0.6-(-0.1)]	0.003
Hepatitis b screening knowledge score			
Incoming intern	0.0	(Reference)	
Outgoing intern	-0.2	(-0.6-0.3)	
Senior resident	0.5	(-0.1-1.5)	
Attending	0.3	(-0.3-1.0)	
Hepatitis b vaccination knowledge score			
Incoming intern	0.0	(Reference)	
Outgoing intern	0.1	(-0.8-1.2)	
Senior resident	0.1	(0.2-2.8)	
Attending	0.1	(-0.4-2.2)	

<sup>1</sup>All estimates adjusted for age (continuous years); <sup>2</sup>The P value < 0.05.

and foreign-born Americans) reported screening rates similar to persons who are at low risk, and 52% of foreign-born API reported having never been tested for CHB<sup>[32]</sup>.

Studies demonstrate that, in general, PCPs provide counseling and preventive services at alarmingly low rates. In a national survey, less than one-third of PCPs report routine assessment and vaccination of adults at risk of CHB infection<sup>[17]</sup>. In a survey by Daley et al, one-quarter of PCP respondents rate identification and vaccination of HBV a "low priority"<sup>[17]</sup>. While most physicians we surveyed recognized CHB as being vaccine-preventable, less than half recognized the importance of immunizing uninfected household contacts of CHB patients.

Studies consistently demonstrate poor follow-up in HBsAg positive patients by providers. Jung *et al*<sup>[16]</sup> reports less than 30% of CHB patients received further evaluation of their infection; in fact, this study revealed that API ethnicity was instead significantly associated with not receiving treatment. In another study of 2238 CHB patients, only 32% reported having been referred to a gastroenterologist or hepatologist<sup>[14]</sup>. Our data corroborates current literature by suggesting that low rates of treatment, liver cancer screening, and referral may be due to poor knowledge about appropriate follow-up procedure following CHB detection; only 16% of respondents were able to accurately describe the appropriate next steps for a patient previously diagnosed as a "healthy hepatitis B carrier". Only 34% were able to identify the appropriate screening tests for liver cancer according to the AASLD guidelines and just 11% of respondents knew how to report new hepatitis B diagnoses to local infectious disease control agencies. Perhaps this is due to the poor understanding of the devastating consequences of

untreated and unmonitored CHB infection. Most providers significantly underestimated the mortality risk associated with CHB.

To date, we are the only group to examine provider knowledge and practice based on training level. Across all training levels, from interns to attending physicians, most providers expressed a lack of confidence regarding which populations should be screened for CHB infection. Only 39% of providers surveyed were confident that they knew when to refer patients with chronic hepatitis B. Alarmingly, 80% of providers did not feel adequately prepared by their medical training to manage patients with CHB infection. This is corroborated by our observation that no differences in CHB knowledge and screening practices were observed between interns, senior residents, and attending physicians. This not only demonstrates a failure in our current model of physician training in chronic hepatitis B management, but suggests similar deficiencies may exist with other public health concerns.

We postulated whether poor CHB screening was indicative of poor preventive practices by providers in general, rather than due to poor HBV knowledge and awareness. Thus we separately analyzed those providers who self-reported good preventive medicine practices. We included internal control questions asking providers to select the most appropriate screening tests for breast and colon cancer, as well as, to report how frequently they counsel patients regarding smoking cessation. Providers who always advised patients to quit smoking and correctly identified breast and colon cancer screening practices were deemed "Good Screeners". However, Good Screeners were not significantly associated with increased knowledge, proper identification of HBsAg as the most appropriate CHB screening test, or improved screening of asymp-

tomatic individuals for CHB.

We believe this study is representative of overall deficiencies in provider knowledge, training, and practices as it pertains to CHB infection. Limitations of this study may include selection bias as all physicians were recruited from a single geographic region (Northern California) and trainees from a single academic medical center. However, we do not believe this region reflects a training model that is significantly different from national trends. In addition, this region has a larger API population among physicians and patients, which we would expect to result in slightly improved knowledge and sensitivity towards CHB screening. As with all surveys, generalizability is limited and conclusions are based on associations of responses. Respondents were assumed to have answered truthfully (*i.e.*, accurate reporting of level of training, ethnicity, field of practice), to carefully read and interpret the questions, and answers reflect actual practice.

Providers routinely cite inadequate knowledge as a barrier to appropriate management of CHB<sup>[18,31,33]</sup> which has been demonstrated by our study and others. In addition, we report low levels of provider knowledge that persist across increased levels of provider training. This is suggestive of the inadequate education provided by current medical training practices in regards to chronic hepatitis B infection, a disease which affects over one million Americans with an additional 40000 imported new cases each year<sup>[5]</sup>. We found that trainee confidence in their HBV-related knowledge remains low throughout graduate medical education, with no significant difference between training cohorts; 79% of respondents indicated that they did not feel their training adequately prepared them to manage patients with CHB infection. Foster *et al.*<sup>[11]</sup> examined how different information sources influence health behaviors among Asian immigrants. After controlling for age, sex, education, English proficiency, insurance coverage, proportion of life spent in the United States, and family history of CHB, learning about HBV from physicians had the strongest direct effect on positive screening behavior (*i.e.*, perceived benefits, severity, self-efficacy, and knowledge). The Department of Health and Human Service's Viral Hepatitis Action Plan (VHAP) identifies educating providers as a top priority to reduce the health disparity associated with CHB infection in the API population<sup>[34]</sup>. Better provider knowledge about CHB is associated with increased rates of screening and vaccination for CHB<sup>[10,18,20]</sup> and even decreased mortality from liver cancer and liver disease<sup>[35,36]</sup>. Toy reported that life long monitoring of CHB is cost effective, and adherence to monitoring and treatment could reduce CHB related deaths by 83%<sup>[36]</sup>. However, our current medical training paradigm is failing our providers and their patients.

In 2010, the Institute of Medicine released a landmark report identifying CHB as an important

public health issue requiring national attention. As a result of this report and the ratification of the Patient Protection and Affordable Care Act (ACA) in 2011, the Department of Health and Human Services released the VHAP, which highlights strategies to prevent, care for, and treat CHB and related liver-disease<sup>[6,34]</sup>. In 2014, the USPSTF also issued recommendation for hepatitis B screening of asymptomatic, non-pregnant adolescents and adults including those born in regions with  $\geq 2\%$  CHB prevalence and United States born persons who were unvaccinated at birth whose parents were born in high endemic countries including Asia<sup>[8]</sup>. This means that hepatitis B screening of the at risk population would be among the preventive services covered in the ACA.

Both the IOM report and the VHAP call for improved physician education. Our study highlights poor provider CHB screening practices and we suggest this is directly related to the lack of CHB education provided during formal medical training. It is imperative that we reform health care education to reduce CHB-related morbidity and mortality among our nation's rapidly growing immigrant communities.

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## COMMENTS

### Background

Chronic hepatitis B (CHB) affects 1.25 million Americans, CHB disproportionately impacting the Asian population. The Centers for Disease Control and Prevention recommends routine preventive screening for high-risk populations.

### Research frontiers

The authors have conducted a 20-question survey in 219 physicians in Santa Clara County, CA with regard of Knowledge about screening and managing patients with CHB.

### Innovations and breakthroughs

Physicians do not know who to screen for CHB - Physicians do not know how to manage patients who test positive for CHB - Physician knowledge is poor and does not improve during medical training

### Applications

This study calls on the reform health care education to reduce CHB-related morbidity and mortality among our nation's rapidly growing immigrant communities.

### Terminology

CHB is a viral infection caused by chronic hepatitis B virus that primarily infects liver.

### Peer-review

Insufficiency of medical training in the prevention and treatment of hepatitis B virus infection is one of the worldwide health problems. The data of this paper demonstrates that the same problem happens in United States. This topic is very important and useful for many countries as well as United States.

## REFERENCES

- 1 World Health Organization Hepatitis B fact sheet. Updated June

2014. Available from: URL: <http://www.who.int/mediacentre/factsheets/fs204/en/>
- 2 **Ott JJ**, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 2012; **30**: 2212-2219 [PMID: 22273662 DOI: 10.1016/j.vaccine.2011.12.116]
- 3 **Lavanchy D**. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *J Clin Virol* 2005; **34** Suppl 1: S1-S3 [PMID: 16461208]
- 4 **Kowdley KV**, Wang CC, Welch S, Roberts H, Brosgart CL. Prevalence of chronic hepatitis B among foreign-born persons living in the United States by country of origin. *Hepatology* 2012; **56**: 422-433 [PMID: 22105832 DOI: 10.1002/hep.24804]
- 5 **Chang ET**, So SK. Re: "Ten largest racial and ethnic health disparities in the United States based on Healthy People 2010 objectives". *Am J Epidemiol* 2007; **166**: 1105-1106; author reply 1106-1107 [PMID: 17881381]
- 6 **Institute of Medicine (US) Committee on the Prevention and Control of Viral Hepatitis Infection**; Colvin HM, Mitchell AE, editors. Hepatitis and Liver Cancer: A National Strategy for Prevention and Control of Hepatitis B and C. Washington (DC): National Academies Press (US), 2010 [PMID: 25032367]
- 7 **Weinbaum CM**, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, Neitzel SM, Ward JW. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *MMWR Recomm Rep* 2008; **57**: 1-20 [PMID: 18802412]
- 8 **LeFevre ML**. Screening for hepatitis B virus infection in nonpregnant adolescents and adults: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2014; **161**: 58-66 [PMID: 24863637 DOI: 10.7326/M14-1018]
- 9 **Hutton DW**, Tan D, So SK, Brandeau ML. Cost-effectiveness of screening and vaccinating Asian and Pacific Islander adults for hepatitis B. *Ann Intern Med* 2007; **147**: 460-469 [PMID: 17909207]
- 10 **Chu D**, Yang JD, Lok AS, Tran T, Martins EB, Fagan E, Rousseau F, Kim WR. Hepatitis B screening and vaccination practices in asian american primary care. *Gut Liver* 2013; **7**: 450-457 [PMID: 23898386 DOI: 10.5009/gnl.2013.7.4.450]
- 11 **Foster T**, Hon H, Kanwal F, Han S, Spiegel B. Screening high risk individuals for hepatitis B: physician knowledge, attitudes, and beliefs. *Dig Dis Sci* 2011; **56**: 3471-3487 [PMID: 22001940 DOI: 10.1007/s10620-011-1928-z]
- 12 **Ferrante JM**, Winston DG, Chen PH, de la Torre AN. Family physicians' knowledge and screening of chronic hepatitis and liver cancer. *Fam Med* 2008; **40**: 345-351 [PMID: 18465284]
- 13 **Lok AS**, McMahon BJ; Practice Guidelines Committee, American Association for the Study of Liver Diseases (AASLD). Chronic hepatitis B: update of recommendations. *Hepatology* 2004; **39**: 857-861 [PMID: 14999707 DOI: 10.1002/hep.20110]
- 14 **Lin SY**, Chang ET, So SK. Why we should routinely screen Asian American adults for hepatitis B: a cross-sectional study of Asians in California. *Hepatology* 2007; **46**: 1034-1040 [PMID: 17654490]
- 15 **Lin SY**, Chang ET, So SK. Stopping a silent killer in the underserved asian and pacific islander community: a chronic hepatitis B and liver cancer prevention clinic by medical students. *Asian Pac J Cancer Prev* 2009; **10**: 383-386 [PMID: 19640178]
- 16 **Jung CW**, Tan J, Tan N, Kuo MN, Ashok A, Eells SJ, Miller LG. Evidence for the insufficient evaluation and undertreatment of chronic hepatitis B infection in a predominantly low-income and immigrant population. *J Gastroenterol Hepatol* 2010; **25**: 369-375 [PMID: 19929923 DOI: 10.1111/j.1440-1746.2009.06023.x]
- 17 **Daley MF**, Hennessey KA, Weinbaum CM, Stokley S, Hurley LP, Crane LA, Beaty BL, Barrow JC, Babbel CI, Dickinson LM, Kempe A. Physician practices regarding adult hepatitis B vaccination: a national survey. *Am J Prev Med* 2009; **36**: 491-496 [PMID: 19362798 DOI: 10.1016/j.amepre.2009.01.037]
- 18 **Khalili M**, Guy J, Yu A, Li A, Diamond-Smith N, Stewart S, Chen M, Nguyen T. Hepatitis B and hepatocellular carcinoma screening among Asian Americans: survey of safety net healthcare providers. *Dig Dis Sci* 2011; **56**: 1516-1523 [PMID: 21046247 DOI: 10.1007/s10620-010-1439-3]
- 19 **Taylor VM**, Yasui Y, Burke N, Nguyen T, Chen A, Acorda E, Choe JH, Jackson JC. Hepatitis B testing among Vietnamese American men. *Cancer Detect Prev* 2004; **28**: 170-177 [PMID: 15225896]
- 20 **Coronado GD**, Taylor VM, Tu SP, Yasui Y, Acorda E, Woodall E, Yip MP, Li L, Hislop TG. Correlates of hepatitis B testing among Chinese Americans. *J Community Health* 2007; **32**: 379-390 [PMID: 17940869]
- 21 **Taylor VM**, Bastani R, Burke N, Talbot J, Sos C, Liu Q, Carey Jackson J, Yasui Y. Factors associated with hepatitis B testing among Cambodian American men and women. *J Immigr Minor Health* 2012; **14**: 30-38 [PMID: 22002705 DOI: 10.1007/s10903-011-9536-8]
- 22 **Nguyen TT**, McPhee SJ, Stewart S, Gildengorin G, Zhang L, Wong C, Maxwell AE, Bastani R, Taylor VM, Chen MS. Factors associated with hepatitis B testing among Vietnamese Americans. *J Gen Intern Med* 2010; **25**: 694-700 [PMID: 20306150 DOI: 10.1007/s11606-010-1285-1]
- 23 **Nguyen TT**, Taylor V, Chen MS, Bastani R, Maxwell AE, McPhee SJ. Hepatitis B awareness, knowledge, and screening among Asian Americans. *J Cancer Educ* 2007; **22**: 266-272 [PMID: 18067441]
- 24 **Taylor VM**, Choe JH, Yasui Y, Li L, Burke N, Jackson JC. Hepatitis B awareness, testing, and knowledge among Vietnamese American men and women. *J Community Health* 2005; **30**: 477-490 [PMID: 16370056]
- 25 **Hu KQ**. Hepatitis B virus (HBV) infection in Asian and Pacific Islander Americans (APIAs): how can we do better for this special population? *Am J Gastroenterol* 2008; **103**: 1824-1833 [PMID: 18479498 DOI: 10.1111/j.1572-0241.2008.01878.x]
- 26 **Strong C**, Lee S, Tanaka M, Juon HS. Ethnic differences in prevalence and barriers of HBV screening and vaccination among Asian Americans. *J Community Health* 2012; **37**: 1071-1080 [PMID: 22302652]
- 27 **Chen H**, Tu SP, Teh CZ, Yip MP, Choe JH, Hislop TG, Taylor VM, Thompson B. Lay beliefs about hepatitis among North American Chinese: implications for hepatitis prevention. *J Community Health* 2006; **31**: 94-112 [PMID: 16737171]
- 28 **Choe JH**, Chan N, Do HH, Woodall E, Lim E, Taylor VM. Hepatitis B and liver cancer beliefs among Korean immigrants in Western Washington. *Cancer* 2005; **104**: 2955-2958 [PMID: 16276533]
- 29 **Thompson MJ**, Taylor VM, Jackson JC, Yasui Y, Kuniyuki A, Tu SP, Hislop TG. Hepatitis B knowledge and practices among Chinese American women in Seattle, Washington. *J Cancer Educ* 2002; **17**: 222-226 [PMID: 12556060]
- 30 **Centers for Disease Control and Prevention**. Hepatitis B FAQs for Health Professionals. 2008. Available from: URL: <http://www.cdc.gov/hepatitis/HBV/HBVfaq.htm>
- 31 **Upadhyaya N**, Chang R, Davis C, Conti MC, Salinas-Garcia D, Tang H. Chronic hepatitis B: perceptions in Asian American communities and diagnosis and management practices among primary care physicians. *Postgrad Med* 2010; **122**: 165-175 [PMID: 20861600 DOI: 10.3810/pgm.2010.09.2213]
- 32 **Hu DJ**, Xing J, Tohme RA, Liao Y, Pollack H, Ward JW, Holmberg SD. Hepatitis B testing and access to care among racial and ethnic minorities in selected communities across the United States, 2009-2010. *Hepatology* 2013; **58**: 856-862 [PMID: 23359276 DOI: 10.1002/hep.26286]
- 33 **Lai CJ**, Nguyen TT, Hwang J, Stewart SL, Kwan A, McPhee SJ. Provider knowledge and practice regarding hepatitis B screening in Chinese-speaking patients. *J Cancer Educ* 2007; **22**: 37-41 [PMID: 17570807]
- 34 **US Department of Health and Human Services**. Combating the Silent Epidemic of Viral Hepatitis: Action Plan for the Prevention, Care, and Treatment of Viral Hepatitis. 2011. Available from: URL: <http://hepb.org/pdf/Viral-Hepatitis-Action-plan-2011.pdf>
- 35 **Sarkar M**, Stewart S, Yu A, Chen MS, Nguyen TT, Khalili M. Hepatocellular carcinoma screening practices and impact on

survival among hepatitis B-infected Asian Americans. *J Viral Hepat* 2012; **19**: 594-600 [PMID: 22762144 DOI: 10.1111/j.1365-2893.2011.01577.x]

36 Toy M, Salomon JA, Jiang H, Gui H, Wang H, Wang J, Richardus

JH, Xie Q. Population health impact and cost-effectiveness of monitoring inactive chronic hepatitis B and treating eligible patients in Shanghai, China. *Hepatology* 2014; **60**: 46-55 [PMID: 24990105 DOI: 10.1002/hep.26934]

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## Retrospective Study

# Prognostic angiogenic markers (endoglin, VEGF, CD31) and tumor cell proliferation (Ki67) for gastrointestinal stromal tumors

Rodrigo Panno Basilio-de-Oliveira, Vera Lucia Nunes Pannain

Rodrigo Panno Basilio-de-Oliveira, Pathology Post-Graduate Program, Federal University of Rio de Janeiro, Rio de Janeiro 21941-901, Brazil

Rodrigo Panno Basilio-de-Oliveira, Department of Pathology and Clinical Support, Federal University of Rio de Janeiro State, 22280-110 Rio de Janeiro, Brazil

Vera Lucia Nunes Pannain, Department of Pathology, Pathology Post-Graduate Program, Federal University of Rio de Janeiro, Rio de Janeiro 21941-901, Brazil

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**Conflict-of-interest:** None of the authors of this study has received fees for serving as a speaker, a consultant and/or an advisory board member. None has received research funding from organization(s).

**Data sharing:** Technical appendix, statistical code, and dataset available from the corresponding author at [rodrigopboliveira@gmail.com](mailto:rodrigopboliveira@gmail.com). Participant gave informed consent for data sharing.

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**Correspondence to:** Rodrigo Panno Basilio-de-Oliveira, Associate Professor, Department of Pathology and Clinical Support, Federal University of Rio de Janeiro State, Street Alvaro Ramos 71/505, Rio de Janeiro 22280-110, Brazil. [rodrigopboliveira@gmail.com](mailto:rodrigopboliveira@gmail.com)

Telephone: +55-212-2041365

Fax: +55-212-5695605

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## Abstract

**AIM:** To evaluate the correlation between the immunoexpression of angiogenic markers [CD31, CD105 and vascular endothelial growth factor (VEGF)], proliferative index (Ki67), and prognosis of patients with gastrointestinal stromal tumors (GIST).

**METHODS:** This is a retrospective study of 54 GIST cases. Medical records were searched to obtain the GIST patients' demographic and clinical data, and paraffin-embedded blocks of tumor samples were retrieved from the hospital archives to conduct a new immunohistochemical evaluation. The tumor samples of GIST patients were subject to immunohistochemical evaluation for endoglin (CD105), CD31, VEGF, and Ki67 expression. The CD105 and CD31 intratumoral microvascular density (IMVD) was measured using automated analysis. We determined the correlation between the immunoexpression of CD105, CD31, VEGF, Ki67 and prognosis. In addition, we conducted a cutoff analysis using the receiver-operating characteristic curve. VEGF positivity was classified as either null/weak or strong. Ki67 was evaluated using a cutoff of 5% positive cells. The prognosis was classified as good (patient alive without recurrence) or poor (patient with recurrence/death).

**RESULTS:** The distribution of tumor sites among the 54 analyzed samples was as follows: 27 (50%) in the stomach, 20 (37.1%) in the small intestine, 6 (11.1%) in the colon, and 1 (1.8%) in the esophagus. The size of the tumors ranged from 2 to 33 cm (median: 8 cm); in 12 cases (22.2%), the tumor was below 5 cm at the largest diameter, but in 42 cases (77.7%), the tumor was larger than 5 cm. The means of CD105 and CD31 were significantly higher in the group with poor prognosis ( $P < 0.001$ ). The cut-off values of CD105 ( $> 1.2\%$ ) and CD31 ( $> 2.5\%$ ) in the receiver-operating characteristic curve were related to a poorer prognosis. Cases with a better prognosis showed significantly null/weak staining for VEGF ( $P < 0.001$ ). Ki-67 expression of  $\geq 5\%$  was strongly correlated with a worse prognosis ( $P < 0.001$ ). In the multivariate analysis, CD105 was the variable that most strongly correlated with prognosis.

**CONCLUSION:** The IMVD cutoff values for the angiogenic markers CD105 and CD31, may be prognostic factors for GIST, in addition to VEGF and Ki67.

**Key words:** Angiogenesis; Immunohistochemistry, CD105; CD31; Gastrointestinal stromal tumors; Vascular endothelial growth factor; Ki-67

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**Core tip:** Prognosis of gastrointestinal stromal tumors (GIST) is a longstanding challenge. Association of angioimmunomarkers with poor prognosis has recently been demonstrated, but few studies have evaluated the relevance of vascular endothelial growth factor (VEGF) and CD31, and none has analyzed the role of CD105 expression in prognosis. Our results suggest that angiogenic markers (intratumoral microvascular density cut-off of CD105 and CD31 besides VEGF) and Ki67 (tumor cell proliferation marker), may be prognostic factors for GIST, besides and Ki67 (tumor cell proliferation). However, further studies are necessary before considering such angiogenic molecules as possible therapeutic targets.

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## INTRODUCTION

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the gastrointestinal tract<sup>[1]</sup>. Although a great deal is already understood about the biology, diagnosis, and treatment of this tumor type, predicting prognosis in some cases

remains a challenge<sup>[2]</sup>. The primary goal of a prognostic determination to discern patients with localized, resectable disease who require only clinical follow-up from those in need of adjuvant therapy<sup>[3]</sup>. Currently, there are five different classifications<sup>[4-8]</sup> used to stratify tumors into groups associated with a greater or lesser risk of tumor recurrence and/or distant metastasis<sup>[9]</sup>. However, these classifications still leave room for doubt, as there are some groups of rare tumors with an insufficient number of cases for effective analysis, and others with highly variable recurrence/tumor progression rates, *e.g.*, from 24% to 73%, leaving a high percentage of tumors with an indefinite prognosis<sup>[10,11]</sup>.

In addition to morphological criteria, some immunohistochemical markers have been used as prognostic markers of GIST. Among these are the proliferating cell nuclear antigen Ki-67, which is widely used with a cut-off point of 5% as an indicator of poor prognosis. More recently, the angiogenic markers, especially CD31 and vascular endothelial growth factor (VEGF)<sup>[12]</sup>, which is considered the main mediator of tumor angiogenesis have also been used as prognostic<sup>[13]</sup>. Some studies<sup>[14-18]</sup> have shown an association between high levels of tissue VEGF and poorer prognosis of patients with GIST.

In addition to VEGF, another molecule that is involved in the process of tumor angiogenesis is the transmembrane glycoprotein endoglin (CD105). Endoglin may or may not be proangiogenic, depending on whether it is bound to the activin receptor-like kinase<sup>[19]</sup>, and is primarily expressed in activated endothelial cells<sup>[20,21]</sup>. Compared with other pan-endothelial markers (*e.g.*, CD34 and CD31), endoglin shows a characteristic property as a "neovessel" marker, *i.e.*, in a state of proliferation. Therefore, CD105 is considered a more specific immunohistochemical marker for tumor neovasculature<sup>[22]</sup>.

Various studies have demonstrated the importance of CD105, using intratumoral microvessel density (IMVD), as a prognostic factor correlated with overall and disease-free survival, tumor recurrence, and the presence of metastasis in different tumor types<sup>[23,24]</sup>. However, to our knowledge, only one study<sup>[25]</sup> has addressed the association of the immunohistochemical marker CD105 in GIST with morphological factors, and no correlation with prognosis was found. Other angiogenic markers have been studied in relation to cancer prognosis, including CD34, CD31, and factor VIII<sup>[26-28]</sup>. However, the results are contradictory, with some studies<sup>[26,29,30]</sup>, including those on GIST<sup>[17]</sup>, that have identified an association, and others that have not<sup>[31]</sup>. In addition, volumetric growth and the development of metastases in cases of GIST appear to be related to the development of a new vascular network<sup>[17]</sup>. Another fact that corroborates the importance of vascularization in the context of GIST is the mechanism of action of the second-generation

drug sunitinib, which is based on the blockade of VEGF activity along with tyrosine kinase receptor blockade that has been used with success in some GIST patients<sup>[32]</sup>. Another key factor used in defining prognosis in cases of GIST is cell cycle markers, especially Ki67, which is an indicator of proliferating cells<sup>[33]</sup>.

In an attempt to better understand the behavior of GIST, we performed immunohistochemical assays to analyze the expression of angiogenic markers (CD105, CD31 and VEGF) as well as the cell proliferation index (Ki-67) and determined their correlation with the clinical progression of patients. The results of this study should provide valuable information with respect to the effectiveness of these markers as prognostic factors in the context of GIST.

## MATERIALS AND METHODS

### Study design and participants

We conducted a retrospective study of all cases of GIST evaluated in the Pathology Laboratory of two university hospitals in Rio de Janeiro, Brazil (Gaffrée and Guinle University Hospital and Clementino Fraga Filho University Hospital). After obtaining approval from the Ethics Committee (protocol number 079/05), medical records were searched to obtain the patients' demographic and clinical data, and paraffin-embedded blocks with tumor samples were retrieved from the hospital archives for a new immunohistochemical evaluation, as described in detail below. The archives were searched for cases of GIST with positivity for the CD117 antibody, with totally resected tumors. Patients with disseminated GIST or with other types of cancer were excluded from the study.

### Variables

From the clinical records, gender, age, tumor site and size, and clinical progression were recorded. The follow-up period of patients was calculated from the date of surgery until the last follow-up visit. The prognosis was classified as good (disease-free survival) or poor (the patient died due to GIST, or survived but had metastases during follow-up). There were no deaths due to other causes in this case series.

The CD105, CD31, VEGF, and Ki67 expression levels were determined by immunohistochemical analysis, according to the methods and criteria described below.

### Immunohistochemistry

Formalin-fixed, paraffin-embedded tissues, sectioned into 5- $\mu$ m-thick slices, were mounted on poly-L-lysine-coated slides (Sigma, St. Louis, MO, United States; code P8920). The sections were deparaffinized by xylene and dehydrated by a graded series of ethanol. The Novolink polymer (Novocastra, Newcastle, United Kingdom) was used. The chromogen was 3,3'-diaminobenzidine tetrahydrochloride, followed by Mayer's hematoxylin counterstain were applied to

the slides. Negative controls lacking primary antibody application were run simultaneously. The following antibodies with respective dilutions were used: anti-Ki-67 (M7240, 1:250; Dako Dk A/S), anti-CD31 (JC70A, 1:50; Dako Dk A/S), anti-VEGF C-1 (sc-7269, 1:6000; Santa Cruz Biotechnology, Heidelberg, Ger), and anti-CD105 (clone 4G11, 1:60; Leica Biosystems Newcastle Ltd, United Kingdom).

### Measurement of IMVD

Two computer tools were used for the CD105 and CD31 immunohistochemical IMVD analysis: Qcapture and ImageLab. The former is an image-capturing system in an Olympus digital 3.3-megapixel camera attached to an Olympus BX-40 microscope. The three vascular fields showing the highest intensity antibody signals, such as vessels or groups of endothelial cells, were searched at a magnification of  $\times 100$  and captured for each case with a  $\times 200$  magnifying lens. The images were then analyzed using the "color function" and area/density measurement function in ImageLab software<sup>[34]</sup>. The percentages of marked areas (hotspots) were calculated in a fixed area of 216  $\mu$ m on each image. The average of the three areas selected for analysis was recorded into an Excel worksheet.

### Evaluation of VEGF and Ki67

The tumors were classified into two categories based on VEGF expression: null/weakly positive or moderate/ markedly positive. The classification was based on the staining intensity of the vascular structures.

Positive cells for Ki-67 were counted in a field of 1000 cells<sup>[33]</sup>. The tumors were classified into those with less than 5% positive cells and those with 5% or more positive cells.

CD31 and CD105 positivity and Ki-67 expression were analyzed in relation to clinical status<sup>[4,10]</sup>. A receiver-operating characteristic (ROC) curve was constructed to determine a cut-off for poor prognosis.

### Statistical analysis

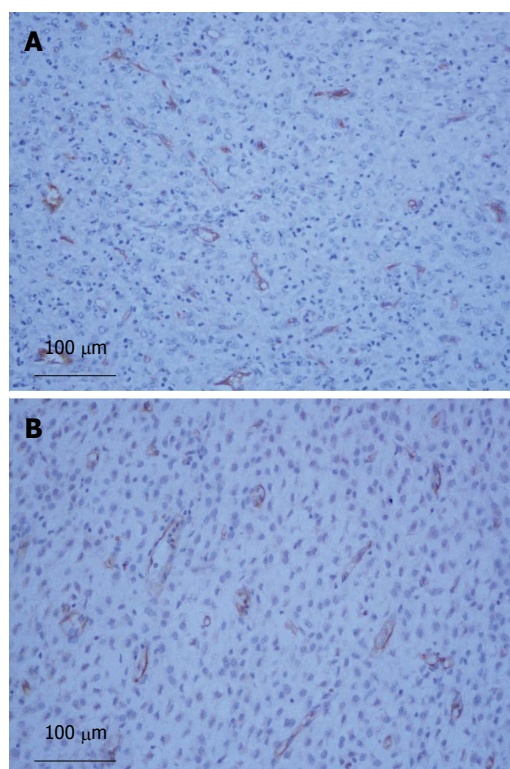
In the univariate analysis, the  $\chi^2$ , Wilks G2, Fisher and Student's *t* tests were used, with a significance threshold value of less than 0.05. In the multivariate analysis, for factors that showed statistical significance in the univariate analysis, we used the Jaccard index, which compares the similarity and diversity of samples. The highest rated factor was the one determined to be most closely linked to prognosis. The statistical methods of this study were reviewed by Mauricio Gama, Associate Professor of Research Department of Clementino Fraga Filho University Hospital.

## RESULTS

### Participants, histological and clinical data

Among the 54 cases of GIST that were studied, the patients' mean age was  $57.34 \pm 13.71$  years (range:





**Figure 1** Gastric gastrointestinal stromal tumor with labeling for CD105 > 1.2% (A), CD31 > 2.5% (B),  $\times 20$ .

24-83 years), and 30 (59.5%) patients were women. The distribution of the tumor sites among patients was as follows: 27 (50%) in the stomach, 20 (37.1%) in the small intestine, 6 (11.1%) in the colon and 1 (1.8%) in the esophagus. The size of the tumors ranged from 2 to 33 cm (median: 8 cm); in 12 cases (22.2%), the tumor was below 5 cm at the largest diameter, but in most cases (42; 77.7%), the tumor was larger than 5 cm.

Of the 54 patients, 33 (61.2%) were alive and disease-free (*i.e.*, good prognosis) and 21 (38.8%) patients were alive with disease or had died due to cancer. The follow-up period ranged from 1 to 242 mo, with a median time of 35 mo.

### Immunohistochemistry results

The mean positivity values of CD105 and CD31 ranged from 0.37% to 4.21% and 0.39% to 7.83%, with median values of 1.14% and 1.96%, respectively (Figure 1).

In relation to VEGF, 21 tumors (38.9%) showed null/weakly positive staining, while 33 (61.1%) showed moderate/strong staining.

The anti-Ki-67 antibody was expressed in 31 (57.4%) tumors with a cell proliferation index of less than 5%, and the remaining 23 tumors (42.6%) had an index greater than or equal to 5%.

### Correlation between prognosis and immunohistochemistry markers

The average value for the CD105 evaluation was

**Table 1** Association among vascular endothelial growth factor, Ki67 and survival status

Immunomarkers	Alive without recurrence	Recurrence/death	P value
VEGF			
Null/weakly <sup>1</sup>	18	3	0.002 <sup>2</sup>
Moderate/strongly <sup>1</sup>	15	18	
Ki67			
< 5%	29	2	< 0.001 <sup>3</sup>
≥ 5%	4	19	

<sup>1</sup>Positive; <sup>2</sup>Fisher exact test, <sup>3</sup>Student's *t*-test. VEGF: Vascular endothelial growth factor.

significantly higher in the group of patients with poor prognosis than in the group of patients who were alive without recurrence (1.98% vs 1.04%,  $P < 0.001$ , Student's *t*-test). Based on the ROC curve for CD105, a cutoff point of 1.21% was determined. This cutoff point was corroborated by the area under the curve value of 0.88, which indicates good power of discrimination.

The average value obtained with CD31 was significantly higher in the poor prognosis group than in the group of patients who survived without recurrence (3.61% vs 1.94%,  $P < 0.001$ , Student's *t*-test). Based on the ROC curve for CD31, a cutoff point of 2.50% was determined. This cutoff point was corroborated by the area under the curve value of 0.92, which indicates strong discriminatory power.

A relatively better patient prognosis was associated with null/weak VEGF expression in the tumors; of the 21 cases with null/weak VEGF expression, only 3 had an unfavorable prognosis ( $P = 0.002$ ). A rate of  $\geq 5\%$  Ki67 expression was strongly associated with reduced overall survival ( $P < 0.001$ ) (Table 1). In the multivariate analysis, the factors that showed statistical significance in the univariate analysis (CD105, CD31, VEGF and Ki-67) were submitted to analysis of similarity (Jaccard index), which revealed that CD105 had the strongest association with prognosis-(Jaccard index value: 0.69278), followed by CD31 (0.66471), Ki67 (0.54286) and VEGF (0.50000).

## DISCUSSION

Determining the prognosis of GIST is essential, given that 60% of tumors are larger than 5 cm, which is associated with a relatively poor prognosis<sup>[5,35,36]</sup>, 50% of patients are considered high-risk<sup>[37]</sup>, and 20%-55% of patients will experience tumor recurrence<sup>[38-40]</sup>.

In our case series, we demonstrated an association between immunohistochemical markers related to angiogenesis and prognosis. CD105 (endoglin) showed an association with prognosis through the IMVD measurement. The plotted ROC curve indicated a cut-off point of 1.2%, which was established as a dividing factor in our series for good and poor prognosis, as



tumors with an IMVD above this value were related to a relatively worse prognosis. No previous study has directly evaluated the role of CD105 expression in prognosis in the context of GIST. The only previously reported study<sup>[25]</sup> on the association between GIST and CD105 demonstrated a link between the strong intensity of immunohistochemical staining with some morphological criteria that is associated with a worse prognosis, such as a mitotic index above 5 mitoses per 50 high-power fields and a high degree of risk.

The average IMVD value of CD31 was higher in tumors from patients with a worse prognosis than in those with a good prognosis, demonstrating a clear relationship between this marker and prognosis, as has been previously reported<sup>[17,18,41]</sup>. Although these previous studies all established a correlation between the IMVD of CD31 and prognosis, there is marked variation among the cut-off values reported for malignancy, which might be due to the different methods used for evaluation and analysis<sup>[17,18]</sup>. There are also some differences between the methods adopted in our study and others. For example, the count of vascular structures was conducted in a semi-automated manner using a computer program, which significantly reduces bias in counting. In addition, the cut-off point value of 2.50% found in our series was validated through analysis of a ROC curve.

VEGF expression was null or weakly positive in 21 tumors, and only 3 of these cases showed poor prognosis, while the 33 patients with strong VEGF positivity in tumors (approximately 60%) experienced recurrence or died. This association between VEGF and GIST prognosis has also been found in other studies<sup>[15-18]</sup>. In addition, high VEGF expression has been associated with a poorer prognosis, independent of the tumor genotype, and with a low therapeutic response to imatinib mesylate<sup>[42]</sup>. In other tumors, such as those of the lung and breast<sup>[26,27]</sup>, high IMVD is associated with a reduction in survival and a shorter time to tumor recurrence<sup>[22,29]</sup>.

We also found a strong association between a marker for nuclear antigen cell proliferation (Ki67) and prognosis in our series, similar to previous studies<sup>[43,44]</sup>. Therefore, the present results demonstrate the high prognostic potential of Ki-67 for GIST. Of the 31 patients with an index of less than 5%, 29 were alive without recurrence and only 2 had progressed to a poor prognosis. In contrast, of the 23 tumors with an index greater than 5%, 19 progressed to metastasis/death. There are other advantages of using Ki-67 as a prognostic marker: unlike the mitotic index, which shows an association with the topography of the tumor<sup>[43]</sup>, it can be used independently of the location of the tumor. It also constitutes a potential discriminator in localized and/or disseminated disease<sup>[43]</sup>. Therefore, despite its inability to determine risk levels in all classifications proposed, we believe that Ki-67 is an important parameter in the prognosis of GIST and that it should not be neglected as a

prognostic marker.

Our results suggest that angiogenic markers, including the CD105 and CD31 IMVD cut-off values, VEGF and the tumor cell proliferation marker Ki67, may be useful prognostic factors in GIST. However, further studies are necessary before considering such angiogenic molecules as possible therapeutic targets.

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## COMMENTS

### Background

Angiogenesis contributes to the growth and spread of gastrointestinal stromal tumors (GIST). Endoglin, vascular endothelial growth factor (VEGF) and CD31 are directly involved in this process. However to date the use of these molecules as prognostic factors remains controversial.

### Research frontiers

Previous studies have shown that high levels of VEGF and CD31 are associated with a poor prognosis. However no studies have addressed the prognostic potential of endoglin. In the present study, independent prognostic implications of endoglin were investigated.

### Innovations and breakthroughs

Tumors with intratumoral microvessel density above the cut-off found in the respective receiver-operating characteristic curves were directly associated with a poor prognosis. In the univariate and multivariate analyses, vascular markers, especially endoglin were associated with prognosis.

### Applications

The results of this study suggest that high levels of molecules related to angiogenesis (endoglin, VEGF and CD31) are an independent indicator of poor prognosis in GIST.

### Terminology

Endoglin is a type I membrane glycoprotein located on cell surfaces and is part of the TGFβ receptor complex. It has a crucial role in angiogenesis and is an important protein for tumor growth, survival and metastasis

### Peer-review

This is a retrospective study in which the authors analyzed the main molecules of angiogenesis (CD31, VEGF and endoglin) and related them to disease prognosis. It is noteworthy that endoglin has not been previously studied in the context of GIST. The results are interesting and suggest that tumors that demonstrate a high microvessel vascular intratumoral had a worse prognosis.

## REFERENCES

- 1 **Miettinen M**, Lasota J. Histopathology of gastrointestinal stromal tumor. *J Surg Oncol* 2011; **104**: 865-873 [PMID: 22069171 DOI: 10.1002/jso.21945]
- 2 **Rubin BP**. Gastrointestinal stromal tumours: an update. *Histopathology* 2006; **48**: 83-96 [PMID: 16359540]
- 3 **Boichuk S**, Parry JA, Makielski KR, Litovchick L, Baron JL, Zewe JP, Wozniak A, Mehalek KR, Korzeniewski N, Seneviratne DS, Schöffski P, Debiec-Rychter M, DeCaprio JA, Duensing A. The DREAM complex mediates GIST cell quiescence and is a novel therapeutic target to enhance imatinib-induced apoptosis. *Cancer Res* 2013; **73**: 5120-5129 [PMID: 23786773 DOI: 10.1158/0008-5472.CAN-13-0579]
- 4 **Fletcher CD**, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 2002; **33**: 459-465 [PMID: 12094370]

- 5 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis. *Arch Pathol Lab Med* 2006; **130**: 1466-1478 [PMID: 17090188]
- 6 **Joensuu H**. Predicting recurrence-free survival after surgery for GIST. *Lancet Oncol* 2009; **10**: 1025 [PMID: 19793677 DOI: 10.1016/S1470-2045(09)70267-0.]
- 7 **Huang H**, Liu YX, Zhan ZL, Liang H, Wang P, Ren XB. Different sites and prognoses of gastrointestinal stromal tumors of the stomach: report of 187 cases. *World J Surg* 2010; **34**: 1523-1533 [PMID: 20145924 DOI: 10.1007/s00268-010-0463-y]
- 8 **Goh BK**, Chow PK, Yap WM, Kesavan SM, Song IC, Paul PG, Ooi BS, Chung YF, Wong WK. Which is the optimal risk stratification system for surgically treated localized primary GIST? Comparison of three contemporary prognostic criteria in 171 tumors and a proposal for a modified Armed Forces Institute of Pathology risk criteria. *Ann Surg Oncol* 2008; **15**: 2153-2163 [PMID: 18546045 DOI: 10.1245/s10434-008-9969-z]
- 9 **Wong NA**. Gastrointestinal stromal tumours--an update for histopathologists. *Histopathology* 2011; **59**: 807-821 [PMID: 21668468 DOI: 10.1111/j.1365-2559.2011.03812.x]
- 10 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol* 2006; **23**: 70-83 [PMID: 17193820]
- 11 **Joensuu H**, Vehtari A, Riihimäki J, Nishida T, Steigen SE, Bräbäck P, Plank L, Nilsson B, Cirilli C, Braconi C, Bordoni A, Magnusson MK, Linke Z, Suflarsky J, Federico M, Jonasson JG, Dei Tos AP, Rutkowski P. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. *Lancet Oncol* 2012; **13**: 265-274 [PMID: 22153892 DOI: 10.1016/S1470-2045(11)70299-6]
- 12 **Takahashi R**, Tanaka S, Hiyama T, Ito M, Kitadai Y, Sumii M, Haruma K, Chayama K. Hypoxia-inducible factor-1 $\alpha$  expression and angiogenesis in gastrointestinal stromal tumor of the stomach. *Oncol Rep* 2003; **10**: 797-802 [PMID: 12792726]
- 13 **Shibuya M**. Differential roles of vascular endothelial growth factor receptor-1 and receptor-2 in angiogenesis. *J Biochem Mol Biol* 2006; **39**: 469-478 [PMID: 17002866]
- 14 **Kerbel RS**. Tumor angiogenesis. *N Engl J Med* 2008; **358**: 2039-2049 [PMID: 18463380 DOI: 10.1056/NEJMra0706596]
- 15 **Miao R**, Liu N, Wang Y, Li L, Yu X, Jiang Y, Li J. Coexpression of cyclooxygenase-2 and vascular endothelial growth factor in gastrointestinal stromal tumor: possible relations to pathological parameters and clinical behavior. *Hepatogastroenterology* 2008; **55**: 2012-2015 [PMID: 19260469]
- 16 **Wang TB**, Qiu WS, Wei B, Deng MH, Wei HB, Dong WG. Serum vascular endothelial growth factor and angiogenesis are related to the prognosis of patients with gastrointestinal stromal tumors. *Ir J Med Sci* 2009; **178**: 315-320 [PMID: 19367428 DOI: 10.1007/s11845-009-0315-7]
- 17 **Imamura M**, Yamamoto H, Nakamura N, Oda Y, Yao T, Kakeji Y, Baba H, Maehara Y, Tsuneyoshi M. Prognostic significance of angiogenesis in gastrointestinal stromal tumor. *Mod Pathol* 2007; **20**: 529-537 [PMID: 17334345]
- 18 **Takahashi R**, Tanaka S, Kitadai Y, Sumii M, Yoshihara M, Haruma K, Chayama K. Expression of vascular endothelial growth factor and angiogenesis in gastrointestinal stromal tumor of the stomach. *Oncology* 2003; **64**: 266-274 [PMID: 12697968]
- 19 **Koleva RI**, Conley BA, Romero D, Riley KS, Marto JA, Lux A, Vary CP. Endoglin structure and function: Determinants of endoglin phosphorylation by transforming growth factor- $\beta$  receptors. *J Biol Chem* 2006; **281**: 25110-25123 [PMID: 16785228]
- 20 **Dallas NA**, Samuel S, Xia L, Fan F, Gray MJ, Lim SJ, Ellis LM. Endoglin (CD105): a marker of tumor vasculature and potential target for therapy. *Clin Cancer Res* 2008; **14**: 1931-1937 [PMID: 18381930 DOI: 10.1158/1078-0432.CCR-07-4478]
- 21 **Li C**, Issa R, Kumar P, Hampson IN, Lopez-Novoa JM, Bernabeu C, Kumar S. CD105 prevents apoptosis in hypoxic endothelial cells. *J Cell Sci* 2003; **116**: 2677-2685 [PMID: 12746487]
- 22 **Duff SE**, Li C, Garland JM, Kumar S. CD105 is important for angiogenesis: evidence and potential applications. *FASEB J* 2003; **17**: 984-992 [PMID: 12773481]
- 23 **Svatek RS**, Karam JA, Roehrborn CG, Karakiewicz PI, Slawin KM, Shariat SF. Preoperative plasma endoglin levels predict biochemical progression after radical prostatectomy. *Clin Cancer Res* 2008; **14**: 3362-3366 [PMID: 18519764 DOI: 10.1158/10780432.CCR-07-4707]
- 24 **Bernabeu C**, Lopez-Novoa JM, Quintanilla M. The emerging role of TGF- $\beta$  superfamily coreceptors in cancer. *Biochim Biophys Acta* 2009; **1792**: 954-973 [PMID: 19607914 DOI: 10.1016/j.bbdis.2009.07.003]
- 25 **Gromova P**, Rubin BP, Thys A, Cullis P, Erneux C, Vanderwinden JM. ENDOGLIN/CD105 is expressed in KIT positive cells in the gut and in gastrointestinal stromal tumours. *J Cell Mol Med* 2012; **16**: 306-317 [PMID: 21435173 DOI: 10.1111/j.1582-4934.2011.01315.x]
- 26 **Uzzan B**, Nicolas P, Cucherat M, Perret GY. Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. *Cancer Res* 2004; **64**: 2941-2955 [PMID: 15126324]
- 27 **Mineo TC**, Ambrogio V, Baldi A, Rabitti C, Bollero P, Vincenzi B, Tonini G. Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. *J Clin Pathol* 2004; **57**: 591-597 [PMID: 15166262]
- 28 **Sharma S**, Sharma MC, Sarkar C. Morphology of angiogenesis in human cancer: a conceptual overview, histopathologic perspective and significance of neoangiogenesis. *Histopathology* 2005; **46**: 481-489 [PMID: 15842629]
- 29 **Weidner N**, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. *N Engl J Med* 1991; **324**: 1-8 [PMID: 1701519]
- 30 **Weidner N**. The importance of tumor angiogenesis: the evidence continues to grow. *Am J Clin Pathol* 2004; **122**: 675-677 [PMID: 15491962]
- 31 **Medri L**, Nanni O, Volpi A, Scarpi E, Dubini A, Riccobon A, Becciolini A, Bianchi S, Amadori D. Tumor microvessel density and prognosis in node-negative breast cancer. *Int J Cancer* 2000; **89**: 74-80 [PMID: 10719734]
- 32 **Chen YY**, Yeh CN, Cheng CT, Chen TW, Rau KM, Jan YY, Chen MF. Sunitinib for Taiwanese patients with gastrointestinal stromal tumor after imatinib treatment failure or intolerance. *World J Gastroenterol* 2011; **17**: 2113-2119 [PMID: 21547131 DOI: 10.3748/wjg.v17.i16.2113]
- 33 **Nakamura N**, Yamamoto H, Yao T, Oda Y, Nishiyama K, Imamura M, Yamada T, Nawata H, Tsuneyoshi M. Prognostic significance of expressions of cell-cycle regulatory proteins in gastrointestinal stromal tumor and the relevance of the risk grade. *Hum Pathol* 2005; **36**: 828-837 [PMID: 16084954]
- 34 **Hadlich MS**, Oliveira GM, Feijóo RA, Azevedo CF, Tura BR, Ziemer PG, Blanco PJ, Pina G, Meira M, Souza e Silva NA. Free and open-source software application for the evaluation of coronary computed tomography angiography images. *Arq Bras Cardiol* 2012; **99**: 944-951 [PMID: 23033110]
- 35 **Nilsson B**, Bümming P, Meis-Kindblom JM, Odén A, Dortok A, Gustavsson B, Sablinska K, Kindblom LG. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era--a population-based study in western Sweden. *Cancer* 2005; **103**: 821-829 [PMID: 15648083]
- 36 **Miettinen M**, Makhlof H, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the jejunum and ileum: a clinicopathologic, immunohistochemical, and molecular genetic study of 906 cases before imatinib with long-term follow-up. *Am J Surg Pathol* 2006; **30**: 477-489 [PMID: 16625094]
- 37 **Corless CL**, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. *Nat Rev Cancer* 2011; **11**: 865-878 [PMID: 22089421 DOI: 10.1038/nrc3143]
- 38 **Ahmed I**, Welch NT, Parsons SL. Gastrointestinal stromal tumours (GIST) - 17 years experience from Mid Trent Region

- (United Kingdom). *Eur J Surg Oncol* 2008; **34**: 445-449 [PMID: 17320340]
- 39 **Hassan I**, You YN, Shyyan R, Dozois EJ, Smyrk TC, Okuno SH, Schleck CD, Hodge DO, Donohue JH. Surgically managed gastrointestinal stromal tumors: a comparative and prognostic analysis. *Ann Surg Oncol* 2008; **15**: 52-59 [PMID: 18000711]
  - 40 **Oliveira RPB**, Pannain VL, Portari Filho PE, Salomão AR, Iglesias AC, Oliveira CAB. Gastrointestinal stromal tumor: analysis of factors related to the prognostic. *Rev Col Bras Cir* 2007; **34**: 374-380 [DOI: 10.1590/S0100-69912007000600004]
  - 41 **Zhao Y**, Wang Q, Deng X, Zhao Y. Altered angiogenesis gene expression in gastrointestinal stromal tumors: potential use in diagnosis, outcome prediction, and treatment. *Neoplasia* 2012; **59**: 384-392 [PMID: 22489693 DOI: 10.4149/neo\_2012\_050]
  - 42 **McAuliffe JC**, Lazar AJ, Yang D, Steinert DM, Qiao W, Thall PF, Raymond AK, Benjamin RS, Trent JC. Association of intratumoral vascular endothelial growth factor expression and clinical outcome for patients with gastrointestinal stromal tumors treated with imatinib mesylate. *Clin Cancer Res* 2007; **13**: 6727-6734 [PMID: 18006774]
  - 43 **Belev B**, Brčić I, Prejac J, Golubić ZA, Vrbanc D, Božikov J, Alerić I, Boban M, Razumović JJ. Role of Ki-67 as a prognostic factor in gastrointestinal stromal tumors. *World J Gastroenterol* 2013; **19**: 523-527 [PMID: 23382631 DOI: 10.3748/wjg.v19.i4.523]
  - 44 **Avanzolini G**, Barbini P, Cappello A, Cevenini G. Real-time tracking of parameters of lung mechanics: emphasis on algorithm tuning. *J Biomed Eng* 1990; **12**: 489-495 [PMID: 2266745]

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## Retrospective Study

# Assessment of liver fibrosis by Fibroscan as compared to liver biopsy in biliary atresia

Qiu-Long Shen, Ya-Jun Chen, Zeng-Meng Wang, Ting-Chong Zhang, Wen-Bo Pang, Jun Shu, Chun-Hui Peng

Qiu-Long Shen, Ya-Jun Chen, Zeng-Meng Wang, Ting-Chong Zhang, Wen-Bo Pang, Jun Shu, Chun-Hui Peng, Department of Pediatric General Surgery, Beijing Children's Hospital, Capital Medical University, Beijing 100045, China

**Author contributions:** Chen YJ and Shen QL designed the study and wrote the manuscript; Wang ZM and Pang WB analyzed the data; Zhang TC, Shu J and Peng CH were involved in editing the manuscript.

**Ethics approval:** The study was reviewed and approved by the Beijing Children Hospital's Institutional Review Board.

**Informed consent:** All study participants' legal guardian provided informed written consent prior to study enrollment.

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**Data sharing:** No additional data are available.

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**Correspondence to:** Ya-Jun Chen, MD, PHD, Department of Pediatric General Surgery, Beijing Children's Hospital, Capital Medical University, 56 South Lishi Road, Beijing 100045, China. [shenqlyishi@163.com](mailto:shenqlyishi@163.com)  
Telephone: +86-10-59616161

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## Abstract

**AIM:** To evaluate liver stiffness measurement (LSM) using non-invasive transient elastography (Fibroscan) in comparison with liver biopsy for assessment of liver fibrosis in children with biliary atresia (BA).

**METHODS:** Thirty-one children with BA admitted to the Department of Pediatric Surgery of Beijing Children's Hospital from March 2012 to February 2013 were included in this study. Their preoperative LSM, liver biopsy findings, and laboratory results were studied retrospectively.

**RESULTS:** The grade of liver fibrosis in all 31 patients was evaluated according to the METAVIR scoring system, which showed that 4 cases were in group F2, 20 in group F3 and 7 in group F4. There were 24 non-cirrhosis cases (F2-F3) and 7 cirrhosis cases (F4). In groups F2, F3 and F4, the mean LSM was  $9.10 \pm 3.30$  kPa,  $11.02 \pm 3.31$  kPa and  $22.86 \pm 12.43$  kPa, respectively. LSM was statistically different between groups F2 and F4 ( $P = 0.002$ ), and between groups F3 and F4 ( $P = 0.000$ ), however, there was no statistical difference between groups F2 and F3 ( $P = 0.593$ ). The area under the receiver operating characteristic curve of LSM for  $\geq F4$  was 0.866. The cut-off value of LSM was 15.15 kPa for  $\geq F4$ , with a sensitivity, specificity, positive predictive value and negative predictive value of 0.857, 0.917, 0.750 and 0.957, respectively.

**CONCLUSION:** Fibroscan can be used as a non-invasive technique to assess liver fibrosis in children with BA. The cut-off value of LSM (15.15 kPa) can distinguish cirrhotic patients from non-cirrhotic patients.

**Key words:** Biliary atresia; Transient elastography; Fibroscan; Liver fibrosis; Liver biopsy



**Core tip:** This study was designed to evaluate liver fibrosis using non-invasive transient elastography (Fibrosan) in comparison with liver biopsy in children with biliary atresia (BA). According to the METAVIR scoring system, in groups F2, F3 and F4, the mean liver stiffness measurement (LSM) was  $9.10 \pm 3.30$  kPa,  $11.02 \pm 3.31$  kPa and  $22.86 \pm 12.43$  kPa, respectively. The AUC of LSM for  $\geq$  F4 was 0.886. The cut-off value of LSM was 15.15 kPa for  $\geq$  F4, with high sensitivity, specificity, positive predictive value and negative predictive value. In conclusion, Fibrosan can be effectively used as a non-invasive technique to assess liver fibrosis in children with BA.

Shen QL, Chen YJ, Wang ZM, Zhang TC, Pang WB, Shu J, Peng CH. Assessment of liver fibrosis by Fibrosan as compared to liver biopsy in biliary atresia. *World J Gastroenterol* 2015; 21(22): 6931-6936 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6931.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6931>

## INTRODUCTION

Biliary atresia (BA) is a unique pediatric liver disease characterized by progressive inflammatory obliterative cholangiopathy. If left untreated, fibrosclerosing obliteration progresses in both intrahepatic and extrahepatic bile ducts, which inevitably leads to liver cirrhosis<sup>[1]</sup>. The incidence of BA in Asia is reported to be as high as approximately 1 in 5000 live births<sup>[2]</sup>, and in Western Europe is approximately 1 in 19000-15000 live births<sup>[3,4]</sup>.

Assessment of liver fibrosis in BA is pivotal in treatment choice, evaluation of the results of the Kasai procedure and assessment of prognosis. Developed in France by Echosens, transient elastography (Fibrosan) is a novel, noninvasive technique which is used to assess the degree of liver fibrosis. The basic principle of Fibrosan involves a one-dimensional transient elastographic wave, which has a distinguishable traveling speed in different media, and can be translated into various degrees of fibrosis. When the liver is hard, the transient elastographic wave travels faster, resulting in a higher Liver Stiffness Measurement (LSM) value (kPa). Current studies concerning the use of Fibrosan seldom include BA patients. Recently, LSM was applied to predict liver related events in BA after Kasai hepatopuertoenterostomy<sup>[5]</sup>, especially oesophageal varices<sup>[6,7]</sup>. This study evaluated the cut-off value for Fibrosan to assess the degree of liver fibrosis in children with BA, and aimed to provide related evidence for the application of Fibrosan in pediatric medicine.

## MATERIALS AND METHODS

### Patients

Thirty-one patients (10 males and 21 females) with BA admitted to the Pediatric Surgery Department of Beijing Children's Hospital from March 2012 to February 2013 were included in this study. Regular blood tests, blood biochemistry tests and LSM were obtained three days before the Kasai procedure. Liver biopsy specimens, obtained during surgery, were well-preserved. The Japanese Society of Pediatric Surgery divided BA into three types: I, atresia of the common bile duct; II, atresia of the hepatic duct; and III, atresia of the porta hepatis. It was found that all the patients under study had type III BA. The age at operation ranged from 34 to 121 d, with a mean age of  $75.58 \pm 21.84$  d.

### Assessment of liver fibrosis

Liver biopsy specimens were obtained from the front right area (5 or 6 segment of the Couinaud segment) of the liver during the Kasai procedure. All samples were preserved at  $-80^{\circ}\text{C}$ . The 10% neutral formalin-fixed and paraffin-embedded liver biopsy sections were stained with Masson trichromatic stain, which was carried out at the Department of Pathology of Beijing Friendship Hospital Affiliated to Capital Medical University. These sections were assessed using microscopy by two experienced pathologists according to the Metavir scoring system. A third pathologist was consulted when disagreement arose. According to the METAVIR scoring system<sup>[8]</sup>, patients were divided into a non-cirrhosis group (F0-F3) and a cirrhosis group (F4).

### LSM

Fibrosan (Echosens, France) was used to assess liver stiffness, and an experienced operator was responsible for obtaining the Fibrosan value. A probe (size S) was placed vertically on the skin surface between the right lower ribs. Ten values were then obtained avoiding major vessels. A median value calculated by the Statistics Analyze System was chosen as the final value, and the interquartile median ratio was less than 0.3.

### Calculation of aspartate aminotransferase to platelet ratio index

The aspartate aminotransferase to platelet ratio index (APRI) was calculated using the following equation:  $\text{APRI} = [\text{AST} / (\text{ULN}) / \text{platelet counts} (10^9/\text{L})] \times 100$ , rounded to the third decimal place.

### Statistical analysis

The data were analyzed using PASW software version 19.0. One-way analysis of variance was performed for the groups with different degrees of fibrosis. Spearman correlation and Logistic regression analyses were used to determine the relationship between LSM value and

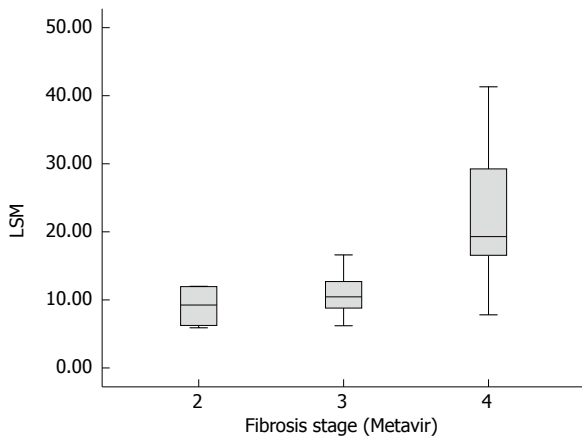


Figure 1 Box-plot of liver stiffness measurement for each fibrosis grade (Metavir).

pathological grade of liver fibrosis. The accuracy of Fibrosan in differentiating fibrosis from cirrhosis was assessed using the area under the receiver operating characteristic (ROC) curve (AUC). An AUC between 0.7 and 0.9 represented a moderate accuracy, while that above 0.9 represented a high accuracy. The Youden index enabled selection of the optimal threshold value between liver fibrosis and liver cirrhosis, and predicted the upper limit of sensitivity and specificity of the different tests. A  $P$  value  $< 0.05$  was considered statistically significant.

## RESULTS

### General information

Before the Kasai procedure, all 31 patients underwent Fibrosan elastography and the success rate was  $95.16\% \pm 6.54\%$ . According to the METAVIR scoring system, no cases were classified in group F0 or F1, 4 cases were in group F2, 20 in group F3, and 7 in group F4. Thus, 24 cases were in the non-cirrhosis group (F0-F3) and 7 cases were in the cirrhosis group (F4). In groups F2, F3 and F4, the mean LSM was  $9.10 \pm 3.30$  kPa,  $11.02 \pm 3.31$  kPa and  $22.86 \pm 12.43$  kPa, respectively. The APRI was  $1.76 \pm 1.12$ ,  $2.34 \pm 1.93$  and  $2.72 \pm 2.36$ , respectively. The blood test results are presented in Table 1.

### Relationship between LSM and liver fibrosis grade

The LSM in groups F2, F3 and F4 was analyzed by one-way analysis of variance, which revealed that the average LSM value in each group was statistically different ( $P = 0.001 < 0.01$ ). Figure 1 is a box-plot of liver fibrosis stage (METAVIR) and LSM. A further comparison between F2 and F4, and between F3 and F4, revealed statistical differences ( $P < 0.01$  for all), while group F2 compared to F3 showed no statistical difference ( $P = 0.593$ ). Using Spearman rank correlation analysis, a positive correlation between LSM and liver fibrosis stage ( $R = 0.544$ ,  $P = 0.002 < 0.01$ ) was observed.

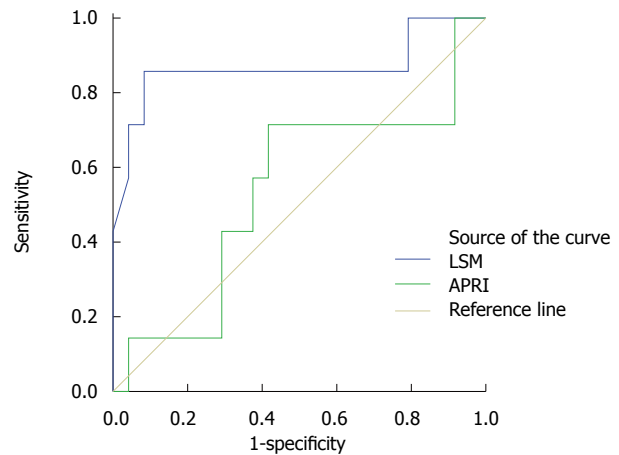


Figure 2 Receiver operating characteristic curves of liver stiffness measurement and aspartate aminotransferase to platelet ratio index.

### Analysis of LSM value

LSM value was determined in 24 patients in the non-cirrhosis group (F2-F3) and in 7 in the cirrhosis group (F4). After Logistic regression analysis, the coefficient of regression of the LSM value for diagnosing cirrhosis was 0.367 and the constant was -6.439 ( $P = 0.021 < 0.05$ ). This regression equation model was tested by  $\chi^2$  analysis ( $P = 0.056$ ) and was found to be insufficient to demonstrate a significant difference, but it was sufficient to indicate that the regression equation model was suitable as the  $P$  value was very close to 0.05. This showed that with increasing LSM value, the probability of cirrhosis increased.

### Cut-off value for cirrhosis

The AUC of LSM was 0.866 for the diagnosis of liver cirrhosis in group F4. The AUC of APRI was 0.536 (Figure 2). The value which corresponded to the maximum Youden index was considered the optimal threshold value. Therefore, the optimal cut-off values of LSM and APRI to diagnose cirrhosis were 15.15 kPa and 1.855, respectively. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 0.857, 0.917, 0.750, 0.957, and 0.903 for LSM, and 0.714, 0.583, 0.357, 0.882, and 0.645 for APRI, respectively.

## DISCUSSION

As a novel noninvasive technique that allows quantification of the degree of liver fibrosis, Fibrosan has been used in adult patients with chronic hepatic diseases. It is not commonly used in children, mainly because there is no suitable probe. However, size S probe has recently been developed by Echosen, France, which can be used in children<sup>[9,10]</sup>.

Some studies have suggested that if at least 10 values were obtained using Fibrosan and the success rate was higher than 65%, it was considered reliable<sup>[11,12]</sup>. In our study, size S probe (Echosen,

**Table 1** Relevant information of different liver fibrosis stages

	F2 (n = 4)	F3 (n = 20)	F4 (n = 7)	P-value
LSM (kPa)	9.10 ± 3.30	11.02 ± 3.31	22.86 ± 12.43	0.001
AST (IU/L)	251.25 ± 173.05	286.59 ± 147.11	328.01 ± 192.54	0.732
ALT (IU/L)	152.50 ± 142.69	180.54 ± 109.90	181.19 ± 92.99	0.893
TBIL (μmol/L)	176.98 ± 43.13	184.81 ± 37.06	194.81 ± 59.87	0.791
DBIL (μmol/L)	89.93 ± 27.46	86.21 ± 13.51	91.64 ± 27.04	0.790
IBIL (μmol/L)	87.05 ± 16.61	98.60 ± 37.29	103.17 ± 38.92	0.773
ALP (U/L)	471.75 ± 92.69	611.37 ± 310.79	626.44 ± 245.08	0.636
GGT (U/L)	631.00 ± 522.17	736.62 ± 587.59	569.89 ± 272.86	0.756
TBA (μmol/L)	103.73 ± 34.29	107.07 ± 35.01	144.09 ± 53.60	0.105
APRI	1.76 ± 1.12	2.34 ± 1.93	2.72 ± 2.36	0.741
Age at operation (d)	73.00 ± 18.67	70.70 ± 20.25	91.00 ± 23.49	0.100

LSM: Liver stiffness measurement; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TBIL: Total bilirubin; DBIL: Direct bilirubin; IBIL: Indirect bilirubin; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transpeptidase; TBA: Total bile acid; APRI: Aspartate aminotransferase to platelet ratio index.

France) was used in all 31 patients to measure liver stiffness. At least 10 values were obtained for each patient, and the success rate was 95.16% ± 6.54%.

As a noninvasive technique for assessing the degree of liver fibrosis, Fibroscan has been widely studied in adults, however, no conclusion regarding the cut-off value for different degrees of fibrosis was reached. According to the Metavir scoring system, the LSM cut-off value for  $F \geq 2$ ,  $F \geq 3$  and  $F = 4$  is 6.9-7.9 kPa, 8.8-12.5 kPa and 11.9-19.5 kPa, respectively<sup>[13-16]</sup>. Based on the pathological stage of the liver specimen (Metavir scoring system) obtained during the Kasai operation, the 31 patients included in the present study were divided into different groups, and using single-factor variance analysis, a significant difference in LSM value was observed between F2 and F4, and between F3 and F4. Furthermore, ROC analysis indicated that the AUC for LSM to diagnose fibrosis or cirrhosis in groups F2, F3 and F4 was 0.259, 0.339 and 0.866, respectively. Although the AUC obtained in this study was smaller than that observed by Sporea *et al.*<sup>[13]</sup>, the overall trend was the same. That is, Fibroscan had a relatively high accuracy in differentiating cirrhosis and non-cirrhosis, although it did not provide sufficient information to determine the degree of fibrosis in non-cirrhosis patients<sup>[17]</sup>. An analysis of related risk factors for liver cirrhosis using Logistic regression indicated that LSM was the only related risk factor. This indicates that with increased LSM, the possibility of liver cirrhosis is higher. Therefore, we consider that LSM can be an assistant tool to distinguish liver cirrhosis from non-cirrhosis in children with BA.

In the analysis of the optimal cut-off value for LSM, 15.15 kPa was considered the best value to determine F4 stage. The sensitivity, specificity, positive predictive value and negative predictive value were 0.857, 0.917, 0.750 and 0.957, respectively. Compared with the positive predictive value, a higher negative predictive value indicated that BA children with an LSM value less than 15.15 kPa were less likely to have liver cirrhosis. As there are no studies on the optimal LSM

cut-off value for liver cirrhosis in BA, we were unable to make comparisons. However, the cut-off value used in this study was relatively consistent with that in adult studies: 17.3 kPa for viral C cirrhosis<sup>[13]</sup>, 17.5 kPa for nonalcoholic steatohepatitis<sup>[18]</sup>, and 19.5 kPa for alcoholic cirrhosis<sup>[16]</sup>.

When comparing Fibroscan (LSM > 15.15 kPa) with the gold standard (pathology) for the diagnosis of cirrhosis, 28 of 31 patients (90.3%) were accurately diagnosed by LSM. While the two techniques were moderately correlated, this was not the expected result. The AUC of LSM used to determine F4 was between 0.7 and 0.9, which was slightly lower than that found in other related studies<sup>[19]</sup>. The reasons for this may be as follows: (1) the sample size in this study was relatively small. The incidence of BA in mainland China has not yet been reported. This in turn added an obstacle in estimating sample capacity, therefore it is hard to estimate that 31 samples were enough or not. We are already working on a large sample size study, but it needs time; (2) different parts of the liver had different stage of fibrosis, therefore the specimen obtained may not have represented the overall stage of fibrosis. In order to minimize this error, we required that all fibroscan examinations and the liver specimens must be taken on the same location; and (3) LSM was affected by other factors<sup>[20-22]</sup>, such as bilirubin, alanine aminotransferase and hemodynamics. In this study, we had done some statistical analyses about the relation between blood tests (AST, ALT, TBIL, DBIL, IBIL, ALP, GGT, and TBA), operation age and LSM, but there was no statistical significance (Table 1). Thus, we believe that the results of this study is reliable.

In this study, APRI was chosen as a representative noninvasive assessment method and was compared with Fibroscan for the accurate diagnosis of liver fibrosis. APRI was expressed as  $[\text{AST}(\text{ULN})/\text{platelet counts} (10^9/\text{L})] \times 100$ , which was developed in 2003 by Wai *et al.*<sup>[23]</sup>. The results suggested that no significant difference in APRI was observed between groups F2, F3 and F4. The AUC to determine liver

cirrhosis in children with BA was 0.536, and if the threshold value was 1.855, the sensitivity, specificity, positive predictive value and negative predictive value were 71.4%, 58.3%, 35.7% and 88.2%, respectively, which was obviously lower than the accuracy of the LSM value. These results are consistent with those of a study on the use of APRI and Fibrosan for the assessment of liver fibrosis in hepatitis B virus (HBV)-infected patients by Bonnard *et al.*<sup>[24]</sup>. However, the results are not consistent with those obtained in a study assessing liver fibrosis and cirrhosis using APRI in children with BA by Kim *et al.*<sup>[25]</sup>. The reasons for this may be as follows: (1) the primitive statistics in this model were too simple and were easily affected by various related factors; (2) the sample size in both studies was relatively small; and (3) errors may have occurred during blood testing, as the sample from each patient was examined separately and the agents used were not from the same batch.

In conclusion, Fibrosan has significant value in diagnosing liver cirrhosis in children with BA. A further study with a larger sample size aimed at obtaining accurate cut-off values to determine liver fibrosis and cirrhosis is required.

## COMMENTS

### Background

Assessment of liver fibrosis in biliary atresia is pivotal in treatment choice, evaluation of the results of the Kasai procedure and assessment of prognosis. Non-invasive methods for fibrosis assessment, such as Fibrosan, are being accepted more and more, replacing the invasive methods.

### Research frontiers

Many studies have been published regarding evaluation of liver fibrosis with Fibrosan in adults, but only a few have been published in biliary atresia. No study was about the cut-off value of liver stiffness measurement (LSM) to distinguish the different levels of liver fibrosis. This study was designed to evaluate liver fibrosis using non-invasive transient elastography (Fibrosan) in comparison with liver biopsy in children with biliary atresia.

### Innovations and breakthroughs

This study used Fibrosan to evaluate liver fibrosis in biliary atresia in comparison with liver biopsy histopathology results. The cut-off value of LSM was 15.15 kPa for  $\geq F_4$ , with a sensitivity, specificity, positive predictive value and negative predictive value of 0.857, 0.917, 0.750 and 0.957, respectively.

### Applications

Fibrosan can be effectively used as a non-invasive technique to assess liver fibrosis in children with biliary atresia. The cut-off value of LSM (15.15 kPa) can distinguish cirrhotic patients from non-cirrhotic patients.

### Terminology

Transient elastography (Fibrosan) is a novel, noninvasive technique which is used to assess the degree of liver fibrosis. The basic principle of Fibrosan involves a one-dimensional transient elastographic wave, which has a distinguishable traveling speed in different media, and can be translated into various degrees of fibrosis. When the liver is hard, the transient elastographic wave travels faster, resulting in a higher LSM value (kPa).

### Peer-review

This is a retrospective analysis of transient elastography (Fibrosan) in the assessment of liver fibrosis in children with biliary atresia. This paper was concise, clear, and well-written. The results are interesting and suggest that Fibrosan can be used as a non-invasive technique to assess liver fibrosis in children with biliary atresia.

## REFERENCES

- Hartley JL, Davenport M, Kelly DA. Biliary atresia. *Lancet* 2009; **374**: 1704-1713 [PMID: 19914515 DOI: 10.1016/S0140-6736(09)60946-6]
- Hsiao CH, Chang MH, Chen HL, Lee HC, Wu TC, Lin CC, Yang YJ, Chen AC, Tiao MM, Lau BH, Chu CH, Lai MW. Universal screening for biliary atresia using an infant stool color card in Taiwan. *Hepatology* 2008; **47**: 1233-1240 [PMID: 18306391 DOI: 10.1002/hep.22182]
- McKiernan PJ, Baker AJ, Kelly DA. The frequency and outcome of biliary atresia in the UK and Ireland. *Lancet* 2000; **355**: 25-29 [PMID: 10615887 DOI: 10.1016/S0140-6736(99)03492-3]
- Chardot C, Carton M, Spire-Bendelac N, Le Pommelet C, Golmard JL, Auvert B. Epidemiology of biliary atresia in France: a national study 1986-96. *J Hepatol* 1999; **31**: 1006-1013 [PMID: 10604573 DOI: 10.1016/S0168-8278(99)80312-2]
- Hahn SM, Kim S, Park KI, Han SJ, Koh H. Clinical benefit of liver stiffness measurement at 3 months after Kasai hepatoportoenterostomy to predict the liver related events in biliary atresia. *PLoS One* 2013; **8**: e80652 [PMID: 24260445 DOI: 10.1371/journal.pone.0080652]
- Colecchia A, Di Biase AR, Scafoli E, Predieri B, Iughetti L, Reggiani ML, Montrone L, Ceccarelli PL, Vestito A, Viola L, Paolucci P, Festi D. Non-invasive methods can predict oesophageal varices in patients with biliary atresia after a Kasai procedure. *Dig Liver Dis* 2011; **43**: 659-663 [PMID: 21596631 DOI: 10.1016/j.dld.2011.04.006]
- Chang HK, Park YJ, Koh H, Kim SM, Chung KS, Oh JT, Han SJ. Hepatic fibrosis scan for liver stiffness score measurement: a useful preendoscopic screening test for the detection of varices in postoperative patients with biliary atresia. *J Pediatr Gastroenterol Nutr* 2009; **49**: 323-328 [PMID: 19633573 DOI: 10.1097/MPG.0b013e31819de7ba]
- Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293 [PMID: 8690394 DOI: 10.1002/hep.510240201]
- Ferraioli G, Lissandrin R, Zicchetti M, Filice C. Assessment of liver stiffness with transient elastography by using S and M probes in healthy children. *Eur J Pediatr* 2012; **171**: 1415; author reply 1417 [PMID: 22729244 DOI: 10.1007/s00431-012-1777-6]
- Engelmann G, Gebhardt C, Wenning D, Wühl E, Hoffmann GF, Selmi B, Grulich-Henn J, Schenk JP, Teufel U. Feasibility study and control values of transient elastography in healthy children. *Eur J Pediatr* 2012; **171**: 353-360 [PMID: 21861093 DOI: 10.1007/s00431-011-1558-7]
- Wang JH, Changchien CS, Hung CH, Eng HL, Tung WC, Kee KM, Chen CH, Hu TH, Lee CM, Lu SN. FibroScan and ultrasonography in the prediction of hepatic fibrosis in patients with chronic viral hepatitis. *J Gastroenterol* 2009; **44**: 439-446 [PMID: 19308312 DOI: 10.1007/s00535-009-0017-y]
- Jang HW, Kim SU, Park JY, Ahn SH, Han KH, Chon CY, Park YN, Choi EH, Kim do Y. How many valid measurements are necessary to assess liver fibrosis using FibroScan® in patients with chronic viral hepatitis? An analysis of subjects with at least 10 valid measurements. *Yonsei Med J* 2012; **53**: 337-345 [PMID: 22318821 DOI: 10.3349/ymj.2012.53.2.337]
- Sporea I, Sirli R, Deleanu A, Tudora A, Popescu A, Curescu M, Bota S. Liver stiffness measurements in patients with HBV vs HCV chronic hepatitis: a comparative study. *World J Gastroenterol* 2010; **16**: 4832-4837 [PMID: 20939112 DOI: 10.3748/wjg.v16.i38.4832]
- Ogawa E, Furusyo N, Toyoda K, Takeoka H, Otaguro S, Hamada M, Murata M, Sawayama Y, Hayashi J. Transient elastography for patients with chronic hepatitis B and C virus infection: Non-invasive, quantitative assessment of liver fibrosis. *Hepatol Res* 2007; **37**: 1002-1010 [PMID: 17608672 DOI: 10.1111/j.1872-034X.2007.00160.x]



- 15 **Muñoz R**, Ramírez E, Fernandez I, Martin A, Romero M, Romero E, Dominguez-Gil B, Hernandez A, Morales E, Andres A, Castellano G, Morales JM. Correlation between fibroscan, liver biopsy, and clinical liver function in patients with hepatitis C virus infection after renal transplantation. *Transplant Proc* 2009; **41**: 2425-2426 [PMID: 19715940 DOI: 10.1016/j.transproceed.2009.06.103]
- 16 **Nguyen-Khac E**, Chatelain D, Tramier B, Decrombecque C, Robert B, Joly JP, Brevet M, Grignon P, Lion S, Le Page L, Dupas JL. Assessment of asymptomatic liver fibrosis in alcoholic patients using fibroscan: prospective comparison with seven non-invasive laboratory tests. *Aliment Pharmacol Ther* 2008; **28**: 1188-1198 [PMID: 18705692 DOI: 10.1111/j.1365-2036.2008.03831.x]
- 17 **Poynard T**, de Ledinghen V, Zarski JP, Stanciu C, Munteanu M, Vergniol J, France J, Trifan A, Le Naour G, Vaillant JC, Ratzu V, Charlotte F. Relative performances of FibroTest, Fibroscan, and biopsy for the assessment of the stage of liver fibrosis in patients with chronic hepatitis C: a step toward the truth in the absence of a gold standard. *J Hepatol* 2012; **56**: 541-548 [PMID: 21889468 DOI: 10.1016/j.jhep.2011.08.007]
- 18 **Yoneda M**, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo S, Nakajima A. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008; **40**: 371-378 [PMID: 18083083 DOI: 10.1016/j.dld.2007.10.019]
- 19 **Alkhoufi N**, Sedki E, Alisi A, Lopez R, Pinzani M, Feldstein AE, Nobili V. Combined paediatric NAFLD fibrosis index and transient elastography to predict clinically significant fibrosis in children with fatty liver disease. *Liver Int* 2013; **33**: 79-85 [PMID: 23146095 DOI: 10.1111/liv.12024]
- 20 **Yashima Y**, Tsujino T, Masuzaki R, Nakai Y, Hirano K, Tateishi R, Sasahira N, Isayama H, Tada M, Yoshida H, Kawabe T, Omata M. Increased liver elasticity in patients with biliary obstruction. *J Gastroenterol* 2011; **46**: 86-91 [PMID: 20814804 DOI: 10.1007/s00535-010-0290-9]
- 21 **Trifan A**, Sfarti C, Cococariu C, Dimache M, Cretu M, Hutanasu C, Stanciu C. Increased liver stiffness in extrahepatic cholestasis caused by choledocholithiasis. *Hepat Mon* 2011; **11**: 372-375 [PMID: 22087164]
- 22 **Harata M**, Hashimoto S, Kawabe N, Nitta Y, Murao M, Nakano T, Arima Y, Shimazaki H, Ishikawa T, Okumura A, Ichino N, Osakabe K, Nishikawa T, Yoshioka K. Liver stiffness in extrahepatic cholestasis correlates positively with bilirubin and negatively with alanine aminotransferase. *Hepatol Res* 2011; **41**: 423-429 [PMID: 21435129 DOI: 10.1111/j.1872-034X.2011.00797.x]
- 23 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]
- 24 **Bonnard P**, Sombié R, Lescure FX, Bougouma A, Guiard-Schmid JB, Poynard T, Calès P, Housset C, Callard P, Le Pendevan C, Drabo J, Carrat F, Pialoux G. Comparison of elastography, serum marker scores, and histology for the assessment of liver fibrosis in hepatitis B virus (HBV)-infected patients in Burkina Faso. *Am J Trop Med Hyg* 2010; **82**: 454-458 [PMID: 20207872 DOI: 10.4269/ajtmh.2010.09-0088]
- 25 **Kim SY**, Seok JY, Han SJ, Koh H. Assessment of liver fibrosis and cirrhosis by aspartate aminotransferase-to-platelet ratio index in children with biliary atresia. *J Pediatr Gastroenterol Nutr* 2010; **51**: 198-202 [PMID: 20531020 DOI: 10.1097/MPG.0b013e3181da1d98]

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## Retrospective Study

# Clinical impact of preoperative acute pancreatitis in patients who undergo pancreaticoduodenectomy for periampullary tumors

Yong-Hua Chen, Si-Ming Xie, Hao Zhang, Chun-Lu Tan, Neng-Wen Ke, Gang Mai, Xu-Bao Liu

Yong-Hua Chen, Si-Ming Xie, Hao Zhang, Chun-Lu Tan, Neng-Wen Ke, Gang Mai, Xu-Bao Liu, Department of Hepatobiliopancreatic Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

**Author contributions:** Liu XB and Mai G contributed equally to this work and are considered as joint corresponding authors; Liu XB, Chen YH and Mai G designed the research; Chen YH, Liu XB, Mai G, Xie SM, Zhang H, Tan CL and Ke NW performed the research; Chen YH, Xie SM and Zhang H analyzed the data; Chen YH and Xie SM wrote the paper; all authors read and approved the final manuscript.

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**Correspondence to:** Xu-Bao Liu, MD, Department of Hepatobiliopancreatic Surgery, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu 610041, Sichuan Province, China. [xbliu@medmail.com.cn](mailto:xbliu@medmail.com.cn)

**Telephone:** +86-28-85422477

**Fax:** +86-28-85164035

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## Abstract

**AIM:** To investigate the impact of preoperative acute pancreatitis (PAP) on the surgical management of periampullary tumors.

**METHODS:** Fifty-eight patients with periampullary tumors and PAP were retrospectively analyzed. Thirty-four patients who underwent pancreaticoduodenectomy (PD) and 4 patients who underwent total pancreatectomy were compared with a control group of 145 patients without PAP during the same period.

**RESULTS:** The preoperative waiting time was significantly shorter for the concomitant PAP patients who underwent a resection (22.4 d vs 54.6 d,  $P < 0.001$ ) compared to those who did not. The presence of PAP significantly increased the rate of severe complications (Clavien grade 3 or higher) (17.6% vs 4.8%,  $P = 0.019$ ) and lengthened the hospital stay (19.5 d vs 14.5 d,  $P = 0.006$ ). A multivariate logistic regression analysis revealed that PAP was an independent risk factor for postoperative pancreatic fistula (OR = 2.91; 95%CI: 1.10-7.68;  $P = 0.032$ ) and severe complications (OR = 4.70; 95%CI: 1.48-14.96;  $P = 0.009$ ) after PD. There was no perioperative mortality.

**CONCLUSION:** PAP significantly increases the incidence of severe complications and lengthens the

hospital stay following PD. PD could be safely performed in highly selective patients with PAP.

**Key words:** Pancreaticoduodenectomy; Complications; Preoperative pancreatitis; Pancreatic fistula

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**Core tip:** To date, it remains unclear how preoperative acute pancreatitis (PAP) affects the surgical management of perampullary tumors. We analyzed patients with perampullary tumors and concomitant PAP who were treated in a high-volume center. In the present study, we showed that PAP delays the resection of perampullary tumors and significantly increases the incidence of severe complications and lengthens the hospital stay following pancreaticoduodenectomy (PD). The study results suggest that PD could be safely performed in highly selective patients with PAP.

Chen YH, Xie SM, Zhang H, Tan CL, Ke NW, Mai G, Liu XB. Clinical impact of preoperative acute pancreatitis in patients who undergo pancreaticoduodenectomy for perampullary tumors. *World J Gastroenterol* 2015; 21(22): 6937-6943. Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6937.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6937>

## INTRODUCTION

There are a large number of etiological factors involved in the development of acute pancreatitis (AP). Excluding common etiologies, such as alcohol and gallstones, it is well known that AP may occur in association with a perampullary neoplasm, which is increasingly being recognized, especially in individuals with idiopathic AP<sup>[1-3]</sup>. Furthermore, post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis is the most common cause of preoperative acute pancreatitis (PAP)<sup>[4]</sup>.

Generally, perampullary tumor patients with AP should initially be managed conservatively<sup>[5]</sup>. However, surgical resection is the sole curative measure for pancreatic cancer, and conservative treatments can significantly delay the need for the cancer operation<sup>[1]</sup>. Therefore, a dilemma arises: Can an operation successfully be performed in a patient with perampullary tumors with AP?

We analyzed patients with perampullary tumors and concomitant PAP who were treated in a high-volume center and assessed the impact of PAP on the surgical management of the perampullary tumors.

## MATERIALS AND METHODS

We performed a retrospective analysis of patients from our database with perampullary tumors and concomitant PAP at the Pancreatic Surgery Center of

West China Hospital between January 1, 2009 and December 31, 2013. This study followed the ethical guidelines of the Helsinki Declaration of 1975 (revised in 1983). The data on the patient demographics, severity of the pancreatitis, tumor stage, applied treatments, and morbidity and mortality rates were analyzed retrospectively using a prospective pancreatic database. Additional information was obtained by contacting the referring physician/hospital. The data were extracted from medical records by 2 reviewers who were blinded to the case-control status. The two reviewers independently assessed these data, and disagreements were resolved by discussion with a third reviewer. The tumor staging and lymph node status evaluation were performed based on the pathological findings.

The diagnosis of AP<sup>[6,7]</sup> was made based on the clinical symptoms (new onset or increased abdominal pain that necessitated an unplanned admission of an outpatient for more than one night or prolonged hospitalization of an inpatient), biochemical analyses (3 times the upper limit of the normal value or a significant elevation of the serum amylase and/or lipase concentrations), as well as contrast-enhanced abdominal computed tomography (CT) and/or endoscopic ultrasonography (EUS)-guided fine needle aspiration (FNA) when necessary. As controls, during the same period, consecutive patients with pancreatic and perampullary disease who did not have AP but underwent PD were also included in the study. We screened patients who had no evidence of distant metastasis or local vascular involvement (which was defined as a tumor surrounding the portal or mesenteric vessels for more than 180 degrees of their circumference or an irregular vessel margin) on a CT and/or magnetic resonance imaging (MRI) scan<sup>[8]</sup>. The interval between the CT and/or MRI examination and admission was set at less than one week. Patients with a serious coexisting illness, active bleeding, ongoing cholangitis, distant metastasis, local vascular involvement or previous preoperative biliary drainage beyond 2 wk were subsequently excluded. Patients with concomitant cholangitis or a pancreatitis episode after an ERCP were included.

From this database, we identified 58 patients with perampullary tumors and concomitant PAP. During the preoperative period, all of the patients were hospitalized and received conservative medical treatment until they were deemed operable. None of the patients required percutaneous radiological or surgical drainage prior to the tumor resection. All of the patients underwent CT and/or MRI prior to the operation, and their resectability was redetermined based on these tests. All of the patients who underwent PD were divided into two groups, which consisted of a "non-AP" group (145 patients who did not have AP) and an "AP" group (34 patients with clinical PAP).

### Definition of postoperative complications

General and surgery-related complications, including postoperative pancreatic fistula (POPF), delayed gastric emptying, intra-abdominal infection, abdominal

**Table 1** Distribution of acute pancreatitis patients who had surgery for a planned laparotomy *n* (%)

Parameter	Resected ( <i>n</i> = 38)	Not resected ( <i>n</i> = 20)	<i>P</i> value
Age (yr), mean ± SD	55.92 ± 11.01	20.8 ± 12.18	0.11
Male	16 (57.1)	16 (80)	0.098
Cause of acute pancreatitis			0.049
ERCP	10 (26.3)	1 (5)	
Unknown	28 (73.7)	19 (95)	
Balthazar CT score	2.05 ± 0.70	2.9 ± 0.85	< 0.001
Grade of severity			0.283
Mild acute pancreatitis	12 (35.3)	6 (30)	
Moderately severe acute pancreatitis	19 (55.9)	9 (45)	
Severe acute pancreatitis	3 (8.8)	5 (25)	
Time to surgery (d), mean ± SD	22.3 ± 17.0	54.6 ± 26.3	< 0.001
Type of operation			
Pancreaticoduodenectomy	34 (89.5)		
Total pancreatectomy	4 (10.5)		
Bypass procedure		7 (35)	
Exploratory laparotomy		13 (65)	

ERCP: Endoscopic retrograde cholangiopancreatography; CT: Computed tomography.

abscess formation, pneumonia, postpancreatectomy hemorrhage, and anastomotic leakage, were analyzed retrospectively using a prospective pancreatic database. The complications, including POPF<sup>[9]</sup>, delayed gastric emptying<sup>[10]</sup>, and postpancreatectomy hemorrhage<sup>[11]</sup>, were defined by standards adopted by the International Study Group of Pancreatic Surgery. The postoperative complications were recorded and graded according to the Clavien classification<sup>[12]</sup>. Severe complications were defined in this study as conditions that were grade 3 or higher based on the Clavien classification<sup>[12]</sup>. The in-hospital death of a patient for any reason was recorded.

### Statistical analysis

All of the data were collected and analyzed using the SPSS statistical program for Windows, Version 13.0 (SPSS Inc., Chicago, United States). The patient demographic and clinical characteristics across the groups were compared using the  $\chi^2$  test (or Fisher's exact test) for the categorical measures and using the *t*-test for the continuous data. Factors with *P* < 0.10 were included in the multivariate analysis. A multivariable analysis of the primary outcomes was completed using logistic regression. The final multivariate model was determined using logistic regression with backward selection in order to identify independent predictors of POPF. *P*-values less than 0.05 were considered statistically significant. The logistic model results are reported as odds ratios (ORs), two-sided 95% confidence intervals (CIs), and *P*-values.

## RESULTS

### Characteristics of the patients with periampullary tumors and concomitant PAP

Fifteen-eight patients underwent surgery. Of these,

20 (34.5%) patients were considered to have either a non-resectable pancreatic lesion or metastasis, including the following: encasement of major vessels (*i.e.*, the portal vein, superior mesenteric vessels, and vena cava) in 8 (40%) patients; presence of distant metastases in 9 (45%); and presence of distant nodal metastases in 3 (15%). A gastroenteric bypass procedure was the surgical intervention in 7 patients. Thirteen-eight (65.5%) patients were found to have lesions considered to be resectable for cure during surgery. The PD procedure was performed in 34 (58.7%) patients, and a total pancreatectomy was performed in 4 (6.9%).

The patients' characteristics are summarized in Table 1. The age and gender distribution were similar for the patients who did and did not undergo resection. Moreover, the cause of the AP and the grades of severity were comparable between the two groups. There were significantly lower Balthazar CT scores in the patients who underwent resection (mean 2.05 vs 2.9, *P* < 0.001). The preoperative waiting time was significantly shorter for the patients who underwent resection (mean 22.4 d vs 54.6 d, *P* < 0.001). The presence of neoplasms and AP was confirmed pathologically in all of the cases. A frozen section analysis of the pancreatic margin was obtained in all of the patients in whom a pancreaticojejunostomy was performed. Although all of the pancreatic resection margins were devoid of tumor and necrosis, microscopic signs of acute/subacute inflammation were noted to some degree in all of the patients.

### Demographic and intraoperative PD data

The data for the patients in each study arm are presented in Table 2. The majority of the variables did not significantly differ between the AP and non-AP groups. The mean difference between the groups in the delay to surgery was 12.4 d: the delay was 6.0 d in the AP group vs 18.4 d in the non-AP group (*P* < 0.001). After the resection, the incidence of tumor-positive lymph nodes (N1) was significantly higher in the AP group (53.3% in the AP group vs 30.2% in the non-AP group, *P* = 0.017).

### Postoperative PD data

The details of the postoperative data from the PD patients are summarized in Table 3. Complications occurred in 38% of patients in the study. Among the entire patient population, the POPF rate was 15.1% (*n* = 27). The overall incidence of POPF was significantly higher in the AP group (29.4% in the AP group vs 11.7% in the non-AP group, *P* = 0.009). Moreover, a statistical comparison of the subsets showed that there were significantly more cases of grades B and C POPF (AP vs non-AP: 20.6% vs 6.2%, *P* = 0.015) in the AP group compared to the non-AP group. There were no substantial differences in the overall complications or mortality between the two groups. However, the rate of severe complications (Clavien



**Table 2** Demographic data, operation and pathologic characteristics of pancreaticoduodenectomy patients *n* (%)

Parameter	AP ( <i>n</i> = 34)	Control ( <i>n</i> = 145)	<i>P</i> value
Patient variables			
Age (yr), mean ± SD	55.9 ± 11.26	59.0 ± 10.92	0.131
Male	21 (61.8)	93 (64.1)	0.796
Weight loss	17 (50)	59 (40.7)	0.323
Comorbid disease	9 (26.5)	47 (32.4)	0.501
Preoperative jaundice	11 (32.4)	68 (43.9)	0.124
Pancreas remnant (soft)	29 (85.3)	109 (75.2)	0.206
Pancreatic duct diameter (< 3 mm)	22 (64.7)	92 (63.4)	0.891
Treatment variables			
Preoperative biliary drainage	6 (17.6)	11 (7.6)	0.099
Time to surgery (d), mean ± SD	18.4 ± 10.76	6.0 ± 2.5	< 0.001
Intraoperative blood transfusion	11 (32.4)	62 (35.9)	0.700
Vein resection	1 (2.9)	7 (4.8)	0.632
Pathological variables			
Characteristics of resectable tumors			0.062
Pancreatic carcinoma	21 (61.8)	58 (40)	
Ampullary carcinoma	4 (11.8)	19 (13.1)	
Duodenal carcinoma	2 (5.9)	35 (24.1)	
Distal bile duct carcinoma	3 (8.8)	18 (12.4)	
Other diagnosis	4 (11.8)	15 (10.3)	
Benign: malignant ratio	4/30	130/15	1.000
Tumor-positive lymph nodes (N1)	16 (53.3)	39 (30.2)	0.017
Microscopically residual disease (R1)	1 (2.9)	2 (1.4)	0.523

grades 3 to 5) was significantly higher in the AP group (17.6%) than in the non-AP group (4.8%; *P* = 0.019). The incidences of intra-abdominal abscess, postpancreatectomy hemorrhage and intestinal fistula were also significantly higher in the patients with PAP than in the patients without. There were no significant differences between the two groups in regards to the incidences of other postoperative complications, such as delayed gastric emptying, bile leakage, and pulmonary complications. The mean hospital stay for the patients with PAP was longer than for that the control group (mean 17.4 d for the AP group vs 13.7 d for the non-AP group, *P* = 0.006).

### Risk factors influencing POPF

Univariate and multivariate analyses were used to reveal the risk factors influencing POPF after PD. As shown in Table 4, four factors were extracted as being useful for discriminating between the patients with and without POPF after PD. A multivariate logistic regression analysis revealed that the most powerful predictor was the pancreatic remnant texture. Patients with soft pancreatic remnants had a much higher likelihood of developing POPF than those with firm pancreatic remnants (OR = 9.82, 95%CI: 1.22-79.31; *P* = 0.032). We observed a much higher likelihood of developing POPF in the patients who presented with AP (OR = 2.91, 95%CI: 1.10-7.68; *P* = 0.032). Finally, there was a greater risk of developing POPF in the patients with intraoperative blood transfusion and preoperative biliary drainage requirements (OR = 2.69,

**Table 3** Postoperative data of pancreaticoduodenectomy patients *n* (%)

Parameter	AP ( <i>n</i> = 34)	Control ( <i>n</i> = 145)	<i>P</i> value
Medical complications	15 (44.1)	53 (36.6)	0.413
Grade of complications			0.086
0-1	24	116	
2	5	22	
3	4	4	
4	2	3	
5 Mortality	0	0	
Severe complications (grade 3 or more)	6 (17.6)	7 (4.8)	0.019
Pancreatic fistula	10 (29.4)	17 (11.7)	0.009
Grade A	3 (8.8)	8 (5.5)	
Grade B	2 (5.9)	7 (4.8)	
Grade C	5 (14.7)	2 (1.8)	
Grade B/C	7 (20.6)	9 (6.2)	0.015
Biliary leak	1 (2.9)	1 (0.7)	0.345
Delayed gastric emptying	10 (29.4)	28 (19.3)	0.195
Intra-abdominal collection or abscess	7 (20.6)	8 (5.5)	0.010
Hemorrhage after pancreatectomy	5 (14.7)	4 (2.8)	0.013
Gastrointestinal hemorrhage	3 (8.8)	2 (1.4)	0.048
Intra-abdominal hemorrhage	1 (2.9)	2 (1.4)	0.471
Wound infection	8 (23.5)	18 (12.4)	0.098
Intestinal fistula	2 (5.9)	0	0.035
Septicemia	1 (2.9)	5 (3.4)	1.000
Pulmonary complications	5 (14.7)	16 (11)	0.557
Postoperative length of stay (d), mean ± SD	17.4 ± 9.8	13.7 ± 6.1	0.006

95%CI: 1.08-6.71, *P* = 0.034; OR = 3.40, 95%CI: 1.05-10.99; *P* = 0.041, respectively).

### Risk factors influencing severe postoperative complications

Univariate and multivariate analyses were used to reveal the risk factors influencing severe postoperative complications (grade 3 or higher) after PD (including 4 cases in TP from the AP group). Table 5 shows the results of 9 parameters that were univariately examined as potential risk factors for 15 patients with severe postoperative complications (grade 3 or higher) after PD vs 168 patients without. Three factors were extracted as being useful for discriminating between the patients with and without severe postoperative complications: preoperative AP (OR = 4.70, 95%CI: 1.48-14.96; *P* = 0.009), comorbid disease (OR = 3.72, 95%CI: 1.18-11.75; *P* = 0.025) and intraoperative blood transfusion (OR = 3.50, 95%CI: 1.11-10.97 *P* = 0.032). The most powerful predictor was preoperative AP (OR = 4.70, 95%CI: 1.48-14.96; *P* = 0.009).

## DISCUSSION

This retrospective analysis was performed using data from 58 consecutive patients with periampullary tumors and concomitant PAP from January 2009 to December 2013. The preoperative waiting time was significantly shorter for the patients who underwent resection (22.4 d vs 54.6 d, *P* < 0.001) compared to those who did not. In the present study, 183 consecutive patients

**Table 4 Risk factors influencing pancreatic fistula after pancreaticoduodenectomy by univariate and multivariate logistic regression analyses**

Parameter	Univariate <sup>1</sup>		Multivariate <sup>1</sup>	
	OR (95%CI)	P value	OR (95%CI)	P value
Age ( $\geq 70$ yr <i>vs</i> $< 70$ yr)	0.74 (0.24-2.30)	0.738		
Sex (male <i>vs</i> female)	0.57 (0.23-1.43)	0.228		
Weight loss (yes <i>vs</i> no)	1.10 (0.48-2.51)	0.821		
Preoperative jaundice (yes <i>vs</i> no)	1.55 (0.64-3.76)	0.334		
Acute pancreatitis (yes <i>vs</i> no)	3.14 (1.28-7.67)	0.012	2.91 (1.10-7.68)	0.032
Blood transfusion (yes <i>vs</i> no)	1.23 (0.53-2.81)	0.632		
Comorbid disease (yes <i>vs</i> no)	1.36 (0.58-3.19)	0.485		
Preoperative biliary drainage (yes <i>vs</i> no)	4.97 (1.70-14.54)	0.003	3.40 (1.05-10.99)	0.041
Intraoperative blood transfusion (yes <i>vs</i> no)	2.28 (0.99-5.24)	0.052	2.69 (1.08-6.71)	0.034
Histopathologic diagnosis		0.090		NS
Pancreatic carcinoma	1.0			
Ampullary carcinoma	1.86 (0.53-6.53)	0.333		
Duodenal carcinoma	2.54 (0.83-7.73)	0.022		
Distal bile duct carcinoma	3.83 (1.28-11.45)	0.016		
Pancreatic texture (soft <i>vs</i> hard)	9.29 (1.22-70.68)	0.031	9.82 (1.22-79.31)	0.032
Pancreatic duct ( $< 3$ mm <i>vs</i> $\geq 3$ mm)	2.22 (0.85-5.82)	0.105		

<sup>1</sup>Logistic regression. NS: Not significant.**Table 5 Risk factors influencing severe complications after pancreaticoduodenectomy by univariate and multivariate logistic regression analyses**

Parameter	Univariate <sup>1</sup>		Multivariate <sup>1</sup>	
	OR (95%CI)	P value	OR (95%CI)	P value
Age ( $\geq 70$ yr <i>vs</i> $< 70$ yr)	0.68 (0.15-3.17)	0.623		
Sex (male <i>vs</i> female)	2.46 (0.67-9.06)	0.175		
Weight loss (yes <i>vs</i> no)	1.14 (0.40-3.28)	0.810		
Preoperative jaundice (yes <i>vs</i> no)	0.85 (0.29-2.49)	0.762		
Preoperative acute pancreatitis (yes <i>vs</i> no)	3.87 (1.30-11.46)	0.015	4.70 (1.48-14.96)	0.009
Preoperative biliary drainage (yes <i>vs</i> no)	2.55 (0.65-10.1)	0.181		
Comorbid disease (yes <i>vs</i> no)	2.78 (0.95-8.07)	0.061	3.72 (1.18-11.75)	0.025
Intraoperative blood transfusion (yes <i>vs</i> no)	2.92 (0.99-8.61)	0.052	3.50 (1.11-10.97)	0.032
Histology (pancreatic <i>vs</i> other)	0.74 (0.26-2.13)	0.578		

<sup>1</sup>Logistic regression. NS: Not significant.

underwent PD following our standard protocol of digestive reconstruction<sup>[13]</sup>. After the resection, the incidence of tumor-positive lymph nodes (N1) was significantly higher in the AP group (53.3% *vs* 30.2%,  $P = 0.017$ ). There was a significant difference in the frequency of overall POPF between the AP and non-AP groups (29.4% *vs* 11.7%,  $P = 0.009$ ). Moreover, the frequency of severe complications (Clavien grade 3 or higher) in the AP group was significantly higher than that in the non-AP group (17.6% *vs* 4.8%,  $P = 0.019$ ). The development of preoperative AP has been recognized as an important risk factor for both POPF and severe complications (Clavien grade 3 or higher) after PD.

When considered in connection with periampullary tumors, PAP may considerably influence the patient's management<sup>[5]</sup>. There is often an immediate and lasting inflammatory response that may induce pancreatic or fatty necrosis and other fluid collection. The resulting adhesion between the parenchyma of the pancreas and the peripancreatic tissues can blur tissue boundaries,

making the surgical procedure more difficult. As a result, the association that exists should initially be managed conservatively. In contrast, surgical resection is the sole curative measure for periampullary tumors. A prolonged delay in surgery may result in a missed opportunity for radical resection of malignancies, especially pancreatic adenocarcinoma, which is generally a very aggressive, fast growing tumor<sup>[1]</sup>. Therefore, a dilemma arises: Can an operation successfully be performed in a patient with periampullary tumors in the setting of AP?

The management decisions related to patients requiring PD following AP include (1) determining the timing of the operation; (2) maximizing the curability of any surgical resection; and (3) avoiding complications, such as POPF and intra-abdominal infections. In our cohort, the mean interval from the diagnosis of AP to the operation was 33.4 d in our 58 cases. This is consistent with the report by Erkan *et al*<sup>[5]</sup>, in which the median interval from the diagnosis of AP to the operation was 34 d in four patients with periampullary tumors followed by mild to moderate AP

who underwent PD, while the median time was 31 d in six patients who underwent TP. In contrast, the median time was 111 d in six patients with periampullary tumors followed by severe AP who underwent PD<sup>[4]</sup>. According to Tummala's<sup>[2]</sup> study of 218 patients with AP who underwent endoscopic ultrasound-guided FNA, 38 pancreatic cancer cases were diagnosed, and their resection rate was 39%. This is consistent with the report by Mujica *et al.*<sup>[1]</sup>, with a curative resection rate of 27%. In contrast, the number of patients found to have a lesion considered to be resectable for cure at the time of surgery was more than 50% in our series (resection rate, 38 of the 58 cases, 65.5%). This finding may account for the timely operation (mean 18.4 d) or the pathological entity, with only 61.8% of pancreatic carcinoma cases, leading to a delay in the resection of these tumors with a lower resection rate. After resection, the incidence of tumor-positive lymph nodes (N1) was significantly higher in the AP group. This finding may account for a longer delay in surgery, leading to the delayed resection of these tumors with a greater number of tumor-positive lymph nodes (N1). Moreover, in the mouse model, AP can accelerate the initiation and progression to pancreatic cancer<sup>[14,15]</sup>. Therefore, a timely diagnosis and the proper treatment of pancreatic cancer with AP may potentially reduce the morbidity and complications and may likely also improve the oncological outcomes<sup>[1,2]</sup>.

In the present study, the PAP and pancreatic texture have been recognized as important risk factors for POPF. It is clear that the pancreatic texture is a major contributing factor, especially when trying to perform a fine duct to mucosa anastomosis on a soft pancreatic remnant. A soft pancreas is very vulnerable to ischemia and actively produces exocrine secretions<sup>[16]</sup>. Patients with PAP generally have a softer/fragile pancreatic texture. In 4 cases from the AP group, the texture of the gland was not suitable (*e.g.*, fragile pancreas due to inflammation) for a safe pancreaticojejunostomy; therefore, the procedure was converted from PD to TP during the surgery. Conversely, in 34 patients, the texture of the pancreas appeared to be safe for an anastomosis. The frozen section analysis of the resection margins of these 34 patients showed varying degrees of inflammation but no necrosis. Erkan *et al.*<sup>[5]</sup> demonstrated that the intraoperative findings of the pancreatic texture determine whether or not a pancreaticojejunostomy should be performed. It could also be theorized that the pancreatic duct diameter might contribute to POPF formation. Our data indicate no difference in the pancreatic duct diameter between the two cohorts. This finding may account for the fact that patients with PAP generally have a softer/fragile pancreatic texture, although they have a large pancreatic duct diameter.

The occurrence of preoperative AP was clarified to be the independent risk factor for severe complications after PD. Two previous studies reported that the occurrence of preoperative AP significantly increased the postoperative

complications, including pancreatic fistula<sup>[4,5]</sup>. In our present study, preoperative AP significantly increased the occurrence of POPF, postpancreatectomy hemorrhage and intra-abdominal infection. The increased postpancreatectomy hemorrhage rate in the AP group compared to the controls indicates that recent pancreatic inflammation may increase the postoperative bleeding in general<sup>[5]</sup>. Many pancreatic surgeons believe that postpancreatectomy hemorrhage after PD is a secondary effect caused by POPF or intra-abdominal abscess<sup>[17,18]</sup>.

Nevertheless, the presence of PAP increased the severe complications and extended the hospitalization time, but there was no mortality. This rate was well within the range seen in previous studies in the literature, where POPF rates ranged from 10% to 28.5%<sup>[19]</sup>, and severe postpancreatectomy hemorrhage rates from 5.8% to 9.2%<sup>[18,20]</sup>. Patients with PAP can be managed conservatively until the timely operation. Therefore, PD may be safely performed in highly selective patients with PAP, although the absence of necrosis or inflammation at the pancreatic resection margin should be evaluated in a larger group of patients to assess its value in predicting anastomotic insufficiency.

In conclusion, PAP significantly increases the incidence of POPF and severe complications after PD. PD can be safely performed in highly selective patients with PAP.

## COMMENTS

### Background

It is well known that acute pancreatitis (AP) may occur in association with a periampullary neoplasm. However, to date, it remains unclear how preoperative AP (PAP) affects the surgical management of periampullary tumors.

### Research frontiers

The current research hotspot is how to manage periampullary tumors with PAP.

### Innovations and breakthroughs

In the present study, the authors showed that PAP delays the resection of periampullary tumors. PAP significantly increases the incidence of postoperative pancreatic fistula and severe complications after pancreaticoduodenectomy (PD).

### Applications

The study results suggest that PD can be safely performed in highly selective patients with PAP.

### Terminology

Idiopathic AP is a term commonly used to describe patients with AP after the common etiologies of AP have been excluded, such as alcohol and gallstones.

### Peer-review

This is an interesting study in which the authors analyzed the effect of PAP on tumors.

## REFERENCES

- 1 **Mujica VR**, Barkin JS, Go VL. Acute pancreatitis secondary to pancreatic carcinoma. Study Group Participants. *Pancreas* 2000; **21**: 329-332 [PMID: 11075985]
- 2 **Tummala P**, Tariq SH, Chibnall JT, Agarwal B. Clinical predictors of pancreatic carcinoma causing acute pancreatitis. *Pancreas* 2013; **42**: 108-113 [PMID: 22722258 DOI: 10.1097/MPA.0b013e318254f473]
- 3 **Mao C**, Howard JM. Pancreatitis associated with neuroendocrine (islet cell) tumors of the pancreas. *Am J Surg* 1996; **171**: 562-564

- [PMID: 8678200 DOI: 10.1016/S0002-9610(96)00032-3]
- 4 **Asari S**, Matsumoto I, Ajiki T, Shinzeki M, Goto T, Fukumoto T, Ku Y. Perioperative management for pancreatoduodenectomy following severe acute pancreatitis in patients with periampullary cancer: our experience with six consecutive cases. *Surg Today* 2015; **45**: 181-188 [PMID: 24799280 DOI: 10.1007/s00595-014-0900-x]
  - 5 **Erkan M**, Kleeff J, Reiser C, Hinz U, Esposito I, Friess H, Büchler MW. Preoperative acute pancreatitis in periampullary tumors: implications for surgical management. *Digestion* 2007; **75**: 165-171 [PMID: 17684367 DOI: 10.1159/000106799]
  - 6 **Banks PA**, Freeman ML. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 2006; **101**: 2379-2400 [PMID: 17032204 DOI: 10.1111/j.1572-0241.2006.00856.x]
  - 7 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]
  - 8 **Eshuis WJ**, van der Gaag NA, Rauws EA, van Eijck CH, Bruno MJ, Kuipers EJ, Coene PP, Kubben FJ, Gerritsen JJ, Greve JW, Gerhards MF, de Hingh IH, Klinkenbijl JH, Nio CY, de Castro SM, Busch OR, van Gulik TM, Bossuyt PM, Gouma DJ. Therapeutic delay and survival after surgery for cancer of the pancreatic head with or without preoperative biliary drainage. *Ann Surg* 2010; **252**: 840-849 [PMID: 21037440 DOI: 10.1097/SLA.0b013e3181fd36a2]
  - 9 **Bassi C**, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J, Neoptolemos J, Sarr M, Traverso W, Buchler M. Postoperative pancreatic fistula: an international study group (ISGPF) definition. *Surgery* 2005; **138**: 8-13 [PMID: 16003309 DOI: 10.1016/j.surg.2005.05.001]
  - 10 **Wente MN**, Bassi C, Dervenis C, Fingerhut A, Gouma DJ, Izbicki JR, Neoptolemos JP, Padbury RT, Sarr MG, Traverso LW, Yeo CJ, Büchler MW. Delayed gastric emptying (DGE) after pancreatic surgery: a suggested definition by the International Study Group of Pancreatic Surgery (ISGPS). *Surgery* 2007; **142**: 761-768 [PMID: 17981197 DOI: 10.1016/j.surg.2007.05.005]
  - 11 **Wente MN**, Veit JA, Bassi C, Dervenis C, Fingerhut A, Gouma DJ, Izbicki JR, Neoptolemos JP, Padbury RT, Sarr MG, Yeo CJ, Büchler MW. Postpancreatectomy hemorrhage (PPH): an International Study Group of Pancreatic Surgery (ISGPS) definition. *Surgery* 2007; **142**: 20-25 [PMID: 17629996 DOI: 10.1016/j.surg.2007.02.001]
  - 12 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]
  - 13 **Chen Y**, Tan C, Zhang H, Mai G, Ke N, Liu X. Novel entirely continuous running suture of two-layer pancreaticojejunostomy using only one polypropylene monofilament suture. *J Am Coll Surg* 2013; **216**: e17-e21 [PMID: 23195205 DOI: 10.1016/j.jamcollsurg.2012.10.009]
  - 14 **Carrière C**, Young AL, Gunn JR, Longnecker DS, Korc M. Acute pancreatitis accelerates initiation and progression to pancreatic cancer in mice expressing oncogenic Kras in the nestin cell lineage. *PLoS One* 2011; **6**: e27725 [PMID: 22140463 DOI: 10.1371/journal.pone.0027725]
  - 15 **Carrière C**, Young AL, Gunn JR, Longnecker DS, Korc M. Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. *Biochem Biophys Res Commun* 2009; **382**: 561-565 [PMID: 19292977 DOI: 10.1016/j.bbrc.2009.03.068]
  - 16 **Imaizumi T**, Hatori T, Tobita K, Fukuda A, Takasaki K, Makuuchi H. Pancreaticojejunostomy using duct-to-mucosa anastomosis without a stenting tube. *J Hepatobiliary Pancreat Surg* 2006; **13**: 194-201 [PMID: 16708294]
  - 17 **Roulin D**, Cerantola Y, Demartines N, Schäfer M. Systematic review of delayed postoperative hemorrhage after pancreatic resection. *J Gastrointest Surg* 2011; **15**: 1055-1062 [PMID: 21267670 DOI: 10.1007/s11605-011-1427-8]
  - 18 **Yamashita Y**, Taketomi A, Fukuzawa K, Tsujita E, Harimoto N, Kitagawa D, Kuroda Y, Kayashima H, Wakasugi K, Maehara Y. Risk factors for and management of delayed intraperitoneal hemorrhage after pancreatic and biliary surgery. *Am J Surg* 2007; **193**: 454-459 [PMID: 17368288 DOI: 10.1016/j.amjsurg.2006.09.008]
  - 19 **Bassi C**, Butturini G, Molinari E, Mascetta G, Salvia R, Falconi M, Gumbs A, Pederzoli P. Pancreatic fistula rate after pancreatic resection. The importance of definitions. *Dig Surg* 2004; **21**: 54-59 [PMID: 14707394 DOI: 10.1159/000075943]
  - 20 **Welsch T**, Eisele H, Zschäbitz S, Hinz U, Büchler MW, Wente MN. Critical appraisal of the International Study Group of Pancreatic Surgery (ISGPS) consensus definition of postoperative hemorrhage after pancreatoduodenectomy. *Langenbecks Arch Surg* 2011; **396**: 783-791 [PMID: 21611815 DOI: 10.1007/s00423-011-0811-x]

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## Retrospective Study

# Study of celiac artery variations and related surgical techniques in gastric cancer

Yuan Huang, Guang-Chuan Mu, Xin-Gan Qin, Zhi-Bai Chen, Jin-Ling Lin, Yan-Jun Zeng

Yuan Huang, Guang-Chuan Mu, Xin-Gan Qin, Zhi-Bai Chen, Jin-Ling Lin, Department of Gastrointestinal Surgery, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China  
Yan-Jun Zeng, Beijing University of Technology, Beijing 100022, China

**Author contributions:** Huang Y and Lin JL performed the majority of experiments; Zeng YJ and Chen ZB provided analytical tools and were also involved in editing the manuscript; Huang Y designed the study; and Mu GC wrote the manuscript.

**Ethics approval:** This study has been approved by the medical ethics committee of the First Affiliated Hospital of Guangxi Medical University.

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**Data sharing:** No additional data are available.

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**Correspondence to:** Yuan Huang, MB, Department of Gastrointestinal Surgery, the First Affiliated Hospital of Guangxi Medical University, No. 6 Shuangyong Street, Nanning 530021, Guangxi Zhuang Autonomous Region, China. [huangyuan\\_09@163.com](mailto:huangyuan_09@163.com)  
Telephone: +86-771-5356701  
Fax: +86-771-5356559

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## Abstract

**AIM:** To investigate celiac artery variations in gastric cancer patients and the impact on gastric cancer surgery, and also to discuss the value of the ultrasonic knife in reducing the risk caused by celiac artery variations.

**METHODS:** A retrospective analysis was conducted to investigate the difference in average operation time, intraoperative blood loss, number of harvested lymph nodes, average postoperative drainage within 3 d, and postoperative hospital stay between the group with vascular variations and no vascular variations, and between the ultrasonic harmonic scalpel and conventional electric scalpel surgery group.

**RESULTS:** One hundred and fifty-eight cases presented with normal celiac artery, and 80 presented with celiac artery variation (33.61%). The average operation time, blood loss, average drainage within 3 d after surgery in the celiac artery variation group were significantly more than in the no celiac artery variation group ( $215.7 \pm 32.7$  min vs  $204.2 \pm 31.3$  min,  $220.0 \pm 56.7$  mL vs  $163.1 \pm 52.3$  mL,  $193.6 \pm 41.4$  mL vs  $175.3 \pm 34.1$  mL, respectively,  $P < 0.05$ ). In celiac artery variation patients, the average operation time, blood loss, average drainage within 3 d after surgery in the ultrasonic harmonic scalpel group were significantly lower than in the conventional electric scalpel surgery group ( $209.5 \pm 34.9$  min vs  $226.9 \pm 29.4$  min,  $207.5 \pm 57.1$  mL vs  $235.6 \pm 52.9$  mL,  $184.4 \pm 38.2$  mL vs  $205.0 \pm 42.9$  mL, respectively,  $P < 0.05$ ), and the number of lymph node dissections was significantly higher than in the conventional surgery group ( $25.5 \pm 9.2$  vs  $19.9 \pm 7.8$ ,  $P < 0.05$ ).

**CONCLUSION:** Celiac artery variation increases the

difficulty and risk of radical gastrectomy. Preoperative imaging evaluation and the application of ultrasonic harmonic scalpel are conducive to radical gastrectomy.

**Key words:** Celiac artery; Variation; MSCTA; Ultrasonic harmonic scalpel; Gastric cancer

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**Core tip:** Celiac artery variation is quite common in gastric cancer patients, and may obviously increase the difficulty and risk of radical gastrectomy with D2 lymphadenectomy. With the development of imaging techniques, not only the accuracy of preoperative staging, but also the individualized image information about celiac artery variation will be improved. Meanwhile, the application of new technology such as ultrasonic harmonic scalpel is conducive to radical gastrectomy with D2 lymphadenectomy and could reduce the risk caused by celiac artery variation; therefore, its utilization is to be recommended.

Huang Y, Mu GC, Qin XG, Chen ZB, Lin JL, Zeng YJ. Study of celiac artery variations and related surgical techniques in gastric cancer. *World J Gastroenterol* 2015; 21(22): 6944-6951 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6944.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6944>

## INTRODUCTION

Gastric cancer is one of the most common malignant tumors in China. Its position has increased to third most common from the previous number four, and is preceded only by lung cancer and breast cancer. In 2010 alone, 404565 cases with new onset of gastric cancer were reported, and 287851 patients died from gastric cancer. The mortality is number three among various malignant cancers<sup>[1-3]</sup>. At present, surgery remains the main treatment for gastric cancer, wherein D2 radical gastrectomy has already become the standard operation for gastric cancer at the progression stage. Focus and difficulty of D2 radical gastrectomy are dissection of lymph nodes around vessels such as celiac trunk, left gastric artery and hepatic artery. As reported in the literature, a high rate of celiac artery variation has been found in liver transplantation, especially in the hepatic arterial system; the rate of variation is up to 24.3%<sup>[4,5]</sup>. Presence of celiac artery variation will definitely increase surgical difficulty and risk. In addition, relevant studies on celiac artery variation among gastric cancer patients are still lacking. Meanwhile, gastric cancer treatment guidelines do not state how to handle an abnormal vessel and its surrounding lymph nodes. This study aims at analyzing retrospectively celiac artery variation

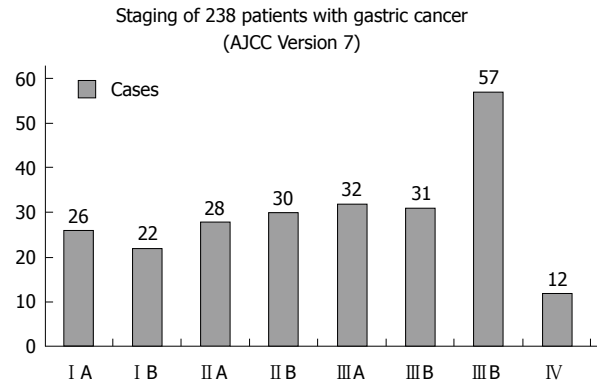


Figure 1 Staging of 238 patients with gastric cancer (AJCC Version 7).

and the effect of vascular variation on gastric cancer surgery outcome among 238 patients receiving radical gastrectomy, meanwhile addressing the efficacy of ultrasound harmonic scalpel in minimizing risk due to vascular variation, so as to provide a reference for guiding gastric cancer treatment in clinical practice.

## MATERIALS AND METHODS

### General information

Two hundred and thirty-eight patients undergoing D2 radical gastrectomy by well experienced general surgeons in our department from January 2009 to May 2014 were included; the detailed information of tumor staging can be seen in Figure 1. All patients provided informed consent, and signed agreements. All the patients were preoperatively examined, through upper abdominal 64 multi-slice computed tomography angiography (MSCTA), to determine whether there was variation in the celiac trunk and its branches, wherein the abnormal hepatic artery was classified with reference to Hiatt's<sup>[4]</sup> classification.

### Inclusion and exclusion criteria

**Inclusion criteria:** (1) preoperative pathology *via* gastroscopic biopsy indicated gastric cancer; (2) preoperative MSCTA was taken; (3) preoperative assessment showed indications for D2 radical surgery; (4) preoperative assessment showed no evident surgical contraindication; and (5) D2 or D2+ radical surgery was performed.

**Exclusion criteria:** (1) no MSCTA was taken pre-operatively; (2) preoperative imaging examination indicated distant metastasis or infiltration into surrounding tissue and organ, which was confirmed by laparoscopy; (3) preoperative assessment showed severe diseases of cardiopulmonary and other systems, and the patient might not be able to tolerate the operation; and (4) intraoperative exploration found extensive metastasis, malignant ascites, infiltration into surrounding tissue in the abdominal cavity, so that neither D2 or D2+radical surgery was practical.

**Table 1** Hepatic artery categories of 238 patients with gastric cancer (according to Hiatt's classification criteria) *n* (%)

Hiatt's classification	In Hiatt's study	In this study	$\chi^2$ value	<i>P</i> value
I	757 (75.70)	170 (71.43)	1.865	0.172
II	97 (9.70)	33 (13.87)	3.549	0.060
III	106 (10.60)	14 (5.88)	4.888	0.027
IV	23 (2.30)	7 (2.94)	0.334	0.563
V	15 (1.50)	6 (2.52)	0.668	0.414
VI	2 (0.20)	0 (0.00)	0.000	1.000
Others	0 (0.00)	8 (3.36)	27.831	0.000
In total	1000 (100.00)	238 (100.00)		

The remaining eight cases of abnormal hepatic artery were: three cases of left or right hepatic artery deriving directly from the common hepatic artery, two cases of left or right hepatic artery deriving directly from the celiac trunk, and one case of complicated variation: the common hepatic artery derived from the superior mesenteric artery, and the left gastric and splenic arteries derived from the abdominal aorta, respectively.

### Operative method and observational indices

D2 surgery for gastric cancer was undertaken according to Japanese gastric cancer treatment guidelines (Ver. 3)<sup>[6]</sup>. TNM staging was in accordance with the 7<sup>th</sup> AJCC classification criteria. Indices including average operation time, intraoperative blood loss, total number of lymph nodes dissected, time required for postoperative recovery of bowel function, time to ambulation after surgery, postoperative hospital stay, mean drainage amount at 3 d after operation, as well as presence of postoperative complications such as bleeding, anastomosis fistula, lymphatic fistula, postoperative pancreatitis, incision infection, postoperative pneumonia, *etc.*, were recorded for the vascular variation group and no vascular variation group, as well as ultrasonic harmonic scalpel surgery subgroup and conventional electric scalpel surgery subgroup in the vascular variation group. Indices were analyzed *via* comparison between the two groups described above, to evaluate the influence of vascular variation on operative safety and postoperative recovery, as well as to explore the effect of the ultrasonic harmonic scalpel in terms of decreasing risk due to abnormal vessels.

### Statistical analysis

Statistical analysis was carried out with statistical software SPSS16.0.  $\chi^2$  test was used to compare general data construction between two groups, and *t*-test was used to analyze differences in various statistical parameters between the vascular variation group and no vascular variation group, as well as ultrasonic harmonic scalpel surgery subgroup and conventional electric scalpel subgroup in the vascular variation group. *P* < 0.05 was considered to be a statistically significant difference. The statistical methods of this study were reviewed by Huang Gao-Ming from the College of Public Hygiene of Guangxi Medical University.

## RESULTS

### Celiac artery variation

This study showed that there were 158 cases with normal celiac artery and 80 cases with celiac artery variation, with a variation rate of 33.61%. Among them, 68 cases had abnormal hepatic artery, and were classified and compared according to Hiatt's classification criteria (Table 1).

Five cases had abnormal left gastric artery: two cases had left gastric artery deriving from the abdominal aorta, one case deriving from splenic artery, one case with left gastric artery absence, and one case with two left gastric arteries deriving from the celiac trunk, specifically sharing the same trunk from 4 mm and subsequently branching into two left gastric arteries.

Additionally, two cases had abnormal splenic arteries deriving from superior mesenteric artery; two cases had celiac trunks which shared the same trunk with the superior mesenteric artery and derived from the abdominal aorta; two cases had gastroduodenal arteries deriving from the celiac trunk; and one case had right gastric artery deriving from the gastroduodenal artery.

### Results from comparing observational indices in each group

There were significant differences between the 80 patients with vascular variation and 158 patients with no vascular variation in distribution of general data. All the patients received uneventful surgery, without occurrence of severe intraoperative or postoperative complications. There were 12 cases with postoperative complications, including incision infection and postoperative pneumonia. No postoperative anastomosis fistula or anastomotic bleeding occurred (Table 2). Comparing results of the study showed that celiac artery variation prolonged operation duration significantly and increased intraoperative bleeding and postoperative drainage amount, but the total number of lymph nodes dissected, time required for postoperative recovery of bowel function, time to ambulation after surgery, postoperative hospital stay, and total hospitalization costs were not significantly different (Table 3). Among patients with celiac artery variation, operation duration, intraoperative blood loss, and postoperative amount of drainage in the ultrasonic harmonic scalpel group were significantly decreased compared to the conventional electric scalpel surgery group, and total number of lymph nodes dissected was increased, whereas time required for postoperative recovery of bowel function, time to ambulation after surgery, postoperative hospital stay, and total hospitalization cost were not significantly different (Table 4). The relative results can be seen in the comparison of observations between the ultrasonic harmonic scalpel subgroup and the electrical unit

**Table 2** Comparison of general data between vascular variation group and no vascular variation group (case)

Clinical data	Vascular variation group	No vascular variation group	Statistics value	P value
Total cases	80	158		
Age (yr)	58.3 ± 12.5	56.3 ± 12.4	1.195	0.233
Sex			0.633	0.426
Male	61	112		
Female	19	46		
Tumor location			0.115	0.944
Fundus ventriculi	17	33		
Corpus ventriculi	13	24		
Gastric antrum	50	101		
Gastric cancer staging			1.343	0.246
Stage I - II	31	75		
Stage III-IV	49	83		
Postoperative complications			0.086	0.770
Yes	5	7		
No	75	151		

**Table 3** Comparison of observations between vascular variation group and no vascular variation group

Observations index	Vascular variation group	No vascular variation group	t value	P value
Operation time (min)	215.7 ± 32.7	204.2 ± 31.3	2.624	0.009
Intraoperative blood loss (mL)	220.0 ± 56.7	163.1 ± 52.3	7.674	0.000
Total number of lymph nodes dissected	23.0 ± 9.1	21.2 ± 8.5	1.505	0.134
Time required for postoperative recovery of bowel function (d)	3.0 ± 1.2	3.2 ± 1.4	1.260	0.209
Time to ambulation (d)	3.9 ± 0.8	4.1 ± 1.0	2.760	0.060
Postoperative hospitalization (d)	7.6 ± 1.2	8.1 ± 0.9	1.356	0.121
Mean drainage amount in 3 d after operation (mL)	193.6 ± 41.4	175.3 ± 34.1	1.639	0.032
Total hospitalization cost (RMB)	35862.8 ± 2965.3	36759.3 ± 2732.5	1.356	0.130

subgroup among the no vascular variation population in Table 5. Images of the preoperative MSCTA examination and lymph node dissection by ultrasonic harmonic scalpel can be seen in Figures 2-4.

## DISCUSSION

Gastric cancer is the third most common and the malignant tumor with the second highest mortality in Asia<sup>[7]</sup>. The incidence of gastric cancer has an increasing trend in China. It possesses a great threat to the mental and physical health of people, and is an important public health issue in China, as well as exerting a heavy economic burden on the Chinese healthcare community<sup>[8,9]</sup>. At present, radical

**Table 4** Comparison of observations between ultrasonic harmonic scalpel subgroup and electrical unit subgroup among vascular variation population

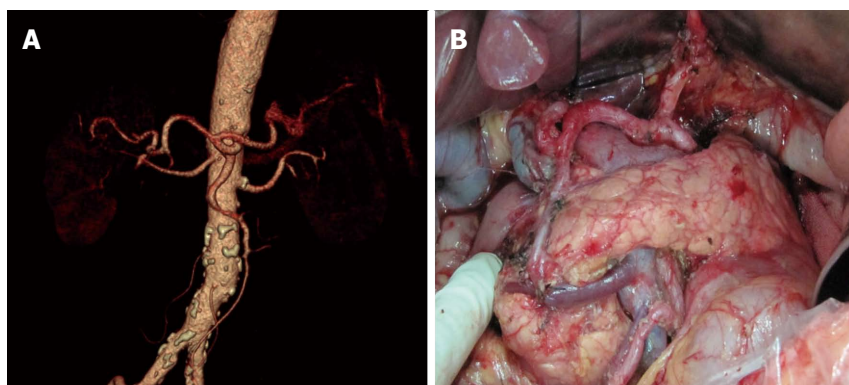
Observation	Ultrasonic harmonic scalpel group	Electrical unit subgroup	t value	P value
Cases (persons)	44	36		
Operation duration (min)	209.5 ± 34.9	226.9 ± 29.4	1.168	0.046
Intraoperative blood loss (mL)	207.5 ± 57.1	235.6 ± 52.9	2.242	0.028
Total number of lymph nodes dissected	25.5 ± 9.2	19.9 ± 7.8	2.903	0.005
Time required for postoperative recovery of bowel function (d)	3.0 ± 0.9	3.1 ± 0.8	0.442	0.660
Time to ambulation (d)	4.0 ± 0.9	3.9 ± 0.8	0.291	0.772
Postoperative hospitalization (d)	7.4 ± 1.3	8.1 ± 1.1	1.251	0.101
Mean drainage amount in 3 d after operation (mL)	184.4 ± 38.2	205.0 ± 42.9	2.255	0.027
Total hospitalization cost (RMB)	36167.3 ± 2845.6	35246.4 ± 2789.5	1.236	0.102

**Table 5** Comparison of observations between ultrasonic harmonic scalpel subgroup and electrical unit subgroup among no vascular variation population

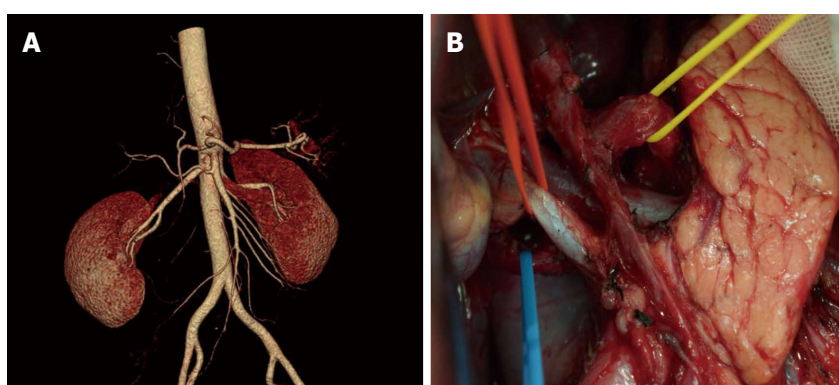
Observation	Ultrasonic harmonic scalpel group	Electrical unit subgroup	t value	P value
Cases (persons)	81	77		
Operation duration (min)	188.4 ± 25.4	220.4 ± 28.3	7.481	0.000
Intraoperative blood loss (mL)	168.1 ± 49.6	158.0 ± 55.3	1.210	0.228
Total number of lymph nodes dissected	23.3 ± 7.9	19.2 ± 8.6	3.085	0.002
Time required for postoperative recovery of bowel function (d)	3.3 ± 1.1	3.1 ± 0.9	1.359	0.176
Time to ambulation (d)	4.4 ± 1.0	4.2 ± 1.0	1.330	0.185
Postoperative hospitalization (d)	7.3 ± 1.5	7.9 ± 1.2	1.251	0.132
Mean drainage amount in 3 d after operation (mL)	191.3 ± 37.0	187.7 ± 30.0	2.349	0.080
Total hospitalization cost (RMB)	35917.6 ± 2741.6	36186.9 ± 2717.3	1.016	0.161

gastrectomy with D2 lymphadenectomy is widely accepted by an increasing number of surgeons as a standard of surgery for advanced gastric cancer<sup>[10]</sup>. Japanese gastric cancer treatment guidelines (Ver. 3) also specifies D2 lymphadenectomy as the standard operation<sup>[6]</sup>. Focus and difficulty of D2 radical surgery

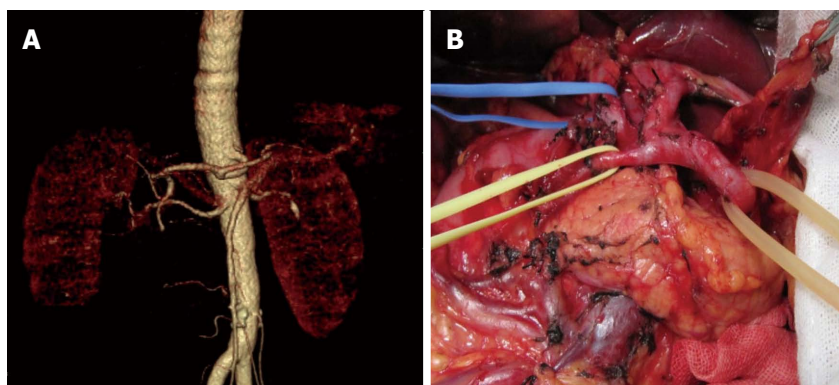




**Figure 2 Normal celiac artery branches.** A: Preoperative multi-slice computed tomography angiography: Normal celiac trunk gives off LGA, splenic artery and common hepatic artery; B: Intraoperative finding was consistent with preoperative findings.



**Figure 3 Replaced right hepatic artery.** A: Preoperative multi-slice computed tomography angiography: Replaced right hepatic artery deriving from superior mesenteric artery; B: Intraoperative finding: Replaced right hepatic artery deriving from superior mesenteric artery in the same patient.



**Figure 4 Left gastric artery absence.** A: Preoperative multi-slice computed tomography angiography found left gastric artery absence; B: Intraoperative dissection did not find left gastric artery existence in the same patient.

is to skeletonize vessels at the left gastric artery, celiac trunk, hepatic artery and hepatoduodenal ligament, and to dissect the corresponding group of lymph nodes. Any branch of the celiac artery could be a variation, which definitely will increase the difficulty and risk of operation. Therefore, preoperative imaging assessment of the presence of celiac artery variation and route of the normal branch gives importance guidance for the operation. Meanwhile, gastric cancer treatment guidelines do not state whether

lymph nodes around an abnormal vessel should be dissected. Therefore, study of celiac artery variation among gastric cancer patients has important clinical implications for guiding gastric cancer surgical strategy development.

The celiac artery has a high variation rate. Our data showed the variation rate is up to 33.61%, wherein, hepatic artery variation is the most common with a rate of 28.57%, which is slightly higher than 20.4% reported in the literature<sup>[11]</sup>. This may be attributed

to the fact that the previous studies mainly focused on intraoperative anatomic findings during hepatic implantation, and were subject to ignore small vascular anomalies. The majority were replaced/accessory left hepatic artery, with an incidence of 16.8%, which is similar to that reported in literature<sup>[12-15]</sup>. Replaced/accessory left hepatic artery has important implications in D2 radical gastrectomy. It is necessary during surgery to ligate at the root and cut off the left gastric artery, which may affect the hepatic tissue supplied by the replaced/accessory left hepatic artery deriving from the left gastric artery, thus influencing hepatic function, especially for the replaced right hepatic artery<sup>[16-18]</sup>. A study believed that intraoperative resection of the accessory right hepatic artery was safe for patients without chronic hepatic disease; however, for patients complicated with chronic hepatic disease, it had to be reserved for protecting hepatic function<sup>[19]</sup>. Therefore, accurate preoperative assessment of whether the abnormal left hepatic artery is replaced by the right hepatic artery or accessory right hepatic artery is especially important. Oki *et al*<sup>[20]</sup> believed that preoperative 3DCT and angiography could not differentiate replaced and accessory hepatic artery; therefore, they recommended that all abnormal left hepatic arteries should be preserved intraoperatively. Based on data from this study, results have shown that continuous tracking of vessels flowing into the liver *via* preoperative CTA in combination with thin layer scanning at arterial phase could well differentiate these two. For lymph nodes around normal hepatic arteries deriving from the superior mesenteric artery, results from our previous study indicated no necessity for dissection; however, due to limitations regarding the small sample size, no definite conclusion could be drawn, and studies with larger sample size and prospective comparative studies of influence on outcome are still awaited<sup>[21]</sup>.

In D2 radical gastrectomy, skeletonizing should be performed for vessels around the stomach to dissect corresponding groups of lymph nodes. Vascular variation around the stomach increases difficulty and risk of lymph node dissection<sup>[22,23]</sup>. Our data showed the operation time in the vascular variation group was increased as compared to the no variation group (215.7 min vs 204.2 min). To avoid damaging abnormal vessels and causing unnecessary accessory injury, we should perform more precise dissection for anomalous vessels. In the meantime, for patients at an advanced age and with scleratheroma, we recommend to perform lymph node dissection outside the vascular sheath, to avoid damaging vessels, inducing aneurysm and risking rupture and bleeding. Intraoperative accidental injury of abnormal hepatic artery will influence hepatic function directly<sup>[24]</sup>. Furthermore, prolonged operation duration may also be an important factor of hepatic impairment. A comparative study of laparoscopic radical gastrectomy and conventional laparotomy by Jeong *et al*<sup>[25]</sup> showed the operation

time, BMI index, intraoperative hepatic injury and mistakenly pricking the hepatic artery are important factors influencing postoperative hepatic function. Meanwhile, celiac artery variation could significantly increase intraoperative blood loss, especially in those who lacked effective preoperative assessment of the abdominal artery. Perioperative bleeding could be decreased through preoperative CTA assessment of celiac artery imaging information<sup>[26]</sup>.

Results of this study showed that the celiac artery variation significantly extended operation time and increased intraoperative blood loss and postoperative amount of drainage, and therefore was an important factor influencing successful implementation of D2 radical gastrectomy and postoperative recovery. Recently, innovative devices such as the ultrasonic harmonic scalpel have been introduced to clinical practice, and their scope of use has been extended gradually. With their increasing surgical speed and quality, they thus add new elements to gastric cancer surgery<sup>[27-29]</sup>. Prospective, randomized controlled study on the ultrasonic harmonic scalpel and conventional electric scalpel showed that the ultrasonic harmonic scalpel could significantly reduce radical gastrectomy operation time and perioperative bleeding (209.5 min vs 226.9 min, 207.5 mL vs 235.6 mL, respectively), and had an evident advantage in terms of lymph node dissection<sup>[29]</sup>. The value of the ultrasonic harmonic scalpel during operation among gastric cancer patients with celiac artery variation is worthy of further exploration. This study showed that the ultrasonic harmonic scalpel had an evident advantage during celiac artery variation surgery, as it effectively reduced operation duration and intraoperative blood loss. Ultrasonic harmonic scalpel could coagulate vessels with a diameter up to 5 mm, and greatly decrease additional vascular ligation during transection of the greater omentum, splenogastric ligament and hepatogastric ligament, so as to make the surgery smoother and significantly reduce operation time and perioperative bleeding. Especially during dissection of lymph nodes around an abnormal hepatic artery deriving from the superior mesenteric artery and reserving abnormal left hepatic artery deriving from the left gastric artery, the operation could be achieved along the vascular surface with the thinner tip of an ultrasonic harmonic scalpel, facilitating vascular skeletonizing, so as to not only achieve more complete lymph node dissection, but also to find the root of the abnormal vessel easily. Thus accessory injury and additional bleeding due to damaging the abnormal hepatic artery could be avoided. Meanwhile, the ultrasonic harmonic scalpel could close arteries and veins, capillaries and lymph vessels in the wound bed, and intraoperative bleeding and postoperative amount of abdominal drainage is significantly decreased.

In conclusion, celiac artery variation is common in clinical practice, and therefore great attention should be paid to it. Vascular variation increases difficulty

and risk of radical gastrectomy. Application of new techniques such as preoperative imaging assessment and the ultrasonic harmonic scalpel could facilitate successful implementation of D2 radical gastrectomy and efficiently decrease risk due to celiac artery variation, meanwhile hospitalization days and cost are not increased significantly. Therefore, they are recommended to be applied widely. However, this study could not arrive at definite conclusions since it was limited as a retrospective study; a larger number of cases in larger studies need to be examined and randomized controlled trial studies need to be carried out.

## COMMENTS

### Background

Gastric cancer is one of the most common malignant tumors in China. At present, surgery remains the main treatment for gastric cancer, wherein D2 radical gastrectomy has already become the standard operation for gastric cancer at the progression stage. Focus and difficulty of D2 radical gastrectomy are dissection of lymph nodes around vessels such as the celiac trunk, left gastric artery and hepatic artery. As reported in the literature, a high rate of celiac artery variation was found in liver transplantation, especially in the hepatic arterial system. The presence of celiac artery variation will definitely increase surgical difficulty and risk. In addition, relevant studies on celiac artery variation among gastric cancer patients are still lacking. Meanwhile, gastric cancer treatment guidelines do not state how to handle an abnormal vessel and its surrounding lymph nodes. This study aims at analyzing retrospectively celiac artery variation and effects of vascular variation on gastric cancer surgery among 238 patients receiving radical gastrectomy, meanwhile addressing efficacy of ultrasound harmonic scalpel regarding risk due to vascular variation, so as to provide a reference for guiding gastric cancer treatment in clinical practice.

### Research frontiers

Although D2 radical gastrectomy has already become the standard surgery for gastric cancer at the progression stage in the east and west, vascular variation around the stomach has been the focus and difficulty of gastric cancer surgery, and gastric cancer treatment guidelines do not state how to handle an abnormal vessel and its surrounding lymph nodes; lymph node metastasis around abnormal vascular will be one of hotspots in the future.

### Innovations and breakthroughs

The main innovation of this study was that it mainly investigated celiac artery variation among gastric cancer patients and also discussed the values of ultrasonic knife in reducing the risk caused by celiac artery variations. Celiac artery variation increases the difficulty and risk of radical gastrectomy with D2 lymphadenectomy. Preoperative imaging evaluation and the application of new technology such as ultrasonic harmonic scalpel are conducive to radical gastrectomy with D2 lymphadenectomy and could reduce the risk caused by celiac artery variation, and are therefore applications worthy of promotion.

### Applications

Celiac artery variation is common in gastric cancer patients. The authors should pay attention to celiac artery variations and undertake preoperative imaging evaluation because celiac artery variations may increase the difficulty and risk of radical gastrectomy with D2 lymphadenectomy. However, the ultrasonic harmonic scalpel could reduce the risk caused by celiac artery variation.

### Peer-review

This is a prospective and controlled clinical study that emphasizes one of the important clinical problems surgeons face. It is important to show the prevalence of Celiac Artery Variations and to test whether the ultrasonic knife is a better tool than conventional methods. Both aspects of the study have clinical applicability.

- report on status of cancer in China, 2010. *Chin J Cancer Res* 2014; **26**: 48-58 [PMID: 24653626 DOI: 10.3978/j.issn.1000-9604]
- 2 **Jing JJ**, Liu HY, Hao JK, Wang LN, Wang YP, Sun LH, Yuan Y. Gastric cancer incidence and mortality in Zhuanghe, China, between 2005 and 2010. *World J Gastroenterol* 2012; **18**: 1262-1269 [PMID: 22468091]
- 3 **Yan S**, Li B, Bai ZZ, Wu JQ, Xie DW, Ma YC, Ma XX, Zhao JH, Guo XJ. Clinical epidemiology of gastric cancer in Hehuang valley of China: a 10-year epidemiological study of gastric cancer. *World J Gastroenterol* 2014; **20**: 10486-10494 [PMID: 25132766 DOI: 10.3748/wjg.v20.i30.10486]
- 4 **Hiatt JR**, Gabbay J, Busuttil RW. Surgical anatomy of the hepatic arteries in 1000 cases. *Ann Surg* 1994; **220**: 50-52 [PMID: 8024358]
- 5 **Kobayashi S**, Otsubo T, Koizumi S, Ariizumi S, Katagiri S, Watanabe T, Nakano H, Yamamoto M. Anatomic variations of hepatic artery and new clinical classification based on abdominal angiographic images of 1200 cases. *Hepatogastroenterology* 2014; **61**: 2345-2348 [PMID: 25699380]
- 6 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer* 2011; **14**: 113-123 [PMID: 21573742 DOI: 10.1007/s10120-011-0042-4]
- 7 **Rahman R**, Asombang AW, Ibdah JA. Characteristics of gastric cancer in Asia. *World J Gastroenterol* 2014; **20**: 4483-4490 [PMID: 24782601 DOI: 10.3748/wjg.v20.i16.4483]
- 8 **Yang L**. Incidence and mortality of gastric cancer in China. *World J Gastroenterol* 2006; **12**: 17-20 [PMID: 16440411]
- 9 **Fock KM**, Ang TL. Epidemiology of *Helicobacter pylori* infection and gastric cancer in Asia. *J Gastroenterol Hepatol* 2010; **25**: 479-486 [PMID: 20370726]
- 10 **Mu GC**, Huang Y, Liu ZM, Lin JL, Zhang LL, Zeng YJ. Clinical research in individual information of celiac artery CT imaging and gastric cancer surgery. *Clin Transl Oncol* 2013; **15**: 774-779 [PMID: 23359186]
- 11 **Yang Y**, Jiang N, Lu MQ, Xu C, Cai CJ, Li H, Yi SH, Wang GS, Zhang J, Zhang JF, Chen GH. [Anatomical variation of the donor hepatic arteries: analysis of 843 cases]. *Nan Fang Yi Ke Da Xue Xue Bao* 2007; **27**: 1164-1166 [PMID: 17715016]
- 12 **Matsuki M**, Kani H, Tatsugami F, Yoshikawa S, Narabayashi I, Lee SW, Shinohara H, Nomura E, Tanigawa N. Preoperative assessment of vascular anatomy around the stomach by 3D imaging using MDCT before laparoscopy-assisted gastrectomy. *AJR Am J Roentgenol* 2004; **183**: 145-151 [PMID: 15208129]
- 13 **Nakamura H**, Uchida H, Kuroda C, Yoshioka H, Tokunaga K, Kitatani T, Sato T, Ohi H, Hori S. Accessory left gastric artery arising from left hepatic artery: angiographic study. *AJR Am J Roentgenol* 1980; **134**: 529-532 [PMID: 6766620]
- 14 **Celik A**, Celik AS, Altinli E, Beykal O, Caglayan K, Koksall N. Left gastric and right hepatic artery anomalies in a patient with gastric cancer: images for surgeons. *Am J Surg* 2011; **202**: e13-e16 [PMID: 21810494]
- 15 **Abid B**, Douard R, Chevallier JM, Delmas V. [Left hepatic artery: anatomical variations and clinical implications]. *Morphologie* 2008; **92**: 154-161 [PMID: 19008142 DOI: 10.1016/j.morpho.2008.10.001]
- 16 **Shinohara T**, Ohyama S, Muto T, Yanaga K, Yamaguchi T. The significance of the aberrant left hepatic artery arising from the left gastric artery at curative gastrectomy for gastric cancer. *Eur J Surg Oncol* 2007; **33**: 967-971 [PMID: 17418995]
- 17 **Friesen SR**. The significance of the anomalous origin of the left hepatic artery from the left gastric artery in operations upon the stomach and esophagus. *Am Surg* 1957; **23**: 1103-1108 [PMID: 13488026]
- 18 **Lurie AS**. The significance of the variant left accessory hepatic artery in surgery for proximal gastric cancer. *Arch Surg* 1987; **122**: 725-728 [PMID: 3579588]
- 19 **Huang CM**, Chen QY, Lin JX, Zheng CH, Li P, Xie JW, Wang JB, Lu J. Short-term clinical implications of the accessory left hepatic artery in patients undergoing radical gastrectomy for gastric cancer. *PLoS One* 2013; **8**: e64300 [PMID: 23717589 DOI: 10.1371/journal.pone.0064300]
- 20 **Oki E**, Sakaguchi Y, Hiroshige S, Kusumoto T, Kakeji Y, Maehara

## REFERENCES

- 1 **Chen W**, Zheng R, Zhang S, Zhao P, Zeng H, Zou X, He J. Annual

- Y. Preservation of an aberrant hepatic artery arising from the left gastric artery during laparoscopic gastrectomy for gastric cancer. *J Am Coll Surg* 2011; **212**: e25-e27 [PMID: 21398157 DOI: 10.1016/j.jamcollsurg.2011.01]
- 21 **Huang Y**, Liu C, Lin JL, Mu GC, Zeng Y. Is it necessary to dissect the lymph nodes around an abnormal hepatic artery in D2 lymphadenectomy for gastric cancer? *Clin Transl Oncol* 2013; **15**: 472-476 [PMID: 23143952 DOI: 10.1007/s12094-012-0955-3]
  - 22 **Huang Y**, Liu C, Lin JL. Clinical significance of hepatic artery variations originating from the superior mesenteric artery in abdominal tumor surgery. *Chin Med J (Engl)* 2013; **126**: 899-902 [PMID: 23489799]
  - 23 **Gielecki J**, Zurada A, Sonpal N, Jabłońska B. The clinical relevance of coeliac trunk variations. *Folia Morphol (Warsz)* 2005; **64**: 123-129 [PMID: 16228946]
  - 24 **Nakanishi R**, Endo K, Yoshinaga K, Saeki H, Morita M, Kakeji Y, Maehara Y. Unique variation of the hepatic artery identified on preoperative three-dimensional computed tomography angiography in surgery for gastric cancer: report of a case. *Surg Today* 2010; **40**: 967-971 [PMID: 20872202]
  - 25 **Jeong GA**, Cho GS, Shin EJ, Lee MS, Kim HC, Song OP. Liver function alterations after laparoscopy-assisted gastrectomy for gastric cancer and its clinical significance. *World J Gastroenterol* 2011; **17**: 372-378 [PMID: 21253398 DOI: 10.3748/wjg.v17.i3.372]
  - 26 **Miyaki A**, Imamura K, Kobayashi R, Takami M, Matsumoto J, Takada Y. Preoperative assessment of perigastric vascular anatomy by multidetector computed tomography angiogram for laparoscopy-assisted gastrectomy. *Langenbecks Arch Surg* 2012; **397**: 945-950 [PMID: 22562645 DOI: 10.1007/s00423-012-0956-2]
  - 27 **Massarweh NN**, Cosgriff N, Slakey DP. Electrosurgery: history, principles, and current and future uses. *J Am Coll Surg* 2006; **202**: 520-530 [PMID: 16500257]
  - 28 **Sun ZC**, Xu WG, Xiao XM, Yu WH, Xu DM, Xu HM, Gao HL, Wang RX. Ultrasonic dissection versus conventional electrocautery during gastrectomy for gastric cancer: a meta-analysis of randomized controlled trials. *Eur J Surg Oncol* 2015; **41**: 527-533 [PMID: 25690648 DOI: 10.1016/j.ejso.2015.01.025]
  - 29 **Inoue K**, Nakane Y, Michiura T, Yamada M, Mukaide H, Fukui J, Miki H, Ueyama Y, Nakatake R, Tokuhara K, Iwamoto S, Yanagimoto H, Toyokawa H, Satoi S, Kwon AH. Ultrasonic scalpel for gastric cancer surgery: a prospective randomized study. *J Gastrointest Surg* 2012; **16**: 1840-1846 [PMID: 22833440]

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## Clinical Trials Study

# Prevalence, significance and predictive value of antiphospholipid antibodies in Crohn's disease

Nora Sipeki, Laszlo Davida, Eszter Palyu, Istvan Altorjay, Jolan Harsfalvi, Peter Antal Szalmas, Zoltan Szabo, Gabor Veres, Zakera Shums, Gary L Norman, Peter L Lakatos, Maria Papp

Nora Sipeki, Laszlo Davida, Eszter Palyu, Istvan Altorjay, Maria Papp, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, H-4032 Debrecen, Hungary

Jolan Harsfalvi, Clinical Research Center, Faculty of Medicine, University of Debrecen, H-4032 Debrecen, Hungary

Peter Antal Szalmas, Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, H-4032 Debrecen, Hungary

Zoltan Szabo, Division of Emergency Medicine, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, H-4032 Debrecen, Hungary

Gabor Veres, Peter L Lakatos, 1<sup>st</sup> Department of Medicine, Semmelweis University, H-1083 Budapest, Hungary

Zakera Shums, Gary L Norman, Inova Diagnostics, Inc., San Diego, California, CA 92131, United States

**Author contributions:** Papp M, Lakatos PL, Antal Szalmas P, Szabo Z and Veres G designed research; Sipeki N, Papp M, Davida L, Palyu E and Altorjay I performed research; Harsfalvi J, Antal Szalmas P, Shums Z and Norman GL contributed new reagents/analytic tools; Papp M, Sipeki N and Lakatos PL analyzed data; Papp M, Sipeki N and Lakatos PL wrote paper.

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**Correspondence to:** Maria Papp, MD, PhD, Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Nagyerdei krt. 98, H-4032 Debrecen, Hungary. [papp.maria@med.unideb.hu](mailto:papp.maria@med.unideb.hu)

Telephone: +36-52-255152

Fax: +36-52-255152

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## Abstract

**AIM:** To assess the prevalence and stability of different antiphospholipid antibodies (APLAs) and their association with disease phenotype and progression in inflammatory bowel diseases (IBD) patients.

**METHODS:** About 458 consecutive patients [Crohn's disease (CD): 271 and ulcerative colitis (UC): 187] were enrolled into a follow-up cohort study in a tertiary IBD referral center in Hungary. Detailed clinical phenotypes were determined at enrollment by reviewing the patients' medical charts. Disease activity, medical treatment and data about evolvement of complications or surgical interventions were determined prospectively during the follow-up. Disease course (development of complicated disease phenotype and need for surgery), occurrence of thrombotic events, actual state of disease

activity according to clinical, laboratory and endoscopic scores and accurate treatment regime were recorded during the follow-up, (median, 57.4 and 61.6 mo for CD and UC). Sera of IBD patients and 103 healthy controls (HC) were tested on individual anti- $\beta$ 2-Glycoprotein-I (anti- $\beta$ 2-GPI IgA/M/G), anti-cardiolipin (ACA IgA/M/G) and anti-phosphatidylserine/prothrombin (anti-PS/PT IgA/M/G) antibodies and also anti-*Saccharomyces cerevisiae* antibodies (ASCA IgA/G) by enzyme-linked immunosorbent assay (ELISA). In a subgroup of CD ( $n = 198$ ) and UC patients ( $n = 103$ ), obtaining consecutive samples over various arbitrary time-points during the disease course, we evaluated the intraindividual stability of the APLA status. Additionally, we provide an overview of studies, performed so far, in which significance of APLAs in IBD were assessed.

**RESULTS:** Patients with CD had significantly higher prevalence of both ACA (23.4%) and anti-PS/PT (20.4%) antibodies than UC (4.8%,  $P < 0.0001$  and 10.2%,  $P = 0.004$ ) and HC (2.9%,  $P < 0.0001$  and 15.5%,  $P = \text{NS}$ ). No difference was found for the prevalence of anti- $\beta$ 2-GPI between different groups (7.2%-9.7%). In CD, no association was found between APLA and ASCA status of the patients. Occurrence of anti- $\beta$ 2-GPI, ACA and anti-PS/PT was not different between the group of patients with active *vs* inactive disease state according to appropriate clinical, laboratory and endoscopic scores in CD as well as in UC patients. All subtypes of anti- $\beta$ 2-GPI and ACA IgM status were found to be very stable over time, in contrast ACA IgG and even more ACA IgA status showed significant intraindividual changes. Changes in antibody status were more remarkable in CD than UC (ACA IgA: 49.9% *vs* 23.3% and ACA IgG: 21.2% *vs* 5.8%). Interestingly, 59.1% and 30.1% of CD patients who received anti-TNF therapy showed significant negative to positive changes in ACA IgA and IgG antibody status respectively. APLA status was not associated with the clinical phenotype at diagnosis or during follow-up, medical therapy, or thrombotic events and it was not associated with the probability of developing complicated disease phenotype or surgery in a Kaplan-Meier analysis.

**CONCLUSION:** The present study demonstrated enhanced formation of APLAs in CD patients. However, presence of different APLAs were not associated with the clinical phenotype or disease course.

**Key words:** Crohn's disease; Ulcerative colitis; Disease progression; Antiphospholipid antibodies; Anti- $\beta$ 2-Glycoprotein-I antibodies; Anti-phosphatidylserine/prothrombin; Anti-cardiolipin antibodies; Thrombosis

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**Core tip:** Enhanced serological antibody formation is a well-known feature of inflammatory bowel diseases. Antiphospholipid antibodies (APLAs) are a

prothrombotic group of antibodies acquired in various inflammatory diseases. However their association with clinical phenotype and disease progression is still unclear in inflammatory bowel diseases (IBD). In the present study we report enhanced formation of APLAs in patients with Crohn's disease, which was not associated with clinical phenotype or disease course during follow-up in a tertiary referral IBD center from Hungary.

Sipeki N, Davida L, Palyu E, Altorjay I, Harsfalvi J, Antal Szalmas P, Szabo Z, Veres G, Shums Z, Norman GL, Lakatos PL, Papp M. Prevalence, significance and predictive value of antiphospholipid antibodies in Crohn's disease. *World J Gastroenterol* 2015; 21(22): 6952-6964 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6952.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6952>

## INTRODUCTION

Enhanced serological antibody formation is a well-known feature of inflammatory bowel diseases (IBD). A wide range of anti-microbial and autoantibodies have been reported to be associated with either Crohn's disease (CD) or ulcerative colitis (UC)<sup>[1]</sup> as well as with complicated disease course. Anti-microbial antibodies are formed against different surface carbohydrate (anti-glycans<sup>[2]</sup>) or protein antigens of various gut microbes<sup>[3]</sup>. The first and still most relevant anti-microbial antibody is the ASCA (anti-*Saccharomyces cerevisiae* antibody). Autoantibodies are directed against various host proteins. Based on recent findings, their existence might also be related to enhanced microbial challenge to the gut<sup>[4,5]</sup> due to a disturbed gut innate immune system and may trigger an exaggerated adaptive immune response. Furthermore, these serological antibodies may also be actively involved in the pathophysiology of inflammation in IBD<sup>[6,7]</sup>.

Antiphospholipid antibodies (APLAs) are a prothrombotic group of autoantibodies and established as the serological hallmark of antiphospholipid syndrome (APS)<sup>[8]</sup>. These antibodies comprise anti-cardiolipin (ACA), anti- $\beta$ 2-Glycoprotein-I (anti- $\beta$ 2-GPI), and anti-phosphatidylserine/prothrombin antibodies (anti-PS/PT). APLAs, however, are also found in a variety of disorders (chronic inflammatory diseases<sup>[9-12]</sup> or post infectious conditions<sup>[13-16]</sup>) not necessarily exhibiting prothrombotic activity. Even if non-prothrombotic, they may have certain pathogenetic roles in several diseases as well<sup>[17,18]</sup>.

In IBD, available cross-sectional, mainly single-time point studies assessing different aspects of APLAs, came to discrepant conclusions regarding formation, prevalence, and stability of these antibodies. Their clinical significance, including association to thrombotic events in IBD is also unclear<sup>[19-33]</sup> (Table 1). Thus a comprehensive evaluation of the primary APLAs in a

Table 1 Prevalence and clinical significance of antiphospholipid antibodies in inflammatory bowel disease - review of the literature

Ref.	Study design	Group	n	ACA prevalence (%)				Anti-β2-GPI prevalence (%)				ACA				Anti-β2-GPI			
				Total	IgG	IgM	IgA	Total	IgG	IgM	IgA	Disease activity	Association with disease phenotype	Location	Disease activity	TE event	IS therapy	Complicated disease behaviour	Need for surgery
[19]	R	HC	136	4.0															
		CD	73	20.0								No		No	No	No	No		
		UC	63	10.0								No		No	No	No	No		
[20]	P	AS	19	5.3	5.3			0	0	0									
		CD	63	8.0	3.0			0	0	0		No			No				
[21]	CS	CD	22	36.0								No		No					
		UC	30	20.0								No		No					
[22]	CS	CD	50	22.0	10.0	6.0	6					No		No	No	No	No		
[23]	R	HC	40	5.0								No							
		CD	41	26.8								No							
		UC	19	26.3								No							
[24]	CS	HC	261	1.2	1.2							No		No	No	No	No		
		UC	80	10.0	17.5							No							
[25]	R&CS	HC	100	3.0	2.0	1.0		0	0	0									
		CD	45	15.6	8.9	6.7		4.4	2.2	2.2		No		No	No	No	No	No	No
		UC	83	18.1	13.3	4.8		10.8	8.4	2.4		+		No	No	No	No	No	No
[26]	CS	HC	118	2.5															
		CD	138	11.0								No							
[27]	CS	HC	40																
		CD	7	3.9	0														
		UC	19																
[28]	P	CD	4	0												No			
		UC	8	12.5															
[29]	R	HC	102																
		CD	24	6.6															
		UC	21																
[30]	R	HC	53	0	0														
		CD	29	9.4	6.9														
		UC	24	12.5															
[31]	R	HC	100																
		CD	26	0	0														
		UC	75	1.3	1.3														
[32]	CS	IBD	20	30.0															
[33]	CS	HC	27	0	0														
		CD	5	7.4	3.7														
		UC	22																

ACA: Anti-cardiolipin antibody; β2-GPI: β2-Glycoprotein-I; CS: Cross-sectional; P: Prospective; R: Retrospective; TE: Thromboembolic; HC: Healthy control; CD: Crohn's disease; UC: Ulcerative colitis; IS: Immunosuppressive.

**Table 2 Clinical characteristics of inflammatory bowel disease patients at time of inclusion *n* (%)**

	CD ( <i>n</i> = 271)		UC ( <i>n</i> = 187)	
Male/female ( <i>n</i> )	115/156		86/101	
Age (yr) <sup>1</sup>	31 (24.0-41.0)		40 (29.0-52.0)	
Age at presentation (yr) <sup>1</sup>	25 (19.0-33.0)		33 (23.0-43.0)	
Duration (yr) <sup>1</sup>	4 (1.0-9.0)		4 (1.0-11.0)	
Familial IBD	12 (4.4)		6 (3.2)	
Location				
L1	46 (17.0)	Proctitis	30 (16.0)	
L2	67 (24.7)	Left-sided	104 (55.6)	
L3	157 (57.9)	Extensive	53 (28.3)	
L4 only	1 (0.4)			
All L4	18			
Behavior				
B1	154 (56.5)	Remitting	174 (93.0)	
B2	56 (20.7)	Continuous	12 (6.4)	
B3	61 (22.5)	Prolonged remission	1 (0.5)	
Perianal disease	76 (27.5)		-	
Arthritis	54 (19.9)		26 (13.9)	
Ocular manifestations	65 (24.0)		12 (6.4)	
Cutaneous manifestation	35 (12.9)		16 (8.6)	
PSC	9 (3.3)		8 (4.3)	
Steroid use/refractory	242 (89.3)/ 32 (13.2)		144 (77.0)/ 11 (7.6)	
Azathioprine use	196 (72.3)		66 (35.3)	
Surgery/multiple in CD	54 (19.6)/ 19 (7.0)		7 (3.7)	
Biological use	106 (39.1)		25 (13.4)	
Smoking habits				
never	219 (80.8)		167 (89.3)	
yes	47 (17.3)		18 (9.6)	
previous	5 (1.8)		2 (1.1)	
Disease activity				
Inactive HBI ≤ 4	199 (73.4)	Inactive partial Mayo ≤ 3 Active partial Mayo > 4	135 (72.2)	
Active HBI ≥ 5	72 (26.6)		52 (27.8)	

<sup>1</sup>Median (IQR). Location: L1: Ileal; L2: Colonic; L3: Ileocolonic; L4: Upper gastrointestinal disease; Behavior: B1: Inflammatory; B2: Stenosing; B3: Penetrating; PSC: Primary sclerosing cholangitis; Surgery: CD-related abdominal surgery and colectomy in UC; HBI: Harvey-Bradshaw Index; CD: Crohn's disease; UC: Ulcerative colitis.

large prospectively followed-up IBD cohort is required.

Current advances may add a new spark to the investigation of the role of anti-β2-GPI antibodies in the pathomechanism of IBD. The presence of cross-reactive epitopes on *Saccharomyces cerevisiae* and β2-GPI<sup>[34]</sup> has been reported in APS and raises the possibility that ASCA alone or by cross-reactivity with anti-β2-GPI exaggerate the pathologic intestinal microvascular processes in IBD and interfering with the inhibitory effect of β2-GPI on von Willebrand factor-dependent platelet adhesion and aggregation. Inflammation and coagulation are closely linked, interdependent processes in the microvasculature. Coagulation abnormalities at the mucosal level result in microthrombi formation, which are well-known

features of CD and thought to be involved in the disease pathogenesis and progression<sup>[35]</sup>. Theoretically, impairment in the function of β2-GPI due to the presence of anti-β2-GPI may deteriorate certain innate immune functions as well. A novel function of the β2-GPI protein with important relevance to innate immunity, is its ability to bind and scavenge lipopolysaccharide (LPS) through a direct interaction between domain 5 (D5) of β2-GPI and LPS<sup>[36,37]</sup>.

The aims of this study were to investigate in a large IBD cohort: (1) the prevalence and type of APLAs; (2) associations between the presence of APLAs and clinical phenotype of the disease or its activity; and (3) whether the presence of APLAs is associated with the disease course or development of thrombosis during prospective follow-up. Additionally, we also provide an overview of studies, performed over the last 24 years, in which significance of APLAs in IBD were assessed.

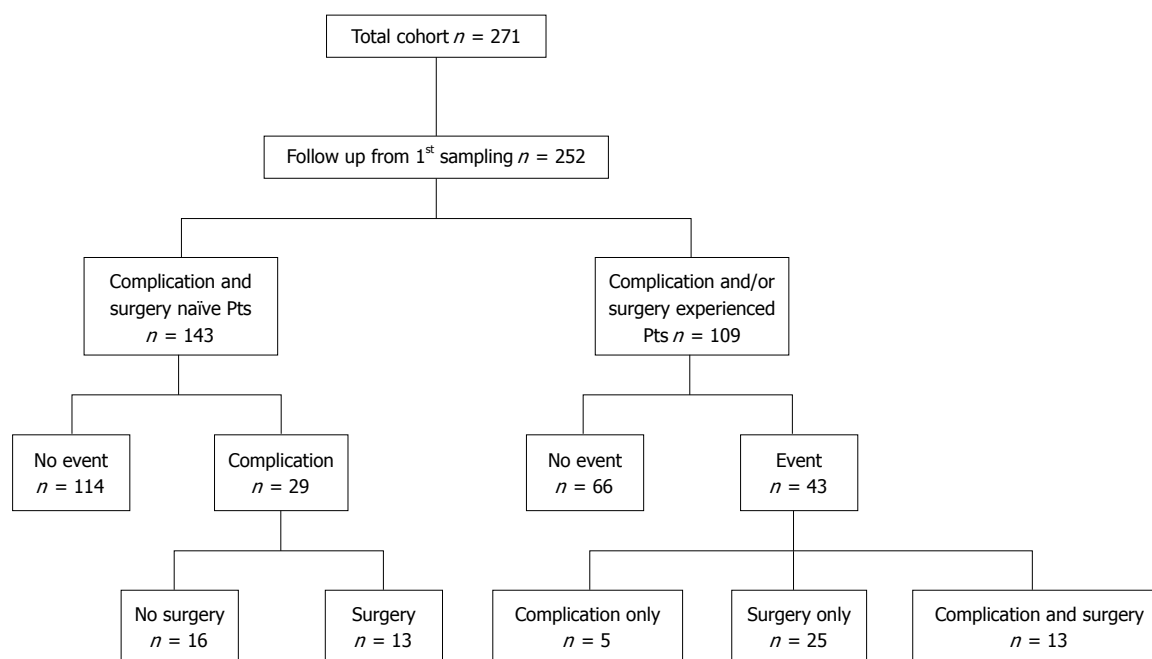
## MATERIALS AND METHODS

### Patient population

We performed a cohort study among adult CD and UC patients in a Hungarian tertiary IBD referral center (Division of Gastroenterology, Department of Internal Medicine, University of Debrecen). In all, 458 well-characterized, unrelated, consecutive IBD patients with a complete clinical follow-up CD: 271 (male/female: 120/140, age at presentation: 27.7 ± 11.6 years, disease duration: 6.0 ± 6.7 years) and UC: 187 (male/female: 86/101, age at presentation: 34.0 ± 13.2 years, disease duration: 7.4 ± 8.6 years) seen at our outpatient clinic were included between January 1, 2005 and June 1, 2010. Serum samples were obtained at enrollment from each patient and frozen at -80°C until testing. The clinical characteristics of the patients at time of inclusion and sample procurement are presented in Table 2.

Diagnosis of IBD was based on the Lennard-Jones criteria<sup>[38]</sup>. The disease phenotype (age at onset, duration, location, and behaviour) was determined according to the Montreal Classification<sup>[39]</sup>. Clinical disease activity was calculated according to the Harvey-Bradshaw Index (HBI)<sup>[40]</sup> in CD and the partial Mayo score in UC<sup>[41]</sup>. In this study we followed the European Crohn's and Colitis Organisation guidelines<sup>[42]</sup> and defined HBI ≤ 4 as a state of remission and ≥ 5 as a state of active disease. In case of UC, ≤ 3 was defined as a state of remission and > 4 as a state of active disease. Endoscopic activity was determined according to the Simple Endoscopic Score for Crohn's Disease (SES-CD) in CD<sup>[43]</sup> and the endoscopic component of the Mayo score in UC<sup>[44]</sup>. SES-CD defines endoscopic activity as ≥ 3 points and inactive disease ≤ 2 in CD, meanwhile in UC a state of active disease was defined as ≥ 1 points according to invasive partial Mayo score. Detailed clinical phenotypes were determined by thorough review of patients' medical records, which





**Figure 1** Flow chart of the patients with Crohn's disease in the cohort study. Event: Complication and/or surgery; Complication: Stricture development and/or internal penetration and/or perianal penetration; Surgery: Crohn's disease-related surgery (resection only). Pts: Patients.

had been collected in a uniform format. Medical records that documented the presence of extraintestinal manifestations [for example, arthritis: peripheral and axial; ocular manifestations: conjunctivitis, uveitis, iridocyclitis; skin lesions: erythema nodosum, pyoderma gangrenosum; arterial (AT) and venous thrombosis (VT) or pregnancy loss; and hepatic manifestations: primary sclerosing cholangitis (PSC)], frequency of flare-ups (frequent flare-up: > 1 clinical relapse/year)<sup>[45]</sup>, medication use (e.g., steroid, immunosuppressive and/or biological use at any time), need for surgery (resection in CD and colectomy in UC), the presence of familial IBD, smoking habits, and perianal involvement were retrospectively analyzed for the period prior to the prospective follow-up.

#### **Phenotypical characterization of IBD patients during prospective follow-up**

252 of 271 CD patients and 173 of 187 UC patients were available to be enrolled into a prospective follow-up study, where the treating IBD physicians registered laboratory data, endoscopic and imaging findings, disease activity, medical treatment, date and type of complications, surgery and thrombosis during regular and extraordinary outpatient follow-up visits and inpatient stays. In Hungary, a follow-up visit is usually scheduled for every 6 mo at a specialized gastroenterology center (the actual interval varies between 3-6 mo). Collected data were transferred and stored in a database for analysis. In October 1, 2013, all patients' charts and database were reviewed and updated for the data points mentioned above. Follow-up for a particular patient was terminated if there was no further record available. Median follow-up was

57.4 mo (IQR: 40.9-80.1) for CD and 61.6 mo (IQR: 46.5-81.3) for UC patients. In CD, complicated disease behavior was defined as the occurrence of stenosis or internal penetration. Perianal fistulizing disease was distinguished from internal penetrating disease and evaluated separately. Need for surgery was defined as CD-related abdominal surgery (resection). Figure 1 summarizes flow chart of the patients with CD in the cohort study. In UC, complicated disease behavior was defined as progression of the disease extent or need for colectomy.

The control group consisted of 103 age- and gender- matched healthy blood donors (male/female: 46/57, age: 35.3 ±12.6 years old). The control subjects did not have any gastrointestinal and/or liver disease and were selected from consecutive blood donors in Debrecen.

#### **Serological analysis**

Anti-β2-GPI, ACA and anti-PS/PT levels in serum samples were tested using the semiquantitative QUANTA Lite™ aβ2-GPI, ACAIII and aPS/PT IgA, IgG and IgM kits (INOVA Diagnostics, San Diego, California). These enzyme-linked immunosorbent assay (ELISA) kits detect IgA, IgG and IgM antibodies against β2-GPI, cardiolipin and the PS/PT complex in human serum. Plastic microwell plate wells are coated with purified β2-GPI, cardiolipin or PS/PT complex. Upon incubation, serum β2-GPI, cardiolipin and PS/PT IgA, IgG or IgM antibodies bind to β2-GPI, cardiolipin or the PS/PT complex. Unbound protein is removed by washing, while bound antibodies are detected by human IgA, IgG or IgM horseradish peroxidase-labelled conjugate. A peroxidase substrate is then

added. The presence of anti- $\beta$ 2-GPI, ACA and aPS/PT antibodies is determined spectrophotometrically by measuring the signal intensity of each sample compared to a five-point calibration curve. All assays were performed according to the manufacturer's instructions and were considered positive when titers were above the manufacturer's pre-established cut-off points (for anti- $\beta$ 2-GPI and ACA assays,  $\geq 20$  units for all the IgA, IgG and IgM, and for anti-PS/PT assays  $\geq 30$  units for both the IgG and IgM). In case of anti-PS/PT IgA the results are presented as OD due to lack of established calibrators. Values above the OD cut-off 0.795 were considered positive for anti-PS/PT IgA. This cut-off OD value represented the mean  $\pm$  SD values of the healthy controls. The results were documented in absolute OD values and in frequency of positivity. Of the 458 IBD samples obtained at enrollment, serologic analysis was technically successful in 451 of the 458 IBD cases.

ASCA antibody evaluation in CD patients was performed in our previous study<sup>[46]</sup> by ELISA (QUANTA Lite™, INOVA Diagnostics, San Diego, CA) according to the manufacturers' instructions. The results are presented as arbitrary units, and values above the cut-off of 25 units were considered as positive. The results were documented in absolute values and in frequency of positivity.

All the serological assays were performed in a blinded fashion without prior knowledge of the patients' diagnosis or other clinical information.

#### **Detection of NOD2/CARD15 SNP8, 12, 13 mutations**

NOD2/CARD15 SNP8, SNP12, and SNP13 genotypes were performed previously<sup>[46]</sup> in CD patients ( $n = 235$ ), but not in UC patients. NOD2/CARD15 variants were detected by denaturing high-performance liquid chromatography (dHPLC, Wave DNA Fragment Analysis System, Transgenomic Limited, United Kingdom). Sequence variation, observed in the dHPLC profile, was sequenced on both strands to confirm the alteration. Sequencing reactions were performed with the ABI BigDye Terminator Cycle Sequencing Kit v1.1 (Applied Biosystems, Foster City, CA) and samples were sequenced on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). All investigated polymorphisms were in Hardy-Weinberg equilibrium (data not shown).

#### **Review of the literature**

We performed a systematic review of studies reporting on APLAs in IBD. Papers were eligible if they presented original research in adult IBD patients and reported occurrence of APLAs and their possible association with disease activity, medication, clinical phenotype of the disease, need for surgery and thromboembolic events. Case series or case reports were not included. Studies had to have been published in peer-reviewed journals. We started searching PubMed using the following

search terms: "antiphospholipid antibodies" OR "anticardiolipin antibodies" OR "anti- $\beta$ 2-Glycoprotein-I antibodies" OR "phosphatidylserine-dependent anti-prothrombin antibodies" AND "inflammatory bowel disease" OR "Crohn's disease" OR "ulcerative colitis". Limits were human and time ranging from 1991 until 2014 (30<sup>th</sup> of November). This search revealed 15 articles. In Table 1 we summarize the prevalence and clinical significance of APLAs in IBD based on findings in relevant literature.

#### **Ethical permission**

The study protocol was approved by the regional and national committee for research ethics. Each patient was informed of the nature of the study and signed an informed consent form.

#### **Statistical analysis**

Variables were tested for normality using Shapiro Wilk's  $W$  test. Continuous variables were summarized as mean  $\pm$  SD or as medians (IQR) according to their homogeneity. To evaluate differences between IBD and healthy control group, as well as within subgroups of patients with IBD the following statistical methods were used. Categorical variables were compared with the Fisher's exact test or  $\chi^2$ -test with Yates correction, as appropriate. Continuous variables were compared with Student's  $t$  test, one-way analysis of variance (ANOVA), or Mann-Whitney's  $U$  test. Kaplan-Meier survival curves were plotted for analyzing the association between categorical clinical variables or APLAs and complicated disease outcomes during follow-up with LogRank and Breslow tests. Associations are given as OR and HR with a 95%CI. A 2-sided probability value  $< 0.05$  was considered to be significant. For statistical analysis, GraphPad Prism 6 (San Diego, CA) and SPSS 15.0 (SPSS Inc, Chicago, IL) programs were used. The statistical methods of this study were reviewed by Elek Dinya from Semmelweis University, Institute of Health Informatics, Development and Further Training.

## **RESULTS**

#### **Frequency of APLA Markers in IBD**

The prevalence rates of anti- $\beta$ 2-GPI, ACA and anti-PS/PT antibodies are presented in Table 3. ACA positivity was associated with increased risk for CD compared to the controls (OR<sub>ACA</sub> = 10.18, 95%CI: 3.12-33.24). Of the different isotypes, ACA IgA (OR<sub>ACA IgA</sub> = 49.70, 95%CI: 3.04-813.8,  $\chi^2$ -test with Yates correction) had the highest association to CD. ACA positivity was also significantly different between CD and UC. While the prevalence of anti-PS/PT was significantly different between CD and UC, there was not a significant difference between CD and the controls. No difference was found for the prevalence of anti- $\beta$ 2-GPI in different groups.

**Table 3** Anti-phospholipid antibodies in patients with Crohn's disease, ulcerative colitis, and healthy controls *n* (%)

	CD ( <i>n</i> = 265)	UC ( <i>n</i> = 186)	HC ( <i>n</i> = 103)
Anti-β2-GPI IgG	5 (1.9)	4 (2.2)	2 (1.9)
Anti-β2-GPI IgM	8 (3.0)	12 (6.5)	3 (2.9)
Anti-β2-GPI IgA	7 (2.6)	7 (3.8)	3 (2.9)
Any anti-β2-GPI	19 (7.2)	18 (9.7)	8 (7.8)
ACA IgG	27 (10.2) <sup>bd</sup>	4 (2.2) <sup>d</sup>	2 (1.9) <sup>b</sup>
ACA IgM	8 (3.0)	4 (2.2)	1 (1.0)
ACA IgA	51 (19.2) <sup>bd</sup>	1 (0.5) <sup>d</sup>	0 (0.0) <sup>b</sup>
Any ACA	62 (23.4) <sup>bd</sup>	9 (4.8) <sup>d</sup>	3 (2.9) <sup>b</sup>
Anti-PS/PT IgG	20 (7.5)	9 (4.8)	9 (8.7)
Anti-PS/PT IgM	25 (9.4) <sup>bc</sup>	8 (4.3) <sup>c</sup>	1 (1.0) <sup>b</sup>
Anti-PS/PT IgA	24 (9.1) <sup>c</sup>	7 (3.8) <sup>c</sup>	9 (8.7)
Any anti-PS/PT	54 (20.4) <sup>d</sup>	19 (10.2) <sup>d</sup>	16 (15.5)

<sup>b</sup>*P* < 0.01, CD *vs* controls; <sup>c</sup>*P* < 0.05, <sup>d</sup>*P* < 0.01, CD *vs* UC. Using  $\chi^2$ -test with Yates correction. APLA: Antiphospholipid antibodies; β2-GPI: Beta2-glycoprotein-I; ACA: Anti-cardiolipin antibody; PS/PT: Phosphatidylserine/prothrombin complex; CD: Crohn's disease; UC: Ulcerative colitis.

#### Association between APLA positivity, and other serologic markers or NOD2/CARD15 genotypes in CD

In CD, no association was found between APLA and ASCA status of the patients. Neither ACA IgA nor IgG positivity differed significantly according to presence or absence of ASCA IgA and ASCA IgG. Similarly, the prevalence of ACA was not associated with the presence of major *NOD2/CARD15* mutations. *NOD2/CARD15* genotypes were available in 235 CD patients. The prevalence of any ACA was not different between patients with or without *NOD2/CARD15* mutations (16.1% and 26.1%, *P* = NS,  $\chi^2$ -test with Yates correction).

#### Association between APLA positivity and actual clinical, laboratory or endoscopic activity of the disease

At the time of enrollment, 26.6% of CD patients and 27.8% of UC patients had active disease according to clinical activity scores (Table 1). Occurrence of anti-β2-GPI, ACA and anti-PS/PT was not different between the group of patients with active (2.8%, 29.2% and 18.1%) and inactive disease state (8.8%, 21.2% and 21.2%, respectively) signified by HBI ≥ 5. Similarly, there was no correlation between the disease activity determined by partial Mayo score > 4 and APLA status in UC (data not shown).

Furthermore, the prevalence of any ACA was similar between CD patients with C-reactive protein (CRP) level > 10 mg/L and those with ≤ 10 mg/L (29.0% and 21.1%, *P* = NS,  $\chi^2$ -test with Yates correction).

A total of 87 CD patients had ileocolonoscopy at enrollment. The prevalence of any ACA was not different according to endoscopic disease activity denoted by a SES-CD cut-off value of ≥ 3 (inactive *vs* active: 25.6% and 29.5%). Likewise, ACA IgA and ACA IgG level was not associated with CRP level, actual HBI or SES-CD score in patients with CD applying

Spearman correlation analysis (data not shown).

20.3% of the CD patients showed frequent relapse during the follow-up. The ACA prevalence was not significantly different between patients with or without frequent relapse (ACA IgA 23.5% *vs* 16.7%, ACA IgG 3.9% *vs* 10.1% and ACA IgM 2.0% *vs* 3.5%, *P* = NS for all).

Lastly, we investigated the association between disease duration and both the presence and magnitude of serologic response in CD. The rate of any ACA positivity was the same in all four disease duration quartile groups (Q1: 26.9%, Q2: 22.7%, Q3: 22.4% and Q4: 23.0%, *P* = NS,  $\chi^2$ -test with Yates correction). The level of ACA IgA and IgG was also not associated with disease duration (Kruskal-Wallis test).

#### APLA markers and disease progression in CD

A total of 154 (56.5%) CD patients had non-stricturing and non-penetrating disease (B1) according to Montreal classification at time of sampling. 143 patients were eligible for a prospective follow-up study. The median follow-up was 53.4 mo (IQR: 38.0-79.3). Among these complication and surgery naïve patients, 20.3% (29/143) experienced a complication during follow-up (31.03% developed strictures, 44.83% internal penetration and 24.14% perianal perforation only). The median time to complication was 21.4 mo (IQR: 8.1-43.1). In all, 9.1% (13/143) had to undergo CD-related abdominal surgery (resection) during the follow-up period (Figure 1). Two patients had surgical intervention without previous complication due to the development of colorectal cancer. In the remaining patients the reason for surgery was the occurrence of a complication (23.1% stenosis, 46.2% internal penetration, 15.4% both). The median time to surgery was 50.0 mo (IQR: 30.5-54.5).

In patients classified as B1, the progression of the disease to a first event defined as stenosis, internal and/or perianal penetration or CD-related surgery (Table 4) was not associated to presence or absence of APLA positivity. Furthermore in Kaplan-Meier analysis the likelihood for earlier progression to a disease event was similar in patients with or without APLA positivity. Of the clinical factors, disease location and smoking were those that associated with time to development of first internal penetrating and/or stricturing complication and frequent relapses to development of perianal penetrating disease (Table 5).

In UC patient group association between APLAs and clinical phenotype or progression of the disease was not evaluated due to the lack of increased prevalence of any APLAs in UC population.

#### APLA markers and thromboembolic events in IBD

In total, 5.1% (23/452) of IBD patients had at least one thromboembolic event (14 CD and 9 UC patients). In CD, 18 events of VT, 1 event of PE and 3 events of AT were diagnosed. In UC, 8 events of VT and 1

**Table 4** Antiphospholipid antibodies and clinical characteristics of complication and surgery naïve Crohn's disease patient cohort *n* (%)

Factor	Follow-up of CD B1 at first sampling total cohort ( <i>n</i> = 143)					
	CD B1 total	No complication	Perianal change only (B1p)	Behaviour change to B2 or B3	No surgery	Surgery
	( <i>n</i> = 143)	( <i>n</i> = 114)	( <i>n</i> = 7)	( <i>n</i> = 22)	( <i>n</i> = 130)	( <i>n</i> = 13)
Male/female ( <i>n</i> )	60/83	45/69	5/2	10/12	55/75	5/8
Age at presentation (yr) <sup>1</sup>	25 (19.0-34.0)	26 (20.0-34.3)	17 (14.0-31.0)	25 (18.8-34.8)	25 (19.0-34.0)	31 (18.0-35.5)
Duration (yr) <sup>1</sup>	3 (1.0-6.0)	2 (1.0-7.0)	3 (1.0-5.0)	3 (1.0-5.0)	2 (1.0-6.0)	3 (1.0-10.0)
Location						
L1	30 (21.0)	24 (21.1)	0 (0.0)	6 (27.3)	26 (20.0)	4 (30.8)
L2	51 (35.7)	44 (38.6)	3 (42.9)	4 (18.2)	48 (36.9)	3 (23.1)
L3	62 (43.3)	46 (40.4)	4 (57.1)	12 (54.5)	56 (43.1)	6 (46.2)
L4 only	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Frequent relapse	27 (18.9)	18 (15.8) <sup>2</sup>	4 (57.1) <sup>2</sup>	5 (22.7)	23 (17.7)	4 (30.8)
Smoking habit yes	21 (16.2)	14 (12.3) <sup>2</sup>	2 (28.6)	8 (36.4) <sup>2</sup>	21 (16.2)	3 (23.1)
Follow up time from first sampling, mo <sup>3</sup>	53.4 (38.0-79.3)	51.7 (36.9-73.9)	79.8 (42.9-91.4)	64.7 (45.6-85.0)	50.9 <sup>3</sup> (37.25-74.2)	84.0 <sup>3</sup> (60.0-91.7)
Positive markers						
Anti-β2-GPI IgG	1.4	1.8	0	0	1.6	0
Anti-β2-GPI IgM	5.0	5.3	16.7	0	5.5	0
Anti-β2-GPI IgA	2.8	1.8	16.7	4.5	3.1	0
Any anti-β2-GPI	8.5	8.8	16.7	4.5	9.4	0
ACA IgG	9.2	11.5	0	0	10.2	0
ACA IgM	2.8	3.5	0	0	3.1	0
ACA IgA	17.0	18.6	33.3	4.5	18.8	0
Any ACA	19.9	22.1	33.3	4.5	21.9	0
Anti-PS/PT IgG	8.5	8.0	0	13.6	7.8	15.4
Anti-PS/PT IgM	7.8	8.8	16.7	0	7.8	7.7
Anti-PS/PT IgA	9.9	9.7	16.7	9.1	10.9	0
Any anti-PS/PT	20.6	19.5	33.3	22.7	20.3	23.1

<sup>1</sup>Median (IQR); <sup>2</sup>Using  $\chi^2$ -test with Yates correction ( $P \leq 0.01$ ); <sup>3</sup>Using Mann-Whitney test ( $P < 0.01$ ). Location: L1: Ileal; L2: Colonic; L3: Ileocolonic; L4: Upper gastrointestinal disease; Behavior: B1: Inflammatory; B2: Stenosing; B3: Penetrating. APLA: Antiphospholipid antibodies; β2-GPI: Beta2-glycoprotein-I; ACA: Anti-cardiolipin antibody; PS/PT: Phosphatidylserine/prothrombin complex; CD: Crohn's disease.

**Table 5** Univariate Kaplan Meier and Log-rank analysis of serologic and clinical factor associated with complicated disease course in B1 Crohn's disease patients (*n* = 143)

Variable	Internal penetrating and/or stricturing complication		Perianal penetrating complication		CD-related abdominal surgery	
	HR (95%CI)	<i>P</i> <sub>LogRank-value</sub>	HR (95%CI)	<i>P</i> <sub>LogRank-value</sub>	HR (95%CI)	<i>P</i> <sub>LogRank-value</sub>
Gender - male	1.10 (0.47-2.55)	0.83	2.69 (0.80-9.03)	0.11	0.91 (0.33-2.55)	0.86
Smoking	3.72 (1.23-11.21) <sup>1</sup>	0.02 <sup>1</sup>	1.61 (0.36-7.19)	0.53	1.64 (0.45-5.97)	0.45
Location (colon only)	0.41 (0.17-0.96) <sup>1</sup>	0.04 <sup>1</sup>	1.00 (0.29-3.43)	0.99	0.61 (0.22-1.74)	0.36
Frequent relapse	1.10 (0.39-3.07)	0.85	7.29 (1.72-30.99) <sup>1</sup>	0.007 <sup>1</sup>	1.64 (0.44-6.10)	0.46
APLA at all	1.36 (0.59-3.16)	0.47	1.30 (0.40-4.27)	0.66	1.37 (0.49-3.80)	0.55
Anti-β2-GPI at all	0.56 (0.13-2.32)	0.42	0.99 (0.13-7.70)	0.99	0.33 (0.06-1.81)	0.20
ACA at all	0.41 (0.13-1.26)	0.12	1.12 (0.23-5.49)	0.89	0.30 (0.07-1.27)	0.10
Anti-PS/PT at all	1.07 (0.39-2.97)	0.89	1.74 (0.38-8.03)	0.48	1.11 (0.29-4.18)	0.88

<sup>1</sup>The *P*<sub>LogRank-value</sub> < 0.05. APLA: Antiphospholipid antibodies; β2-GPI: Beta2-glycoprotein-I; ACA: Anti-cardiolipin antibody; PS/PT: Phosphatidylserine/prothrombin complex.

events of AT were diagnosed. 7 (1.6%) patients had a TE also prior to diagnosis of IBD. 4 (1.5%) CD patients had recurrent TE. In women, pregnancy loss occurred in 6.4% (10/156) of CD and 6.9% (7/101) of UC patients.

CD patients presenting with VT were significantly older than those without (median age: 30.0 years vs 38.5 years,  $P = 0.003$ ). Of the investigated clinical factors and laboratory markers, previous VT event (RR = 23.0, 95%CI: 10.6-50.1) and factor V Leiden

mutation (RR = 8.3, 95%CI: 2.2-31.3) were associated with the risk of VT events. At the same time, in patients with UC, frequent relapse was associated with higher risk of VT event (RR = 6.4, 95%CI: 1.7-24.1). However, none of the APLA markers were associated with increased risk of VT events in IBD.

Table 6 summarizes the patient characteristics, prevalence of APLAs and the known genetic and serologic markers of thrombophilia according to presence and type of thrombotic events in CD patients. We



**Table 6** Antiphospholipid antibodies, thrombophilia markers and clinical characteristics of total Crohn's disease according to presence and type of thrombosis *n* (%)

Factor	Follow-up of CD Total Cohort from diagnosis ( <i>n</i> = 265)				
	No thrombosis ( <i>n</i> = 251)	Arterial thrombosis ( <i>n</i> = 3)	Venous thrombosis ( <i>n</i> = 11)	No pregnancy loss ( <i>n</i> = 146)	Pregnancy loss ( <i>n</i> = 10)
Male/female	104/147	1/2	7/4	146/0	10/0
Age at presentation (yr) <sup>1</sup>	25.0 (19.0-33.0)	40.0 (28.0-42.0)	29.5 (23.3-39.3)	26.0 (20.0-35.0)	25.5 (21.0-35.0)
Frequent relapse	48 (20.2)	0 (0.0)	4 (36.4)	33 (24.1)	2 (20.0)
Previous thrombosis	1 (0.4) <sup>3</sup>	0 (0.0)	4 (36.4) <sup>3</sup>	2 (1.4)	0 (0.0)
Smoking habits yes	48 (19.1)	0 (0.0)	3 (27.3)	22 (15.1)	2 (20.0)
Follow up time from diagnosis, mo <sup>1</sup>	102.2 (63.3-172.8) <sup>2</sup>	149.9 (130.8-219.8)	186.3 (142.0-244.2) <sup>2</sup>	109.0 (61.8-184.6)	136.5 (95.4-180.6)
Positive markers (%)					
Anti-β2-GPI IgG and/or IgM	4.8	0	9.1	4.9	10
Anti-β2-GPI IgA	2.8	0	0	4.9	0
ACA IgG and/or IgM	12.1	0	27.3	13.9	10
ACA IgA	19.0	66.7	0	20.1	10
Anti-PS/PT IgG and/or IgM	14.6	50.0	36.4	18.3	40
Anti-PS/PT IgA	8.9	50.0	9.1	7.7	10
At least 1 APLA pos	48.0	66.7	54.5	52.8	60
At least 2 APLA pos	16.1	33.3	9.1	18.1	10
At least 3 APLA pos	3.2	0	9.1	4.9	0
Thrombophilia markers <sup>3</sup> (%)					
LA	7.5	0	0	5.1	0
PS deficiency (inherited and/or acquired)	8.1	0	25.0	12.7	0
ATIII deficiency (inherited and/or acquired)	0	0	0	0	0
PC deficiency (inherited and/or acquired)	3.0	0	0	4.8	0
FV Leiden	5.9 <sup>4</sup>	0	42.9 <sup>4</sup>	7.8	0
FII20210A	5.9	0	16.7	7.8	0

<sup>1</sup>Median (IQR); <sup>2</sup>Using Kruskal-Wallis test ( $P \leq 0.01$ ); <sup>3</sup>Using  $\chi^2$ -test with Yates correction ( $P \leq 0.05$ ); <sup>4</sup>Serologic and genetic markers of thrombophilia were available in 105 patients. pts: Patients; APLA: Antiphospholipid antibodies; β2-GPI: Beta2-glycoprotein-I; ACA: Anti-cardiolipin antibody; PS/PT: Phosphatidylserine/prothrombin complex.

further investigated the probability for thromboembolic events as a function of positivity for a certain amount of APLAs out of the whole panel. However, neither CD and nor UC patients positive for multiple APLAs showed a higher probability for the development of thromboembolic events.

### Stability of APLA markers

In order to evaluate the stability in the APLA status (positive or negative for a respective antibody), we analyzed samples from same patients over various arbitrary time-points during the disease course. At least two serum samples were taken from a subgroup of CD patients ( $n = 198$ ) and UC patients ( $n = 103$ ). Median time between sample procurements were 13.5 mo (IQR: 6.8-22.9 mo) for CD and 12.1 mo (IQR: 5.7-20.4 mo) for UC patients. Interestingly, the anti-β2-GPI status was very stable over time with respect to all three Ig subtypes. Only 1.5%-5.8% of either CD or UC patients had a change in their anti-β2-GPI antibody status compared to the initial sample procurement. At the same time marked differences were found in case of ACA Ig subtypes. ACA IgM status, similar to anti-β2-GPI, was also stable. In contrast, ACA IgG and even more ACA IgA status showed significant changes over time, mainly from negative to positive. Changes in

antibody status were more remarkable in CD than UC (ACA IgA: 49.9% vs 23.3% and ACA IgG: 21.2% vs 5.8%). Stability data are not available for anti-PS/PT antibodies since measurements were only preformed on the first available serum samples of the patients. APLA changes are summarized in Table 7.

After availability of tumor necrosis factor (TNF) antagonist therapy through National Health reimbursement system in 2008, 43.7% (110/252) of our patients received either infliximab or adalimumab treatment. We assessed the impact of post-enrolment anti-TNF therapy in the induction of new ACA antibody formation. 59.1% (55/93) of patients who received anti-TNF therapy and had negative findings for ACA IgA at the baseline were found to have positive results later on (negative to positive change), which was significantly higher compared to the proportion of 35.6% (36/101) found among patients who did not receive anti-TNF therapy at all ( $P = 0.004$ ). These ratios were 30.1% vs 12.9% ( $P = 0.033$ ) for ACA IgG.

## DISCUSSION

To our knowledge, this is the largest study to investigate prospectively the prevalence, type, and clinical significance of multiple APLAs simultaneously

**Table 7** Stability of antiphospholipid antibodies marker status over time in inflammatory bowel diseases patients with at least 2 samples during the disease course *n* (%)

APLA marker	CD ( <i>n</i> = 198)	UC ( <i>n</i> = 103)
Anti-β2-GPI IgG		
Stable negative	194 (98.0)	97 (94.2)
Stable positive	1 (0.5)	2 (1.9)
Neg. to pos.	0 (0.0)	3 (2.9)
Pos. to neg.	3 (1.5)	1 (1.0)
Anti-β2-GPI IgM		
Stable negative	186 (94.0)	94 (91.3)
Stable positive	4 (2.0)	3 (2.9)
Neg. to pos.	6 (3.0)	5 (4.9)
Pos. to neg.	2 (1.0)	1 (0.9)
Anti-β2-GPI IgA		
Stable negative	190 (96.0)	93 (90.3)
Stable positive	1 (0.5)	4 (3.9)
Neg. to pos.	3 (1.5)	5 (4.9)
Pos. to neg.	4 (2.0)	1 (0.9)
ACA IgG		
Stable negative	139 (70.2)	95 (92.2)
Stable positive	3 (1.5)	1 (1.0)
Neg. to pos.	42 (21.2)	6 (5.8)
Pos. to neg.	14 (7.1)	1 (1.0)
ACA IgM		
Stable negative	173 (87.4)	95 (92.2)
Stable positive	3 (1.5)	1 (1.0)
Neg. to pos.	18 (9.1)	7 (6.8)
Pos. to neg.	4 (2.0)	0 (0.0)
ACA IgA		
Stable negative	73 (36.9)	78 (75.7)
Stable positive	13 (6.6)	0 (0.0)
Neg. to pos.	93 (46.9)	24 (23.3)
Pos. to neg.	19 (9.6)	1 (1.0)

Anti-PS/PT measurements were only preformed on the first available serum samples of the patients. APLA: Antiphospholipid antibodies; β2-GPI: Beta2-glycoprotein-I; ACA: Anti-cardiolipin antibody; PS/PT: Phosphatidylserine/prothrombin complex; CD: Crohn's disease; UC: Ulcerative colitis.

in a cohort of IBD patients to date. Three different antibodies were assessed by ELISA. Contrary to routine laboratory practice, APLAs were identified by anti-IgA secondary antibody in addition to anti-IgG and anti-IgM isotypes. Moreover, in the present study we also provided an overview of relevant APLA studies in IBD.

We demonstrated, for the first time, that enhanced ACA IgA formation is a feature of CD; the presence of ACA IgA was significantly higher as compared to UC and HC. Previously only one study<sup>[22]</sup>, involving 50 CD patients, evaluated the occurrence of ACA IgA. However, the reported prevalence rate was much lower than in the present study (6.0% vs 19.2%). At the same time, occurrence of IgM and IgG class ACA was studied more extensively in IBD, but several studies provide only total prevalence rate for these two ACA subtypes. Reported prevalence of total ACA was widely varied both in CD (0.0%-36%)<sup>[21,22,25]</sup> and UC (1.3%-20.0%)<sup>[30,31]</sup> which was 12.6% and 4.3%, respectively in the present study. Studies evaluating separately these two subtypes of ACA revealed that

the increased ACA prevalence is mainly due to IgG subtype. Except one study<sup>[30]</sup>, occurrence of ACA IgG prevailed over ACA IgM<sup>[20,22,25]</sup>. This is concordant with our results in CD cohort (ACA IgG: 10.2 vs ACA IgM: 3.0%).

What can be the cause of enhanced ACA formation in CD? Animal models, immunization with lipid A and lipoteichoic acid have resulted in induction of ACA and/or lupus anticoagulant (LA) formation<sup>[47]</sup>. Moreover, appearance of ACA antibodies may be due to the sustained exposure to bacterial DNA, which is enriched in unmethylated CpG motifs. These motifs are expressed *e.g.* in *Escherichia coli* DNA<sup>[48]</sup>. Based on these literature findings, the role of bacterial translocation (BT) in the induction of ACA, similar to anti-microbial antibodies, seems plausible<sup>[1]</sup>. At the same time, we did not find any association between the presence of ACA and ASCA, even when assessing according to separate isotypes. This finding implies mechanisms other than BT in the formation of ACA. Moreover, in contrast to anti-microbial antibodies, APLA formation was not influenced by NOD2/CARD15 genotypes. The fact that neither the presence of ACA, nor the titers of the antibodies were affected by actual disease activity and were also not associated with the CD phenotype of frequent relapse contradict the involvement of BT in the formation mechanisms of ACA as well.

Former studies<sup>[19-26]</sup> extensively assessed the link between disease activity and APLA formation either by clinical activity or by CRP level, but found no association. Considering that clinical, laboratory and endoscopic activity are not inevitably congruent in all cases, in the present study, we re-evaluated this relationship in a complex way, applying all the three kinds of activity parameters simultaneously, but came to same conclusion.

The prevalence of anti-β2-GPI IgG/IgM in the present study was comparable to those reported in the study of Koutroubakis *et al.*<sup>[25]</sup> (CD: 5.0% vs 4.4% and UC: 7.5% vs 10.8%). Anti-PS/PT antibodies were not assessed previously in IBD, however we did not find enhanced antibody formation in IBD as compared to HC.

Association of certain APLAs with clinical phenotype of IBD was mainly assessed in the cross-sectional single-time point studies summarized in Table 1. In most of these studies, these antibodies were neither linked to the disease location<sup>[19,21,22,25]</sup>, behavior<sup>[25]</sup> and need for surgery<sup>[19]</sup> in CD patients, nor to the extent of the disease<sup>[19,21,24,25]</sup> and colectomy<sup>[19]</sup> in UC patients. Likewise, different APLAs were not associated with the risk of VTE events in either CD<sup>[19,22,23,25,26]</sup> or in UC<sup>[19,23-25]</sup>. In case of these single point cross sectional studies the serum samples were obtained at different times during the disease course. These approaches combine samples taken before, at the time of and after complications occur and only allow revealing

associations, but do not have predictive capability. In the present study to enhance the potential clinical value of these markers, we applied a prospective study design, which enabled us to evaluate the potential predictive capabilities of APLAs in respect to complicated CD behavior and surgery. Predictive ability for serum markers might be beneficial at any time during the disease course but perform distinctly by chance. Thus we formed two separate groups for our CD cohort (1) complication and surgery naïve CD patients; and (2) complication and/or surgery experienced patients prior to sample procurement. Several observations suggest that the presence of perianal and internal penetrating disease signify distinct phenotypes in CD concerning all the clinical, serologic and genetic point of view<sup>[1,49]</sup>. Accordingly, we evaluated B1p complication separately from B3 complication. However, APLA did not proved as a predictive marker of the complicated disease in neither clinically setting of CD. A clear strength of our study was prospective follow-up design and the application of the widest panel of currently available APLAs.

Due to low frequency of different thromboembolic complications, we assessed the occurrence of these events even from the diagnosis involving the period prior to sample procurement as well. Development of VT, AT and pregnancy loss, however, did not vary according to APLA status.

At the same time, significant changes were found in ACA IgA and ACA IgG positivity over time in patients with CD. In rheumatology disorders, introduction of TNF antagonists (adalimumab, infliximab, and etanercept) was reported to induce production of various types of autoantibodies, such as APLAs<sup>[50]</sup>. However such data are scarce in IBD. In a study by Atzeni *et al*<sup>[20]</sup> involving 63 patients with CD, 5 (8%) had ACA antibodies (mainly IgM) at baseline and two additional patients developed these autoantibodies during infliximab treatment with no apparent clinical effect. Thus we investigated the possible role of the TNF antagonists therapy in our prospective CD cohort. Interestingly, the patients initially negative for ACA developed ACA IgA or IgG positivity significantly more frequently if they received anti-TNF therapy suggesting a causative association. In the non-anti-TNF treated subgroup of patients, the change of the ACA status is less well understood and needs further clarification. The pathogenic mechanism that changes the humoral response leading to development of autoimmunity during anti-TNF inhibitors therapy is unknown, but various hypothesis have been proposed<sup>[51]</sup>. One hypothesis is that binding of anti-TNF antagonists to the transmembrane and soluble TNF, rapidly lowering TNF level and enhancing apoptotic cell death, which triggers the development of autoantibodies. Further investigations are warranted to elucidate whether the new ACA appears associated with a worse clinical outcome.

In conclusion, the present study demonstrated enhanced formation of APLAs in CD patients. The presence of the APLAs however was not associated with the clinical phenotype, disease course, or risk of venous thrombotic events during the prospective follow-up.

## COMMENTS

### Background

Enhanced serological antibody formation is a well-known feature of inflammatory bowel diseases (IBD). A wide range of anti-microbial and autoantibodies have been reported to be associated with either Crohn's disease (CD) or ulcerative colitis (UC) as well as with complicated disease course. Autoantibodies are directed against various host proteins. Based on recent findings, their existence might also be related to enhanced microbial challenge to the gut due to a disturbed gut innate immune system and may trigger an exaggerated adaptive immune response. Furthermore, these serological antibodies may also be actively involved in the pathophysiology of inflammation in IBD. Antiphospholipid antibodies (APLAs) are a prothrombotic group of antibodies acquired in various inflammatory diseases. These antibodies comprise anti-cardiolipin (ACA), anti- $\beta$ 2-Glycoprotein-I (anti- $\beta$ 2-GPI), and anti-phosphatidylserine/prothrombin antibodies (anti-PS/PT). Their association with clinical phenotype and disease progression is still unclear in IBD. Even if non-prothrombotic, they may have certain pathogenetic roles in several diseases as well.

### Research frontiers

In IBD, available cross-sectional, mainly single-time point studies assessing different aspects of APLAs, came to discrepant conclusions regarding formation, prevalence, and stability of these antibodies. Their clinical significance, including association to thrombotic events in IBD is also unclear. Thus a comprehensive evaluation of the primary APLAs in a large prospectively followed-up IBD cohort is required. Current advances may add a new spark to the investigation of the role of anti- $\beta$ 2-GPI antibodies in the pathomechanism of IBD.

### Innovations and breakthroughs

This is the largest study to investigate prospectively the prevalence, type, and clinical significance of multiple APLAs simultaneously in a cohort of IBD patients to date. Three different antibodies were assessed by ELISA. Contrary to routine laboratory practice, APLAs were identified by anti-IgA secondary antibody in addition to anti-IgG and anti-IgM isotypes. Moreover, in the present study the authors also provided an overview of relevant APLA studies in IBD. Although they detected enhanced formation of APLAs in CD patients, the presence of the APLAs was not associated with the clinical phenotype, disease course, or risk of venous thrombotic events during the prospective follow-up. A clear strength of this study was prospective follow-up design and the application of the widest panel of currently available APLAs.

### Applications

Previous studies investigating APLAs in IBD patients had certain shortcomings resulting in an inconclusive, contradictory and confusing viewpoint regarding their pathogenetic and clinical significance, as well as their predictive value. Based on these large scale and extensive evaluation of APLAs they were not proved as a predictive marker of the complicated disease course in neither clinically settings of CD. Thromboembolic events were also not associated to any individual or multiple APLA positivity. Therefore use of APLAs in everyday practice and decision making cannot be recommended.

### Terminology

APLAs are a prothrombotic group of antibodies acquired in various inflammatory diseases. These ACA, anti- $\beta$ 2-GPI, and anti-PS/PT. Bacterial translocation is defined as an enhanced passage of bacteria and/or bacterial products from the intestinal tract to systemic circulation.

### Peer-review

This article relooks at the role of APLAs in IBD and provides a small systematic review of the currently available literature. The authors show the analysis of APLAs in patients with IBD ( $n = 458$ ) using prospective cohort study. The manuscript has extensive information on APLAs. This is a well-designed and conducted study and gives clear conclusion that despite increased occurrence

of APLAs in IBD, they are not associated with disease phenotype including the thrombosis and do not need further study unless a newer aspect comes in future.

## REFERENCES

- Papp M, Lakatos PL. Serological studies in inflammatory bowel disease: how important are they? *Curr Opin Gastroenterol* 2014; **30**: 359-364 [PMID: 24811052 DOI: 10.1097/mog.0000000000000076]
- Lakatos PL, Papp M, Rieder F. Serologic antiglycan antibodies in inflammatory bowel disease. *Am J Gastroenterol* 2011; **106**: 406-412 [PMID: 21245832 DOI: 10.1038/ajg.2010.505]
- Rieder F, Kugathasan S. Circulating antibodies against bacterial wall products: are there arguments for early immunosuppression? *Dig Dis* 2012; **30** Suppl 3: 55-66 [PMID: 23295693 DOI: 10.1159/000342603]
- Terjung B, Söhne J, Lechtenberg B, Gottwein J, Muennich M, Herzog V, Mähler M, Sauerbruch T, Spengler U. p-ANCAs in autoimmune liver disorders recognise human beta-tubulin isotype 5 and cross-react with microbial protein FtsZ. *Gut* 2010; **59**: 808-816 [PMID: 19951907 DOI: 10.1136/gut.2008.157818]
- Papp M, Sipeki N, Vitalis Z, Tornai T, Altörjay I, Tornai I, Udvardy M, Fechner K, Jacobsen S, Teegen B, Sumegi A, Veres G, Lakatos PL, Kappelmayer J, Antal-Szalmas P. High prevalence of IgA class anti-neutrophil cytoplasmic antibodies (ANCA) is associated with increased risk of bacterial infection in patients with cirrhosis. *J Hepatol* 2013; **59**: 457-466 [PMID: 23639483 DOI: 10.1016/j.jhep.2013.04.018]
- Pavlidis P, Romanidou O, Roggenbuck D, Mytilinaiou MG, Al-Sulttan F, Liaskos C, Smyk DS, Koutsoumpas AL, Rigopoulou EI, Conrad K, Forbes A, Bogdanos DP. Ileal inflammation may trigger the development of GP2-specific pancreatic autoantibodies in patients with Crohn's disease. *Clin Dev Immunol* 2012; **2012**: 640835 [PMID: 23118780 DOI: 10.1155/2012/640835]
- Roggenbuck D, Reinhold D, Werner L, Schierack P, Bogdanos DP, Conrad K. Glycoprotein 2 antibodies in Crohn's disease. *Adv Clin Chem* 2013; **60**: 187-208 [PMID: 23724745 DOI: 10.1016/B978-0-12-407681-5.00006-4]
- Ortel TL. Antiphospholipid syndrome: laboratory testing and diagnostic strategies. *Am J Hematol* 2012; **87** Suppl 1: S75-S81 [PMID: 22473619 DOI: 10.1002/ajh.23196]
- Staub HL, Franck M, Ranzolin A, Norman GL, Iverson GM, von Mühlen CA. IgA antibodies to beta2-glycoprotein I and atherosclerosis. *Autoimmun Rev* 2006; **6**: 104-106 [PMID: 17138253 DOI: 10.1016/j.autrev.2006.06.014]
- Gabeta S, Norman GL, Gatselis N, Liaskos C, Papamichalis PA, Garagounis A, Zachou K, Rigopoulou EI, Dalekos GN. IgA anti-b2GPI antibodies in patients with autoimmune liver diseases. *J Clin Immunol* 2008; **28**: 501-511 [PMID: 18551357 DOI: 10.1007/s10875-008-9211-6]
- Ramírez E, Serrano A, García F, Alfaro FJ, Pérez V, Paz-Artal E, Morales JM. Prospective study on autoantibodies against apolipoprotein H (beta2GPI) in several clinical parameters from patients with terminal renal failure and functioning renal transplants. *Transplant Proc* 2009; **41**: 2370-2372 [PMID: 19715922 DOI: 10.1016/j.transproceed.2009.06.104]
- Mankai A, Achour A, Thabet Y, Manoubia W, Sakly W, Ghedira I. Anti-cardiolipin and anti-beta 2-glycoprotein I antibodies in celiac disease. *Pathol Biol (Paris)* 2012; **60**: 291-295 [PMID: 21839587 DOI: 10.1016/j.patbio.2011.07.003]
- Sène D, Piette JC, Cacoub P. Antiphospholipid antibodies, antiphospholipid syndrome and infections. *Autoimmun Rev* 2008; **7**: 272-277 [PMID: 18295729 DOI: 10.1016/j.autrev.2007.10.001]
- García-Carrasco M, Galarza-Maldonado C, Mendoza-Pinto C, Escarcega RO, Cervera R. Infections and the antiphospholipid syndrome. *Clin Rev Allergy Immunol* 2009; **36**: 104-108 [PMID: 19089659 DOI: 10.1007/s12016-008-8103-0]
- Frauenknecht K, Lackner K, von Landenberg P. Antiphospholipid antibodies in pediatric patients with prolonged activated partial thromboplastin time during infection. *Immunobiology* 2005; **210**: 799-805 [PMID: 16325500 DOI: 10.1016/j.imbio.2005.10.012]
- Shin JI, Lee JS, Kim HS. Lupus anticoagulant and IgM anti-phospholipid antibodies in Korean children with Henoch-Schönlein purpura. *Scand J Rheumatol* 2009; **38**: 73-74; author reply 74-75 [PMID: 19191188 DOI: 10.1080/03009740802482469]
- Horstman LL, Jy W, Bidot CJ, Ahn YS, Kelley RE, Zivadinov R, Maghzi AH, Etemadifar M, Mousavi SA, Minagar A. Antiphospholipid antibodies: paradigm in transition. *J Neuroinflammation* 2009; **6**: 3 [PMID: 19154576 DOI: 10.1186/1742-2094-6-3]
- Serrano A, García F, Serrano M, Ramírez E, Alfaro FJ, Lora D, de la Cámara AG, Paz-Artal E, Praga M, Morales JM. IgA antibodies against  $\beta$ 2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality. *Kidney Int* 2012; **81**: 1239-1244 [PMID: 22358146 DOI: 10.1038/ki.2011.477]
- Aichbichler BW, Petritsch W, Reicht GA, Wenzl HH, Eherer AJ, Hinterleitner TA, Auer-Grumbach P, Krejs GJ. Anti-cardiolipin antibodies in patients with inflammatory bowel disease. *Dig Dis Sci* 1999; **44**: 852-856 [PMID: 10219848 DOI: 10.1023/a:1026646816672]
- Atzeni F, Ardizzone S, Sarzi-Puttini P, Colombo E, Maconi G, De Portu S, Carrabba M, Bianchi Porro G. Autoantibody profile during short-term infliximab treatment for Crohn's disease: a prospective cohort study. *Aliment Pharmacol Ther* 2005; **22**: 453-461 [PMID: 16128684 DOI: 10.1111/j.1365-2036.2005.02576.x]
- Caccavo D, Greco B, Caradonna L, Leandro G, Bonomo L, Jirillo E. Antiphospholipid antibodies in patients with inflammatory bowel disease. *Med Sci Res* 1996; **24**: 711-713
- Chamouard P, Grunebaum L, Wiesel ML, Freyssinet JM, Duclos B, Cazenave JP, Baumann R. Prevalence and significance of anticardiolipin antibodies in Crohn's disease. *Dig Dis Sci* 1994; **39**: 1501-1504 [PMID: 8026262 DOI: 10.1007/bf02088055]
- Chiarantini E, Valanzano R, Liotta AA, Cellai AP, Fedi S, Ilari I, Prisco D, Tonelli F, Abbate R. Hemostatic abnormalities in inflammatory bowel disease. *Thromb Res* 1996; **82**: 137-146 [PMID: 9163067 DOI: 10.1016/0049-3848(96)00060-6]
- Dalekos GN, Manoussakis MN, Goussia AC, Tsianos EV, Moutsopoulos HM. Soluble interleukin-2 receptors, antineutrophil cytoplasmic antibodies, and other autoantibodies in patients with ulcerative colitis. *Gut* 1993; **34**: 658-664 [PMID: 8504967 DOI: 10.1136/gut.34.5.658]
- Koutroubakis IE, Petinaki E, Anagnostopoulou E, Kritikos H, Mouzas IA, Kouroumalis EA, Manousos ON. Anti-cardiolipin and anti-beta2-glycoprotein I antibodies in patients with inflammatory bowel disease. *Dig Dis Sci* 1998; **43**: 2507-2512 [PMID: 9824143 DOI: 10.1023/a:1026602803622]
- Lonjon I, Beaugerie L, Deschamps A, Barthet C, Carbonnel F, Ngô Y, Cosnes J, Abuaf N, Gendre JP. [Prevalence and role of anticardiolipin antibodies in Crohn disease]. *Gastroenterol Clin Biol* 1996; **20**: 633-637 [PMID: 8977809]
- Maher MM, Soloma SH. Assessment of thrombophilic abnormalities during the active state of inflammatory bowel disease. *Saudi J Gastroenterol* 2008; **14**: 192-197 [PMID: 19568537 DOI: 10.4103/1319-3767.41743]
- Martinović Z, Perišić K, Pejnović N, Lukacević S, Rabrenović L, Petrović M. Antiphospholipid antibodies in inflammatory bowel diseases. *Vojnosanit Pregl* 1998; **55**: 47-49 [PMID: 9623359]
- Oldenburg B, Van Tuyl BA, van der Griend R, Fijnheer R, van Berge Henegouwen GP. Risk factors for thromboembolic complications in inflammatory bowel disease: the role of hyperhomocysteinaemia. *Dig Dis Sci* 2005; **50**: 235-240 [PMID: 15745078 DOI: 10.1007/s10620-005-1588-y]
- Saibeni S, Vecchi M, Valsecchi C, Faioni EM, Razzari C, de Franchis R. Reduced free protein S levels in patients with inflammatory bowel disease: prevalence, clinical relevance, and role of anti-protein S antibodies. *Dig Dis Sci* 2001; **46**: 637-643 [PMID: 11318545 DOI: 10.1023/a:1005675921664]
- Souto JC, Borrell M, Fontcuberta J, Roca M. Antiphospholipid antibodies in inflammatory bowel disease. *Dig Dis Sci* 1995; **40**: 1524-1525 [PMID: 7628277 DOI: 10.1007/bf02285202]
- Vecchi M, Cattaneo M, de Franchis R, Mannucci PM. Risk of



- thromboembolic complications in patients with inflammatory bowel disease. Study of hemostasis measurements. *Int J Clin Lab Res* 1991; **21**: 165-170 [PMID: 1815761]
- 33 **Yurekli BP**, Aksoy DY, Aybar M, Egesel T, Gurgey A, Hascelik G, Kirazli S, Haznedaroglu IC, Arslan S. The search for a common thrombophilic state during the active state of inflammatory bowel disease. *J Clin Gastroenterol* 2006; **40**: 809-813 [PMID: 17016137 DOI: 10.1097/01.mcg.0000225603.33481.56]
  - 34 **Krause I**, Blank M, Cervera R, Font J, Matthias T, Pfeiffer S, Wies I, Fraser A, Shoenfeld Y. Cross-reactive epitopes on beta2-glycoprotein-I and *Saccharomyces cerevisiae* in patients with the antiphospholipid syndrome. *Ann N Y Acad Sci* 2007; **1108**: 481-488 [PMID: 17894013 DOI: 10.1196/annals.1422.051]
  - 35 **Deban L**, Correale C, Vetrano S, Malesci A, Danese S. Multiple pathogenic roles of microvasculature in inflammatory bowel disease: a Jack of all trades. *Am J Pathol* 2008; **172**: 1457-1466 [PMID: 18458096 DOI: 10.2353/ajpath.2008.070593]
  - 36 **Agar C**, de Groot PG, Mörgelin M, Monk SD, van Os G, Levels JH, de Laat B, Urbanus RT, Herwald H, van der Poll T, Meijers JC.  $\beta$ 2-glycoprotein I: a novel component of innate immunity. *Blood* 2011; **117**: 6939-6947 [PMID: 21454452 DOI: 10.1182/blood-2010-12-325951]
  - 37 **Laplanche P**, Amireault P, Subang R, Dieudé M, Levine JS, Rauch J. Interaction of  $\beta$ 2-glycoprotein I with lipopolysaccharide leads to Toll-like receptor 4 (TLR4)-dependent activation of macrophages. *J Biol Chem* 2011; **286**: 42494-42503 [PMID: 21965665 DOI: 10.1074/jbc.M111.230383]
  - 38 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-9 [PMID: 2617184 DOI: 10.3109/00365528909091339]
  - 39 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhardt AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5A-36A [PMID: 16151544]
  - 40 **Vermeire S**, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation between the Crohn's disease activity and Harvey-Bradshaw indices in assessing Crohn's disease severity. *Clin Gastroenterol Hepatol* 2010; **8**: 357-363 [PMID: 20096379 DOI: 10.1016/j.cgh.2010.01.001]
  - 41 **Lewis JD**, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. *Inflamm Bowel Dis* 2008; **14**: 1660-1666 [PMID: 18623174 DOI: 10.1002/ibd.20520]
  - 42 **Van Assche G**, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, Ochsenkühn T, Orchard T, Rogler G, Louis E, Kupcinkas L, Mantzaris G, Travis S, Stange E. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis* 2010; **4**: 7-27 [PMID: 21122488 DOI: 10.1016/j.crohns.2009.12.003]
  - 43 **Daperno M**, D'Haens G, Van Assche G, Baert F, Bulois P, Maunoury V, Sostegni R, Rocca R, Pera A, Gevers A, Mary JY, Colombel JF, Rutgeerts P. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc* 2004; **60**: 505-512 [PMID: 15472670 DOI: 10.1016/S0016-5107(04)01878-4]
  - 44 **Schroeder KW**, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987; **317**: 1625-1629 [PMID: 3317057 DOI: 10.1056/nejm198712243172603]
  - 45 **Stange EF**, Travis SP, Vermeire S, Beglinger C, Kupcinkas L, Geboes K, Barakauskiene A, Villanacci V, Von Herbay A, Warren BF, Gasche C, Tilg H, Schreiber SW, Schölmerich J, Reinisch W. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *Gut* 2006; **55** Suppl 1: i1-15 [PMID: 16481628 DOI: 10.1136/gut.2005.081950a]
  - 46 **Papp M**, Lakatos PL, Harsfalvi J, Farkas G, Palatka K, Udvardy M, Molnar T, Farkas K, Nagy F, Veres G, Lakatos L, Kovacs A, Dinya T, Kocsis AK, Papp J, Altörjay I. Mannose-binding lectin level and deficiency is not associated with inflammatory bowel diseases, disease phenotype, serology profile, and NOD2/CARD15 genotype in a large Hungarian cohort. *Hum Immunol* 2010; **71**: 407-413 [PMID: 20079790 DOI: 10.1016/j.humimm.2010.01.012]
  - 47 **Gotoh M**, Matsuda J. Induction of anticardiolipin antibody and/or lupus anticoagulant in rabbits by immunization with lipoteichoic acid, lipopolysaccharide and lipid A. *Lupus* 1996; **5**: 593-597 [PMID: 9116702 DOI: 10.1177/096120339600500606]
  - 48 **Bauer M**, Heeg K, Wagner H, Lipford GB. DNA activates human immune cells through a CpG sequence-dependent manner. *Immunology* 1999; **97**: 699-705 [PMID: 10457226 DOI: 10.1046/j.1365-2567.1999.00811.x]
  - 49 **Tang LY**, Rawsthorne P, Bernstein CN. Are perineal and luminal fistulas associated in Crohn's disease? A population-based study. *Clin Gastroenterol Hepatol* 2006; **4**: 1130-1134 [PMID: 16905369 DOI: 10.1016/j.cgh.2006.06.021]
  - 50 **Atzeni F**, Talotta R, Salaffi F, Cassinotti A, Varisco V, Battellino M, Ardizzone S, Pace F, Sarzi-Puttini P. Immunogenicity and autoimmunity during anti-TNF therapy. *Autoimmun Rev* 2013; **12**: 703-708 [PMID: 23207283 DOI: 10.1016/j.autrev.2012.10.021]
  - 51 **Kolarz B**, Majdan M, Darmochwal-Kolarz DA, Dryglewska M. Antiphospholipid antibodies during 6-month treatment with infliximab: a preliminary report. *Med Sci Monit* 2014; **20**: 1227-1231 [PMID: 25027437 DOI: 10.12659/msm.890270]

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## Clinical Trials Study

# Esomeprazole regimens for reflux symptoms in Chinese patients with chronic gastritis

Jing Sun, Yao-Zong Yuan, Xiao-Hua Hou, Duo-Wu Zou, Bin Lu, Min-Hu Chen, Fei Liu, Kai-Chun Wu, Xiao-Ping Zou, Yan-Qing Li, Li-Ya Zhou

Jing Sun, Yao-Zong Yuan, Department of Gastroenterology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Xiao-Hua Hou, Department of Gastroenterology, Union Hospital Medical College, Huazhong University of Science and Technology, Wuhan 430074, Hubei Province, China

Duo-Wu Zou, Department of Gastroenterology, Changhai Hospital, The Second Military Medical University, Shanghai 200433, China

Bin Lu, Department of Gastroenterology, Zhejiang Traditional Chinese Medicine Hospital, Hangzhou 310006, Zhejiang Province, China

Min-Hu Chen, Department of Gastroenterology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, Guangdong Province, China

Fei Liu, Department of Gastroenterology, Shanghai East Hospital, Shanghai 200120, China

Kai-Chun Wu, Department of Gastroenterology, Xijing Hospital, the Fourth Military Medical College, Xi'an 710032, Shaanxi Province, China

Xiao-Ping Zou, Department of Gastroenterology, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing 210093, Jiangsu Province, China

Yan-Qing Li, Department of Gastroenterology, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China

Li-Ya Zhou, Department of Gastroenterology, Peking University Third Hospital, Beijing 100191, China

**Author contributions:** Yuan YZ is the guarantor of the article, who took responsibility for the integrity of the work as a whole, from inception to published article; Sun J, Zou XP, Hou XH and Li YQ helped to perform the research; Zou D and Lu B collected and analyzed the data; Chen MH and Liu F designed the research; Wu KC and Zhou LY contributed to the paper review and the design of the study; all authors approved the final version of the article, including the authorship list.

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**Correspondence to:** Yao-Zong Yuan, MD, PhD, Department of Gastroenterology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, No. 197, Rui Jin Er Road, Shanghai 200025, China. [yyz28@medmail.com.cn](mailto:yyz28@medmail.com.cn)

**Telephone:** +86-21-64150773

**Fax:** +86-21-64333548

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## Abstract

**AIM:** To compare symptom control with esomeprazole regimens for non-erosive reflux disease and chronic gastritis in patients with a negative endoscopy.

**METHODS:** This randomized, open-label study was designed in line with clinical practice in China. Patients with typical reflux symptoms for  $\geq 3$  mo and a negative endoscopy who had a Gastroesophageal Reflux Disease Questionnaire score  $\geq 8$  were randomized to initial treatment with esomeprazole 20 mg once daily either for 8 wk or for 2 wk. Patients with symptom relief could enter another 24 wk of maintenance/on-demand treatment, where further courses of esomeprazole 20 mg once daily were given if symptoms recurred. The primary endpoint was the symptom control rate at week 24 of the maintenance/on-demand treatment period. Secondary endpoints were symptom relief rate, success rate (defined as patients who had symptom relief after initial treatment and after 24 wk of maintenance treatment), time-to-first-relapse and satisfaction rate.

**RESULTS:** Based on the data collected in the modified intention-to-treat population (MITT; patients in the ITT population with symptom relief after initial esomeprazole treatment,  $n = 262$ ), the symptom control rate showed a small but statistically significant difference in favor of the 8-wk regimen (94.9% *vs* 87.3%,  $P = 0.0473$ ). Among the secondary endpoints, based on the data collected in the ITT population ( $n = 305$ ), the 8-wk group presented marginally better results in symptom relief after initial esomeprazole treatment (88.3% *vs* 83.4%,  $P = 0.2513$ ) and success rate over the whole study (83.8% *vs* 72.8%,  $P = 0.0258$ ). The 8-wk regimen was found to provide a 46% reduction in risk of relapse *vs* the 2-wk regimen (HR = 0.543; 95%CI: 0.388-0.761). In addition, fewer unscheduled visits and higher patient satisfaction supported the therapeutic benefits of the 8-wk regimen over the 2-wk regimen. Safety was comparable between the two groups, with both regimens being well tolerated.

**CONCLUSION:** Chinese patients diagnosed with chronic gastritis achieved marginally better control of reflux symptoms with an 8-wk *vs* a 2-wk esomeprazole regimen, with a similar safety profile.

**Key words:** Esomeprazole; Non-erosive reflux disease regimen; Chronic gastritis regimen; Symptom control rate

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**Core tip:** In China, physicians tend to perform endoscopies on patients presenting with typical symptoms of gastro-esophageal reflux disease, such as reflux or heartburn. If endoscopy findings are negative, patients are usually diagnosed with chronic gastritis rather than non-erosive reflux disease (NERD) and treated with a 2-wk course of proton pump inhibitors (PPIs). We compared symptom

control rates when patients were treated with a 2-wk course of PPIs *vs* an 8-wk course, as recommended for patients with NERD. In this multicenter, randomized, open-label study, the 8-wk PPI regimen provided marginally better symptom control and relief rates than the 2-wk regimen, with a similar safety profile.

Sun J, Yuan YZ, Hou XH, Zou DW, Lu B, Chen MH, Liu F, Wu KC, Zou XP, Li YQ, Zhou LY. Esomeprazole regimens for reflux symptoms in Chinese patients with chronic gastritis. *World J Gastroenterol* 2015; 21(22): 6965-6973 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6965.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6965>

## INTRODUCTION

Gastro-esophageal reflux disease (GERD) can be routinely diagnosed on the basis of typical reflux symptoms<sup>[1]</sup>. Approximately 60% of patients with GERD have non-erosive reflux disease (NERD)<sup>[2,3]</sup>, which is characterized by typical reflux symptoms and normal esophageal findings.

Recent studies have shown that the prevalence of GERD in Asia is increasing<sup>[4,5]</sup>. In China, the majority of patients with reflux symptoms are investigated by endoscopy and, if no clear endoscopic findings are identified, they are often diagnosed as having chronic gastritis (CG) rather than NERD because this diagnosis is thought to be more acceptable to the patient. Patients are then treated according to the recommended treatment algorithm for CG in China, which involves a 2-wk course of proton pump inhibitors (PPIs), rather than the treatment algorithm for NERD, which involves a 8-wk course, as proposed by the 2007 Chinese GERD consensus statement<sup>[6]</sup>.

The aim of this study was to compare symptom control rates between the NERD regimen (8 wk of PPI treatment) and the CG regimen (2 wk) in patients who have typical reflux symptoms and a negative endoscopy.

## MATERIALS AND METHODS

### Study design

This multicenter, randomized, open-label phase IV study (clinicaltrials.gov study code: D9612L00127) was conducted in 10 centers in China. The study was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with International Conference on Harmonization/Good Clinical Practice guidelines. All participants gave written informed consent before any study procedures were performed.

### Patients

Adult patients (aged 18-75 years) with a Gastro-esophageal Reflux Disease Questionnaire (GerdQ) score

$\geq 8$  were enrolled if they had typical reflux symptoms (*i.e.*, heartburn, regurgitation or both) as their main gastrointestinal symptoms for  $\geq 3$  mo. Patients were required to have undergone endoscopy in the 2 wk before randomization, without any endoscopic findings. The main exclusion criteria were as follows: involvement in the planning and/or conduct of the study; previous enrollment or randomization in the present study; participation in another clinical study with an investigational product during the last 3 mo; and endoscopically visible reflux esophagitis, esophageal varices, Barrett's esophagus, malignancy or peptic ulcer. Patients were ineligible if they had: unintentional weight loss  $> 3$  kg in the previous 3 mo; hematemesis, melena or per-rectum blood loss in the previous year; progressive dysphagia, anemia or any other symptom suggestive of malignancy; PPI or histamine-2 receptor antagonist (H2RA) therapy in the 2 wk before enrollment; known intolerance/allergy to PPIs; or history of esophageal, gastric or duodenal surgery.

Treatment of *Helicobacter pylori* (*H. pylori*) infection was not allowed during the study. If *H. pylori* infection was detected, treatment could start after the final visit, as assessed by the investigator.

### Treatments

Patients who met the inclusion criteria were randomized into either the 8-wk treatment group or the 2-wk treatment group. Treatment of the 8-wk group was divided into two phases on the basis of the 2007 Chinese consensus on GERD<sup>[6]</sup>: initial treatment phase and maintenance/on-demand phase. Treatment of the 2-wk group was designed to reflect treatment practice in China.

For the initial phase, patients were randomized to treatment with esomeprazole 20 mg once daily for 8 wk or 2 wk. Patients with symptom relief (defined as no more than 1 day with mild symptoms of GERD during the past 7 d) at the end of the initial 2- or 8-wk treatment phase entered a 24-wk maintenance/on-demand phase. The total study period was therefore 26 wk for the CG regimen and 32 wk for the NERD regimen.

During the maintenance/on-demand phase, patients were given esomeprazole if their symptoms relapsed (*i.e.*, they had a recurrence of their symptoms that was sufficient to require treatment in the opinion of the investigator). In the event of symptom relapse in the 8-wk group, patients were instructed to take esomeprazole 20 mg once daily for at least 3 d consecutively until symptom control was reached. If relapse occurred before week 8 of the on-demand/maintenance period, patients received 14 tablets of esomeprazole at each of the scheduled week 8 and week 16 visits. If this was not sufficient, patients were instructed to visit the study center to receive extra tablets. Such visits were not considered unscheduled if their purpose was limited to obtaining on-demand

drugs. If the first relapse occurred after week 8, the patient was withdrawn from the study and treated according to standard clinical practice. In the 2-wk group, patients were treated with another 2-wk course of esomeprazole 20 mg once daily if their symptoms recurred. In this group, there was no limitation on the number of repeated treatments that could be given during the 24-wk maintenance period. As with the 8-wk group, hospital visits were not considered unscheduled if their purpose was to obtain on-demand drugs.

### Assessments

In the 2-wk and 8-wk treatment periods, assessments occurred at screening (0 wk), and then at 2 and 8 wk, respectively. There were three scheduled visits (at 8, 16 and 24 wk) during the 24-wk maintenance/on-demand period. GerdQ<sup>[7]</sup> was administered when the patients entered the study and at the week-8, week-16 and week-24 visits to assess symptom control status in the 7 d before each visit.

The primary endpoint was defined as the symptom control rate at week 24 of the maintenance/on-demand period. Symptom control was defined as a score  $\leq 1$  for GerdQ items relating to frequency of heartburn, regurgitation, nocturnal reflux and rescue medication use.

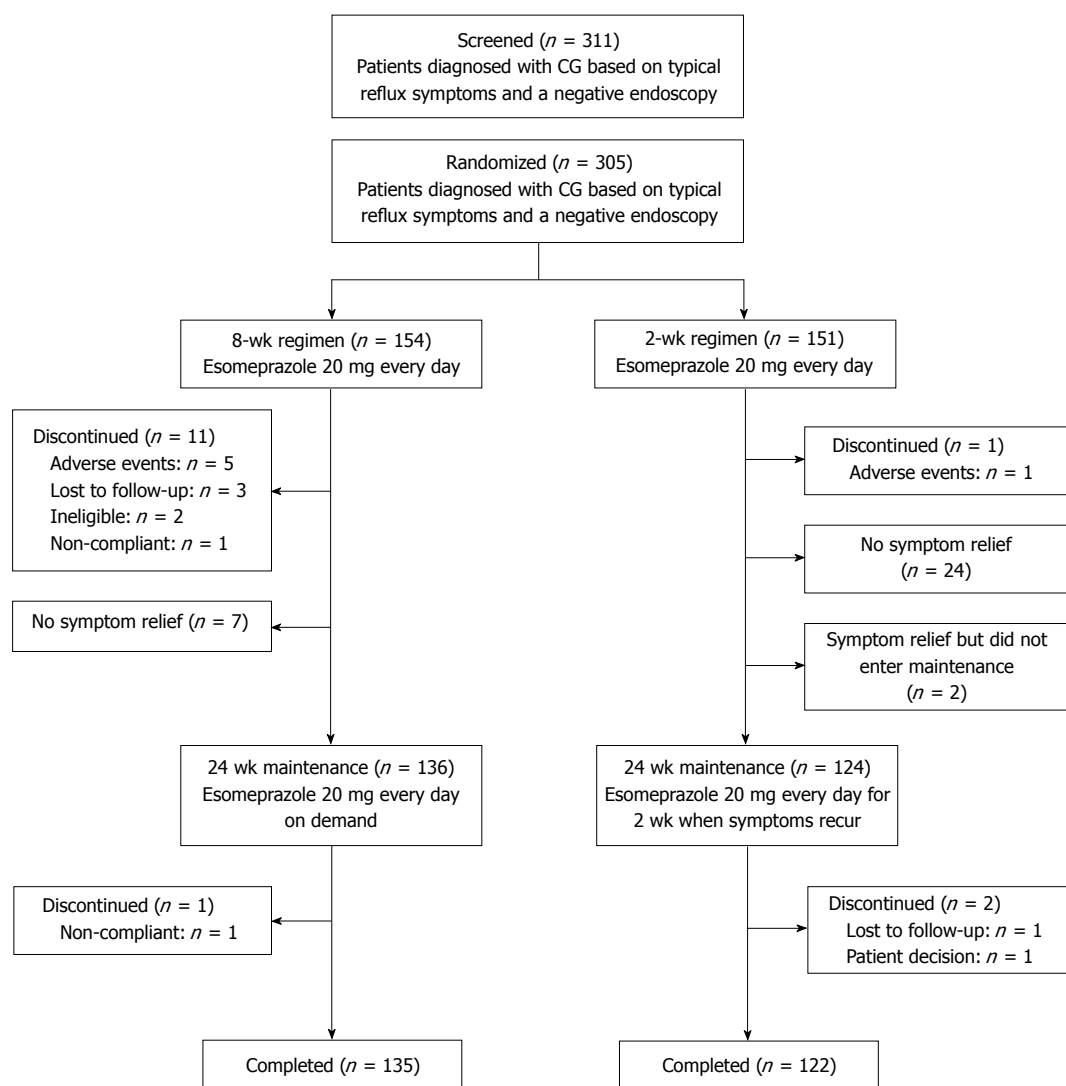
Secondary endpoints included symptom relief rate, success rate, time-to-first-relapse and satisfaction. Symptom relief was defined as no more than 1 d with mild symptoms of GERD during the past 7 d, which was reported retrospectively by the patient. Success was defined as symptom relief after initial treatment and symptom control at 24 wk of maintenance treatment. Symptom relief rate and success rate were used as efficacy variables for initial and overall treatment, respectively. Time-to-first-relapse was defined as the period from the date of the last dose of initial treatment to the date when for the first time the patient visited the investigator owing to symptom relapse. Unscheduled visits were indicated by the recurrence of a patient's symptoms, need for extra treatment or medical consultation. Satisfaction rate was self-reported using a 7-point scale, which showed the patients' subjective attitudes towards both regimens.

Safety assessments included monitoring of serious adverse events (AEs) and discontinuation due to AEs of any severity.

### Statistical analysis

The required sample size was calculated based on clinical experience, as there were insufficient data available from past studies for the symptom control rates in both treatment regimens to be estimated. With a total of 170 evaluable patients, 85 in the 8-wk group and 85 in the 2-wk group, the power would be greater than 80% to detect a difference of 20% in symptom





**Figure 1 Study flow diagram.** Only patients whose symptoms resolved after 8 or 2 wk of treatment entered the 24-wk maintenance/on-demand period. CG: Chronic gastritis.

control rates between two treatment groups at a 2-sided significance level of 0.05 using Fisher's exact test, assuming that symptom control rates were about 66% in the 2-wk group and 86% in the 8-wk group (in reference to data from the as-yet unpublished BU-NEG-0005 study, which used the same definition of symptom control as the present study). Considering PPI response rates of around 70% and drop-out rates of around 20%, approximately 300 patients needed to be randomized.

Analysis on efficacy endpoints was performed for the intention-to-treat (ITT) population (all randomized patients who received at least one dose of study medication), the modified intention-to-treat (MITT) population (patients in the ITT population with symptom relief after initial esomeprazole treatment) and the per-protocol population (patients in the ITT population without significant protocol deviations/violations), with the MITT as the primary analysis

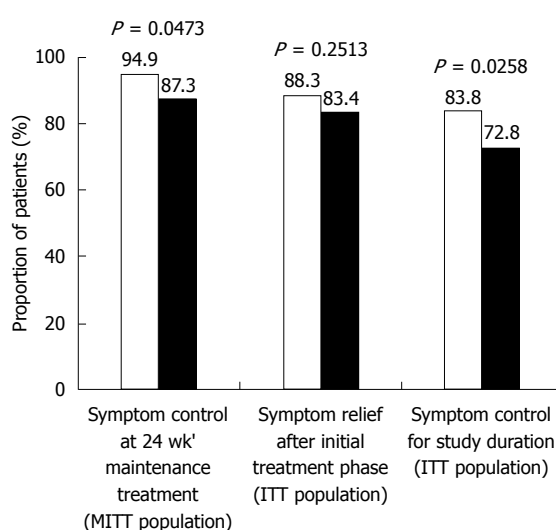
population. The safety population was defined as all patients who took at least one dose of trial medication and for whom post-dose data were collected.

All treatment comparisons were two-sided and the nominal level of significance was 5%. The Fisher's exact test was used to analyze the primary efficacy endpoint of symptom control rate at 24 wk in the MITT population and the secondary efficacy endpoints of symptom relief rate at initial treatment and success rate in the whole regimen, both of which were analyzed using the ITT population. The Kaplan-Meier survival analysis method<sup>[8]</sup> and the log-rank test were used to assess time-to-first-relapse. A weighted least-squares regression analysis was also performed to compare mean number of unscheduled visits between the two regimens. Comparison of patients who were satisfied (scores of 1-4) or very satisfied (scores of 1-2) in the groups at 24 wk was performed using Fisher's exact test.

**Table 1** Patient demographic and baseline clinical characteristics *n* (%)

Parameter	ITT ( <i>n</i> = 305)			MITT ( <i>n</i> = 262)		
	8-wk group	2-wk group	Total	8-wk group	2-wk group	Total
Age, yr, mean $\pm$ SD	45.9 $\pm$ 12.72	44.9 $\pm$ 13.12	45.4 $\pm$ 12.91	45.3 $\pm$ 12.88	45.1 $\pm$ 13.36	45.2 $\pm$ 13.09
Sex						
Men	80 (51.9)	54 (35.8)	134 (43.9)	74 (54.4)	46 (36.5)	120 (45.8)
Women	74 (48.1)	97 (64.2)	171 (56.1)	62 (45.6)	80 (63.5)	142 (54.2)
Symptom						
Heartburn	142 (92.2)	136 (90.1)	278 (91.1)	126 (92.6)	113 (89.7)	239 (91.2)
Regurgitation	138 (89.6)	135 (89.4)	273 (89.5)	124 (91.2)	113 (89.7)	237 (90.5)
Duration, mo, mean $\pm$ SD						
Heartburn	36.3 $\pm$ 56.47	35.8 $\pm$ 49.05	36.1 $\pm$ 52.87	39.0 $\pm$ 59.20	39.1 $\pm$ 52.55	39.0 $\pm$ 56.04
Regurgitation	36.4 $\pm$ 56.72	34.9 $\pm$ 47.41	35.6 $\pm$ 52.24	38.4 $\pm$ 59.28	37.7 $\pm$ 50.71	38.1 $\pm$ 55.24
HP test positive	48 (31.2)	44 (29.1)	92 (30.2)	39 (28.7)	35 (27.8)	74 (28.2)
GerdQ score, mean $\pm$ SD	10.7 $\pm$ 1.78	10.4 $\pm$ 1.89	10.5 $\pm$ 1.84	10.7 $\pm$ 1.76	10.4 $\pm$ 1.94	10.6 $\pm$ 1.85

GerdQ: Gastroesophageal Reflux Disease Questionnaire; ITT: Intention-to-treat; MITT: Modified intention-to-treat.



**Figure 2** Efficacy of 8-wk and 2-wk regimens. ITT: Intention-to-treat; MITT: Modified intention-to-treat.

## RESULTS

In total, 154 patients were randomized into the 8-wk group and 151 into the 2-wk group (Figure 1). The patients were enrolled from April 2010 to June 2011. Six patients discontinued treatment owing to AEs (five in the 8-wk group and one in the 2-wk group; Figure 1). The completion rates for the initial 8- and 2-wk treatment phases were 92.9% (143/154) and 99.3% (150/151), and the completion rates for the whole study were 87.7% (135/154) and 80.8% (122/151), respectively.

Overall, both groups were generally comparable between ITT and MITT populations with respect to baseline GERD characteristics. Patient demographics were well balanced between both regimens except for sex (Table 1).

### Efficacy

The proportions of patients with symptom control after

24 wk of maintenance/on-demand treatment (MITT population) in the 8-wk and 2-wk groups were 94.9% (129/136) and 87.3% (110/126), respectively, with a significant difference favoring the 8-wk regimen ( $P = 0.0473$ ; Figure 2). The proportions of patients with symptom relief after the initial phase of treatment (ITT population) in the 8-wk and 2-wk groups were 88.3% (136/154) and 83.4% (126/151), respectively; this result numerically favored the 8-wk regimen with no significant difference ( $P = 0.2513$ ; Figure 2). With regard to success rates in the whole study (ITT population), 83.8% (129/154) and 72.8% (110/151) of patients in the 8-wk and 2-wk groups, respectively, had successful symptom control, with a significant difference favoring the 8-wk regimen ( $P = 0.0258$ ; Figure 2).

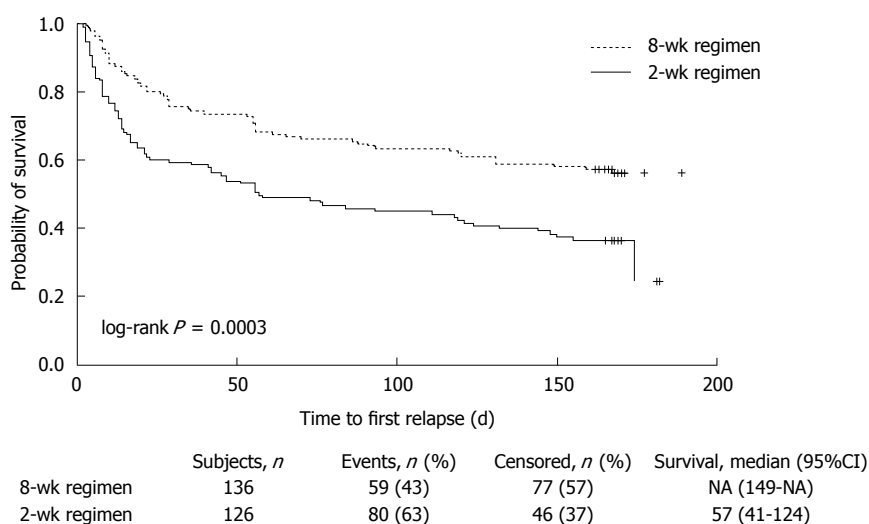
A total of 136 patients in the 8-wk group and 126 patients in the 2-wk group (MITT population) were included in the survival analysis. The median time-to-first-relapse for patients in the 2-wk group was 57 d, and the median for patients in the 8-wk group had not been reached at the end of the study. There were 59 patients with relapse in the 8-wk group (43.4%) and 80 (63.5%) in the 2-wk group. Significantly more patients in the 8-wk regimen stayed relapse-free in the maintenance/on-demand phase than in the 2-wk regimen (68.4% vs 50.6%,  $P = 0.003$  at week 8; 63.2% vs 44.1%,  $P = 0.0016$  at week 16; 56.3% vs 36.6%,  $P = 0.0012$  at week 24). A log-rank test showed that symptom relapse occurred significantly later in the 8-wk group than in the 2-wk group ( $P = 0.0003$ ; Figure 3). To quantify the reduction in risk of relapse with the 8-wk regimen, a *post hoc* Cox regression analysis with the treatment as the only covariate was performed and showed a 46% reduction (HR = 0.543; 95%CI: 0.388-0.761).

The proportions of patients who were satisfied or very satisfied with their symptom control after 24 wk of maintenance were significantly higher in the 8-wk group than in the 2-wk group (100% vs 96%,  $P =$

**Table 2** Adverse events leading to discontinuation of study medication, and their relationship to study medication *n* (%)

	2-wk group ( <i>n</i> = 151)		8 wk group ( <i>n</i> = 154)		Total ( <i>n</i> = 305)	
	Mild	Moderate	Mild	Moderate	Mild	Moderate
Patients with at least one AE	0	1 (0.7)	3 (1.9)	2 (1.3)	3 (1.0)	3 (1.0)
Gastrointestinal disorders	0	0	3 (1.9)	2 (1.3)	3 (1.0)	2 (0.7)
Nausea	0	0	1 (0.6) <sup>1</sup>	2 (1.3) <sup>2</sup>	1 (0.3)	2 (0.7)
Abdominal discomfort	0	0	1 (0.6) <sup>1</sup>	1 (0.6) <sup>1</sup>	1 (0.3)	1 (0.3)
Constipation	0	0	0	1 (0.6) <sup>1</sup>	0	1 (0.3)
Frequent bowel movements	0	0	1 (0.6) <sup>1</sup>	0	1 (0.3)	0
Abdominal pain, upper	0	0	0	1 (0.6) <sup>1</sup>	0	1 (0.3)
Eye disorders	0	0	1 (0.6)	0	1 (0.3)	0
Vision blurred	0	0	1 (0.6)	0	1 (0.3)	0
Musculoskeletal and connective tissue disorders	0	1 (0.7)	0	0	0	1 (0.3)
Back pain	0	1 (0.7)	0	0	0	1 (0.3)
Renal and urinary disorders	0	1 (0.7)	0	0	0	1 (0.3)
Dysuria	0	1 (0.7)	0	0	0	1 (0.3)
Respiratory, thoracic and mediastinal disorders	0	0	0	1 (0.6)	0	1 (0.3)
Cough	0	0	0	1 (0.6)	0	1 (0.3)

<sup>1</sup>Related to study medication; <sup>2</sup>One case related to study medication. One patient was counted at most once per category.

**Figure 3** Kaplan-Meier survival curves showing time-to-first-relapse of gastro-esophageal reflux disease symptoms (modified intention-to-treat population). GERD: Gastro-esophageal reflux disease; MITT: Modified intention-to-treat.

0.0247; 48.5% vs 24.6%,  $P < 0.0001$ , respectively).

### Safety

Both regimens were well tolerated. No serious AEs or deaths were reported in this study (Table 2). A total of five patients from the 8-wk group discontinued owing to AEs that were of mild or moderate intensity, while one patient in the 2-wk group discontinued owing to two AEs of moderate intensity.

## DISCUSSION

This study compared the efficacy of an 8-wk regimen of esomeprazole 20 mg once daily vs a 2-wk regimen in patients with typical reflux symptoms and a negative endoscopy. Our results showed that high rates of symptom control were seen in both groups, but that the 8-wk regimen was significantly superior

to the 2-wk regimen. Several previous studies have suggested that patients with NERD are less responsive to PPIs than patients with reflux esophagitis<sup>[9-11]</sup>; however, the high rate of symptom control found in both arms of our study suggests that patients with NERD can be responsive to highly effective PPIs such as esomeprazole.

The study design for the 8-wk regimen in our study was similar to that in the esomeprazole group of the COMMAND study<sup>[12]</sup>, which compared on-demand treatment with esomeprazole 20 mg with continuous therapy with lansoprazole 15 mg in patients with NERD in the United Kingdom. In the COMMAND study, after 4 wk of initial treatment and 6 mo of on-demand treatment, more than 85% of patients reported to have no or only mild symptoms in the previous 7 d, while the results from the 8-wk arm in our study indicated that 94.9% of patients achieved symptom

control after 24 wk. This suggests the superiority of at least 8 wk of initial treatment with esomeprazole over either 2 or 4 wk of initial treatment.

With regard to symptom relief rate in the present study, a numerically higher symptom relief rate of 88.3% was observed for the 8-wk regimen vs 83.4% in the 2-wk regimen. This finding is in line with the hypothesis raised in a randomized, double-blind study of esomeprazole compared with rabeprazole, which found that by extending the duration of initial therapy beyond 4 wk more patients would have symptom relief<sup>[13]</sup>. In the COMMAND study<sup>[12]</sup>, after the initial 2 wk of treatment, 47% of patients had complete resolution of GERD symptoms for 7 consecutive days. This increased to 77% of patients after treatment for 4 wk<sup>[12]</sup>. Symptom relief rate in our study was higher numerically; however, our definition of symptom relief did allow 1 d with mild symptoms of GERD during the past 7 d, so a higher rate should be expected. Success rate was also analyzed across the whole duration of our study with a statistically significant difference of 10.9% favoring the 8-wk regimen over the 2-wk regimen ( $P = 0.0258$ ).

In clinical practice, therapeutic options are mainly driven by relapse frequency<sup>[14]</sup>, and one possible limitation of on-demand therapy is that it allows symptoms to recur<sup>[15]</sup>. Therefore, relapse has become a crucial predictor of the efficacy of initial treatment for most patients with NERD. In our study, we observed relapse rates of 63.5% and 43.4% in the 2-wk and 8-wk groups, respectively, showing that fewer relapses occurred in the 8-wk group. Time-to-first-relapse was also measured as another quantitative endpoint to analyze the effectiveness of initial treatments. The longer the time-to-first-relapse, as demonstrated in the 8-wk group, the better the efficacy of the regimen was. Moreover, the median time-to-first-relapse for patients in the 8-wk group had not been reached at the time of analysis, supporting the superiority of that treatment regimen.

Patient satisfaction evaluation in NERD studies has been used as an important outcome measure<sup>[16]</sup>. Ideally, this should be evaluated using a validated questionnaire<sup>[17]</sup>. In our study, a questionnaire on patient satisfaction was measured on a 7-point scale, and a higher level of patient satisfaction was reported with the 8-wk regimen than the 2-wk. However, it should be noted that the satisfaction rates were very high in both regimens, with 100% in the 8-wk group and 96.0% in the 2-wk group, so this finding should not be over-interpreted. In the COMMAND study<sup>[12]</sup>, a high satisfaction rate (93.2%) suggested that a transient recurrence of heartburn symptoms (predominantly mild in severity) was only a minor inconvenience to patients. It may also be attributed to the nature of on-demand treatment, which enabled patients to feel more in control of their treatment and tailor it to their own particular needs<sup>[12]</sup>. On-demand

treatment may be more acceptable to patients than the requirement to take medication every day on a long-term basis<sup>[10]</sup>.

In placebo-controlled trials, esomeprazole 20 mg on demand has been shown to be effective and well tolerated in the maintenance of symptom control in patients with NERD<sup>[18,19]</sup>. In the present study, more patients discontinued from the 8-wk than the 2-wk treatment arm, which may have been the result of the different drug exposures in the treatment period and the difference in assigning maintenance/on-demand treatment in both groups. However, it should be noted that few patients discontinued from either arm. Overall, no safety concerns were associated with the use of esomeprazole 20 mg once daily for up to 8 wk followed by a 24-wk esomeprazole 20 mg once daily maintenance/on-demand treatment.

A key strength of this study is that it is the first randomized, comparative, clinical trial of patients with NERD ever conducted in China, and that it was designed on the basis of the 2-phase treatment algorithm proposed by the 2007 Chinese consensus statement on GERD<sup>[6]</sup>. The main limitation of our study lies in the open-label design, which may lead to bias<sup>[20]</sup>. We tried to minimize the possibility of bias by rigorous quality-control management. In terms of the randomization, unlike the COMMAND study<sup>[12]</sup>, where randomization was set after the initial phase, we performed the randomization before the initial phase. We believe the potential bias arising from this randomization is limited as the MITT patients showed a well-balanced demographic and clinical characteristic profile that was similar to that of ITT patients. However, the proportion of women was higher in the 2-wk group than in the 8-wk group. The implications of this are unclear. A further limitation is that satisfaction rates were measured with a patient-reported questionnaire that used a 1-wk recall period and are therefore subject to greater self-reporting bias than if a daily diary had been used<sup>[21]</sup>.

In conclusion, in Chinese patients who have typical reflux symptoms and a negative endoscopy, 8 wk of treatment with esomeprazole provides marginally better symptom control and symptom relief rates than a 2-wk regimen, with a similar safety profile.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Recent studies have shown that the prevalence of gastro-esophageal reflux



disease (GERD) in Asia is increasing. In China, physicians tend to perform endoscopies on patients presenting with typical symptoms of GERD, such as reflux or heartburn. If endoscopy findings are negative, patients are usually diagnosed with chronic gastritis rather than non-erosive reflux disease (NERD) because this diagnosis is thought to be more acceptable to both the doctor and the patient. These patients are then given the recommended treatment regimen for chronic gastritis [a 2-wk course of proton pump inhibitors (PPIs)] rather than the regimen for NERD (an 8-wk course of PPIs)

### Research frontiers

Selection of the most appropriate PPI treatment regimen for GERD symptoms is of importance to patients, physicians and payers. This was the first randomized, comparative, clinical trial of patients with NERD ever conducted in China. The authors compared symptom control when patients were treated with a 2-wk regimen of esomeprazole vs an 8-wk regimen.

### Innovations and breakthroughs

High rates of symptom control were seen in both groups, but the 8-wk regimen of esomeprazole was significantly superior to the 2-wk regimen. The 8-wk regimen was also found to provide a reduction in the risk of relapse vs the 2-wk regimen. In addition, fewer unscheduled visits and higher levels of patient satisfaction supported the therapeutic benefits of the 8-wk regimen over the 2-wk regimen. Safety was similar between the two groups, with both regimens being well tolerated.

### Applications

An initial 8-wk regimen of esomeprazole treatment was found to provide slightly superior control and relief of GERD symptoms compared with an initial 2-wk regimen. The findings of this study may help physicians to optimize future treatment of GERD.

### Terminology

GERD symptoms such as regurgitation and heartburn are caused by reflux of acid from the stomach into the esophagus. Proton pump inhibitors such as esomeprazole reduce the amount of acid produced in the stomach and are the mainstay of pharmacological treatment for GERD.

### Peer-review

This very well designed and performed multicenter, randomized, open-label study considers the comparison of symptom control between the recommended PPI treatment regimens for non-erosive reflux disease (8 wk) and chronic gastritis (2 wks) in 305 Chinese patients who have typical reflux symptoms and negative endoscopy. This study confirms that the 8-wk PPI regimen provided marginally better symptom control and relief rates than the 2-wk regimen, with a similar safety profile. This study is making a great contribution to randomized clinical trial studies leading to optimization of GERD treatment.

## REFERENCES

- Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. [The Montreal definition and classification of gastroesophageal reflux disease: a global, evidence-based consensus paper]. *Z Gastroenterol* 2007; **45**: 1125-1140 [PMID: 18027314 DOI: 10.1111/j.1572-0241.2006.00630.x]
- Hershcovici T, Fass R. Nonerosive Reflux Disease (NERD) - An Update. *J Neurogastroenterol Motil* 2010; **16**: 8-21 [PMID: 20535321 DOI: 10.5056/jnm.2010.16.1.8]
- Labenz J, Jaspersen D, Kulig M, Leodolter A, Lind T, Meyer-Sabellek W, Stolte M, Vieth M, Willich S, Malfertheiner P. Risk factors for erosive esophagitis: a multivariate analysis based on the ProGERD study initiative. *Am J Gastroenterol* 2004; **99**: 1652-1656 [PMID: 15330897 DOI: 10.1111/j.1572-0241.2004.30390.x]
- Goh KL. Changing epidemiology of gastroesophageal reflux disease in the Asian-Pacific region: an overview. *J Gastroenterol Hepatol* 2004; **19** Suppl 3: S22-S25 [PMID: 15324378 DOI: 10.1111/j.1440-1746.2004.03591.x]
- Wong BC, Kinoshita Y. Systematic review on epidemiology of gastroesophageal reflux disease in Asia. *Clin Gastroenterol Hepatol* 2006; **4**: 398-407 [PMID: 16616342 DOI: 10.1016/j.cgh.2005.10.011]
- Chinese Medical Association, GI Branch. Treatment consensus for gastroesophageal reflux disease. *Zhonghua Xiaohua Zazhi* 2007; **27**: 689-690
- Jones R, Junghard O, Dent J, Vakil N, Halling K, Wernersson B, Lind T. Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. *Aliment Pharmacol Ther* 2009; **30**: 1030-1038 [PMID: 19737151 DOI: 10.1111/j.1365-2036.2009.04142.x]
- Klein JP, Moeschberger ML. Survival analysis - Techniques for censored and truncated data. New York: Springer, 2003: 234-238 [DOI: 10.1007/b97377]
- Carlsson R, Dent J, Watts R, Riley S, Sheikh R, Hatlebakk J, Haug K, de Groot G, van Oudvorst A, Dalvåg A, Junghard O, Wiklund I. Gastro-oesophageal reflux disease in primary care: an international study of different treatment strategies with omeprazole. International GORD Study Group. *Eur J Gastroenterol Hepatol* 1998; **10**: 119-124 [PMID: 9581986]
- Galmiche JP, Barthelemy P, Hamelin B. Treating the symptoms of gastro-oesophageal reflux disease: a double-blind comparison of omeprazole and cisapride. *Aliment Pharmacol Ther* 1997; **11**: 765-773 [PMID: 9305487]
- Venables TL, Newland RD, Patel AC, Hole J, Wilcock C, Turbitt ML. Omeprazole 10 milligrams once daily, omeprazole 20 milligrams once daily, or ranitidine 150 milligrams twice daily, evaluated as initial therapy for the relief of symptoms of gastro-oesophageal reflux disease in general practice. *Scand J Gastroenterol* 1997; **32**: 965-973 [PMID: 9361167 DOI: 10.3109/00365529709011211]
- Tsai HH, Chapman R, Shepherd A, McKeith D, Anderson M, Vearer D, Duggan S, Rosen JP. Esomeprazole 20 mg on-demand is more acceptable to patients than continuous lansoprazole 15 mg in the long-term maintenance of endoscopy-negative gastro-oesophageal reflux patients: the COMMAND Study. *Aliment Pharmacol Ther* 2004; **20**: 657-665 [PMID: 15352914 DOI: 10.1111/j.1365-2036.2004.02155.x]
- Fock KM, Teo EK, Ang TL, Chua TS, Ng TM, Tan YL. Rabeprazole vs esomeprazole in non-erosive gastro-esophageal reflux disease: a randomized, double-blind study in urban Asia. *World J Gastroenterol* 2005; **11**: 3091-3098 [PMID: 15918196]
- Tytgat GN. Review article: management of mild and severe gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2003; **17** Suppl 2: 52-56 [PMID: 12786613]
- Talley NJ, Vakil N, Lauritsen K, van Zanten SV, Flook N, Bolling-Sternevald E, Persson T, Björck E, Lind T. Randomized-controlled trial of esomeprazole in functional dyspepsia patients with epigastric pain or burning: does a 1-week trial of acid suppression predict symptom response? *Aliment Pharmacol Ther* 2007; **26**: 673-682 [PMID: 17697201 DOI: 10.1111/j.1365-2036.2007.03410.x]
- Modlin IM, Hunt RH, Malfertheiner P, Moayyedi P, Quigley EM, Tytgat GN, Tack J, Heading RC, Holtman G, Moss SF. Diagnosis and management of non-erosive reflux disease--the Vevey NERD Consensus Group. *Digestion* 2009; **80**: 74-88 [PMID: 19546560 DOI: 10.1159/000219365]
- Coyne KS, Wiklund I, Schmier J, Halling K, Degl' Innocenti A, Revicki D. Development and validation of a disease-specific treatment satisfaction questionnaire for gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2003; **18**: 907-915 [PMID: 14616154]
- Talley NJ, Lauritsen K, Tunturi-Hihnala H, Lind T, Moum B, Bang C, Schulz T, Omland TM, Delle M, Junghard O. Esomeprazole 20 mg maintains symptom control in endoscopy-negative gastro-oesophageal reflux disease: a controlled trial of 'on-demand' therapy for 6 months. *Aliment Pharmacol Ther* 2001; **15**: 347-354 [PMID: 11207509]
- Talley NJ, Venables TL, Green JR, Armstrong D, O'Kane KP, Gaffier M, Bardhan KD, Carlsson RG, Chen S, Hasselgren GS. Esomeprazole 40 mg and 20 mg is efficacious in the long-term management of patients with endoscopy-negative gastro-oesophageal reflux disease: a placebo-controlled trial of on-demand therapy for 6 months. *Eur J Gastroenterol Hepatol* 2002; **14**: 857-863 [PMID: 12172406]

- 20 **Megan B**, Pickering RM, Weatherall M. Design, objectives, execution and reporting of published open-label extension studies. *J Eval Clin Pract* 2012; **18**: 209-215 [PMID: 21040252 DOI: 10.1111/j.1365-2753.2010.01553.x]
- 21 **Massof RW**. A general theoretical framework for interpreting patient-reported outcomes estimated from ordinally scaled item responses. *Stat Methods Med Res* 2014; **23**: 409-429 [PMID: 23427227 DOI: 10.1177/0962280213476380]

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## Clinical Trials Study

# Detection of superficial esophageal squamous cell neoplasia by chromoendoscopy-guided confocal laser endomicroscopy

Jin Huang, Yun-Sheng Yang, Zhong-Sheng Lu, Shuang-Fang Wang, Jing Yang, Jing Yuan

Jin Huang, Department of Gastroenterology and Hepatology, Chinese PLA 153 Hospital, Zhengzhou 450000, Henan Province, China

Yun-Sheng Yang, Zhong-Sheng Lu, Shuang-Fang Wang, Jing Yang, Department of Gastroenterology and Hepatology, Chinese PLA General Hospital, Beijing 100853, China

Jing Yuan, Department of Pathology, Chinese PLA General Hospital, Beijing 100853, China

**Author contributions:** Huang J and Yang YS designed the research; Huang J, Lu ZS, Wang SF and Yang J performed the research; Yuan J was responsible for the histological analysis; Huang J and Lu ZS analyzed the data; and Huang J wrote the paper.

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**Correspondence to:** Yun-Sheng Yang, Professor, Department of Gastroenterology and Hepatology, Chinese PLA General Hospital, No. 28 Fuxing Road, 100853 Beijing, China. [huangjin034@163.com](mailto:huangjin034@163.com)  
Telephone: +86-10-55499005  
Fax: +86-10-55499005

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## Abstract

**AIM:** To evaluate the diagnostic potential of Lugol's

chromoendoscopy-guided confocal laser endomicroscopy (CLE) in detecting superficial esophageal squamous cell neoplasia (ESCN).

**METHODS:** Between December 2008 and September 2010, a total of 52 patients were enrolled at the Chinese PLA General Hospital in Beijing, China. First, Lugol's chromoendoscopy-guided CLE was performed in these patients and the CLE *in vivo* histological diagnosis was recorded. Then, chromoendoscopy-guided biopsy was performed in the same patients by another endoscopist who was blinded to the CLE findings. Based on the biopsy and CLE diagnosis, *en bloc* endoscopic resection was performed. The CLE *in vivo* diagnosis and the histological diagnosis of biopsy of ESCN were compared, using a histological examination of the endoscopic resection specimens as the standard reference.

**RESULTS:** A total of 152 chromoendoscopy-guided biopsies were obtained from 56 lesions. In the 56 lesions of 52 patients, a total of 679 CLE images were obtained *vs* 152 corresponding biopsies. The sensitivity, specificity, negative predictive value and positive predictive value of chromoendoscopy-guided CLE compared with biopsy were 95.7% *vs* 82% ( $P < 0.05$ ), 90% *vs* 70% ( $P < 0.05$ ), 81.8% *vs* 46.7% ( $P < 0.05$ ), and 97.8% *vs* 92.7% ( $P > 0.05$ ), respectively. There was a significant improvement in sensitivity, specificity, negative predictive value, and accuracy when comparing chromoendoscopy-guided CLE with biopsy.

**CONCLUSION:** Lugol's chromoendoscopy-guided CLE is a real-time, non-invasive endoscopic diagnostic technology; the accuracy of the detection of superficial ESCN is equivalent to or may be superior to biopsy histology.

**Key words:** Superficial esophageal neoplasia; Squamous

cell neoplasm; Confocal endomicroscopy; Endoscopic submucosal dissection; Chromoendoscopy

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**Core tip:** The aim of the present study was to determine the diagnostic potential of confocal laser endomicroscopy (CLE) combined with Lugol's iodine chromoendoscopy in detecting superficial esophageal squamous cell neoplasia (ESCN). Lugol's chromoendoscopy-guided CLE was performed in 52 enrolled patients. In the same patients, chromoendoscopy-guided biopsy was performed by another endoscopist. A comparison of the detection rates between the CLE finding and biopsy was performed. The sensitivity, specificity, negative predictive value and positive predictive value of chromoendoscopy-guided CLE were 95.7%, 90%, 81.8% and 97.8%, respectively. There was a statistically significant difference in the detection of dysplasia between chromoendoscopy-guided CLE and biopsy. Lugol's chromoendoscopy-guided CLE is a real-time, non-invasive endoscopic diagnostic technology; the accuracy of the detection of superficial ESCN is equivalent to or may be superior to biopsy histology.

Huang J, Yang YS, Lu ZS, Wang SF, Yang J, Yuan J. Detection of superficial esophageal squamous cell neoplasia by chromoendoscopy-guided confocal laser endomicroscopy. *World J Gastroenterol* 2015; 21(22): 6974-6981 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6974.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6974>

## INTRODUCTION

Esophageal carcinoma is one of the most aggressive tumors and has a poor prognosis; it is the eighth leading cause of cancer-related death worldwide<sup>[1]</sup>. The morbidity and mortality rates associated with esophageal carcinoma are the highest in China. Squamous cell carcinoma and adenocarcinoma are the most common types of esophageal carcinoma. The former has always been the dominant histological type of esophageal cancer in China. Since most patients are diagnosed when the cancer has grown quite large or has spread to lymph nodes or other structures, the prognosis for esophageal squamous cell carcinoma (ESCC) is poor. Superficial esophageal squamous cell neoplasia (ESCN) is usually asymptomatic and curable if detected early, so early detection is the key to reducing the high mortality of ESCC.

Lugol's iodine chromoendoscopy (LIC) is an easy and inexpensive method that improves the detection of dysplastic lesions of the esophagus. This simple technique is highly sensitive for identifying squamous lesions but has a low specificity and requires biopsy

pathology to confirm the diagnosis<sup>[2,3]</sup>.

Confocal laser endomicroscopy (CLE) is a new approach. This technique allows not only the observation of living cells and tissue but also of the vascular networks of the mucosal layer in the gastrointestinal tract during ongoing endoscopy<sup>[4]</sup>. With 1000-fold magnification ability, this technique enables the visualization of cells of the esophageal squamous epithelium and intraepithelial papillary capillary loops (IPCLs).

*En bloc* endoscopic resection (ER) is desirable for accurate histopathological assessment of tissue specimens obtained using endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). ER is well-recognized as the standard treatment for superficial ESCC *in situ*.

The purpose of this study was to determine the diagnostic potential of CLE combined with LIC in detecting superficial ESCN, using histological examination of *en bloc* ER specimens as the standard.

## MATERIALS AND METHODS

### Patients

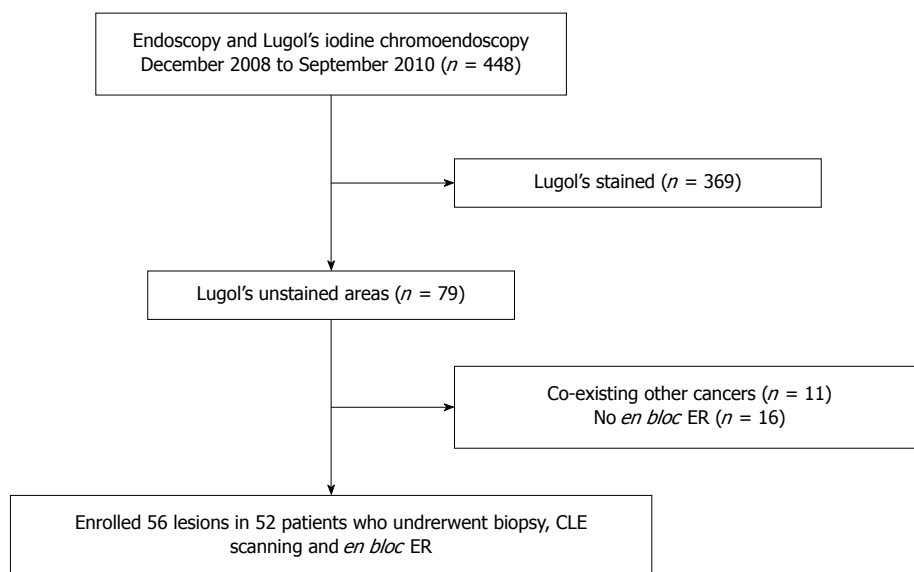
The inclusion criteria were patients aged 18-80 years whose endoscopic appearance of the esophageal lesion was type 0-II according to the Paris classification<sup>[5]</sup>. The exclusion criteria were patients who did not provide informed consent and were allergic to fluorescein, and those who had other co-existing cancers.

The study was performed as a single-center study at the Chinese PLA General Hospital in Beijing, China. The protocol was approved by the medical ethical committee of the Chinese PLA General Hospital and the clinical study was registered as a clinical trial (Registry No. NCT01156064 and No. NCT01378507).

### Endoscopic procedures

Between December 2008 and September 2010, 448 patients underwent upper gastrointestinal (GI) endoscopy and Lugol's iodine test during endoscopy at the Department of Gastroenterology and Hepatology in our hospital. Areas unstained for Lugol's iodine in the esophageal mucosa were revealed in 79 patients. CLE scanning for Lugol-voiding areas was carried out in 52 patients (Figure 1). A single endoscopist was engaged in the procedures of CLE combined with Lugol's staining. The CLE endoscopist had gained experience with the CLE system for at least three months before the initiation of the study (Huang J). The CLE images were collected in real-time during the procedure, and the CLE *in vivo* histological diagnosis was recorded shortly after the procedure. Biopsy combined with Lugol's staining chromoendoscopy (mainframe: CLV-260SL, endoscope: GIF-H260, Tokyo, Japan) was performed by another experienced endoscopist (Lu ZS) who was blinded to the CLE diagnosis. *En bloc* ER





**Figure 1 Flow chart of lesion recruitment.** All the endoscopic procedures were performed with the patients under conscious sedation with intravenous midazolam, propofol, fentanyl, or pethidine. Cardiorespiratory function was continually monitored throughout the procedure. CLE: Confocal laser endomicroscopy; ER: Endoscopic resection.

was performed in 52 patients.

### CLE

The CLE system used was an integrated confocal laser endomicroscope (Pentax Co. Ltd., Mainframe: ISC-1000, endoscopy: EC-3878K, Tokyo, Japan) which combines a confocal laser microscope into the distal tip of a conventional video endoscope, thus enabling confocal microscopy in addition to standard video endoscopy. The scanning rate of the confocal images was 0.8 frames/s ( $1024 \times 1024$  pixels) or 1.6 frame/s ( $1024 \times 512$  pixels); the optical slice thickness of each scan was  $4 \mu\text{m}$ , the resolution was  $0.7 \mu\text{m}$ , and the maximum depth for observation was  $250 \mu\text{m}$ . During the endoscopic examination, 5 mL of 10% fluorescein sodium was administered *via* intravenous injection, and the confocal laser scanning system was used to observe the blood vessels, cellular and subcellular structures of Lugol stained areas. CLE images were collected using a foot pedal. All lesions were diagnosed correctly by endomicroscopy in accordance with the criteria previously described<sup>[6]</sup>. The CLE *in vivo* histological diagnosis was identified as non-neoplasm or neoplasm.

### En bloc ER

Once the diagnosis of ESCN was confirmed by either CLE or biopsy, *en bloc* ER was performed by a single endoscopist (Lu ZS) who had gained experience with more than 50 ESD and 100 EMR of esophageal lesions before performing ESD on any patient in this study; the endoscopist controlled the single-channel endoscope (GIFQ260J; Olympus Optical Co, Ltd, Tokyo, Japan).

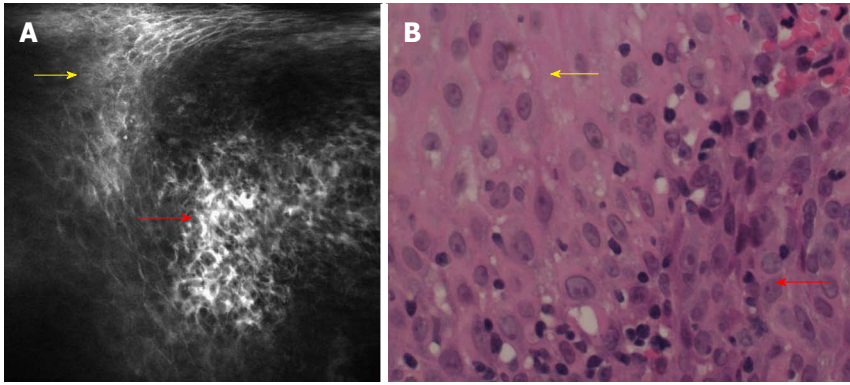
The transparent distal translucent cap EMR (EMR-C) method<sup>[7]</sup> was used to resect lesions  $< 1.5$  cm. ESD

was used to treat neoplastic lesions  $> 1.5$  cm. The typical ESD procedure involved marking, incision, and submucosal dissection with simultaneous hemostasis. After making several marking dots outside the lesion, a saline solution containing epinephrine (0.01 mg/mL) and indigo carmine was injected into the submucosal layer. A circumferential incision was made in the mucosa using a needle-knife (KD-1L; Olympus Optical Co., Ltd., Tokyo, Japan) or an IT-knife (KD-610L; Olympus Optical Co., Ltd., Tokyo, Japan). The submucosal layer was dissected directly, mainly with the IT-knife, until complete removal was achieved. Endoscopic hemostasis was performed either with the knife or hemostatic forceps (FD-410LR; Olympus Optical Co., Ltd., Tokyo, Japan) whenever bleeding was noted. After dissection, preventive endoscopic hemostasis was performed for any oozing or exposed vessel. Only *en bloc* ER was included in order to completely capture the histopathologic examination.

### Histopathologic examination

Biopsies were obtained by standard biopsy forceps from the Lugol-voiding areas. The resected (EMR or ESD) specimen was stretched out and pinned to a soft board with the oral and anal orientations clearly marked, and then measured. The specimen was fixed in a 10% formaldehyde solution, continuously sectioned at 2 mm from the proximal end to the distal end, paraffin embedded, and stained with hematoxylin and eosin (HE). Before sectioning, one side of the specimen was marked with ink. Then, all slides were checked under the microscope from the side with the ink mark to the other side, to observe the histological type.

The biopsy and resected specimens were evaluated by an experienced GI pathologist (Wei LX). The histological diagnosis was identified as inflammation,



**Figure 2** Confocal laser endomicroscopy images of esophageal superficial squamous cell neoplasia. A: Confocal laser endomicroscopy (CLE) scanning; squamous cells (yellow arrow) are homogeneous, while the indicated squamous cells (red arrow) are irregularly arranged with a distinct size and morphology. Capillary leakage of fluorescein sodium is observed; B: Pathological images; the yellow arrow indicates homogeneous cells; the red arrow indicates disordered cell arrangement. The cells have a distinct size and morphology, which is in accordance with CLE.

low-grade intraepithelial neoplasia (LGIN), high-grade intraepithelial neoplasia (HGIN) or ESCC.

### Statistical analysis

Experimental data were recorded and saved in Microsoft Excel 2003. All statistical analyses were performed using the SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL, United States). For descriptive statistics, mean  $\pm$  SD was used in the case of a normal distribution of variables. A Pearson  $\chi^2$  test was applied to compare the sensitivity and specificity of chromoendoscopy-guided CLE and biopsy in the diagnosis of ESCN. A significant difference was assumed for  $P < 0.05$ .

## RESULTS

### Patients

A total of 56 lesions in 52 patients were enrolled for the study, including 16 females and 36 males (mean age, 60.5 years; range, 43-78 years). Five lesions were located in the cervical and upper thoracic esophagus, 40 in the middle thoracic esophagus, and 11 in the lower thoracic esophagus and abdominal esophagus. The mean size of the lesions was 2.3 cm (range, 1.0-6.0 cm).

Inflammation and LGIN by histologic diagnosis and non-neoplasm by CLE *in vivo* diagnosis classified the low-risk group, while HGIN or ESCC by histologic diagnosis and neoplasm by CLE comprised the high-risk group.

### Lugol's chromoendoscopy

Of the 56 demarcated iodine-unstained areas that were studied, 42 lesions were endoscopically diagnosed as neoplasms before endoscopic biopsy and CLE. Among these 42 lesions, 41 were *en bloc* resection specimens histologically confirmed to be neoplasms; one lesion was confirmed to be inflammation. Among the 14 lesions diagnosed as non-neoplasms, nine were histologically confirmed to be non-neoplasms and

five were confirmed to be neoplasms. The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of chromoendoscopy were 89.1%, 90%, 64.3% and 97.6%, respectively.

### CLE

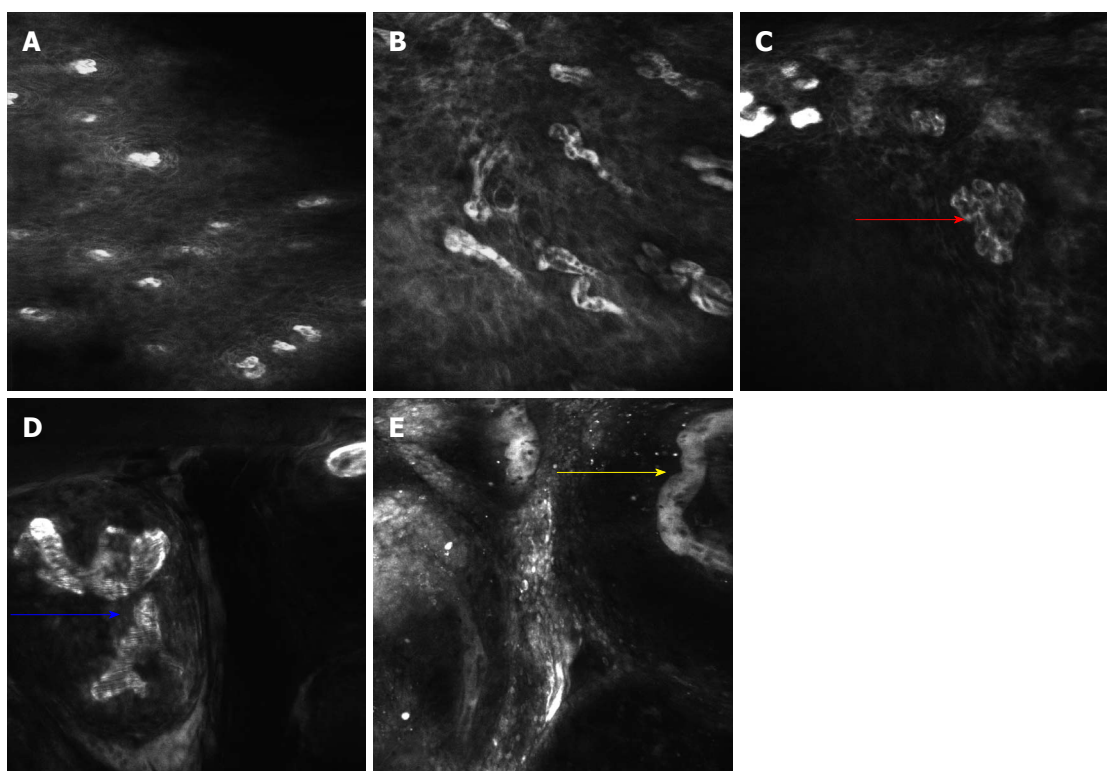
A total of 679 CLE images were obtained from the 56 lesions. Regular squamous epithelium was characterized by regular IPCLs and homogenous epithelial cells with regular architecture and clear borders<sup>[6,8]</sup>. Neoplastic lesions in the esophagus were characterized by irregular IPCLs and heterogeneous epithelial cells with irregular architecture, varying sizes and invisible borders (Figure 2). Various types of irregular IPCLs including dilatation, twist, caliber change and various shape changes were observed in the CLE images (Figure 3). The mean duration of the CLE procedure was  $20 \pm 13$  min, including staining with Lugol's solution in a total of 56 Lugol-voiding areas.

### Histopathology

Histological examination of *en bloc* ER specimens revealed that 46 of the 56 lesions had HGIN or ESCC *in situ*. Inflammation and LGIN of biopsy histologic diagnosis were classified as the non-neoplasms, while HGIN or ESCC *in situ* indicated neoplasms.

A total of 152 chromoendoscopy-guided biopsies were obtained from the 56 lesions. The biopsy histopathologic assessment revealed the presence of neoplasms in 41/56 lesions, which was confirmed by histopathologic analyses of *en bloc* ER specimens in 38/41 lesions.

CLE *in vivo* histological diagnosis revealed non-neoplasms in 11/56 and neoplasms in 45/56 (80.3%), which was confirmed by histopathologic analyses of *en bloc* ER specimens in 9/11 (81.8%) and 44/45 (97.8%) of lesions. The sensitivity, specificity, NPV and PPV of chromoendoscopy-guided CLE were 95.7%, 90.0%, 81.8% and 97.8%, respectively. The results show a significant improvement in sensitivity,



**Figure 3** Confocal laser endomicroscopy images showing different intraepithelial papillary capillary loop changes in the esophageal lesions. A: Regular squamous esophageal epithelium with regular intraepithelial papillary capillary loops (IPCLs) and epithelial cells; B: Some tortuous IPCLs are seen in the non-neoplastic inflammatory lesion; C: Various shapes of twisted IPCLs (red arrow) are seen in the low-grade intraepithelial neoplasia lesion; D: Obvious caliber and shape changes with a larger diameter IPCL (blue arrow) are seen in the high-grade intraepithelial neoplasia lesion; E: Tumor vessels (yellow arrow) are seen in esophageal squamous cell carcinoma.

specificity, NPV, FPR, FNR, and accuracy compared with chromoendoscopy-guided CLE and biopsy.

#### Side effects associated with the procedure

The CLE procedure was generally well-tolerated by all patients. No fluorescein injection-induced side effects were observed, except for mild urine and skin discoloration. In particular, no allergic reactions occurred, because the sensitivity test for fluorescein was undertaken before CLE.

The study included 15 lesions treated by EMR-C and 41 lesions treated by ESD (Figure 4). The duration of the procedure was 27 min (range, 17-39 min) for EMR-C and 113 min (range, 47-175 min) for ESD. Minor bleeding was encountered in all ER procedures, but successful hemostasis was always achieved using thermocoagulation. No patient experienced massive hemorrhage requiring a blood transfusion or postprocedural emergent endoscopy. No delayed hemorrhage occurred. Exposure of the muscular layer during ESD occurred in four cases, but no overt esophageal perforation or the presence of pneumomediastinum was observed by computed tomography scanning.

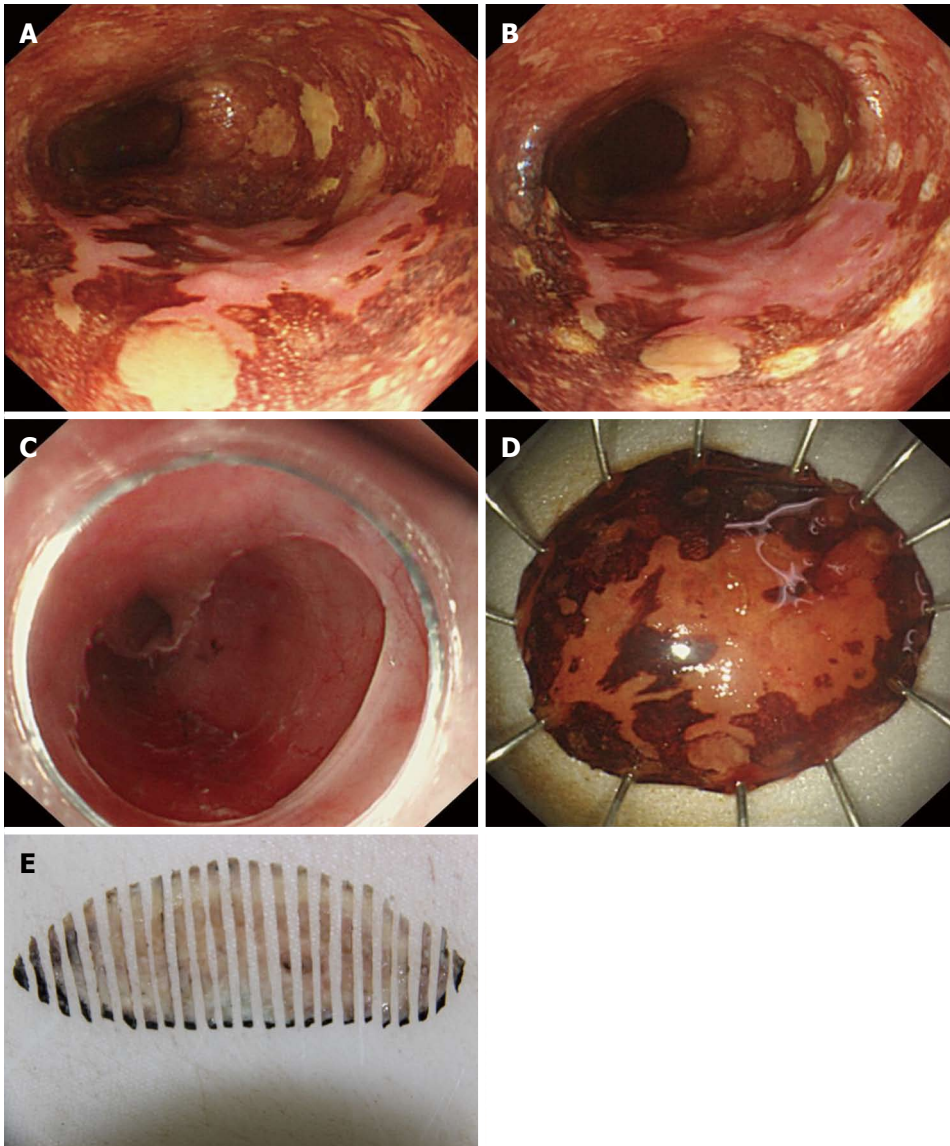
## DISCUSSION

The aim of the present study was to determine the

diagnostic potential of CLE combined with LIC in detecting superficial ESCN. In order to completely capture the histological analysis, histological examination of the *en bloc* ER specimens was used as the standard reference. Our result supports the hypothesis that CLE is non-inferior to biopsy in detecting ESCN.

Lugol's solution is an absorptive stain containing iodine, potassium iodide and distilled water. The solution has an affinity for glycogen in non-keratinized squamous epithelium, and therefore is often used in the esophagus to detect squamous dysplasia and squamous cell carcinoma<sup>[9]</sup>. The absence of staining results from the depletion of glycogen in squamous cells; this occurs in dysplasia, squamous cell carcinoma, Barrett's epithelium, and inflammation<sup>[10]</sup>. Although various techniques for endoscopic diagnosis have been developed, iodine staining is still the most useful screening method for early esophageal carcinoma<sup>[2,11]</sup>. Lugol's staining also improves the visualization of the lateral margins of lesions, which can result in a significant increase in the size of lesions compared with the size estimates obtained by standard endoscopy<sup>[12]</sup>. However, iodine is an irritant that may cause a choking sensation or chest discomfort during or after the procedure, and some patients are hypersensitive to iodine. HGIN and ESCC barely react with iodine due to the small number





**Figure 4** *En bloc* endoscopic resection for esophageal lesions. A: Lugol's iodine chromoendoscopy showed an unstained lesion located in the esophagus; B: The marks surrounding the lesion are at least 5 mm away from the lesion; C: Upper gastrointestinal endoscopy showed an artificial ulceration after ESD; D: *En bloc* resected specimen. 3.0 cm × 3.0 cm; E: The resected specimen was fixed in 10% formaldehyde solution and continuously sliced into 2 mm sections from the proximal end to the distal end for pathology.

of glycogen-containing cells and are therefore seen as completely unstained areas with a reddish color after the brown color of iodine solution has faded. On the other hand, LGIN and areas of inflammation react slightly with iodine and are therefore seen as unstained areas with a yellowish-white color. It is difficult to distinguish HGIN from LGIN without biopsy histological diagnosis even if the pink-color sign is used<sup>[13-16]</sup>. Chromoendoscopy with iodine staining has a low specificity and requires many biopsy specimens. Choosing an adequate biopsy point in a scattered-type esophagus<sup>[17]</sup>, which is characterized by the existence of multiple Lugol-voiding lesions<sup>[18]</sup>, is quite difficult because many of the iodine-unstained lesions are inflammatory areas of the mucosa or LGINs.

CLE enables the endoscopist to perform an *in*

*vivo* histologic examination of the gastrointestinal mucosa and distinguish between neoplastic and non-neoplastic tissues during ongoing endoscopy<sup>[19-22]</sup>. Currently, two confocal imaging systems are available: integrated CLE and probe-based CLE. Integrated CLE was used in our research because the endoscope's working channel can still be used for Lugol staining while confocal images are generated simultaneously with the endoscopic images. CLE images showed a significantly higher proportion of squamous epithelial cells with an irregular arrangement and alteration in IPCLs in ESCC. Squamous cell neoplasia showed dark cells of variable size, no clearly visible borders and an irregular architecture. Various alterations in IPCLs such as increased diameter, tortuous vessels and long branching have been observed<sup>[8,23]</sup>. In this study, CLE



scanning used in Lugol-unstained areas could easily find typical lesions, thus reducing the procedure time and the false positive rate and false negative rate.

Pech *et al*<sup>[6]</sup> compared CLE and biopsy histology; the accuracy was 95%, and the sensitivity and specificity were 100% and 87%, respectively. Unfortunately, a high false positive rate (30%) and false negative rate (17.3%) of biopsy pathology were noted in our research because it was difficult to find typical biopsy lesions in the Lugol-unstained areas. Tissue acquired by biopsy forceps was limited and may be blind; biopsies only reflect limited characteristics of pathological lesions. Endoscopic biopsy also causes mucosal bleeding and consequently makes it difficult to find other target lesions. As a result, it was not possible to observe all pathologic changes, and therefore difficult to perform an objective pathological evaluation on the lesions<sup>[23]</sup>.

In this study, CLE was compared with *en bloc* ER specimen histology. Only *en bloc* ER was used in this study because one of the benefits of *en bloc* resection is the more accurate histological assessment compared to biopsy. All *en bloc* resected specimens were continuously sliced into 2 mm sections from the proximal end to the distal end, such that *en bloc* resection made an entire histopathologic evaluation possible. CLE was able to scan the entire lesion with high accuracy, which not only avoided complications such as bleeding and tissue damage from repeat multiple endoscopy and biopsies, but also reduced the time of histologic examination of the biopsy. Therefore, the diagnostic value of CLE for superficial ESCN is confirmed, but the depth of CLE scanning is limited. Currently, the ability to diagnose esophageal carcinoma is limited to the superficial type; application of these methods to other types of esophageal carcinomas remains to be studied.

ER has become an established standard treatment for patients with superficial ESCN in recent years. In the present study, ESD was used in large lesions (> 1.5 cm), while EMR-C was used in small lesions. We defined the strict criterion of a size of 1.5 cm to ensure *en bloc* resection of the lesions. In our study, ER was performed for either CLE or biopsy-proven ESCN. Five lesions were identified as neoplasms by CLE while LIN/non-neoplasia by biopsy. ER was finally performed in these patients after informed consent was obtained, not only because the patients (including smokers and alcohol users) were highly suspected of having esophageal squamous cell cancer, but because the endoscopic appearance of these lesions was type 0-II according to the Paris classification and Lugol-voiding was obvious. Histological examination of the ESD specimens confirmed the CLE *in vivo* histological diagnosis in these lesions.

Our study had several limitations. First, it was a single-center study. Second, the number of the enrolled cases was limited. Although EMR-C was widely used, the application of ESD was limited due to the

technical difficulty and the high complication rate in China. The CPLA General Hospital was one of the first hospitals to use ESD and CLE in China. Although the number of cases is limited, it is still acceptable due to our strict inclusion criteria. Third, the depth of lesion invasion was not predictable by CLE due to the limited laser penetration depth of integrated CLE.

In conclusion, this study demonstrates that ESCN can be diagnosed reliably by Lugol's chromoendoscopy-guided CLE. The accuracy, sensitivity, specificity, PPV, and NPV were high in our series. CLE combined with LIC is a real-time, non-invasive endoscopic diagnostic technology. Directly progressing to ER without further biopsy procedures may become the standard after further research into CLE in the future.

## COMMENTS

### Background

Superficial esophageal squamous cell neoplasia (ESCN) is usually asymptomatic and curable if detected early. Lugol's iodine chromoendoscopy (LIC) is highly sensitive for identifying squamous lesions, but has a low specificity. Confocal laser endomicroscopy (CLE) can show the cells of the esophageal squamous epithelium and intraepithelial papillary capillary loops *in vivo*, thus contributing to an accurate diagnosis of ESCN when combined with LIC.

### Research frontiers

CLE has been used to investigate inflammatory disease, gastrointestinal cancer and precancer including Barrett's esophagus, gastric intestinal metaplasia, gastric neoplasms and early cancer, colon polyps, colonic neoplasia and cancer, and ulcerative colitis.

### Innovations and breakthroughs

The early detection of esophageal squamous cell carcinoma is the key to reducing the high mortality. This study used CLE combined with LIC in detecting superficial ESCN, first using histological examination of *en bloc* endoscopic resection specimens as the standard pathology.

### Applications

This study has demonstrated that ESCN can be diagnosed reliably by using Lugol's chromoendoscopy-guided CLE. Clinical endoscopists could use CLE combined with LIC to quickly and accurately identify ESCN.

### Terminology

CLE is novel digestive endoscope which enables the endoscopist to perform *in vivo* histologic examination of the gastrointestinal mucosa, and distinguish between neoplastic and non-neoplastic tissues during ongoing endoscopy.

### Peer-review

The authors have demonstrated an effective method for the detection and diagnosis of ESCN by Lugol's chromoendoscopy-guided CLE, which has a higher sensitivity and specificity than conventional endoscopy.

## REFERENCES

- 1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.49.1.33]
- 2 Hashimoto CL, Iriya K, Baba ER, Navarro-Rodriguez T, Zerbini MC, Eisig JN, Barbuti R, Chinzon D, Moraes-Filho JP. Lugol's dye spray chromoendoscopy establishes early diagnosis of esophageal cancer in patients with primary head and neck cancer. *Am J Gastroenterol* 2005; **100**: 275-282 [PMID: 15667482 DOI: 10.1111/j.1572-0241.2005.30189.x]
- 3 Yokoyama A, Ohmori T, Makuuchi H, Maruyama K, Okuyama K, Takahashi H, Yokoyama T, Yoshino K, Hayashida M, Ishii H. Successful screening for early esophageal cancer in alcoholics using endoscopy and mucosa iodine staining. *Cancer* 1995; **76**: 928-934 [PMID: 8625217]

- 4 **Polglase AL**, McLaren WJ, Skinner SA, Kiesslich R, Neurath MF, Delaney PM. A fluorescence confocal endomicroscope for in vivo microscopy of the upper- and the lower-GI tract. *Gastrointest Endosc* 2005; **62**: 686-695 [PMID: 16246680 DOI: 10.1016/j.gie.2005.05.021]
- 5 **Endoscopic Classification Review Group**. Update on the paris classification of superficial neoplastic lesions in the digestive tract. *Endoscopy* 2005; **37**: 570-578 [PMID: 15933932 DOI: 10.1055/s-2005-861352]
- 6 **Pech O**, Rabenstein T, Manner H, Petrone MC, Pohl J, Vieth M, Stolte M, Ell C. Confocal laser endomicroscopy for in vivo diagnosis of early squamous cell carcinoma in the esophagus. *Clin Gastroenterol Hepatol* 2008; **6**: 89-94 [PMID: 18063417 DOI: 10.1016/j.cgh.2007.10.013]
- 7 **Inoue H**, Takeshita K, Hori H, Muraoka Y, Yoneshima H, Endo M. Endoscopic mucosal resection with a cap-fitted panendoscope for esophagus, stomach, and colon mucosal lesions. *Gastrointest Endosc* 1993; **39**: 58-62 [PMID: 8454147 DOI: 10.1016/S0016-5107(93)70012-7]
- 8 **Deinert K**, Kiesslich R, Vieth M, Neurath MF, Neuhaus H. In-vivo microvascular imaging of early squamous-cell cancer of the esophagus by confocal laser endomicroscopy. *Endoscopy* 2007; **39**: 366-368 [PMID: 17427075 DOI: 10.1055/s-2007-966217]
- 9 **Freitag CP**, Barros SG, Krueel CD, Putten AC, Dietz J, Gruber AC, Diehl AS, Meurer L, Breyer HP, Wolff F, Vidal R, Arruda CA, Luz LP, Fagundes RB, Prolla JC. Esophageal dysplasias are detected by endoscopy with Lugol in patients at risk for squamous cell carcinoma in southern Brazil. *Dis Esophagus* 1999; **12**: 191-195 [PMID: 10631911 DOI: 10.1046/j.1442-2050.1999.00046.x]
- 10 **Weinstein WM**. Vital staining of esophageal and gastric mucosa: not vital but may be helpful. *Gastrointest Endosc* 1992; **38**: 723-725 [PMID: 1282116 DOI: 10.1016/S0016-5107(92)70578-1]
- 11 **Fagundes RB**, de Barros SG, Pütten AC, Mello ES, Wagner M, Bassi LA, Bombassaro MA, Gobbi D, Souto EB. Occult dysplasia is disclosed by Lugol chromoendoscopy in alcoholics at high risk for squamous cell carcinoma of the esophagus. *Endoscopy* 1999; **31**: 281-285 [PMID: 10376452 DOI: 10.1055/s-1999-122]
- 12 **Dawsey SM**, Fleischer DE, Wang GQ, Zhou B, Kidwell JA, Lu N, Lewin KJ, Roth MJ, Tio TL, Taylor PR. Mucosal iodine staining improves endoscopic visualization of squamous dysplasia and squamous cell carcinoma of the esophagus in Linxian, China. *Cancer* 1998; **83**: 220-231 [PMID: 9669803]
- 13 **Mori M**, Adachi Y, Matsushima T, Matsuda H, Kuwano H, Sugimachi K. Lugol staining pattern and histology of esophageal lesions. *Am J Gastroenterol* 1993; **88**: 701-705 [PMID: 7683176]
- 14 **Kitamura K**, Kuwano H, Yasuda M, Sonoda K, Sumiyoshi K, Tsutsui S, Kitamura M, Sugimachi K. What is the earliest malignant lesion in the esophagus? *Cancer* 1996; **77**: 1614-1619 [PMID: 8608552]
- 15 **Shimizu Y**, Omori T, Yokoyama A, Yoshida T, Hirota J, Ono Y, Yamamoto J, Kato M, Asaka M. Endoscopic diagnosis of early squamous neoplasia of the esophagus with iodine staining: high-grade intra-epithelial neoplasia turns pink within a few minutes. *J Gastroenterol Hepatol* 2008; **23**: 546-550 [PMID: 17573830 DOI: 10.1111/j.1440-1746.2007.04990.x]
- 16 **Ishihara R**, Yamada T, Iishi H, Kato M, Yamamoto S, Yamamoto S, Masuda E, Tatsumi K, Takeuchi Y, Higashino K, Uedo N, Tatsuta M, Ishiguro S. Quantitative analysis of the color change after iodine staining for diagnosing esophageal high-grade intraepithelial neoplasia and invasive cancer. *Gastrointest Endosc* 2009; **69**: 213-218 [PMID: 18718584 DOI: 10.1016/j.gie.2008.04.052]
- 17 **Shimizu Y**, Tukagoshi H, Fujita M, Hosokawa M, Kato M, Asaka M. Metachronous squamous cell carcinoma of the esophagus arising after endoscopic mucosal resection. *Gastrointest Endosc* 2001; **54**: 190-194 [PMID: 11474389 DOI: 10.1067/mge.2001.116877]
- 18 **Muto M**, Hironaka S, Nakane M, Boku N, Ohtsu A, Yoshida S. Association of multiple Lugol-voiding lesions with synchronous and metachronous esophageal squamous cell carcinoma in patients with head and neck cancer. *Gastrointest Endosc* 2002; **56**: 517-521 [PMID: 12297767 DOI: 10.1067/mge.2002.128104]
- 19 **Kiesslich R**, Gossner L, Goetz M, Dahlmann A, Vieth M, Stolte M, Hoffman A, Jung M, Nafe B, Galle PR, Neurath MF. In vivo histology of Barrett's esophagus and associated neoplasia by confocal laser endomicroscopy. *Clin Gastroenterol Hepatol* 2006; **4**: 979-987 [PMID: 16843068 DOI: 10.1016/j.cgh.2006.05.010]
- 20 **Kakeji Y**, Yamaguchi S, Yoshida D, Tanoue K, Ueda M, Masunari A, Utsunomiya T, Imamura M, Honda H, Maehara Y, Hashizume M. Development and assessment of morphologic criteria for diagnosing gastric cancer using confocal endomicroscopy: an ex vivo and in vivo study. *Endoscopy* 2006; **38**: 886-890 [PMID: 16981104 DOI: 10.1055/s-2006-944735]
- 21 **Li WB**, Zuo XL, Li CQ, Zuo F, Gu XM, Yu T, Chu CL, Zhang TG, Li YQ. Diagnostic value of confocal laser endomicroscopy for gastric superficial cancerous lesions. *Gut* 2011; **60**: 299-306 [PMID: 21193460 DOI: 10.1136/gut.2010.223586]
- 22 **Sanduleanu S**, Driessen A, Gomez-Garcia E, Hameeteman W, de Bruïne A, Masclee A. In vivo diagnosis and classification of colorectal neoplasia by chromoendoscopy-guided confocal laser endomicroscopy. *Clin Gastroenterol Hepatol* 2010; **8**: 371-378 [PMID: 19683597 DOI: 10.1016/j.cgh.2009.08.006]
- 23 **Liu H**, Li YQ, Yu T, Zhao YA, Zhang JP, Zuo XL, Li CQ, Zhang JN, Guo YT, Zhang TG. Confocal laser endomicroscopy for superficial esophageal squamous cell carcinoma. *Endoscopy* 2009; **41**: 99-106 [PMID: 19214886 DOI: 10.1055/s-0028-1119492]

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## Observational Study

# Cardiac autonomic dysfunction in patients with gastroesophageal reflux disease

Branislav Milovanovic, Branka Filipovic, Slavica Mutavdzin, Marija Zdravkovic, Tatjana Gligorijevic, Jovana Paunovic, Marina Arsic

Branislav Milovanovic, Slavica Mutavdzin, Tatjana Gligorijevic, Jovana Paunovic, Marina Arsic, Department of Cardiology, Neurocardiology Laboratory, Clinical and Hospital Center "Bezanijska Kosa", Belgrade 11080, Republic of Serbia

Branislav Milovanovic, Marija Zdravkovic, University of Belgrade, Medical Faculty, Belgrade 11080, Republic of Serbia

Branka Filipovic, Department of Gastroenterology, Clinical and Hospital Center "Bezanijska Kosa", Belgrade 11080, Republic of Serbia

Marija Zdravkovic, Department of Cardiology, Clinical and Hospital Center "Bezanijska Kosa", Belgrade 11080, Republic of Serbia

**Author contributions:** Milovanovic B performed the autonomic function testing; Filipovic B performed gastroenterologic examinations; Mutavdzin S, Gligorijevic T, Paunovic J, and Arsic M collected the data; Milovanovic B, Zdravkovic M, and Mutavdzin S wrote the manuscript.

**Ethics approval:** This research was conducted in the frame work of the Ministry of Science project (No. 32040). Scientific Ethical Committee of Clinical Hospital Center "Bezanijska Kosa" approved all research in the frame work of this project.

**Informed consent:** All the patients were informed about the protocol in detail and provided written consent.

**Conflict-of-interest:** The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Data sharing:** Technical appendix, statistical code, and dataset available from the corresponding author at [slavica.mutavdzin@gmail.com](mailto:slavica.mutavdzin@gmail.com).

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Correspondence to: Slavica Mutavdzin, MD, Department of

Cardiology, Neurocardiology Laboratory, Clinical and Hospital Center "Bezanijska Kosa", Bezanijska kosa b.b., Belgrade 11080, Republic of Serbia. [slavica.mutavdzin@gmail.com](mailto:slavica.mutavdzin@gmail.com)

Telephone: +381-11-3010773

Fax: +381-11-2606520

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## Abstract

**AIM:** To investigate autonomic nervous function in patients with a diagnosis of gastroesophageal reflux disease (GERD).

**METHODS:** The investigation was performed on 29 patients (14 men), aged 18-80 years ( $51.14 \pm 18.34$ ), who were referred to our Neurocardiology Laboratory at the Clinical and Hospital Center "Bezanijska Kosa" with a diagnosis of GERD. One hundred sixteen healthy volunteers matched in age and sex with the examinees served as the control group. The study protocol included the evaluation of autonomic function and hemodynamic status, short-term heart rate variability (HRV) analysis, 24 h ambulatory ECG monitoring with long-term HRV analysis and 24 h ambulatory blood pressure monitoring.

**RESULTS:** Pathologic results of cardiovascular reflex test were more common among patients with reflux compared to the control group. Severe autonomic dysfunction was detected in 44.4% of patients and in 7.9% of controls ( $P < 0.001$ ). Parameters of short-term analysis of RR variability, which are the indicators of

vagal activity, had lower values in patients with GERD than in the control group. Long-term HRV analysis of time-domain parameters indicated lower values in patients with reflux disease when compared to the control group. Power spectral analysis of long-term HRV revealed lower low- and high-frequency values. Detailed 24 h ambulatory blood pressure analysis showed significantly higher values of systolic blood pressure and pulse pressure in the reflux group than in the control group.

**CONCLUSION:** Patients with GERD have distortion of sympathetic and parasympathetic components of the autonomic nervous system, but impaired parasympathetic function appears more congruent to GERD.

**Key words:** Autonomic nervous system; Blood pressure monitoring; Cardiovascular reflex test; ECG monitoring; Gastroesophageal reflux disease

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**Core tip:** Autonomic nervous function was assessed in patients with gastroesophageal reflux disease (GERD) for the purpose of treating patients according to their presenting autonomic pattern. The results demonstrate that autonomic dysfunction is more frequently detected in patients than in controls. Parameters of short-term and long-term analysis of heart rate variability had lower value while blood pressure was higher in patients than in the controls. In conclusion, patients with GERD have distortion of both components of autonomic nervous system, but the impairment of parasympathetic function is more congruent to GERD.

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## INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the most common digestive diseases in the Western world, with a high prevalence in the general population (20%)<sup>[1]</sup>. Heartburn or acid regurgitation is experienced on a weekly basis by nearly 20% of the population<sup>[2]</sup>. The prevalence of GERD symptoms increased approximately 50% until the mid-1990s, when it plateaued. This increase in GERD is not exactly clear, but has been attributed to the increasing prevalence of obesity, changing diet, and perhaps the decreasing prevalence of *Helicobacter pylori* (*H. pylori*) infection<sup>[3,4]</sup>. Recent

publications sustained earlier observations of age-related decline in the number of cholinergic neurons in the enteric nervous system. They also reveal a progressive loss of interstitial cells of Cajal in the stomach and colon throughout adult life. These changes appear to have a surprisingly small effect on gastrointestinal motor function in normal ageing, though gut sensation is impaired and older individuals have an increased susceptibility to gastrointestinal complications from comorbid illnesses<sup>[5]</sup>.

Autonomic nervous dysfunction has frequently been observed in patients with GERD and pathophysiology of GERD has been linked to disturbances in autonomic nervous system activity. The association between gastrointestinal symptoms and cardiac dysrhythmias, as one of the autonomic system impairments in GERD patients, has been described as gastrocardiac syndrome<sup>[6,7]</sup>. Esophageal inflammation is not related to autonomic nervous system dysfunction *per se*, as vagal dysfunction is observed in the presence and absence of inflammatory changes in the esophagus. It has even been suggested that parasympathetic dysfunction is not just the consequence of esophageal inflammation, but the prime factor in the etiology of GERD<sup>[8]</sup>. Disturbances in autonomic nervous system activity affect both contraction and transient relaxation of the lower esophageal sphincter (normally acting as a reflux barrier), leading to the occurrence and progression of GERD<sup>[9]</sup>.

The primary aim of this study is to treat patients according to the type of autonomic pattern and adjustment of autonomic function. We hypothesize that there are significant differences between GERD patients and healthy volunteers in autonomic function as assessed by cardiovascular reflex tests.

## MATERIALS AND METHODS

### Demographic data

The investigation was performed on 29 (14 male and 15 female) patients aged  $51.14 \pm 18.34$  years (range: 18-80 years) who were referred to our Neurocardiology Laboratory of the Clinical and Hospital Center "Bezanijska Kosa" with GERD. All the patients were informed about the protocol in detail and provided written consent. This study was approved by the Scientific Ethical Committee of Clinical Hospital Center "Bezanijska Kosa".

The diagnosis of GERD was established by upper endoscopic examination. Exclusion criteria were a prior history of: coronary artery, atrial fibrillation, secondary arterial hypertension, renal failure (serum creatinine > 1.2 mg/dL), autoimmune disease, or previous treatment with antipsychotics, antidepressants, mood stabilizers, antiarrhythmics, or cimetidine. Patients were asked to stop all medications during the study.

The control group consisted of 116 healthy age- and sex-matched volunteers.



### **Study protocol**

The protocol included the clinical autonomic function tests, short-term heart rate variability (HRV) analysis, 24 h ambulatory ECG monitoring with long-term HRV analysis and 24 h ambulatory blood pressure monitoring. Patients were tested under ideal temperature conditions (23 °C), without any preceding consumption of alcohol, nicotine, or food.

### **Clinical autonomic function tests**

The protocol included five standard Ewing's clinical autonomic function tests, as well as cold pressure and mental stress test. Cardiovascular reflex tests according to Ewing *et al.*<sup>[10]</sup> were the first step in the assessment of autonomic function. This includes two groups of tests: parasympathetic (heart rate response to Valsalva maneuver, deep breathing, and standing) and sympathetic tests [blood pressure (BP) response to standing and sustained handgrip test]. Participants rested in the supine position for 10 min before starting the tests and also rested for 2 min between each test.

### **Parasympathetic tests**

#### **Heart rate response to Valsalva maneuver:**

The patient was asked to maintain a column of mercury at 40 mmHg for 15 s blowing into a modified sphygmomanometer, with ECG recording. The result, expressed as a Valsalva ratio was taken as the maximum RR interval in the 15 s following expiration divided by the minimum RR interval during the maneuver.

**Heart rate response to deep breathing:** Respiratory sinus arrhythmia was assessed by the performance of six deep breaths at 0.1 Hz frequency. The response was taken as the mean of the differences between the maximum and minimum instantaneous heart rates for each cycle.

#### **Heart rate response to standing (30:15 ratio):**

Heart rate response after standing was expressed as a ratio between the longest RR interval corresponding with the 30<sup>th</sup> beat after starting and the shortest RR interval corresponding with the 15<sup>th</sup> beat. The ratio was measured using a ruler and electrocardiograph trace, which was recorded continuously.

### **Sympathetic tests**

**BP response to standing:** Orthostatic BP change was calculated as the difference between the nadir systolic BP 180 s after standing and the systolic BP prior to standing.

**BP response to sustained handgrip test:** Sustained muscle contraction causes a rise in systolic and diastolic BP and heart rate. The test was performed with 30% of maximal voluntary contraction for 5 min with BP measurement. Increment of diastolic BP during this test was taken as result.

### **Cold pressure test**

The hand of the patient was put in iced water for 6 min. Sympathetic failure was diagnosed related to the fall or absence of changes of heart rate and BP during the test.

### **Mental stress test**

Arithmetic calculation (addition of 17 up to 1017) for 6 min with a previous 3-min rest period was used. Sympathetic dysfunction was present related to the absence of rise or changes of heart rate and BP during the mental stimulation.

### **Cardiovascular reflex test results**

Results of all tests were expressed as normal, borderline, or abnormal according to the cutoff values given by Ewing *et al.*<sup>[10]</sup>. Based on the results of the cardiovascular reflex tests, a scoring system was applied and autonomic dysfunction in each patient was qualified as: vagal denervation, vagal and sympathetic damage, or severe autonomic neuropathy<sup>[10]</sup>.

### **Short-term HRV analysis**

Short-term HRV analysis was performed from 512 consecutive RR intervals using commercial software (Schiller AT-10, Austria) according to previously published guidelines<sup>[11]</sup>. Short-term HRV analysis includes time and frequency domain analyses. The following time domain variables were computed for each subject from dRR tachogram: average dRR interval, standard and mean deviations of dRR intervals (SD dRR and MDdRR), square root of the mean of squared differences of two consecutive RR intervals (RMSSD), and percent of beats with consecutive RR interval difference of > 50 ms (pNN50). The following short-term frequency domain indices were determined using Hanning window-type signal limitation before Fourier transformation: very low-frequency power (VLF; 0.016-0.05 Hz), low-frequency power (LF; 0.05-0.15 Hz), high-frequency power (HF; 0.15-0.35 Hz), and LF/HF ratio.

The Task Force Monitor (CNSystems, Graz, Austria) was used to monitor beat-to-beat HR by ECG, beat-to-beat stroke index by an improved method of impedance cardiography, and beat-to-beat BP by the vascular unloading technique, which was corrected automatically to the oscillometric BP measured on the contralateral arm. The Task Force Monitor automatically provides beat-to-beat spectral analysis of heart rate and systolic and diastolic BP variability by applying an autoregressive methodology. The total power (TP) and the power of the three frequency bands (VLF band between 0-0.05 Hz; LF band between 0.05-0.17 Hz; and HF band between 0.17-0.40 Hz) were computed and expressed in absolute values (ms<sup>2</sup>) or normalized units (%). Beat-to-beat analysis of BP enables assessment of baroreceptor reflex sensitivity from spontaneously occurring in a rise and fall of BP,

**Table 1** Distribution of autonomic dysfunction among patients with reflux and controls *n* (%)

	Parasympathetic damage			Sympathetic damage	Combined damage
	Without	Early	Definitive		
Reflux	4 (21.1)	7 (36.8)	8 (42.1)	17 (94.4)	10 (58.8)
Control	24 (31.2)	43 (55.8)	10 (13.0)	55 (72.4)	8 (10.7)

For parasympathetic damage:  $\chi^2 = 8.48$ ,  $df = 3$ ,  $P = 0.014$ .

**Table 2** Autonomic cardiovascular tests reflecting parasympathetic damage *n* (%)

Autonomic cardiovascular reflex tests	Reflux ( <i>n</i> = 19)	Controls ( <i>n</i> = 77)	<i>P</i> value <sup>1</sup>
Valsalva maneuver	8 (42.1)	18 (23.4)	0.015
Heart rate variation during deep breathing	10 (52.6)	8 (10.4)	< 0.001
Heart rate response to standing test	6 (31.6)	36 (47.4) <sup>2</sup>	0.028
Vagal dysfunction	8 (42.1)	10 (13.0)	0.014

<sup>1</sup>Mann-Whitney test; <sup>2</sup>Data from one patient missing.

which are followed by regulatory heart rate interval changes. The following parameters were included in analyses: maximal slope, minimal slope, and mean slope of baroreflex sensitivity (ms/mmHg).

### Twenty-four-hour ambulatory ECG monitoring with long-term HRV analysis

Twenty-four-hour ambulatory ECG recordings were acquired by a 12-lead electrocardiogram with a sampling rate of 1000 Hz (Cardioscan; DMS Software Inc., CA, United States) and analyzed. The time and frequency domain HRV analyses were carried out using the software package present in the system. The Fast Fourier transformation and Hanning window were used for the analysis of the frequency (spectral) domain parameters.

From Time domain HRV analysis, the following time domain variables were computed: mean RR interval for 24 h (mean NN), standard deviation of normal RR intervals (SDNN), standard deviation of all 5-min mean normal RR intervals (SDANN), square root of the mean of the sum of the squares of differences between adjacent RR intervals (r-MSSD), and percentage of adjacent RR intervals differing > 50 ms (pNN50). From Frequency domain HRV analysis, the following 24-h frequency domain indices were determined: total power (TP-0-0.4 Hz), high-frequency power (HF-0.15-0.4 Hz), low-frequency power (LF - 0.04-0.15 Hz), and the LF/HF ratio. Heart rate was measured in ms; variance, which is referred to as the power in a portion of the total spectrum of frequencies, was measured in ms<sup>2</sup>.

### Twenty-four hour ambulatory BP monitoring

Evaluation of the 24 h BP profile was conducted using recorder and commercial software for analysis (Mobil-O-graph; I.E.M., Stolberg, Germany). BP

**Table 3** Autonomic cardiovascular tests reflecting sympathetic damage *n* (%)

Autonomic cardiovascular reflex tests	Reflux ( <i>n</i> = 18)	Controls ( <i>n</i> = 77)	<i>P</i> value <sup>1</sup>
Orthostatic hypotension	1 (5.3)	2 (2.6)	0.822
Hand grip test	16 (88.9)	59 (76.6)	0.481
Sympathetic dysfunction	17 (94.4)	55 (72.4) <sup>2</sup>	0.047

<sup>1</sup>Mann Whitney test; <sup>2</sup>Data from one patient missing.

measurements were performed every 15 min by oscillometry. Sleep BP was defined as the BPs from the time when the subjects went to bed until the time they got out of bed. Awake BP was defined as BPs recorded during the rest of the day. Morning BP was defined as the average of BP during the first hour after waking up. Systolic and diastolic BP variability was defined as standard deviation of systolic and standard deviation of diastolic BP measurements during the awake period and during sleep. Dippers were defined as those who exhibit a reduction in mean systolic BP of < 10 mmHg from daytime to nighttime, and the remaining subjects were classified as nondippers.

### Statistical analysis

The results are expressed as the mean  $\pm$  SD. The Student's *t*-test and Mann Whitney *U* test were used for comparison between the groups. A *P*-value < 0.05 was considered statistically significant. All calculations were performed using a commercially available statistical software program (SPSS 15.0; SPSS Inc., Chicago IL, United States). The statistical methods of this study were reviewed by a biostatistician from the Institute for Oncology and Radiology of Serbia.

## RESULTS

### Cardiovascular reflex tests

Pathologic results of cardiovascular reflex tests were more common among the patients with reflux compared to the control group (Tables 1, 2, 3 and 4), and severe autonomic dysfunction was detected in 8 out of 29 patients and in 6 out of 116 controls ( $P < 0.001$ ) (Table 5).

### Short-term HRV analysis

All spectral and time domain parameters were considerably lower in patients with GERD. Mean and standard deviations of the dRR, square root of the

**Table 4 Complete autonomic dysfunction *n* (%)**

Complete autonomic dysfunction	Reflux	Controls	<i>P</i> value <sup>1</sup>
Absent	7 (41.2)	67 (89.3)	< 0.001
Present	10 (58.8)	8 (10.7)	< 0.001
Total	17 (100)	75 (100)	< 0.001

<sup>1</sup>Mann-Whitney test.**Table 5 Degree of autonomic dysfunction *n* (%)**

Degree of autonomic dysfunction	Reflux	Controls	<i>P</i> value <sup>1</sup>
Normal	0 (0.0)	0 (0.0)	< 0.001
Mild	1 (5.6)	22 (28.9)	< 0.001
Moderate	9 (50.0)	48 (63.2)	< 0.001
Severe	8 (44.4)	6 (7.9)	< 0.001
Total	18 (100)	76 (100)	< 0.001

**Table 6 Short term heart rate variability analysis (mean  $\pm$  SD)**

Parameter	Reflux	Controls	<i>P</i> value <sup>1</sup>
Average dRR (ms)	15.67 $\pm$ 10.35	27.80 $\pm$ 17.49	0.003
SD dRR (ms)	12.48 $\pm$ 7.63	22.42 $\pm$ 13.87	0.001
MD dRR (ms)	9.76 $\pm$ 6.36	17.10 $\pm$ 10.25	0.002
pNN50%	3.62 $\pm$ 6.26	9.82 $\pm$ 10.29	0.009
RMSSD (ms)	19.81 $\pm$ 12.81	35.87 $\pm$ 21.78	0.001
VLF (ms <sup>2</sup> )	67.76 $\pm$ 65.56	129.33 $\pm$ 129.19	0.036
LF (ms <sup>2</sup> )	56.29 $\pm$ 65.64	135.07 $\pm$ 142.90	0.015
HF (ms <sup>2</sup> )	35.62 $\pm$ 51.27	102.52 $\pm$ 115.53	0.011
LF/HF	3.07 $\pm$ 2.34	2.27 $\pm$ 2.82	0.225

<sup>1</sup>*t*-test. SD dRR: Standard deviation of normal RR intervals (SD); MD dRR: Absolute mean of standard deviation; pNN50%: Percentage of adjacent RR intervals differing > 50 ms; RMSSD: Mean square root of the mean of the sum of the squares of differences between adjacent RR intervals; VLF: Very low-frequency power; LF: Low-frequency power; HF: High-frequency power.

mean of squared differences of two consecutive RR intervals, and percent of beats with consecutive RR interval difference of > 50 ms, which are the indicators of vagal activity, had significantly lower values in patients with GERD than in the control group (all *P* < 0.05) (Table 6). The value of HF, reflecting vagal activity, was significantly decreased in patients with GERD (*P* < 0.05). LF spectral parameter, reflecting sympathetic and vagal function, was also lower in GERD. LF/HF ratio, reflecting sympathovagal balance, was higher in the reflux group compared to the control group, but no significant difference was obtained.

#### Beat-to-beat heart rate variability and baroreflex sensitivity

All short-term beat-to-beat spectral parameters (TP, VLF, LF, HF) and the mean value of baroreflex sensitivity were significantly decreased in the GERD patients compared with the control group (all *P* < 0.05) (Table 7).

**Table 7 Beat-to-beat heart rate variability and baroreflex sensitivity (mean  $\pm$  SD)**

Parameter	Reflux	Controls	<i>P</i> value <sup>1</sup>
Heart rate variability			
LFnu-RRI (%)	63.85 $\pm$ 17.27	59.74 $\pm$ 15.98	0.280
HFnu-RRI (%)	36.15 $\pm$ 17.27	39.98 $\pm$ 15.37	0.298
VLF-RRI (ms <sup>2</sup> )	132888.77 $\pm$ 56675.91	745.72 $\pm$ 2409.152	0.021
LF-RRI (ms <sup>2</sup> )	319.18 $\pm$ 347.13	864.78 $\pm$ 1036.92	0.016
HF-RRI (ms <sup>2</sup> )	225.73 $\pm$ 263.42	656.44 $\pm$ 996.35	0.047
Total power (ms <sup>2</sup> )	1383.55 $\pm$ 65646.66	2264.58 $\pm$ 3231.24	0.034
LF/HF	3.32 $\pm$ 2.88	2.83 $\pm$ 3.72	0.561
Baroreflex sensitivity (ms/mmHg)			
Minimal slope	3.62 $\pm$ 3.99	4.43 $\pm$ 3.32	0.317
Maximal slope	40.50 $\pm$ 31.57	47.06 $\pm$ 32.29	0.385
Mean slope	12.11 $\pm$ 7.00	17.11 $\pm$ 9.77	0.024

<sup>1</sup>*t*-test. HF: High-frequency power; LF: Low-frequency power; LFnu-RRI: Percent of normalized LF interval component; HFnu-RRI: Percent of normalized HF interval component; VLF-RRI: Very low-frequency interval component of heart rate variability; LF-RRI: LF interval component of heart rate variability; HF-RRI: HF interval component of heart rate variability.

**Table 8 Holter ECG heart rate and long-term HRV analysis (mean  $\pm$  SD)**

Parameter	Reflux	Controls	<i>P</i> value <sup>1</sup>
Mean RR (ms)	822.59 $\pm$ 82.76	811.08 $\pm$ 79.88	0.559
SDNN (ms)	125.76 $\pm$ 33.54	154.82 $\pm$ 39.72	0.003
SDANNindex (ms)	113.24 $\pm$ 33.71	141.65 $\pm$ 36.28	0.002
SDNN index (ms)	49.71 $\pm$ 17.92	65.26 $\pm$ 16.87	< 0.001
RMSSD (ms)	28.33 $\pm$ 11.72	37.29 $\pm$ 13.20	0.006
pNN50%	8.48 $\pm$ 8.97	14.13 $\pm$ 9.41	0.016
Total power (ms <sup>2</sup> )	2683.56 $\pm$ 2081.23	4446.65 $\pm$ 2151.45	0.001
VLF (ms <sup>2</sup> )	1851.73 $\pm$ 1318.44	2964.81 $\pm$ 1557.53	0.004
LF (ms <sup>2</sup> )	615.72 $\pm$ 624.36	1048.73 $\pm$ 462.23	0.001
HF (ms <sup>2</sup> )	197.15 $\pm$ 203.88	408.51 $\pm$ 291.02	0.002

<sup>1</sup>*t*-test. SDNN: Standard deviation of all the RR intervals; SDNN index: Mean of standard deviation of all RR intervals for all 5-min segments of the entire recording; RMSSD: Square root of the mean of squared differences of two consecutive RR intervals; pNN50%: Percent of beats with consecutive RR interval difference of more than 50 ms; VLF: Very low-frequency interval; LF: Low-frequency interval; HF: High-frequency interval.

#### Twenty-four-hour ambulatory ECG monitoring with long-term HRV analysis

Analysis of the time domain parameters indicated statistical significance for important arrhythmia risk predictors. The standard deviation of normal RR intervals, standard deviation of all 5-min mean normal RR intervals and their indices had considerably lower values in patients with reflux when compared to the control group (Table 8). Power spectral analysis of long-term HRV revealed lower both LF and HF values.

#### Twenty-four-hour ambulatory BP monitoring

Detailed ambulatory BP analysis during 24 h included

**Table 9 Twenty-four-hour ambulatory blood pressure monitoring (mean  $\pm$  SD)**

Parameter	Reflux	Controls	P value <sup>1</sup>
Systolic BP (mmHg)			
24 h	125.65 $\pm$ 14.47	116.17 $\pm$ 8.73	< 0.001
Awake	127.80 $\pm$ 13.57	118.79 $\pm$ 9.02	< 0.001
Sleep	117.11 $\pm$ 17.03	105.64 $\pm$ 11.83	0.001
Diastolic BP (mmHg)			
24 h	74.80 $\pm$ 7.92	72.36 $\pm$ 6.21	0.138
Awake	76.65 $\pm$ 7.49	74.46 $\pm$ 6.23	0.178
Sleep	68.58 $\pm$ 9.51	64.64 $\pm$ 7.04	0.046
Standard deviation of BP			
Awake systolic BP	14.52 $\pm$ 4.04	12.29 $\pm$ 3.15	0.008
Awake diastolic BP	11.12 $\pm$ 3.65	9.25 $\pm$ 1.95	0.002
Sleep systolic BP	13.14 $\pm$ 5.38	9.29 $\pm$ 4.41	0.002
Sleep diastolic BP	10.21 $\pm$ 2.53	8.41 $\pm$ 3.34	0.031
Pulse pressure			
24 h	50.72 $\pm$ 9.52	43.72 $\pm$ 5.20	< 0.001
Awake	51.14 $\pm$ 9.26	44.51 $\pm$ 5.55	< 0.001
Sleep	48.57 $\pm$ 10.77	40.95 $\pm$ 7.59	0.001

<sup>1</sup>t-test. BP: Blood pressure.

mean systolic and diastolic BPs during 24 h, daytime, nighttime, early in the morning, as well as systolic and diastolic BP variability. The results showed significantly higher values of systolic BP and pulse pressure in the reflux group than in the control group (Table 9).

## DISCUSSION

The aim of this study was to assess the role of autonomic system impairment in patients with GERD. Several studies have outlined that parasympathetic dysfunction is highly prevalent in patients with GERD. Esophageal stimulation by either electrical, mechanical, or chemical stimuli increases the vagal modulation of cardiac function, as evidenced by the significant increase in HF of HRV<sup>[8,12]</sup>. The principal mechanism of gastroesophageal reflux is mediated through afferent stimuli from the gastric fundus to the sensory nucleus in the medulla and then through the efferent signals for transient lower esophageal sphincter relaxation. The observed autonomic dysfunction is supposed to cause intrinsic inhibitory reflex disturbances, abnormal fundal accommodation and gastric emptying, and consequently, an increased number of transient lower esophageal sphincter relaxations<sup>[13]</sup>. Some reports also found a decreased sympathetic function or a generalized autonomic decline in patients with GERD<sup>[13,14]</sup>. Campo *et al.*<sup>[13]</sup> outlined that there is some evidence for a slightly decreased sympathetic function in patients with GERD that is inversely correlated with total time reflux. However, decreased sympathetic function may cause dysfunction of intrinsic inhibitory control with increased transient spontaneous lower esophageal sphincter relaxations, resulting in GERD.

In this investigation, parasympathetic dysfunction was observed in about 79% of patients with gastroesophageal reflux, in which about 42% had irreparable

parasympathetic damage. Both parasympathetic and sympathetic dysfunctions have been noted in 59% of GERD individuals. The existence of abnormal vagal function in 40% of examined patients raises the possibility that vagal dysfunction is important in the genesis of gastroesophageal reflux<sup>[15]</sup>.

HRV analysis was used as a noninvasive method of assessing sympathetic-parasympathetic activities<sup>[16]</sup>. HRV with continuous ECG monitoring shows that stimulation of the esophagus by acid can alter the balance between vagal and sympathetic activity and trigger dysrhythmias. Finally, there is evidence that chronic GERD may induce an autoimmune response that contributes to cardiac dysrhythmias, especially atrial fibrillation<sup>[17]</sup>. As a confirmation of their statement, in this clinical study, all analyzed parameters of short-term analysis of RR variability had significantly lower values in GERD patients than in the control group.

Reflux disease of the esophagus occasionally leads to release of inflammatory mediators, which may affect the atrial myocardium and other elements of the cardiac conduction pathways. Inflammation of the esophageal mucosa affects local receptors that may induce afferent-efferent reflex mechanisms of the cardiac rhythm, which can lead to secondary stimulation of the vagal nerves inducing the cardiac dysrhythmias<sup>[18]</sup>. Propagation of the local inflammatory process through the esophageal wall may also cause local pericarditis or atrial myocarditis<sup>[19]</sup>.

Other reports, however, have suggested a strong association between esophageal acid exposure and neurocardiac dysfunction in patients with reflux symptomatology. It was suggested that the treatment of GERD simultaneously benefits the impaired cardiac function<sup>[20]</sup>. Disturbances in autonomic nervous system activity, such as decreased vagal activity, could lead to reduce myogenic control of the lower esophageal sphincter, favor lower esophageal sphincter relaxation, and thus probably increase the frequency of transient relaxations of the lower esophageal sphincter<sup>[21]</sup>.

GERD plays a role in the etiology of asthma, chronic bronchitis, aspiration pneumonia, bronchiectasis, and interstitial lung fibrosis<sup>[22,23]</sup>. Initial episodes of reflux may induce acute esophageal injury resulting in reduced lower esophageal sphincter pressure, delayed acid clearing, and exacerbated reflux. Sensitization of the pulmonary tree may cause the airways to become reactive to other stimuli resulting in bronchospasm through a vagal mechanism<sup>[24]</sup>. Amarasiri *et al.*<sup>[9]</sup> showed that asthmatics with mild, clinically stable asthma have peristaltic dysfunction and increased gastroesophageal reflux, and the individuals with more severe GERD symptoms had pronounced peristaltic esophageal dysfunction. Also, the same authors claimed that asthmatic patients demonstrated a vagal hyper-reactivity rather than a vagal hypofunction. On the other hand, some investigators reported that in GERD patients, there is no correlation between autonomic function and esophageal motility or



esophageal acid exposure<sup>[25]</sup>.

In conclusion, patients with GERD have distortion of both components of autonomic nervous system, sympathetic and parasympathetic. The impairment of parasympathetic function seems to be more congruent to GERD and it may be the result of vagal fiber damage. The mechanism of impairment of parasympathetic function of the patients with GERD is not completely clear, but in all autonomic neuropathies, the first stage of dysfunction is damage of parasympathetic neurons, possibly because the general function of the autonomic nervous system depends on vagal activity.

Further research will include additional patients and study designs that include the use of medications for autonomic function modulation and assessment of the medication effect on GERD and cardiac symptoms. As chronic inflammation, such as that resulting from *H. pylori* inflammation is a cause of autonomic dysfunction, future studies will analyze autonomic function in patients treated with commercially available GERD medications.

## COMMENTS

### Background

Gastroesophageal reflux disease (GERD) is one of the most common digestive diseases in the Western world. The main clinical implication of this study is to treat patients according to the type of autonomic pattern and adjustment of autonomic function. The authors hypothesized that autonomic function in GERD patients differs significantly from healthy volunteers.

### Research frontiers

This study assesses autonomic nervous system function in patients diagnosed with GERD and in healthy volunteers using complete testing of the autonomic nervous system.

### Innovations and breakthroughs

The protocol of investigation included complete testing of the autonomic nervous system, 24-h Holter ECG and ambulatory blood pressure monitoring. All of these tests are noninvasive, simple to perform, and provide a wide range of results.

### Applications

According to the results showing that autonomic dysfunction occurs more frequently in patients with diagnosis of GERD, the authors hypothesized that medications for autonomic function modulation may improve GERD symptoms. Further research will include assessment of the effect of GERD medications on autonomic function and cardiac symptoms.

### Peer-review

In this article, Milovanovic *et al* present the assessment of autonomic nervous function in patients with diagnosis of GERD. This paper shows that patients with GERD have distortion of both components of the autonomic nervous system, but that the impairment of parasympathetic function seems to be more congruent to GERD. This is an interesting report for the clinical practice.

## REFERENCES

- 1 Pleyer C, Bittner H, Locke GR, Choung RS, Zinsmeister AR, Schleck CD, Herrick LM, Talley NJ. Overdiagnosis of gastroesophageal reflux disease and underdiagnosis of functional dyspepsia in a USA community. *Neurogastroenterol Motil* 2014; **26**: 1163-1171 [PMID: 24916517 DOI: 10.1111/nmo.12377]
- 2 Vela MF, Kramer JR, Richardson PA, Dodge R, El-Serag HB. Poor sleep quality and obstructive sleep apnea in patients with GERD and Barrett's esophagus. *Neurogastroenterol Motil* 2014; **26**: 346-352 [PMID: 24460751 DOI: 10.1111/nmo.12265]
- 3 Parasa S, Sharma P. Complications of gastro-oesophageal reflux disease. *Best Pract Res Clin Gastroenterol* 2013; **27**: 433-442 [PMID: 23998980 DOI: 10.1016/j.bpg.2013.07.002]
- 4 Bello B, Zoccali M, Gullo R, Allaix ME, Herbella FA, Gasparaitis A, Patti MG. Gastroesophageal reflux disease and antireflux surgery-what is the proper preoperative work-up? *J Gastrointest Surg* 2013; **17**: 14-20; discussion p. 20 [PMID: 23090280 DOI: 10.1007/s11605-012-2057-5]
- 5 Rayner CK, Horowitz M. Physiology of the ageing gut. *Curr Opin Clin Nutr Metab Care* 2013; **16**: 33-38 [PMID: 23095947 DOI: 10.1097/MCO.0b013e32835acaf4]
- 6 Altomare A, Guarino MP, Cocca S, Emerenziani S, Cicala M. Gastroesophageal reflux disease: Update on inflammation and symptom perception. *World J Gastroenterol* 2013; **19**: 6523-6528 [PMID: 24151376 DOI: 10.3748/wjg.v19.i39.6523]
- 7 Patcharatrakul T, Gonlachanvit S. Gastroesophageal reflux symptoms in typical and atypical GERD: roles of gastroesophageal acid refluxes and esophageal motility. *J Gastroenterol Hepatol* 2014; **29**: 284-290 [PMID: 23926926 DOI: 10.1111/jgh.12347]
- 8 Lee YC, Wang HP, Lin LY, Chuang KJ, Chiu HM, Wu MS, Chen MF, Lin JT. Circadian change of cardiac autonomic function in correlation with intra-esophageal pH. *J Gastroenterol Hepatol* 2006; **21**: 1302-1308 [PMID: 16872314 DOI: 10.1111/j.1440-1746.2006.04147.x]
- 9 Amarasiri DL, Pathmeswaran A, de Silva HJ, Ranasinha CD. Response of the airways and autonomic nervous system to acid perfusion of the esophagus in patients with asthma: a laboratory study. *BMC Pulm Med* 2013; **13**: 33 [PMID: 23724936 DOI: 10.1186/1471-2466-13-33]
- 10 Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J (Clin Res Ed)* 1982; **285**: 916-918 [PMID: 6811067 DOI: 10.1136/bmj.285.6346.916]
- 11 Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; **93**: 1043-1065 [PMID: 8598068 DOI: 10.1161/01.CIR.93.5.1043]
- 12 Dobrek L, Nowakowski M, Mazur M, Herman RM, Thor PJ. Disturbances of the parasympathetic branch of the autonomic nervous system in patients with gastroesophageal reflux disease (GERD) estimated by short-term heart rate variability recordings. *J Physiol Pharmacol* 2004; **55** Suppl 2: 77-90 [PMID: 15608363]
- 13 Campo SM, Capria A, Antonucci F, Martino G, Ciamei A, Rossini PM, Bologna E, Cannata D. Decreased sympathetic inhibition in gastroesophageal reflux disease. *Clin Auton Res* 2001; **11**: 45-51 [PMID: 11503951 DOI: 10.1007/BF02317802]
- 14 Ciccaglione AF, Grossi L, Cappello G, Malatesta MG, Ferri A, Toracchio S, Marzio L. Effect of hyoscine N-butylbromide on gastroesophageal reflux in normal subjects and patients with gastroesophageal reflux disease. *Am J Gastroenterol* 2001; **96**: 2306-2311 [PMID: 11519531 DOI: 10.1111/j.1572-0241.2001.04034.x]
- 15 Chakraborty TK, Ogilvie AL, Heading RC, Ewing DJ. Abnormal cardiovascular reflexes in patients with gastro-oesophageal reflux. *Gut* 1989; **30**: 46-49 [PMID: 2920926 DOI: 10.1136/gut.30.1.46]
- 16 Huikuri HV, Stein PK. Heart rate variability in risk stratification of cardiac patients. *Prog Cardiovasc Dis* 2013; **56**: 153-159 [PMID: 24215747 DOI: 10.1016/j.pcad.2013.07.003]
- 17 Velagapudi P, Turagam MK, Leal MA, Kocheril AG. Atrial fibrillation and acid reflux disease. *Clin Cardiol* 2012; **35**: 180-186 [PMID: 22318757 DOI: 10.1002/clc.21969]
- 18 Rieder F, Cheng L, Harnett KM, Chak A, Cooper GS, Isenberg G, Ray M, Katz JA, Catanzaro A, O'Shea R, Post AB, Wong R, Sivak MV, McCormick T, Phillips M, West GA, Willis JE, Biancani P, Fiocchi C. Gastroesophageal reflux disease-associated esophagitis induces endogenous cytokine production leading to motor abnormalities. *Gastroenterology* 2007; **132**: 154-165 [PMID: 17241868 DOI: 10.1053/j.gastro.2006.10.009]
- 19 Newton M, Kamm MA, Soediono PO, Milner P, Burnham WR, Burnstock G. Oesophageal epithelial innervation in health and

- reflux oesophagitis. *Gut* 1999; **44**: 317-322 [PMID: 10026314 DOI: 10.1136/gut.44.3.317]
- 20 **Cuomo R**, De Giorgi F, Adinolfi L, Sarnelli G, Loffredo F, Efficie E, Verde C, Savarese MF, Usai P, Budillon G. Oesophageal acid exposure and altered neurocardiac function in patients with GERD and idiopathic cardiac dysrhythmias. *Aliment Pharmacol Ther* 2006; **24**: 361-370 [PMID: 16842463 DOI: 10.1111/j.1365-2036.2006.02987.x]
  - 21 **Tougas G**, Spaziani R, Hollerbach S, Djuric V, Pang C, Upton AR, Fallen EL, Kamath MV. Cardiac autonomic function and oesophageal acid sensitivity in patients with non-cardiac chest pain. *Gut* 2001; **49**: 706-712 [PMID: 11600476 DOI: 10.1136/gut.49.5.706]
  - 22 **Ozaydin I**, Annakkaya AN, Ozaydin C, Aydin M. Effects of cruroraphy and laparoscopic Nissen fundoplication procedures on pulmonary function tests in gastroesophageal reflux patients. *Int J Clin Exp Med* 2014; **7**: 431-434 [PMID: 24600501]
  - 23 **Kantar A**, Bernardini R, Paravati F, Minasi D, Sacco O. Chronic cough in preschool children. *Early Hum Dev* 2013; **89** Suppl 3: S19-S24 [PMID: 24008117 DOI: 10.1016/j.earlhumdev.2013.07.018]
  - 24 **Havemann BD**, Henderson CA, El-Serag HB. The association between gastro-oesophageal reflux disease and asthma: a systematic review. *Gut* 2007; **56**: 1654-1664 [PMID: 17682001 DOI: 10.1136/gut.2007.122465]
  - 25 **Cunningham KM**, Horowitz M, Riddell PS, Maddern GJ, Myers JC, Holloway RH, Wishart JM, Jamieson GG. Relations among autonomic nerve dysfunction, oesophageal motility, and gastric emptying in gastro-oesophageal reflux disease. *Gut* 1991; **32**: 1436-1440 [PMID: 1773945 DOI: 10.1136/gut.32.12.1436]

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## Observational Study

# Metabolic surgery and intestinal gene expression: Digestive tract and diabetes evolution considerations

Marcos Ricardo da Silva Rodrigues, Marco Aurelio Santo, Giovani Marino Favero, Elaine Cristina Vieira, Roberto Ferreira Artoni, Viviane Nogaroto, Egberto Gaspar de Moura, Patricia Lisboa, Fabio Quirillo Milleo

Marcos Ricardo da Silva Rodrigues, Marco Aurelio Santo, Giovani Marino Favero, Elaine Cristina Vieira, Roberto Ferreira Artoni, Viviane Nogaroto, Egberto Gaspar de Moura, Patricia Lisboa, Fabio Quirillo Milleo, Laboratório Multidisciplinar da Universidade Estadual de Ponta Grossa-Campus Uvaranas, Avenida General Carlos Cavalcanti, CEP 84030-900 Ponta Grossa, Paraná, Brazil

**Author contributions:** Rodrigues MRS designed the research and wrote the paper; Milleo FQ designed the research, conducted the surgical procedures and collected the data; Vieira EC designed the research, analyzed the data and wrote the paper; Santo MA conducted the surgical procedures and reviewed the paper; Favero GM analyzed data, conducted laboratorial experiments and reviewed the paper; Nogaroto V, Artoni RF, Moura EG and Lisboa P conducted the laboratorial experiments and reviewed the paper.

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**Correspondence to:** Fabio Quirillo Milleo, MD, PhD, Laboratório Multidisciplinar da Universidade Estadual de Ponta Grossa-Campus Uvaranas, Avenida General Carlos Cavalcanti, 474, CEP 84030-900 Ponta Grossa, Paraná, Brazil. [fqmilleo58@gmail.com](mailto:fqmilleo58@gmail.com)  
Telephone: +55-42-32229444

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## Abstract

**AIM:** To investigate the effects of bariatric surgery on metabolic parameters, incretin hormone secretion, and duodenal and ileal mucosal gene expression.

**METHODS:** Nine patients with type 2 diabetes mellitus (T2DM), chronic serum hyperglycemia for more than 2 years, and a body mass index (BMI) of 30-35 kg/m<sup>2</sup> underwent metabolic surgery sleeve gastrectomy with transit bipartition between May 2011 and December 2011. Blood samples were collected pre and 3, 6 and 12 mo postsurgery. Duodenal and ileal mucosa samples were collected pre- and 3 mo postsurgery. Pre- and postoperative blood samples were collected in the fasting state before ingestion of a standard meal (520 kcal) and again 30, 60, 90, and 120 min after the meal

to determine hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) levels and the lipid profile, which consisted of triglyceride and total cholesterol levels. Intestinal gene expression of *p53* and transforming growth factor (TGF)- $\beta$  was analyzed using quantitative reverse-transcription PCR. Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) were quantified using the enzyme-linked immunoassay method and analyzed pre- and postoperatively. Student's *t* test or repeated measurements analysis of variance with Bonferroni corrections were performed as appropriate.

**RESULTS:** BMI values decreased by 15.7% within the initial 3 mo after surgery ( $31.29 \pm 0.73$  vs  $26.398 \pm 0.68$ ,  $P < 0.05$ ) and then stabilized at 22% at 6 mo postoperative, resulting in similar values 12 mo postoperatively ( $20\text{--}25$  kg/m<sup>2</sup>). All of the patients experienced improved T2DM, with 7 patients (78%) achieving complete remission (HbA<sub>1c</sub>  $< 6.5\%$ ), and 2 patients (22%) achieving improved diabetes (HbA<sub>1c</sub>  $< 7.0\%$  with or without the use of oral hypoglycemic agents). At 3 mo postoperatively, fasting plasma glucose had also decreased (59%) ( $269.55 \pm 18.24$  mg/dL vs  $100.77 \pm 3.13$  mg/dL,  $P < 0.05$ ) with no further significant changes at 6 or 12 mo postoperatively. In the first month postoperatively, there was a complete withdrawal of hypoglycemic medications in all patients, who were taking at least 2 hypoglycemic drugs preoperatively. GLP-1 levels significantly increased after surgery ( $149.96 \pm 31.25$  vs  $220.23 \pm 27.55$ ) ( $P < 0.05$ ), while GIP levels decreased but not significantly. *p53* gene expression significantly increased in the duodenal mucosa ( $P < 0.05$ , 2.06 fold) whereas the tumor growth factor- $\beta$  gene expression significantly increased ( $P < 0.05$ , 2.52 fold) in the ileal mucosa after surgery.

**CONCLUSION:** Metabolic surgery ameliorated diabetes in all of the patients, accompanied by increased antiproliferative intestinal gene expression in non-excluded segments of the intestine.

**Key words:** Diabetes mellitus; Hyperglycemia; Intestine; Gene expression; Hyperplasia; Bariatric surgery

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**Core tip:** This study shows an improvement in expression of antiproliferative genes of intestinal mucosa after type 2 diabetes mellitus amelioration promoted by metabolic surgery procedure. We make a link between this outcome and morphological changes in intestinal mucosa on diabetes, that occurs mainly by insufficient negative control of mucosal growth in hyperglycemia. Metabolic and bariatric surgery promotes dramatic amelioration of glucose metabolism in diabetic patients by not completely understood means. This paper highlights for the first time, the intestinal absorptive capacity as the main focus for research of diabetes amelioration after metabolic surgery procedures.

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## INTRODUCTION

It has been recently suggested that diabetes alters the gene expression of various DNA repair genes, including *p53* and transforming growth factor (TGF)- $\beta$ <sup>[1]</sup>. Both of these genes are considered to be cell division down-regulators<sup>[2]</sup>, including for normal intestinal epithelium growth<sup>[3-5]</sup>, and their expression might be altered in patients predisposed or affected by metabolic disease. It was established over 40 years ago that diabetes influences, or can be influenced by, morphological changes in the intestinal mucosa<sup>[6]</sup>. In experimental models of type 2 diabetes mellitus (T2DM), hyperplasia of the intestinal mucosa was observed in the early phases of the metabolic changes<sup>[7]</sup>. This phenomenon may be related to a transient interruption of normal cellular apoptosis<sup>[8]</sup>, indicating altered intestinal regulatory gene functions for disease development.

Rat studies have demonstrated that hypertrophic changes occur primarily in the proximal gastrointestinal (GI) tract and enhance the carbohydrate absorptive capacity of the intestinal mucosa<sup>[9]</sup>. Furthermore, in experimental models of T2DM, postprandial hyperglycemia has been observed before the development of the metabolic disease, concomitantly with the morphofunctional changes in the intestinal epithelium<sup>[7]</sup>. In humans, postprandial hyperglycemia strongly predicts the development of T2DM<sup>[10]</sup>. Interestingly, with the resolution of diabetes, the intestinal epithelium also regains normal function and trophic, although the mechanisms underlying these changes are not understood<sup>[11]</sup>.

Recently, Verdam *et al.*<sup>[12]</sup> suggested that obese patients with T2DM have significantly more intestinal mass than their non-T2DM counterparts, suggesting that the hypertrophic changes previously observed in experimental models might be a cornerstone feature for T2DM development in humans as well.

Santoro *et al.*<sup>[13]</sup> recently published the results of sleeve gastrectomy with transit bipartition (SGTB) or a partial duodenal switch, which is a metabolic surgery procedure. This innovative procedure brings the distal ileum in contact with undigested food through a gastro-ileal anastomosis, without excluding the duodenum from the passage of food; it has shown potential in resolving T2DM.

The present study aimed to investigate intestinal mucosal gene expression [*p53* and tumor growth factor- $\beta$  (TGF- $\beta$ )] before and after SGTB in class I obese [body mass index (BMI)  $30\text{--}35$  kg/m<sup>2</sup>] patients with





**Figure 1 Sleeve gastrectomy with transit bipartition.** At 180 cm proximal to the ileocecal valve, the ileum is anastomosed to the gastric antrum. The biliopancreatic segment is anastomosed to the ileum 80 cm proximal to the ileocecal valve.

T2DM and resolution of hyperglycemia after surgery. Considerations about the role of the GI tract in T2DM are also highlighted.

## MATERIALS AND METHODS

### Patients

This observational study was conducted from May to December 2011. Nine class I obese patients with T2DM who had presented with chronic serum hyperglycemia for > 2 years were enrolled and underwent SGTB. This open access procedure involved a typical sleeve gastrectomy. After the SG, the ileocecal transition is located. A single stitch is used to mark the point at the ileum located 80 cm from the ileocecal valve. The point 260 cm proximal to the ileocecal valve is then located. At this point, the intestinal segment is sectioned. The distal end is brought to the gastric antrum, and a wide laterolateral gastroileal anastomosis is created. In the following sequence, the small bowel cranial to the gastroileal anastomosis is laterally and widely anastomosed to the ileum at an 80 cm distance from the ileocecal valve (previously marked) in a lateral-lateral mode (Figure 1).

All patients provided written informed consent before undergoing the surgery. The study was approved by the Institutional Review Board at the State University of Ponta Grossa, Paraná, Brazil.

Diabetes diagnoses were based on the following American Diabetes Association<sup>[14]</sup> criteria: a fasting plasma glucose level  $\geq 126$  mg/dL (7.0 mmol/L), symptoms of diabetes plus a casual plasma glucose level  $\geq 200$  mg/dL (11.1 mmol/L), or a 2-h postload glucose level  $\geq 200$  mg/dL during a 75-g oral glucose tolerance test. The exclusion criteria included peptide C levels < 0.9 ng/dL, pregnancy, inflammatory bowel disease, drug or alcohol addiction, and psychiatric disturbances that precluded complete understanding of the surgical procedure.

Diabetes remission was defined as a hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) level < 6.5% without the use of diabetes

medications. Diabetes was considered to be improved if the patients had an HbA<sub>1c</sub> level < 7.0% with or without the use of oral hypoglycemic agents. The lipid profile consisted of fasting and postprandial triglyceride and total cholesterol levels. Clinical evolution was analyzed at 3, 6 and 12 postoperative months.

Pre- and postoperative blood samples were collected during the fasting state (8 h fasting) before the patients ingested a standard meal (520 kcal) and again 30, 60, 90, and 120 min after the meal. Blood was collected in tubes with the anticoagulant ethylenediaminetetraacetic acid (1 g/L). Plasma was separated in aliquots of 1 mL per vial and frozen at -80 °C. During the preoperative period (under deep sedation with 50 mg of propofol plus 50 µg of fentanyl), biopsy specimens of the duodenal mucosa (4 specimens, approximately 2 mm<sup>3</sup> each) were obtained using upper digestive endoscopy. Samples of the ileal mucosa (260 cm proximal to the ileocecal valve) were collected prior to the gastro-ileal anastomosis joining at the anastomosis site. After SGTB, access to the ileum and duodenum is possible during an upper endoscopy (Figure 1). Three months after surgery, mucosal samples were collected using endoscopic biopsy forceps with the patients under deep sedation with 5 mL of propofol and 1 mL of fentanyl. The trans- and post-operative tissues were promptly placed in RNAlater solution (Qiagen), with subsequent freezing at -80 °C for storage until qRT-PCR analysis.

### Quantification of gastric inhibitory polypeptide and glucagon-like peptide-1

Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the two primary incretin hormones secreted from the intestine after ingesting glucose or nutrients that stimulate insulin secretion by pancreatic  $\beta$  cells. Quantification of plasma GLP-1 and GIP was performed using the enzyme-linked immunoassay method in the range of 450 nm (Biotek EL800, Winooski, VT, United States). The peptides were quantified using commercial kits from Phoenix Pharmaceuticals, Inc. (Belmont, CA, United States), according to the manufacturer's instructions.

### RNA extraction and quantitative reverse-transcription-PCR assays

Total RNA from the duodenal and ileal samples was isolated using the IllustraRNAspin Mini RNA Isolation Kit (GE Healthcare, Buckinghamshire, United Kingdom), according to the manufacturer's instructions. Reverse transcription of 1 µg of total RNA was performed using the First-Strand cDNA Synthesis Kit (GE Healthcare, Buckinghamshire, United Kingdom), according to the manufacturer's protocol. Gene expression of the target genes was quantified with qRT-PCR in a StratageneMxPro3005P thermocycler (Agilent Technologies, Santa Clara, United States). cDNA was amplified in duplicate PCR reactions using 1× of the

**Table 1** Demographic characteristics of the patients with type 2 diabetes mellitus who underwent sleeve gastrectomy with transit bipartition ( $n = 9$ )

	mean $\pm$ SD	Range
Age (yr)	47.11 $\pm$ 7.84	30.00–53.00
Gender (men), $n$ (%)	4 (44.4)	
Gender (women), $n$ (%)	5.0 <sup>1</sup> (55.6)	
T2DM duration (yr)	5.6	3.00–11.00
Preoperative BMI (kg/m <sup>2</sup> )	31.17 $\pm$ 2.18	26.47–33.39

<sup>1</sup>Non-menopausal. BMI: Body mass index; T2DM: Type 2 diabetes mellitus.

SYBR® Green Master Mix (Stratagen, La Jolla, CA, United States). A negative control was also included for each gene amplification assay. The PCR cycling conditions were as follows: 5 min at 94 °C; 40 cycles of 15 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, with a dissociation curve. The 18S was used as an internal control. The values are expressed as the relative expression in terms of the control levels ( $2^{-\Delta\Delta Ct}$ ).

### Statistical analysis

Data are shown as the mean  $\pm$  SE. Student's  $t$  test or repeated measurements ANOVA with Bonferroni corrections were performed as appropriate, with a level of significance set at  $P < 0.05$ .

## RESULTS

### Clinical characteristics of the patients

The patients (5 women, 4 men) were similar in age (Table 1). The mean BMI was  $31.18 \pm 1.17$  kg/m<sup>2</sup>. All patients had satisfactory postoperative evaluations, with a normal diet, normal bowel movements, and no hospital readmissions.

### Sleeve gastrectomy with transit bipartition surgery leads to improvements in glucose and lipid metabolism

Complete T2DM remission was observed in 7 (78%) patients, and improved diabetes was observed in 2 (22%) patients. Before surgery, all patients were taking at least 2 hypoglycemic drugs, and 6 (66.7%) were insulin dependent. In the first postoperative month, there was a complete withdrawal of hypoglycemic medications in all patients.

There was a statistically significant decrease in BMI at 3 mo (by 15.7%) ( $31.29 \pm 0.73$  vs  $26.39 \pm 0.683$ ,  $P < 0.05$ ), a further decrease at 6 postoperative months (22.2%), and a decrease of 22.8% at 12 postoperative months compared with the pre-operative values (Figure 2A). At 3 postoperative months, the fasting plasma glucose had also decreased (59%) ( $269.55 \pm 18.24$  mg/dL vs  $100.77 \pm 3.13$  mg/dL,  $P < 0.05$ ); there were no further changes in the fasting glucose levels at 6 or 12 postoperative months surgery (Figure 2B). Three months postoperatively, postprandial glycaemia had decreased by 55% ( $334.88 \pm 19.24$  mg/dL vs

$150.75 \pm 6.84$  mg/dL,  $P < 0.05$ ), and this level was maintained at 6 and 12 postoperative months (Figure 2C). The HbA<sub>1c</sub> levels before surgery were 10.7%, and they decreased to 6.8% at 3 postoperative months ( $10.66\% \pm 0.59\%$  vs  $6.78\% \pm 0.10\%$ ,  $P < 0.05$ ) (Figure 2D). Serum triglyceride and cholesterol levels also decreased 3 mo after surgery ( $325.11 \pm 60.29$  mg/dL vs  $144.25 \pm 10.11$  mg/dL and  $214.33 \pm 10.26$  mg/dL vs  $139.12 \pm 7.63$  mg/dL (55% and 27%, respectively) and remained at this level at 6 and 12 postoperative months (Figure 2E and F).

### Sleeve gastrectomy with transit bipartition surgery leads to increased glucagon-like peptide-1 levels in patients with type 2 diabetes mellitus

Eight patients completed the fasting and postprandial blood sample collection protocol. There was a significant increase in the postoperative GLP-1 levels of each patient [compared with the preoperative levels ( $P < 0.05$ ; Figure 3A)] when analyzed with the area under the curve (AUC) analysis. There was a slight decrease in the GIP concentrations, but this decrease was not significant (Figure 3B).

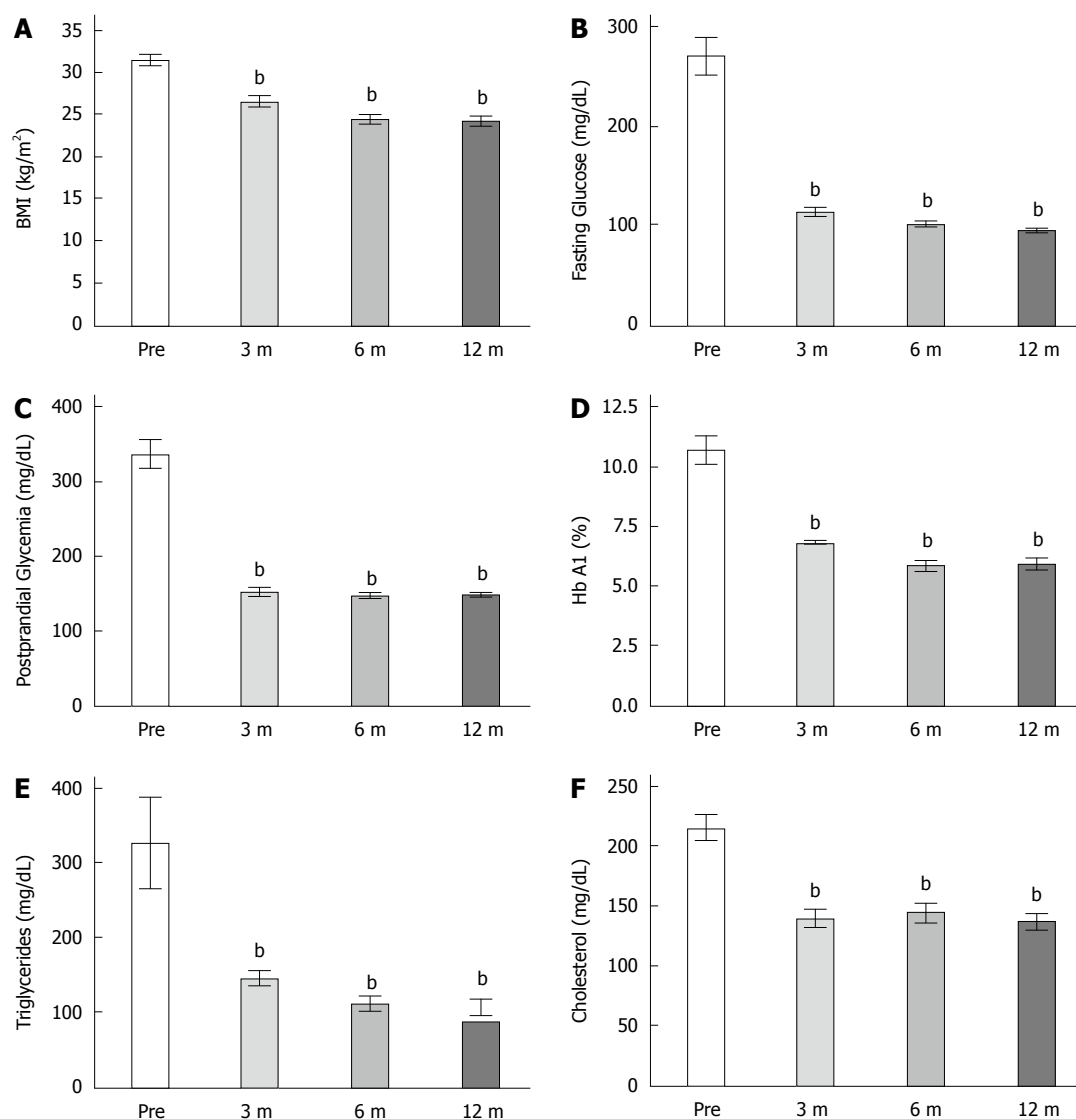
### Sleeve gastrectomy with transit bipartition surgery leads to an increase in duodenal p53 gene expression and ileal-transforming growth factor-beta expression

Three months after surgery, the *p53* expression significantly increased in the duodenum ( $P < 0.05$ ; Figure 4A), and there was a trend of increased expression in the ileal mucosa ( $P = 0.1$ ; Figure 4B). The *TGF- $\beta$*  expression levels did not change in the duodenum (Figure 5A). However, *TGF- $\beta$*  expression significantly increased in the ileum (Figure 5B) at 3 postoperative months.

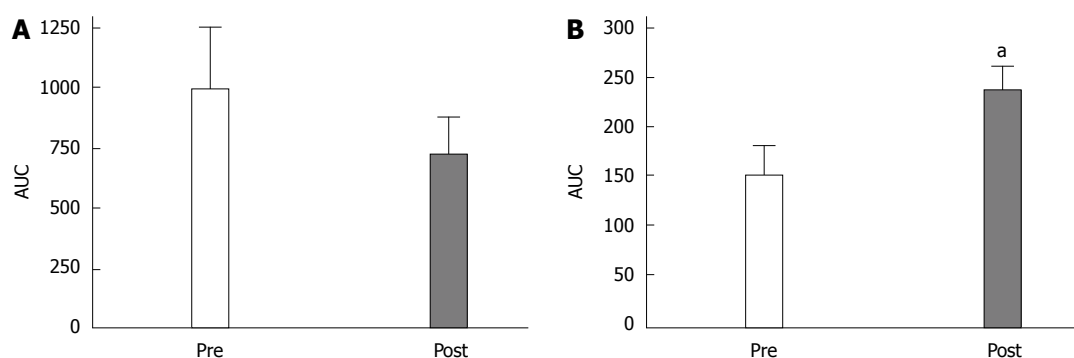
## DISCUSSION

The present study demonstrated that SGTB improves glucose and lipid metabolism in patients with T2DM and leads to complete T2DM remission in the majority of patients. In addition, there was increased expression of the anti-proliferative intestinal genes after metabolic improvement, suggesting that these genes play a possible role in the intestinal mucosa with T2DM progression.

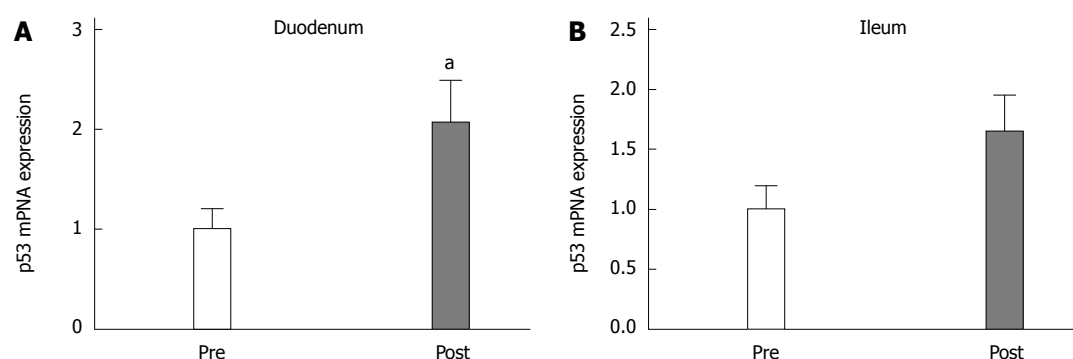
Surgeries that are performed in the digestive tract, particularly bariatric or metabolic surgical procedures, are reportedly effective not only in treating obesity<sup>[15]</sup> but also for T2DM<sup>[16–18]</sup>, although the specific mechanisms underlying the latter finding are not completely understood. Two different theories have been posited, namely the foregut and hindgut hypothesis<sup>[19]</sup>. Research has also been conducted to understand how improvements in T2DM occur even before weight loss following these surgeries<sup>[20]</sup>. The effects of GI surgery on metabolic processes reinforce the importance of the GI tract in T2DM development.



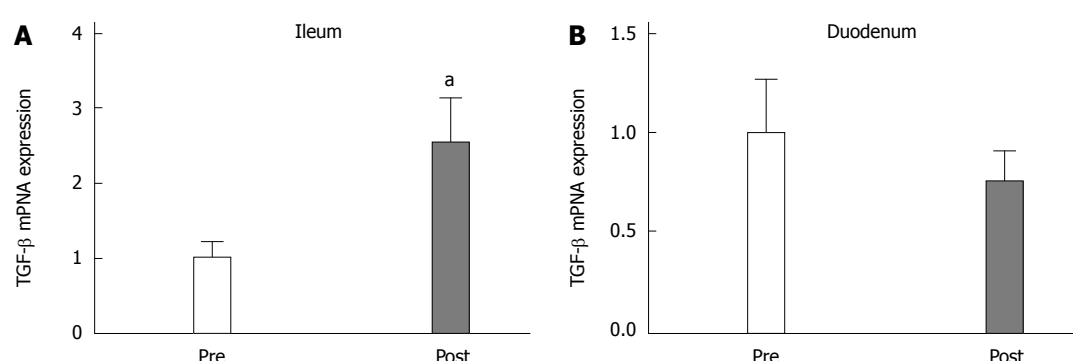
**Figure 2** Changes in metabolic parameters following sleeve gastrectomy with transit bipartition in patients with type 2 diabetes mellitus. A: Body mass index; B: Fasting glycemia; C: Postprandial glycemia; D: Hemoglobin A1c; E: Plasma triglycerides; F: Plasma total cholesterol. The gray bars show data before the sleeve gastrectomy with transit bipartition (SGTB), and the black bars show data after the surgery ( $n = 9$ ). Data are expressed as the mean  $\pm$  SEM. <sup>b</sup> $P < 0.01$  vs Pre. Pre: Pre-surgery; 3 m: 3 mo post-surgery; 6 m: 6 mo post-surgery; 12 m: 12 mo post-surgery.



**Figure 3** Changes in gastric inhibitory polypeptide and glucagon-like peptide-1 levels following sleeve gastrectomy with transit bipartition in patients with type 2 diabetes mellitus. A: Area under the curve (AUC) for the plasma glucagon-like peptide-1 levels pre- and post-surgery while fasting and after ingesting a standard meal (520 kcal). Blood samples were collected 30, 60, 90, and 120 min after the meal; B: Sum of the individual AUCs of plasma gastric inhibitory polypeptide levels pre- and post-surgery while fasting and after ingesting a standard meal (520 kcal). Blood samples were collected 30, 60, 90, and 120 min after the meal. Data are expressed as the mean  $\pm$  SE. <sup>a</sup> $P < 0.05$  vs Pre. Pre: Pre-surgery; Post: Post-surgery.



**Figure 4** Gene expression profile of p53 in the intestinal mucosa before and after sleeve gastrectomy with transit bipartition in patients with type 2 diabetes mellitus. A: p53 mRNA expression in the duodenum; B: p53 mRNA expression in the ileum. The gray bars show data before the gastrectomy with transit bipartition, and the black bars show data 3 mo postoperatively. Data are expressed as the mean  $\pm$  SE. <sup>a</sup> $P < 0.05$  vs Pre. Pre: Pre-surgery; Post: Post-surgery.



**Figure 5** Gene expression profile of transforming growth factor tumor growth factor- $\beta$  in the intestinal mucosa before and after sleeve gastrectomy with transit bipartition in patients with type 2 diabetes mellitus. A: Tumor growth factor- $\beta$  (TGF- $\beta$ ) mRNA expression in the duodenum; B: TGF- $\beta$  mRNA expression in the ileum. The gray bars show data before the sleeve gastrectomy with transit bipartition (SGTB), and the black bars show data 3 mo postoperatively. Data are expressed as the mean  $\pm$  SE. <sup>a</sup> $P < 0.05$  vs Pre. Pre: Pre-surgery; Post: Post-surgery.

In morbidly obese patients, bariatric surgery results in up to a 70% loss of excess weight<sup>[21]</sup>. In the present study, the mean postoperative BMI was  $< 25 \text{ kg/m}^2$ , a result that we considered to be attributed to a 100% loss of excess weight. In addition, we observed significant improvements in other metabolic parameters, such as triglyceride and total cholesterol levels. Different bariatric surgery procedures promote amelioration of dyslipidemia, potentially because of the ingestion of less food and the improved insulin resistance<sup>[22]</sup>.

There were significant improvements in fasting and postprandial glycemia after surgery. Pancreatic beta cell behavior depends on serum glucose levels, and phasic or chronic hyperglycemia may lead to desensitization, exhaustion, and apoptosis of the beta cells (glucotoxicity)<sup>[23,24]</sup>. Loss of beta cell mass is a cornerstone feature for the development of chronic hyperglycemia<sup>[25]</sup>. Hyperabsorption in the epithelium in diabetes patients promotes postprandial hyperglycemia, an event that is potentially toxic to beta cell mass. Studies in different experimental models have demonstrated improvements in beta cell function after metabolic surgery<sup>[26]</sup>, and this effect might be a result not only from improved incretin hormone function but also from less glucotoxicity

in the beta cells, which are frequently exposed to postprandial hyperglycemia caused by the consumption of high glycemic foods. In the present group of T2DM patients, lower levels of postprandial glycemia might have been promoted by partial or complete deviation of hyperplastic and hyper absorption in the proximal segments of the intestine; however, further research is required to clarify this hypothesis.

GIP and GLP-1 are the two primary incretin hormones secreted from the intestine, and T2DM patients generally have lower levels of GLP-1<sup>[27]</sup>. The post-operative improvement in T2DM in the present study, at least partially, might be a result of enhanced GLP-1. Schirra *et al.*<sup>[28]</sup> have demonstrated that the load of ingested carbohydrates must overcome the absorptive capacity of the proximal intestine to enhance the GLP-1 secretion by mucosal L cells, which are primarily located in the distal small intestine. The intestinal hyperabsorption in T2DM patients might prevent food from reaching these distal segments of the intestine. When direct contact of the duodenal and proximal jejunal epithelium with food is avoided through an endoluminal plastic prostheses (EndoBarrier® GI Dynamics), improvements are observed in insulin resistance, fasting glycemic levels, and postprandial glycemic levels<sup>[29,30]</sup>; these outcomes are similar to



those obtained from bariatric surgery. These results, in addition to increased GLP-1 secretion, are also observed in glucose intolerant patients through the inhibition of disaccharidase alpha by orally administered acarbose, which partially prevents the absorption of carbohydrates in the proximal intestine<sup>[31]</sup>. Similarly, studies have shown that metabolic surgery improves GLP-1 secretion, mainly by stimulating the distal intestine with undigested food<sup>[32,33]</sup>, and SGTB promotes stimulation of the ileum by ingested food through a gastroileal anastomosis. Isolated sleeve gastrectomy without transit bipartition also elevates postprandial GLP-1 levels and improves glucose metabolism, although it does not alter the intestinal flow. After sleeve gastrectomy, gastric emptying is accelerated<sup>[34]</sup>, and this acceleration might prevent complete absorption of food in the proximal intestine. The proximal intestine absorption capacity and flow intensity (amount of substrate/time) are important tasks for distal intestine stimulations and GLP-1 secretion<sup>[28]</sup>.

In contrast, the GIP levels tended to decrease in the postoperative period in the present study. In T2DM patients, there is a resistance to GIP action<sup>[35]</sup>, and surgery might influence its function. Højberg *et al.*<sup>[36]</sup>, while studying rats, demonstrated that resolution of hyperglycemia restores GIP function by stimulating the expression of GIP receptors in pancreatic tissue. However, there is considerable variation in the reported results regarding GIP secretion after bariatric and metabolic surgery; thus, the relationship remains unclear<sup>[37]</sup>.

Comparing two groups of patients with the same weight loss (9.5 kg) achieved through bariatric surgery and a hypocaloric diet, Laferrère<sup>[38]</sup> shows better metabolic results (incretin secretion, postprandial glucose levels) in operated patients instead of having the same amount of fat tissue loss. This result reinforces the importance of metabolic surgery or the rearrangement of the gastrointestinal tract on metabolic profile amelioration.

According to Osborne *et al.*<sup>[11]</sup>, diabetes promotes morphological changes in the intestinal mucosa, and hyperglycemia correction reverses the hyperplasia. The mechanism underlying hyperplasia in T2DM appears to differ from that of other models of intestinal hyperplasia. In rats subjected to Roux-en-Y gastric bypass, the hyperplasia was only observed on the roux limb and not on the biliopancreatic limb, suggesting that overstimulation of the distal segments of the bowel by undigested food, previously poorly stimulated, might be the primary mechanism for the hyperplasia<sup>[39]</sup>. In T2DM models, the hyperplasia occurs as an early step in the evolution of diabetes in non-surgically modified intestines, suggesting different mechanisms of hyperplasia induction<sup>[7]</sup>. Furthermore, Noda *et al.*<sup>[8]</sup> predicted that overfeeding is a necessary condition to intestinal hypertrophy, a serious concern in modern society<sup>[40]</sup>, particularly with the increased consumption of high glycemic

index foods, which parallels the increase in T2DM incidence<sup>[41]</sup>. Food rich in carbohydrates is the primary stimulant for the growth of intestinal cells<sup>[42]</sup>. The inability of the anti-proliferative genes in the intestinal mucosa to counteract the stimulation for growth promoted by food ingestion could be one factor in the pathophysiology of intestinal hypertrophy in diabetes. *p53* and *TGF-β* are important down-regulators of epithelial growth<sup>[3-5]</sup> and are influenced by diabetes<sup>[10]</sup>. Alvarado-Vásquez *et al.*<sup>[43]</sup> have reported lower levels of *p53* in the endothelial cells of the umbilical cords of mothers with a strong family predisposition for T2DM. Interestingly, a cohort study of 55000 subjects demonstrated an association between the development of hyperglycemia and *p53* polymorphisms, leading us to suggest a possible link between the expression levels of this gene and the progression of T2DM<sup>[44]</sup>.

Few studies have analyzed the gene expression profile of intestinal epithelial cells in the presence of metabolic disease. In the present study, we analyzed the mucosal gene expression of *p53* and *TGF-β* in non-excluded segments of the intestine and at the same intestinal site, pre- and postoperatively. Both genes were down-regulated before surgery (before improvements in T2DM). Interestingly, *p53* expression was enhanced postoperatively, and this enhancement was more pronounced in the duodenum than in the ileum. At the same time, the expression of *TGF-β* was not altered in the duodenum but was significantly enhanced in the ileum. The small sample size may have resulted in these expression differences based on the intestinal site. However, lower levels of the anti-proliferative genes in T2DM (as in the preoperative state) could be explained by the direct effect of diabetes on *p53* and *TGF-β*<sup>[11]</sup>. In normal and tumor cells, *p53* regulates the energy source, and it favors phosphorylative oxidation instead of glycolytic pathways. One method by which *p53* favors phosphorylative oxidation is the down-regulation of the expression of glucose transporter (GLUT)-1 receptors<sup>[45]</sup>, which are responsible for basolateral glucose transport. *p53* also influences the expression of GLUT 3 and GLUT 4 receptors<sup>[46]</sup>. A recent report has indicated that bariatric surgery significantly enhances mucosal expression of GLUT-1 receptors<sup>[47]</sup>, thereby enhancing glucose consumption by enterocytes and acting as a primary factor for glucose homeostasis after surgery.

This study has certain limitations. One important limitation is the small sample size, as well as the lack of morphological experiments of the epithelium before and after surgery. Despite these limitations, we believe that our results will encourage future research regarding the relationship between the digestive tract and T2DM, particularly relating to higher proximal intestine absorptive capacity.

In summary, our data demonstrated that SGTB improves T2DM in a group of class I obese patients, and the metabolic improvements were accompanied

by increased expression of the anti-proliferative intestinal genes. We propose a new theory regarding the resolution of T2DM after metabolic surgery, focusing on the hyper absorption capacity of the proximal intestine.

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## COMMENTS

### Background

Bariatric surgery, by not completely understood means, promotes type 2 diabetes mellitus (T2DM) resolution. Hyperplasia of intestinal epithelium in diabetic subjects can enhance the absorptive capacity and might be a cornerstone in the evolution of diabetes. The authors highlight the differential expression of antiproliferative genes of intestinal mucosa in T2DM patients as a marker for metabolic disease development.

### Research frontiers

Gene expression is influenced by hyperglycemia. In patients predisposed to developing T2DM, underexpression of antiproliferative genes on intestinal mucosa might promote morphofunctional intestinal alterations that influence disease development. The correlation between enhanced absorptive capacity of proximal intestine and bariatric surgery capacity in promoting T2DM resolution should be an important research task.

### Innovations and breakthroughs

In this study, the authors analysis expression of antiproliferative genes on intestinal mucosa, particularly *p53* and *TGF-β*, on the hyperglycemic state and after resolving metabolic disease with metabolic surgery. The authors highlight and correlate gene expression with intestinal morphofunctional behavior as a main pathophysiology target in T2DM research.

### Applications

The study suggests that altered gene expression on intestinal mucosa might be a predictor of T2DM development. The authors discuss the role of the absorptive capacity of the proximal intestine in T2DM as a target for disease treatment, and they also propose an alternative explanation for the ability of bariatric surgery to resolve T2DM.

### Terminology

Sleeve gastrectomy with transit bipartition, a metabolic surgery procedure that involves a gastroileal anastomosis in the antrum after sleeve gastrectomy; nutrient transit is maintained in the duodenum, thereby avoiding blind loops and minimizing malabsorption. The stomach retains 2 outflow pathways. A lateral enteroanastomosis connects both segments at 80 cm proximal to the cecum.

### Peer-review

This study examines the metabolic markers before and after bariatric surgery in T2DM patients and also focuses on differential intestinal mucosal gene expression as a predictor of metabolic improvement. This study is useful, although the cohort size is too small to draw definitive conclusions.

## REFERENCES

- Ye C, Li X, Wang Y, Zhang Y, Cai M, Zhu B, Mu P, Xia X, Zhao Y, Weng J, Gao X, Wen X. Diabetes causes multiple genetic alterations and downregulates expression of DNA repair genes in the prostate. *Lab Invest* 2011; **91**: 1363-1374 [PMID: 21647090 DOI: 10.1038/labinvest.2011.87]
- Hong S, Lee HJ, Kim SJ, Hahm KB. Connection between inflammation and carcinogenesis in gastrointestinal tract: focus on TGF-beta signaling. *World J Gastroenterol* 2010; **16**: 2080-2093 [PMID: 20440848 DOI: 10.3748/wjg.v16.i17.2080]
- Li L, Li J, Rao JN, Li M, Bass BL, Wang JY. Inhibition of polyamine synthesis induces p53 gene expression but not apoptosis. *Am J Physiol* 1999; **276**: C946-C954 [PMID: 10199827]
- Li L, Rao JN, Guo X, Liu L, Santora R, Bass BL, Wang JY. Polyamine depletion stabilizes p53 resulting in inhibition of normal intestinal epithelial cell proliferation. *Am J Physiol Cell Physiol* 2001; **281**: C941-C953 [PMID: 11502571]
- Liu L, Santora R, Rao JN, Guo X, Zou T, Zhang HM, Turner DJ, Wang JY. Activation of TGF-beta-Smad signaling pathway following polyamine depletion in intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G1056-G1067 [PMID: 12855402 DOI: 10.1152/ajpgi.00151.2003]
- Schedl HP, Wilson HD. Effects of diabetes on intestinal growth in the rat. *J Exp Zool* 1971; **176**: 487-495 [PMID: 5569235 DOI: 10.1002/jez.1401760410]
- Fujita Y, Kojima H, Hidaka H, Fujimiya M, Kashiwagi A, Kikkawa R. Increased intestinal glucose absorption and postprandial hyperglycaemia at the early step of glucose intolerance in Otsuka Long-Evans Tokushima Fatty rats. *Diabetologia* 1998; **41**: 1459-1466 [PMID: 9867213 DOI: 10.1007/s001250051092]
- Noda T, Iwakiri R, Fujimoto K, Yoshida T, Utsumi H, Sakata H, Hisatomi A, Aw TY. Suppression of apoptosis is responsible for increased thickness of intestinal mucosa in streptozotocin-induced diabetic rats. *Metabolism* 2001; **50**: 259-264 [PMID: 11230775 DOI: 10.1053/meta.2001.21030]
- Adachi T, Mori C, Sakurai K, Shihara N, Tsuda K, Yasuda K. Morphological changes and increased sucrase and isomaltase activity in small intestines of insulin-deficient and type 2 diabetic rats. *Endocr J* 2003; **50**: 271-279 [PMID: 12940455 DOI: 10.1507/endocrj.50.271]
- Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Mott DM, Bennett PH. The natural history of impaired glucose tolerance in the Pima Indians. *N Engl J Med* 1988; **319**: 1500-1506 [PMID: 3054559 DOI: 10.1056/NEJM198812083192302]
- Osborne DL, Payne SC, Russ RD, Tobin B. Comparison of therapeutic regimens in the amelioration of alterations in rat gastrointestinal mucosal DNA, RNA and protein induced by streptozotocin diabetes mellitus. *Life Sci* 2000; **66**: 2405-2417 [PMID: 10864102]
- Verdam FJ, Greve JW, Roosta S, van Eijk H, Bouvy N, Buurman WA, Rensen SS. Small intestinal alterations in severely obese hyperglycemic subjects. *J Clin Endocrinol Metab* 2011; **96**: E379-E383 [PMID: 21084402 DOI: 10.1210/jc.2010-1333]
- Santoro S, Castro LC, Velhote MC, Malzoni CE, Klajner S, Castro LP, Lacombe A, Santo MA. Sleeve gastrectomy with transit bipartition: a potent intervention for metabolic syndrome and obesity. *Ann Surg* 2012; **256**: 104-110 [PMID: 22609843 DOI: 10.1097/SLA.0b013e31825370c0]
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2012; **35** Suppl 1: S64-S71 [PMID: 22187472 DOI: 10.2337/dc12-s064]
- Christou NV, Sampalis JS, Liberman M, Look D, Auger S, McLean AP, MacLean LD. Surgery decreases long-term mortality, morbidity, and health care use in morbidly obese patients. *Ann Surg* 2004; **240**: 416-423; discussion 423-424 [PMID: 15319713 DOI: 10.1097/01.sla.0000137343.63376.19]
- Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, Thomas S, Abood B, Nissen SE, Bhatt DL. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 2012; **366**: 1567-1576 [PMID: 22449319 DOI: 10.1056/NEJMoa1200225]
- Sasaki A, Wakabayashi G, Yonei Y. Current status of bariatric surgery in Japan and effectiveness in obesity and diabetes. *J Gastroenterol* 2014; **49**: 57-63 [PMID: 23595611 DOI: 10.1007/s00535-013-0802-5]
- Pok EH, Lee WJ. Gastrointestinal metabolic surgery for the treatment of type 2 diabetes mellitus. *World J Gastroenterol* 2014; **20**: 14315-14328 [PMID: 25339819 DOI: 10.3748/wjg.v20.i39.14315]
- Rubino F, Gagner M, Gentileschi P, Kini S, Fukuyama S, Feng J, Diamond E. The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose

- metabolism. *Ann Surg* 2004; **240**: 236-242 [PMID: 15273546 DOI: 10.1097/01.sla.0000133117.12646.48]
- 20 **Liu S**, Zhang G, Wang L, Sun D, Chen W, Yan Z, Sun Y, Hu S. The entire small intestine mediates the changes in glucose homeostasis after intestinal surgery in Goto-Kakizaki rats. *Ann Surg* 2012; **256**: 1049-1058 [PMID: 23001083 DOI: 10.1097/SLA.0b013e31826c3866]
  - 21 **Buchwald H**, Estok R, Fahrbach K, Banel D, Jensen MD, Pories WJ, Bantle JP, Sledge I. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009; **122**: 248-256.e5 [PMID: 19272486 DOI: 10.1016/j.amjmed.2008.09.041]
  - 22 **Kaul A**, Sharma J. Impact of bariatric surgery on comorbidities. *Surg Clin North Am* 2011; **91**: 1295-312, ix [PMID: 22054155 DOI: 10.1016/j.suc.2011.08.003]
  - 23 **Donath MY**, Halban PA. Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications. *Diabetologia* 2004; **47**: 581-589 [PMID: 14767595 DOI: 10.1007/s00125-004-1336-4]
  - 24 **Poitout V**, Robertson RP. Minireview: Secondary beta-cell failure in type 2 diabetes--a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 2002; **143**: 339-342 [PMID: 11796484 DOI: 10.1210/endo.143.2.8623]
  - 25 **Nolan CJ**, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. *Lancet* 2011; **378**: 169-181 [PMID: 21705072 DOI: 10.1016/S0140-6736(11)60614-4]
  - 26 **Bradley D**, Magkos F, Klein S. Effects of bariatric surgery on glucose homeostasis and type 2 diabetes. *Gastroenterology* 2012; **143**: 897-912 [PMID: 22885332 DOI: 10.1053/j.gastro.2012.07.114]
  - 27 **Vilsbøll T**, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001; **50**: 609-613 [PMID: 11246881 DOI: 10.2337/diabetes.50.3.609]
  - 28 **Schirra J**, Katschinski M, Weidmann C, Schäfer T, Wank U, Arnold R, Göke B. Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest* 1996; **97**: 92-103 [PMID: 8550855 DOI: 10.1172/JCI118411]
  - 29 **de Moura EG**, Orso IR, Martins BC, Lopes GS, de Oliveira SL, Galvão-Neto Mdos P, Mancini MC, Santo MA, Sakai P, Ramos AC, Garrido-Júnior AB, Halpern A, Cecconello I. Improvement of insulin resistance and reduction of cardiovascular risk among obese patients with type 2 diabetes with the duodenojejunal bypass liner. *Obes Surg* 2011; **21**: 941-947 [PMID: 21442376 DOI: 10.1007/s11695-011-0387-0]
  - 30 **Escalona A**, Pimentel F, Sharp A, Becerra P, Slako M, Turiel D, Muñoz R, Bambs C, Guzmán S, Ibáñez L, Gersin K. Weight loss and metabolic improvement in morbidly obese subjects implanted for 1 year with an endoscopic duodenal-jejunal bypass liner. *Ann Surg* 2012; **255**: 1080-1085 [PMID: 22534421 DOI: 10.1097/SLA.0b013e31825498c4]
  - 31 **Chiasson JL**, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet* 2002; **359**: 2072-2077 [PMID: 12086760]
  - 32 **Cummings DE**. Endocrine mechanisms mediating remission of diabetes after gastric bypass surgery. *Int J Obes (Lond)* 2009; **33** Suppl 1: S33-S40 [PMID: 19363506 DOI: 10.1038/ijo.2009.15]
  - 33 **DePaula AL**, Macedo AL, Schraibman V, Mota BR, Vencio S. Hormonal evaluation following laparoscopic treatment of type 2 diabetes mellitus patients with BMI 20-34. *Surg Endosc* 2009; **23**: 1724-1732 [PMID: 18830747 DOI: 10.1007/s00464-008-0168-6]
  - 34 **Braghetto I**, Davanzo C, Korn O, Csendes A, Valladares H, Herrera E, Gonzalez P, Papapietro K. Scintigraphic evaluation of gastric emptying in obese patients submitted to sleeve gastrectomy compared to normal subjects. *Obes Surg* 2009; **11**: 1515-1521 [DOI: 10.1007/s11695-009-9954-z]
  - 35 **Theodorakis MJ**, Carlson O, Muller DC, Egan JM. Elevated plasma glucose-dependent insulinotropic polypeptide associates with hyperinsulinemia in impaired glucose tolerance. *Diabetes Care* 2004; **27**: 1692-1698 [PMID: 15220248]
  - 36 **Højberg PV**, Vilsbøll T, Rabøl R, Knop FK, Bache M, Krarup T, Holst JJ, Madsbad S. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. *Diabetologia* 2009; **52**: 199-207 [PMID: 19037628 DOI: 10.1007/s00125-008-1195-5]
  - 37 **Rao RS**, Kini S. GIP and bariatric surgery. *Obes Surg* 2011; **21**: 244-252 [PMID: 21082290 DOI: 10.1007/s11695-010-0305-x]
  - 38 **Laferrière B**, Teixeira J, McGinty J, Tran H, Egger JR, Colarusso A, Kovack B, Bawa B, Koshy N, Lee H, Yapp K, Olivan B. Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2008; **93**: 2479-2485 [PMID: 18430778 DOI: 10.1210/jc.2007-2851]
  - 39 **Mumphrey MB**, Patterson LM, Zheng H, Berthoud HR. Roux-en-Y gastric bypass surgery increases number but not density of CCK-, GLP-1-, 5-HT-, and neurotensin-expressing enteroendocrine cells in rats. *Neurogastroenterol Motil* 2013; **25**: e70-e79 [PMID: 23095091 DOI: 10.1111/nmo.12034]
  - 40 **Gupta D**, Krueger CB, Lastra G. Over-nutrition, obesity and insulin resistance in the development of  $\beta$ -cell dysfunction. *Curr Diabetes Rev* 2012; **8**: 76-83 [PMID: 22229253]
  - 41 **Gross LS**, Li L, Ford ES, Liu S. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *Am J Clin Nutr* 2004; **79**: 774-779 [PMID: 15113714]
  - 42 **Weser E**, Babbitt J, Vandeventer A. Relationship between enteral glucose load and adaptive mucosal growth in the small bowel. *Dig Dis Sci* 1985; **30**: 675-681 [PMID: 3924534]
  - 43 **Alvarado-Vásquez N**, Zapata E, Alcázar-Leyva S, Massó F, Montañó LF. Reduced NO synthesis and eNOS mRNA expression in endothelial cells from newborns with a strong family history of type 2 diabetes. *Diabetes Metab Res Rev* 2007; **23**: 559-566 [PMID: 17385193]
  - 44 **Burgdorf KS**, Grarup N, Justesen JM, Harder MN, Witte DR, Jørgensen T, Sandbæk A, Lauritzen T, Madsbad S, Hansen T, Pedersen O. Studies of the association of Arg72Pro of tumor suppressor protein p53 with type 2 diabetes in a combined analysis of 55,521 Europeans. *PLoS One* 2011; **6**: e15813 [PMID: 21283750 DOI: 10.1371/journal.pone.0015813]
  - 45 **Schwartzberg-Bar-Yoseph F**, Armoni M, Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. *Cancer Res* 2004; **64**: 2627-2633 [PMID: 15059920]
  - 46 **Vousden KH**, Ryan KM. p53 and metabolism. *Nat Rev Cancer* 2009; **9**: 691-700 [PMID: 19759539 DOI: 10.1038/nrc2715]
  - 47 **Saeidi N**, Meoli L, Nestoridi E, Gupta NK, Kvas S, Kucharczyk J, Bonab AA, Fischman AJ, Yarmush ML, Stylopoulos N. Reprogramming of intestinal glucose metabolism and glycemic control in rats after gastric bypass. *Science* 2013; **341**: 406-410 [PMID: 23888041 DOI: 10.1126/science.1235103]

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## Prospective Study

# Assessment of the correlation between serum prolidase and alpha-fetoprotein levels in patients with hepatocellular carcinoma

Sevil Uygun Ilikhan, Muammer Bilici, Hatice Sahin, Ayşe Semra Demir Akca, Murat Can, Ibrahim Ilker Oz, Berrak Guven, M Cagatay Buyukuysal, Yucel Ustundag

Sevil Uygun Ilikhan, Muammer Bilici, Hatice Sahin, Department of Internal medicine, Bülent Ecevit University Faculty of Medicine, Zonguldak 67100, Turkey

Ayşe Semra Demir Akca, Department of Family Medicine, Bülent Ecevit University Faculty of Medicine, Zonguldak 67100, Turkey

Murat Can, Berrak Guven, Department of Biochemistry, Bülent Ecevit University Faculty of Medicine, Zonguldak 67100, Turkey  
Ibrahim Ilker Oz, Department of Radiology, Bülent Ecevit University Faculty of Medicine, Zonguldak 67100, Turkey

M Cagatay Buyukuysal, Department of Biostatistic, Bülent Ecevit University Faculty of Medicine, Zonguldak 67100, Turkey  
Yucel Ustundag, Bülent Ecevit University Faculty of Medicine, Department of Gastroenterology, Zonguldak 67100, Turkey

**Author contributions:** Uygun Ilikhan S, Bilici M, Sahin H and Ustundag Y designed the research; Can M, Guven B and Oz I performed the research; Buyukuysal MC analyzed the data; Uygun Ilikhan S, Bilici M, Sahin H and Demir Akca AS wrote the paper.

**Ethics approval:** The study was reviewed and approved by the Clinical Research Ethics Board of the Bülent Ecevit University.  
**Clinical trial registration:** The study was reviewed and approved by the Clinical Research Ethics Board of the Bülent Ecevit University.

**Informed consent:** All study participants provided informed written consent prior to study enrollment.

**Conflict-of-interest:** There are no conflicts of interest to report for any study authors.

**Data sharing:** Technical appendix, statistical code, and dataset available from the corresponding author at [aysesemra@hotmail.com](mailto:aysesemra@hotmail.com). The presented data cannot be linked to individuals and risk of personal identification is minimal as such.

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**Correspondence to:** Dr. Ayşe Semra Demir Akca, Department of Family Medicine, Bülent Ecevit University Faculty of Medicine, Esenköy, Kozlu, Zonguldak 67100, Turkey. [aysesemra@hotmail.com](mailto:aysesemra@hotmail.com)

**Telephone:** +90-372-2612000

**Fax:** +90-372-2610155

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## Abstract

**AIM:** To determine the predictive value of increased prolidase activity that reflects increased collagen turnover in patients with hepatocellular carcinoma (HCC).

**METHODS:** Sixty-eight patients with HCC (mean age of  $69.1 \pm 10.1$ ), 31 cirrhosis patients (mean age of  $59.3 \pm 6.3$ ) and 33 healthy volunteers (mean age of  $51.4 \pm 12.6$ ) were enrolled in this study. Univariate and multivariate analysis were used to evaluate the association of serum  $\alpha$ -fetoprotein (AFP) values with HCC clinicopathological features, such as tumor size, number and presence of vascular and macrovascular invasion. The patients with HCC were divided into groups according to tumor size, number and presence of vascular invasion (diameters;  $\leq 3$  cm, 3-5 cm



and  $\geq 5$  cm, number; 1, 2 and  $\geq 3$ , macrovascular invasion; yes/no). Barcelona-clinic liver cancer (BCLC) criteria were used to stage HCC patients. Serum samples for measurement of prolidase and alpha-fetoprotein levels were kept at  $-80^{\circ}\text{C}$  until use. Prolidase levels were measured spectrophotometrically and AFP concentrations were determined by a *chemiluminescence* immunometric commercial diagnostic assay.

**RESULTS:** In patients with HCC, prolidase and AFP values were evaluated according to tumor size, number, presence of macrovascular invasion and BCLC staging classification. Prolidase values were significantly higher in patients with HCC compared with controls ( $P < 0.001$ ). Prolidase levels were significantly associated with tumor size and number ( $P < 0.001$ ,  $P = 0.002$ , respectively). Prolidase levels also differed in patients in terms of BCLC staging classification ( $P < 0.001$ ). Furthermore the prolidase levels in HCC patients showed a significant difference compared with patients with cirrhosis ( $P < 0.001$ ). In HCC patients grouped according to tumor size, number and BCLC staging classification, AFP values differed separately ( $P = 0.032$ ,  $P = 0.038$ ,  $P = 0.015$ , respectively). In patients with HCC, there was a significant correlation ( $r = 0.616$ ;  $P < 0.001$ ) between prolidase and AFP values in terms of tumor size, number and BCLC staging classification, whereas the presence of macrovascular invasion did not show a positive association with serum prolidase and AFP levels.

**CONCLUSION:** Considering the levels of both serum prolidase and AFP could contribute to the early diagnosing of hepatocellular carcinoma.

**Key words:** Alpha-fetoprotein; Hepatocellular carcinoma; Prolidase; Cirrhosis; Macrovascular invasion

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**Core tip:** Prolidase cleaves dipeptide bonds containing proline, playing a vital role in collagen turnover, matrix remodeling and cell growth. Neoplastic transformation results in deregulation of tissue collagen metabolism, in which metastatic tumor cells produce enhanced amounts of proteases to penetrate basement membranes and the extracellular matrix. Therefore, tumor progression might depend on the breakdown of collagen and other extracellular matrix proteins. The role of prolidase in neoplastic tissues is unknown. Herein, serum prolidase levels in hepatocellular carcinoma (HCC) patients were significantly associated with tumor size and number, Barcelona-clinic liver cancer staging and  $\alpha$ -fetoprotein (AFP). Considering the levels of both serum prolidase and AFP could contribute to early diagnosis of HCC.

II, Guven B, Buyukuysal MC, Ustundag Y. Assessment of the correlation between serum prolidase and alpha-fetoprotein levels in patients with hepatocellular carcinoma. *World J Gastroenterol* 2015; 21(22): 6999-7007 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/6999.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.6999>

## INTRODUCTION

Despite recent developments in surgery and medical therapy, which have significantly improved the outcome of patients with operable and advanced hepatocellular carcinoma (HCC), HCC remains a major health problem worldwide. The majority of HCC cases occur in patients with chronic liver disease, such as hepatitis B-virus (HBV), hepatitis C-virus (HCV) infection, alcoholic liver diseases and non-alcoholic fatty liver diseases<sup>[1,2]</sup>. The complex nature of the disease and its high resistance to conventional systemic therapies, results in poor prognosis for advanced HCC patients<sup>[1]</sup>. Despite regular surveillance to detect small HCCs in these patients, HCC is often diagnosed at an advanced stage, after the symptoms related HCC have appeared, and the 5-year relative survival rate for patients is only 7%<sup>[1]</sup>. If HCC could be diagnosed at an early stage, potentially curative options, such as resection, ablation, and transplantation may be considered<sup>[3]</sup>. Early diagnose may serve as a long-term control in patients. Thus, the regular follow-up of patients with risk factors for HCC seems very important.

Surveillance strategies, including ultrasound imaging and serum  $\alpha$ -fetoprotein (AFP) concentration measurements, have been recommended to detect HCC at earlier stages, without pathological confirmation. AFP is the most commonly used serological marker worldwide to diagnose hepatocellular carcinoma<sup>[4]</sup>. HCC differentiation, size and macrovascular invasion are strongly associated with AFP; poor differentiation and HCC size  $\geq 10$  cm are independent predictors of elevated AFP<sup>[5]</sup>.

Collagen is the main component of connective tissue. Deregulation of tissue collagen metabolism is one of the consequences of neoplastic transformation. Metalloproteinases initiate the breakdown of collagen; however, the final step of collagen degradation is mediated by prolidase<sup>[6,7]</sup>. Prolidase is an important enzyme that cleaves the bonds of dipeptides containing proline (X-Pro), and plays a vital role in collagen turnover, matrix remodeling and cell growth. Metastatic tumor cells produce enhanced amounts of proteases that enable them to penetrate basement membranes and the extracellular matrix (ECM)<sup>[8]</sup>. Therefore, tumor progression might depend critically on the breakdown of collagen and other ECM proteins<sup>[9]</sup>. Prolidase seems a rate-limiting factor in the regulation of collagen biosynthesis because of its role in the last step of

collagen degradation. The role of prolidase activity in neoplastic tissues is not yet known.

In the present study, we aimed to assess the correlation between the serum prolidase and AFP levels in patients with hepatocellular carcinoma.

## MATERIALS AND METHODS

### Ethics

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the *a priori* approval by the Clinical Research Ethics Board of the Bülent Ecevit University. Informed consent was obtained from all individuals.

### Patient population

Ninety-four patients with HCC, 54 cirrhosis patients and 33 healthy volunteers, admitted to the gastroenterology clinic of Bulent Ecevit University Medical Faculty between March 2014 and August 2014, were enrolled in this study. Twenty-six patients with HCC and 23 patients with cirrhosis were excluded from the study according to the exclusion criteria. Exclusion criteria were: dilated cardiomyopathy, uncontrolled hypertension, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, psoriasis, connective tissue disease, chronic obstructive pulmonary disease, chronic pancreatitis, bipolar disorder and thalassemia major. All patients underwent a baseline evaluation, including a detailed medical history, typical physical examination and blood tests. For all patients in this study, the diagnosing of HCC and cirrhosis was made by histopathology and/or based on typical imaging findings for HCC in three-phase multidetector computed tomography (Activision 16-row scanner computed tomography, Toshiba Medical Systems, Otawara, Japan) or dynamic contrast-enhanced magnetic resonance imaging (Intera Master Gyroscan, Philips Medical Systems, Best, the Netherlands) with increased serum AFP concentrations ( $> 400 \mu\text{g/L}$ ). Barcelona-Clinic Liver Cancer (BCLC) criteria were used to stage the patients with HCC<sup>[10]</sup>. The patients were divided into groups according to their tumor size, number and presence of macrovascular invasion (diameters;  $\leq 3 \text{ cm}$ , 3-5 cm and  $\geq 5 \text{ cm}$ , number; 1, 2 and 3  $\leq$ , macrovascular invasion; yes/no). Macrovascular invasion was defined as portal vein thrombosis and was demonstrated by any imaging modality. Distinction of malignant portal vein thrombosis from benign thrombosis was made by contrast enhancement pattern at computed tomography or magnetic resonance imaging.

### Blood samples

In total, 8-10 cc blood samples were withdrawn from all the participants and the sera were separated by centrifugation at 3000 rpm for 10 min. Sera were stored at  $-80^\circ\text{C}$  until the day of measurement.

### Prolidase measurement

Serum was diluted 6-fold with 1 mmol/L  $\text{Mn}^{2+}$ , 50 mmol/L Tris HCl buffer (pH 7.8) and preincubated at  $37^\circ\text{C}$  for 2 h. The reaction mixture containing 94 mmol/L gly-pro, 50 mmol/L Tris HCl buffer (pH 7.8) and preincubation serum was incubated at  $37^\circ\text{C}$  for 30 min. The reaction was stopped by adding 1.0 mL of 0.45 mol/L trichloroacetic acid solution. Prolidase activity was measured in the supernatant samples using the method described by Myara *et al.*<sup>[11]</sup>, which is a modification of Chinard's method and was calculated against proline standards. All reagents were purchased from Sigma. The intra- and inter-assay coefficients of variation were less than 10 %. Prolidase activity was reported as U/L.

### AFP measurement

Serum AFP levels were measured using a chemiluminescence immunometric assay in a UniCell DXI 600 (Beckman Coulter, CA, United States) hormone analyzer. The intra-assay and inter-assay coefficients of variation (CV) were both less than  $< 3.22\%$  (the within run CVs were 3.2%, 2.88%, 2.71% and the between run CVs were 3.22%, 2.04%, 2.07% for the levels of 6.53, 72.1 and 1672.88 ng/mL, respectively) and the measuring range was 0.5-3000 ng/mL. The normal range is less than 10 ng/mL in an adult.

### Statistical methods

SPSS 19.0 for Windows was used for statistical analysis. Categorical variables were given with frequency and percent. Numerical variables were shown as the median, with minimum and maximum values. The Shapiro-Wilk test was used for normality tests. For nonparametric variables, the Mann Whitney *U* test was used for two group comparisons and the Kruskal Wallis test was used for comparisons of three or more groups. Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value of prolidase and AFP regarding tumor size, number and controls. For all statistical analyses, a *P* value  $< 0.05$  indicated statistical significance.

### Statistical analysis

The statistical analysis of the study was performed by author Dr. Çağatay Büyükuysal, a biostatistician and expert on data analysis. His approval of the methods are documented *via* his inclusion as a senior author of the manuscript.

## RESULTS

Demographic and clinical data of the patients with hepatocellular carcinoma, cirrhosis patients and the healthy control group are shown in Table 1. Of the 68 patients with HCC, 31 cirrhosis patients and 33 healthy adults, 80 were males (60.6%) and 52 were females

**Table 1** Demographics and clinical variables of the groups

Characteristic	Patients with HCC ( <i>n</i> = 68)	Cirrhosis patients ( <i>n</i> = 31)	Controls ( <i>n</i> = 33)
Age (yr), mean $\pm$ SD	69.1 $\pm$ 10.1	59.3 $\pm$ 6.3	51.4 $\pm$ 12.6
Sex% (M/F)	47 (69.1)/21 (30.9)	17 (54.8)/14 (45.2)	16 (48.5)/17 (51.5)
Platelet ( $\times 1000/\text{mm}^3$ )	175 (27-413)	88 (45-120)	256 (181-396)
Hb (g/dL)	11.92 $\pm$ 1.80	9.2 $\pm$ 1.40	12.67 $\pm$ 1.63
INR	1.18 (0.10-2.10)	1.10 (0.90-1.60)	-
Total bilirubin (mg/dL)	2.0 (0.30-16.90)	1.17 (0.90-3.00)	0.80 (0.30-2.00)
Albumin (g/dL)	3.2 (2-4.6)	3.49 (2.3-4.4)	4.4 (4-5.3)
Prolidase (U/L) median (min-max)	1179 (1080-1600)	913 (811-1011)	880 (816-969)
AFP (ng/mL) median (min-max)	650 (2.5-2300)	14 (2-40)	4.2 (1-16)
Underlying liver disease			
HBV	34	11	
HCV	18	6	
NASH	12	10	
Alcohol	4	4	

HCC: Hepatocellular carcinoma; Hb: Hemoglobin; AFP:  $\alpha$ -fetoprotein; HBV: Hepatitis B virus; HCV: Hepatitis C virus; INR: International normalized ratio; NASH: Nonalcoholic steatohepatitis.

(39.4%). Prolidase and AFP values of the patients with HCC were evaluated according to tumor size, number, presence of macrovascular invasion and BCLC staging classification (Table 2). Prolidase values were significantly higher in patients with HCC compared with controls ( $P < 0.001$ ). Prolidase levels were significantly associated with tumor size and number ( $P < 0.001$ ,  $P = 0.002$ , respectively). Serum prolidase level in tumors  $\leq 3$  cm was significantly lower than those with tumor size 3-5 cm ( $P = 0.006$ ) and with tumor size  $\geq 5$  cm ( $P < 0.001$ ). In addition, the serum prolidase level in patients with tumor number  $\geq 3$  cm was significantly higher than those with two tumors ( $P = 0.008$ ) and with one tumor ( $P = 0.002$ ). However, prolidase levels showed no positive relation with presence of macrovascular invasion in patients with HCC ( $P = 0.575$ ). In the BCLC staging classification, prolidase levels at various stages were significantly different in patients with HCC ( $P < 0.001$ ), with stage B having the highest level of prolidase. However, a significant difference was observed when the prolidase levels in patients with HCC were compared with cirrhosis patients ( $P < 0.001$ ). There was no significant difference between cirrhotic patients and the control group ( $P = 0.067$ ) (Table 3).

In terms of tumor size and number, a significant relation was found among AFP values ( $P = 0.032$ ,  $P = 0.038$ , respectively). The AFP value in tumors  $\geq 5$  cm was significantly higher ( $P = 0.006$ ) than in tumors  $\leq 3$  cm ( $P = 0.013$ ), whereas there was no significant difference between patients with tumor  $\geq 5$  cm and with tumors of 3-5 cm ( $P = 0.171$ ). In

**Table 2** Serum prolidase and  $\alpha$ -fetoprotein levels of different tumor related factors in patients with hepatocellular carcinoma

Variables	<i>n</i> = 68	AFP (ng/L) median (min-max)	<i>P</i> value	Prolidase (U/L) median (min-max)	<i>P</i> value
Tumor size (cm)					
$\leq 3$	19	440 (2.5-2000)	0.032	1120 (1080-1250)	$< 0.001$
3-5	12	548 (45-1740)		1178 (1123-1260)	
$\geq 5$	37	850 (73-2300)		1219 (1113-1600)	
Tumor number					
1	29	547 (2.5-2248)	0.038	1150 (1080-1310)	0.002
2	13	550 (9-1430)		1142 (1110-1265)	
$\geq 3$	26	1160 (84-2300)		1222 (1120-1600)	
Macrovascular invasion					
No	59	597 (2.5-2300)	0.502	1174 (1080-1600)	0.575
Yes	9	770 (110-1750)		1208 (1120-1276)	
BCLC staging					
A	20	470 (2.5-2000)	0.015	1128 (1080-1260)	$< 0.001$
B	29	862 (78-2300)		1219 (1123-1600)	
C	8	867 (110-1645)		1174 (1120-1276)	
D	11	615 (73-2050)		1150 (1113-1286)	

AFP: Alpha fetoprotein; BCLC: The Barcelona-Clinic Liver Cancer Group; HCC: Hepatocellular carcinoma.

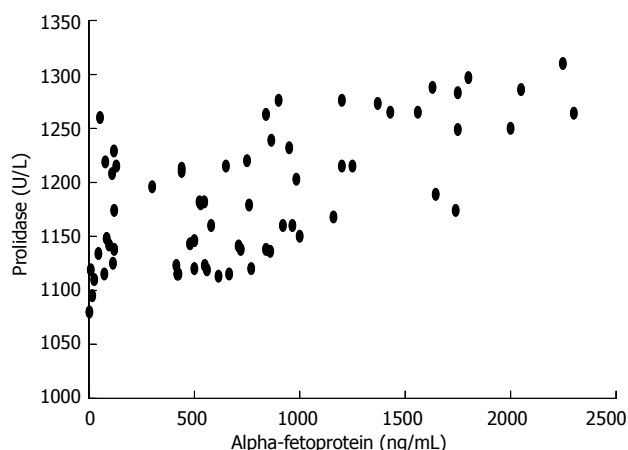
**Table 3** Comparison of the groups for serum prolidase levels

	Prolidase (U/L) median (min-max)	<i>P</i> value
Patients with HCC ( <i>n</i> = 68)	1179 (1080-1600)	$< 0.001$
Cirrhosis patients ( <i>n</i> = 31)	913 (811-1011)	0.067
Controls ( <i>n</i> = 33)	880 (816-969)	0.067

HCC: Hepatocellular carcinoma.

addition, the AFP level in patients with tumor number  $\geq 3$  was significantly higher than those with one tumor ( $P = 0.028$ ) and with two tumors ( $P = 0.030$ ). However there was no positive relation between AFP and presence of macrovascular invasion in patients with HCC ( $P = 0.502$ ). AFP values also significantly differed in patients at various stages of BCLC staging classification ( $P = 0.015$ ): tumors at stage B had the highest levels of AFP. AFP values were significantly higher in the A stage of BCLC compared with stage B and C ( $P = 0.002$ ,  $P = 0.028$ , respectively), whereas, there was no significant relation between stage A and D ( $P = 0.113$ ). In terms of serum AFP values, there was a significant difference between HCC patients and cirrhotic patients ( $P < 0.001$ ). AFP values of both HCC and cirrhotic patients were significantly higher than the healthy control group ( $P < 0.001$ ,  $P < 0.001$ , respectively).

In patients with HCC, there was a significant correlation ( $r = 0.616$ ;  $P < 0.001$ ) between prolidase and AFP values regarding tumor size, number and BCLC staging classification (Figure 1), whereas the presence of macrovascular invasion [yes ( $r = 0.276$ ;  $P = 0.472$ )/no ( $r = 0.646$ ;  $P < 0.001$ )] did not show a



**Figure 1** Relationship between serum prolidase and alpha fetoprotein levels in patients with hepatocellular carcinoma ( $r = 0.616$ ;  $P < 0.01$ ).

positive relation with serum prolidase and AFP levels (Figure 2). The correlation between prolidase and AFP regarding tumor size [diameters;  $\leq 3$  cm ( $r = 0.746$ ;  $P < 0.001$ ), 3-5 cm ( $r = 0.119$ ;  $P = 0.712$ ) and  $\geq 5$  cm ( $r = 0.683$ ;  $P < 0.001$ ), number [1 ( $r = 0.503$ ;  $P = 0.005$ ), 2 ( $r = 0.694$ ;  $P = 0.008$ ) and 3  $\leq$  ( $r = 0.662$ ;  $P < 0.001$ )] and BCLC staging classification [stage A ( $r = 0.600$ ;  $P = 0.005$ ), B ( $r = 0.668$ ;  $P < 0.001$ ), C ( $r = 0.419$ ;  $P = 0.301$ ) and D ( $r = 0.492$ ;  $P = 0.124$ )] are shown in Figure 2.

Additionally, prolidase values were significantly higher in HCC patients ( $n = 9$ ) with low AFP levels (less than 80 ng/mL) compared with both cirrhotic patients and controls ( $P < 0.001$ ,  $P < 0.001$ , respectively). Unfortunately, the small number of patients that had low AFP levels was not enough to perform powerful analysis for the value of prolidase.

Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive value of prolidase in terms of tumor size (diameters;  $\leq 3$  cm and  $> 3$  cm) and number (number;  $\leq 1$  and  $\geq 2$ ). The cut off values of prolidase for tumor size and tumor number were 1138 U/L, 1189 U/L, respectively. For prolidase, the areas under the curve (AUC) regarding tumor size and tumor number [AUC: 0.810 (sensitivity 83.6%; specificity 68.4%), AUC: 0.678 (sensitivity 57.8%; specificity 72.4%), respectively] are shown in Figure 3. The cut off values of AFP for tumor size (diameters;  $\leq 3$  cm and  $> 3$  cm) is 580 ng/mL. For AFP, the areas under the curve (AUC) regarding tumor size [AUC: 0.680 (sensitivity 62.5%; specificity 73.6%)], are shown in Figure 3A. However, The AFP level was not discriminative in terms of tumor number (number;  $\leq 1$  and  $\geq 2$ ) by performing ROC curve analysis (Figure 3B). The AUC for prolidase was higher than the AUC for AFP regarding tumor size (diameters;  $\leq 3$  cm and  $> 3$  cm).

## DISCUSSION

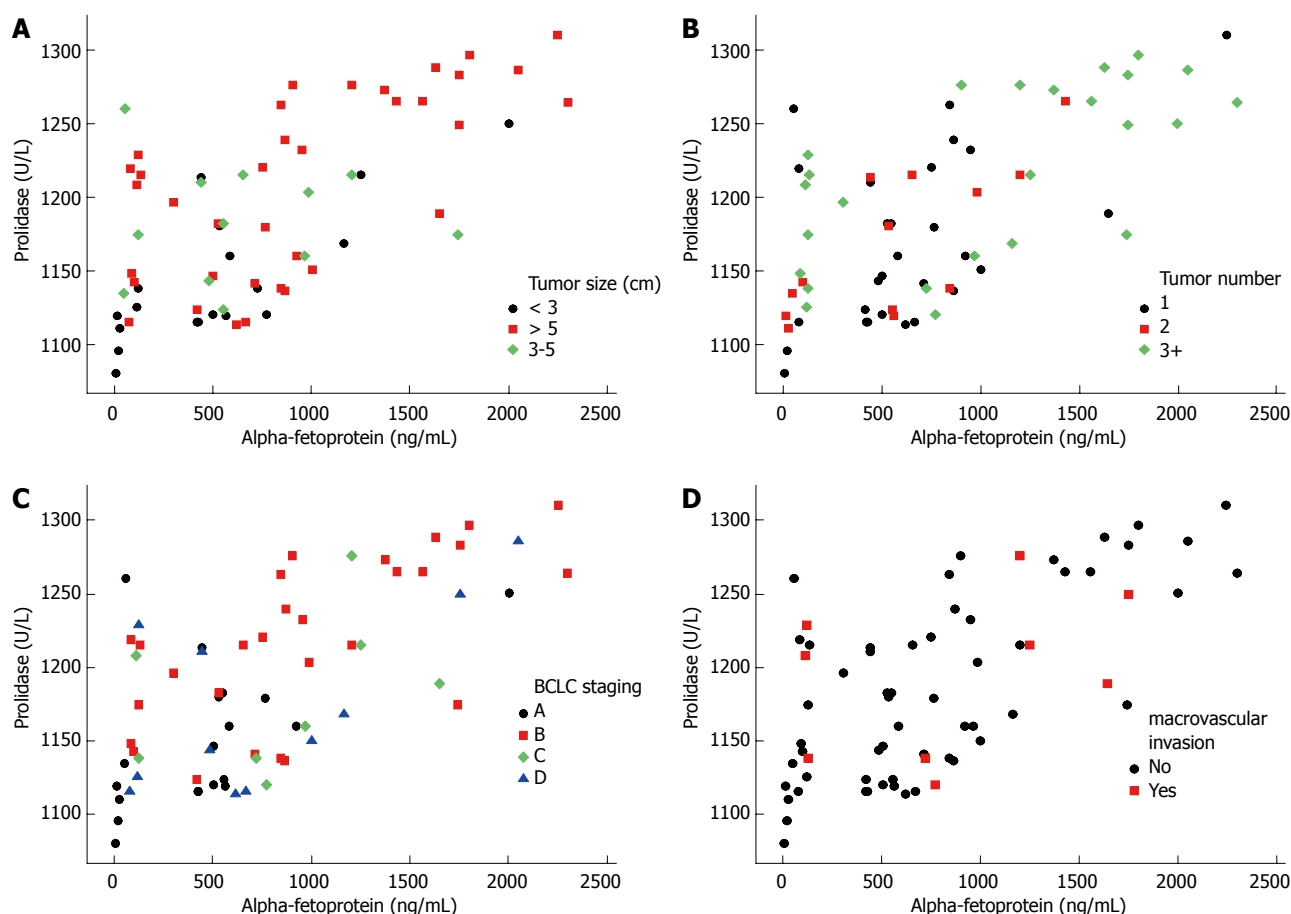
Prolidase enzyme is a cytosolic exopeptidase that

cleaves imidodi- and imidotripeptides with C terminal proline or hydroxyproline. Prolidase plays an important role in collagen metabolism, matrix remodeling and cell growth<sup>[12]</sup>. By releasing proline or hydroxyproline, prolidase helps make them available for collagen resynthesis. Although extracellular collagenases initiate the breakdown of collagen, the final step of collagen degradation is catalyzed by intracellular prolidase. Moreover, it has been suggested that prolidase activity may be a rate-limiting factor in the regulation of collagen biosynthesis<sup>[7]</sup>. Collagen is the major component of the extracellular matrix (ECM), which represents a major barrier against invasion by neoplastic cells. Tumor cells can produce proteolytic enzymes that catalyze the breakdown of tissue barriers, which enables them to penetrate basement membranes and the ECM<sup>[8,13]</sup>. Therefore, tumor progression depends critically on the degradation of collagen and other ECM proteins<sup>[14]</sup>. The final step of collagen degradation is catalyzed by intracellular prolidase; therefore, it may be associated with neoplastic transformation. Increased prolidase activities have been observed in some of cancers, such as lung cancer<sup>[15]</sup>, breast cancer<sup>[16]</sup>, endometrial cancer<sup>[17]</sup>, stomach cancer<sup>[18]</sup>, renal cell cancer<sup>[19]</sup> and ovarian cancer<sup>[20]</sup>. On the other hand, Palka *et al*<sup>[6]</sup> showed reduced levels of prolidase in pancreatic cancer.

Myara *et al*<sup>[21]</sup> used an experimental animal model that demonstrated hepatic damage in rats by chronic CCl<sub>4</sub> intoxication. Consequently, they observed a relationship between elevated prolidase values and hepatic fibrosis. In another study, Myara *et al*<sup>[11]</sup> demonstrated increased prolidase activity in chronic liver disease. Elevated prolidase levels in some common conditions, such as hepatitis B infection, hepatitis C infection, nonalcoholic steatohepatitis and alcoholic liver disease, which cause hepatic damage and hepatic fibrosis, have also been reported previously<sup>[22-25]</sup>. Although numerous non-invasive markers have been described that predict the severity of hepatic fibrosis<sup>[26]</sup>, liver biopsy remains a gold standard method for assessing the severity of liver fibrosis and cirrhosis<sup>[27]</sup>.

To date, prolidase levels have not been evaluated in HCC. In the present study, we assessed serum prolidase levels in patients with HCC and asked whether prolidase activity might show a correlation with AFP; thus contributing to early diagnosis of HCC while following up patients. We observed that serum prolidase levels were higher in HCC than in healthy volunteers, and elevated prolidase levels showed a significant relationship between size and number of HCCs. This might be a consequence of tumor pathogenesis, which might reflect progression of HCC. Moreover, there are few studies that have revealed a correlation between the serum prolidase levels and stage of liver fibrosis according to the results of liver biopsy<sup>[24,28-30]</sup>. In contrast, Duygu *et al*<sup>[22,23]</sup> did not





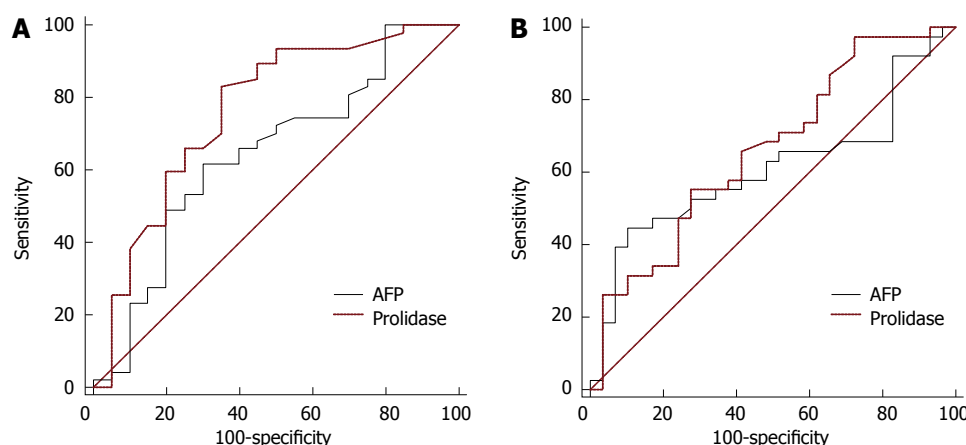
**Figure 2** Relationship between serum prolidase and alpha fetoprotein levels in patients with hepatocellular carcinoma with regard to tumor size (A), number (B), barcelona-clinic liver cancer staging (C) and macrovascular invasion (D).

observe a relationship between the degree of liver fibrosis and serum prolidase levels in patients with chronic hepatitis B and C. In this study, we observed a significant difference when the prolidase levels in cirrhosis patients were compared with patients with HCC. This might indicate enhanced turnover of collagen synthesis in patients with HCC, which can be affected by the degree of neoplastic transformation during the development of HCC, on the basis of the cirrhotic process. By contrast, we did not observe a significant difference between cirrhosis patients and healthy volunteers. Hence, our study needs to be validated by further large population studies to illustrate the changing prolidase levels among patients with liver diseases.

However, the diagnostic value of is being questioned because of poor sensitivity and specificity. The diagnosis of HCC without pathological confirmation is achieved by analyzing serum AFP levels combined with imaging techniques, including ultrasonography, magnetic resonance imaging and computerized tomography<sup>[5]</sup>. The relationship between serum AFP levels and tumor characteristics, such as tumor size, tumor number or macrovascular invasion, has been evaluated in many studies<sup>[31-34]</sup>. Liu *et al.*<sup>[5]</sup> showed a relationship between AFP levels and tumor size, however, AFP concentrations

were not correlated with tumor number in their study. Wang *et al.*<sup>[35]</sup> and Kasahara *et al.*<sup>[36]</sup> have also reported a significant relationship between tumor size and serum AFP levels. Furihata *et al.*<sup>[37]</sup> observed a significant correlation between serum AFP levels and both size and number of HCCs. Furthermore, AFP elevation in HCC was associated with macrovascular invasion in some studies<sup>[38,39]</sup>. In the present study, serum AFP levels were correlated with tumor size, which is consistent with the results of previous studies<sup>[5,35,36]</sup>. There was also a relationship between AFP levels and the tumor number, similar to the other studies<sup>[37,40]</sup>. Moreover, macrovascular invasion was not associated with high AFP levels, contrary to previous studies<sup>[38,39]</sup>. There were only nine patients (13.2%) with macrovascular invasion, which was insufficient to produce powerful statistical results.

In the present study, we observed elevated levels of serum prolidase and AFP in patients with HCC, although the absence of an increase in these markers does not exclude the diagnosis of HCC. Notably, a similar relationship between AFP concentrations and the size and number of tumors was observed for serum prolidase levels. Furthermore, in terms of tumor size (diameters;  $\leq 3$  cm and  $> 3$  cm) and number (number;  $\leq 1$  and  $\geq 2$ ), serum prolidase activity



**Figure 3** Receiver operating characteristic curves of prolidase and alpha fetoprotein levels. A: Predictive value of prolidase and  $\alpha$ -fetoprotein (AFP) regarding tumor size (diameters;  $\leq 3$  cm and  $> 3$  cm); B: Predictive value of prolidase and AFP regarding tumor number (number;  $\leq 1$  and  $\geq 2$ ).

exhibited higher sensitivity and specificity than AFP values in HCC patients. We believe that tumor burden and aggressiveness are the main characteristics that may explain the elevated prolidase levels in HCC patients.

The findings of this study suggested that increased serum prolidase levels might reflect increased collagen turnover associated with the tumor burden in HCC patients. Increased prolidase activity may, in part, play a role in the pathogenesis of HCC. Therefore, consideration of the levels of both serum prolidase and AFP would contribute to the early diagnosis of HCC. Our study has several limitations, such as the low number of patients and no long-term outcomes of HCC patients regarding prolidase levels. Nevertheless, future comprehensive studies covering larger populations are needed to determine the value of prolidase activity during follow-up of the patients with chronic liver diseases.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer death. Despite recent developments in surgery and medical therapy that have significantly improved the outcome of patients with operable and advanced HCC, HCC remains a major health problem worldwide. Screening strategies including ultrasound imaging and serum  $\alpha$ -fetoprotein (AFP) concentration are useful to detect the early stage of HCC development. Via neoplastic transformation, tumor cells acquire the ability to penetrate basement membranes and the extracellular matrix (ECM), involving breakdown of collagen and other ECM proteins. Prolidase is a matrix metalloproteinase that cleaves the bonds of dipeptides containing proline (X-Pro) during collagen metabolism. Several investigators have reported enhanced serum levels of prolidase in certain of cancers, such as lung cancer, breast cancer, endometrial cancer, stomach cancer, renal cell cancer and ovarian cancer.

### Research frontiers

Prolidase is an important enzyme that plays a vital role in collagen turnover,

matrix remodeling and cell growth. It is thought that the growth, invasion and spread of tumor cells depend on the breakdown of collagen and other ECM proteins. This behavior of tumor cells suggests that prolidase activity may reflect the tumor burden and aggressiveness. AFP is the most commonly used serological marker worldwide with radiological imaging for diagnosing hepatocellular carcinoma. Measuring the prolidase activity as complementary test is worth exploring for possible use in detecting HCC at earlier stages. In conclusion, a comprehensive study with a larger samples size is needed to elucidate the value of prolidase activity during follow-up of the patients with chronic liver diseases.

### Innovations and breakthroughs

The authors assessed the correlation between serum prolidase and AFP levels with regard to the features of HCC in patients. The present study had a large enough sample size such statistically relevant results could be obtained. For clinical practice, this article may be beneficial for physicians by permitting detection of HCC at earlier stages, as long as the serum prolidase results are verified by other diagnosis techniques.

### Applications

The present study assessed the predictive relationship between prolidase activity and HCC progression, which may represent a promising approach to detect HCC at earlier stages, allowing physicians to make appropriate clinical decisions.

### Terminology

Prolidase is an important enzyme that cleaves the bonds of dipeptides containing proline (X-Pro) and plays a vital role in collagen turnover, matrix remodeling and cell growth. Metalloproteinases initiate the breakdown of collagen; however, the final step of collagen degradation is mediated by prolidase (E.C.3.4.13.9). AFP is a glycoprotein and is mainly expressed in the fetal yolk sac and liver, but not in normal adult tissues. Serum AFP elevation is accompanies by some kinds of cancer, such as gastric cancer, lung cancer, pancreatic cancer, testicular carcinoma and, particularly, HCC.

### Peer-review

This preliminary study, having a large enough sample, concerns the possible value of prolidase activity to detect HCC at earlier stages, as long as the serum prolidase results are verified by other diagnosis techniques. It carries a good message for the reader and may represent a valuable contribution to the literature.

## REFERENCES

- 1 **Shin JW**, Chung YH. Molecular targeted therapy for hepatocellular carcinoma: current and future. *World J Gastroenterol* 2013; **19**: 6144-6155 [PMID: 24115810 DOI: 10.3748/wjg.v19.i37.6144]
- 2 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]
- 3 **Forner A**, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects.

- Semin Liver Dis* 2010; **30**: 61-74 [PMID: 20175034 DOI: 10.1055/s-0030-1247133]
- 4 **Sato Y**, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y, Nagataki S. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. *N Engl J Med* 1993; **328**: 1802-1806 [PMID: 7684823]
- 5 **Liu C**, Xiao GQ, Yan LN, Li B, Jiang L, Wen TF, Wang WT, Xu MQ, Yang JY. Value of  $\alpha$ -fetoprotein in association with clinicopathological features of hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 1811-1819 [PMID: 23555170 DOI: 10.3748/wjg.v19.i11.1811]
- 6 **Palka J**, Surazynski A, Karna E, Orlowski K, Puchalski Z, Pruszyński K, Laszkiewicz J, Dzienis H. Prolidase activity dysregulation in chronic pancreatitis and pancreatic cancer. *Hepatogastroenterology* 2002; **49**: 1699-1703 [PMID: 12397770]
- 7 **Surazynski A**, Miltky W, Palka J, Phang JM. Prolidase-dependent regulation of collagen biosynthesis. *Amino Acids* 2008; **35**: 731-738 [PMID: 18320291 DOI: 10.1007/s00726-008-0051-8]
- 8 **Nicolson GL**, Poste G. Tumor cell diversity and host responses in cancer metastasis--part II--host immune responses and therapy of metastases. *Curr Probl Cancer* 1983; **7**: 1-42 [PMID: 6340978]
- 9 **Bolon I**, Gouyer V, Devouassoux M, Vandenbunder B, Wernert N, Moro D, Brambilla C, Brambilla E. Expression of c-ets-1, collagenase 1, and urokinase-type plasminogen activator genes in lung carcinomas. *Am J Pathol* 1995; **147**: 1298-1310 [PMID: 7485393]
- 10 **Llovet JM**, Fuster J, Bruix J. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. *Liver Transpl* 2004; **10**: S115-S120 [PMID: 14762851]
- 11 **Myara I**, Myara A, Mangeot M, Fabre M, Charpentier C, Lemonnier A. Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. *Clin Chem* 1984; **30**: 211-215 [PMID: 6692525]
- 12 **Jackson SH**, Dennis AW, Greenberg M. Iminodipeptiduria: a genetic defect in recycling collagen; a method for determining prolidase in erythrocytes. *Can Med Assoc J* 1975; **113**: 759, 762-763 [PMID: 803128]
- 13 **Chen WT**. Membrane proteases: roles in tissue remodeling and tumour invasion. *Curr Opin Cell Biol* 1992; **4**: 802-809 [PMID: 1419057]
- 14 **Kleiner DE**, Stetler-Stevenson WG. Matrix metalloproteinases and metastasis. *Cancer Chemother Pharmacol* 1999; **43** Suppl: S42-S51 [PMID: 10357558]
- 15 **Karna E**, Surazynski A, Palka J. Collagen metabolism disturbances are accompanied by an increase in prolidase activity in lung carcinoma planoepitheliale. *Int J Exp Pathol* 2000; **81**: 341-347 [PMID: 11168680]
- 16 **Cechowska-Pasko M**, Pałka J, Wojtukiewicz MZ. Enhanced prolidase activity and decreased collagen content in breast cancer tissue. *Int J Exp Pathol* 2006; **87**: 289-296 [PMID: 16875494]
- 17 **Arioz DT**, Camuzcuoglu H, Toy H, Kurt S, Celik H, Aksoy N. Serum prolidase activity and oxidative status in patients with stage I endometrial cancer. *Int J Gynecol Cancer* 2009; **19**: 1244-1247 [PMID: 19823062 DOI: 10.1111/IGC.0b013e3181af711e]
- 18 **Guszczyński T**, Sobolewski K. Dereglulation of collagen metabolism in human stomach cancer. *Pathobiology* 2004; **71**: 308-313 [PMID: 15627841]
- 19 **Pirinççi N**, Kaba M, Geçit I, Günes M, Yüksel MB, Tanik S, Arslan A, Demir H. Serum prolidase activity, oxidative stress, and antioxidant enzyme levels in patients with renal cell carcinoma. *Toxicol Ind Health* 2013; Epub ahead of print [PMID: 24081636 DOI: 10.1177/0748233713498924]
- 20 **Camuzcuoglu H**, Arioz DT, Toy H, Kurt S, Celik H, Aksoy N. Assessment of preoperative serum prolidase activity in epithelial ovarian cancer. *Eur J Obstet Gynecol Reprod Biol* 2009; **147**: 97-100 [PMID: 19695763 DOI: 10.1016/j.ejogrb.2009.07.012]
- 21 **Myara I**, Miech G, Fabre M, Mangeot M, Lemonnier A. Changes in prolidase and prolidase activity during CCl<sub>4</sub> administration inducing liver cytolysis and fibrosis in rat. *Br J Exp Pathol* 1987; **68**: 7-13 [PMID: 3814502]
- 22 **Duygu F**, Aksoy N, Cicek AC, Butun I, Unlu S. Does prolidase indicate worsening of hepatitis B infection? *J Clin Lab Anal* 2013; **27**: 398-401 [PMID: 24038226 DOI: 10.1002/jcla.21617]
- 23 **Duygu F**, Koruk ST, Karsen H, Aksoy N, Taskin A, Hamidanoglu M. Prolidase and oxidative stress in chronic hepatitis C. *J Clin Lab Anal* 2012; **26**: 232-237 [PMID: 22811354 DOI: 10.1002/jcla.21510]
- 24 **Kayadibi H**, Gültepe M, Yasar B, Ince AT, Ozcan O, Ipcioglu OM, Kurdas OO, Bolat B, Benek YZ, Guveli H, Atalay S, Ozkara S, Keskin O. Diagnostic value of serum prolidase enzyme activity to predict the liver histological lesions in non-alcoholic fatty liver disease: a surrogate marker to distinguish steatohepatitis from simple steatosis. *Dig Dis Sci* 2009; **54**: 1764-1771 [PMID: 18989777 DOI: 10.1007/s10620-008-0535-0]
- 25 **Brosset B**, Myara I, Fabre M, Lemonnier A. Plasma prolidase and prolidase activity in alcoholic liver disease. *Clin Chim Acta* 1988; **175**: 291-295 [PMID: 3416488]
- 26 **Afdhal NH**. Biopsy or biomarkers: is there a gold standard for diagnosis of liver fibrosis? *Clin Chem* 2004; **50**: 1299-1300 [PMID: 15277345]
- 27 **Gabrielli GB**, Capra F, Casaril M, Squarzone S, Tognella P, Dagradi R, De Maria E, Colombari R, Corrocher R, De Sandre G. Serum laminin and type III procollagen in chronic hepatitis C. Diagnostic value in the assessment of disease activity and fibrosis. *Clin Chim Acta* 1997; **265**: 21-31 [PMID: 9352126]
- 28 **Horoz M**, Aslan M, Bolukbas FF, Bolukbas C, Nazligil Y, Celik H, Aksoy N. Serum prolidase enzyme activity and its relation to histopathological findings in patients with non-alcoholic steatohepatitis. *J Clin Lab Anal* 2010; **24**: 207-211 [PMID: 20486204 DOI: 10.1002/jcla.20334]
- 29 **Büyükhathipoğlu H**, Etkar İ, Eren MA, Demir M, Taşkın A, Aksoy N. The relationship between prolidase enzyme activity and ultrasonographic grading in hepatosteatohepatitis. *J Harran University Med Facul* 2010; **7**: 54-57
- 30 **Tarçın O**, Gedik N, Karakoyun B, Tahan V, Sood G, Celikel C, Tözün N. Serum prolidase and IGF-1 as non-invasive markers of hepatic fibrosis during four different periods after bile-duct ligation in rats. *Dig Dis Sci* 2008; **53**: 1938-1945 [PMID: 17999185 DOI: 10.1007/s10620-007-0073-1]
- 31 **Yamamoto K**, Imamura H, Matsuyama Y, Hasegawa K, Beck Y, Sugawara Y, Makuuchi M, Kokudo N. Significance of alpha-fetoprotein and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma undergoing hepatectomy. *Ann Surg Oncol* 2009; **16**: 2795-2804 [PMID: 19669841 DOI: 10.1245/s10434-009-0618-y]
- 32 **Oishi K**, Itamoto T, Amano H, Fukuda S, Ohdan H, Tashiro H, Shimamoto F, Asahara T. Clinicopathologic features of poorly differentiated hepatocellular carcinoma. *J Surg Oncol* 2007; **95**: 311-316 [PMID: 17326126]
- 33 **Sakata J**, Shirai Y, Wakai T, Kaneko K, Nagahashi M, Hatakeyama K. Preoperative predictors of vascular invasion in hepatocellular carcinoma. *Eur J Surg Oncol* 2008; **34**: 900-905 [PMID: 18343084 DOI: 10.1016/j.ejso.2008.01.031]
- 34 **Lu XY**, Xi T, Lau WY, Dong H, Xian ZH, Yu H, Zhu Z, Shen F, Wu MC, Cong WM. Pathobiological features of small hepatocellular carcinoma: correlation between tumor size and biological behavior. *J Cancer Res Clin Oncol* 2011; **137**: 567-575 [PMID: 20508947 DOI: 10.1007/s00432-010-0909-5]
- 35 **Wang CS**, Lin CL, Lee HC, Chen KY, Chiang MF, Chen HS, Lin TJ, Liao LY. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6115-6119 [PMID: 16273636]
- 36 **Kasahara A**, Hayashi N, Fusamoto H, Kawada Y, Imai Y, Yamamoto H, Hayashi E, Ogihara T, Kamada T. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. *Dig Dis Sci* 1993; **38**: 2170-2176 [PMID: 7505217]
- 37 **Furihata T**, Sawada T, Kita J, Iso Y, Kato M, Rokkaku K, Shimoda M, Kubota K. Serum alpha-fetoprotein level per tumor volume reflects prognosis in patients with hepatocellular carcinoma after curative hepatectomy. *Hepatogastroenterology* 2008; **55**: 1705-1709 [PMID: 18989777 DOI: 10.1007/s10620-008-0535-0]

- 19102374]
- 38 **Peng SY**, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC. High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus infection, age, p53 and beta-catenin mutations. *Int J Cancer* 2004; **112**: 44-50 [PMID: 15305374]
  - 39 **Hakeem AR**, Young RS, Marangoni G, Lodge JP, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2012; **35**: 987-999 [PMID: 22429190 DOI: 10.1111/j.1365-2036.2012.05060.x]
  - 40 **Carr BI**, Kanke F, Wise M, Satomura S. Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. *Dig Dis Sci* 2007; **52**: 776-782 [PMID: 17253135]

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## Randomized Controlled Trial

# Efficacy of poly-unsaturated fatty acid therapy on patients with nonalcoholic steatohepatitis

Yun-Hua Li, Lu-Hua Yang, Kai-Hui Sha, Tong-Gang Liu, Li-Guo Zhang, Xian-Xian Liu

Yun-Hua Li, Tong-Gang Liu, Li-Guo Zhang, Xian-Xian Liu, Department of Infectious Diseases, Binzhou Medical University Hospital, Binzhou 256603, Shandong Province, China  
Lu-Hua Yang, Binzhou Medical University Hospital Outpatient Department, Binzhou Medical University Hospital, Binzhou 256603, Shandong Province, China  
Kai-Hui Sha, Binzhou Medical University School of Nursing, Binzhou 256603, Shandong Province, China

**Author contributions:** Li YH, Yang LH, Sha KH, Zhang LG and Liu XX performed the study; Liu TG performed the statistic analyses; Yang LH wrote the article; and Li YH designed the study.

**Ethics approval:** The study was reviewed and approved by the Binzhou Medical University Hospital Institutional Review Board.

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

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**Correspondence to:** Yun-Hua Li, MD, Department of Infectious Diseases, Binzhou Medical University Hospital, No. 661 Huanghe Erlu, Binzhou 256603, Shandong Province, China. [yunhuali123@126.com](mailto:yunhuali123@126.com)  
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## Abstract

**AIM:** To examine whether poly-unsaturated fatty acid (PUFA) therapy is beneficial for improving nonalcoholic steatohepatitis (NASH).

**METHODS:** In total, 78 patients pathologically diagnosed with NASH were enrolled and were randomly assigned into the control group and the PUFA therapy group (added 50 mL PUFA with 1:1 ratio of EHA and DHA into daily diet). At the initial analysis and after 6 mo of PUFA therapy, parameters of interest including liver enzymes, lipid profiles, markers of inflammation and oxidation, and histological changes were evaluated and compared between these two groups.

**RESULTS:** At the initial analysis, in patients with NASH, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were slightly elevated. Triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol levels, markers of systemic inflammation [C-reactive protein (CRP)] and oxidation [malondialdehyde (MDA)], as well as fibrosis parameters of type IV collagen and pro-collagen type III pro-peptide were also increased beyond the normal range. Six months later, ALT and AST levels were significantly reduced in the PUFA group compared with the control group. In addition, serum levels of TG and TC, CRP and MDA, and type IV collagen and pro-collagen type III pro-peptide were also simultaneously and significantly reduced. Of note, histological evaluation showed that steatosis grade, necro-inflammatory grade, fibrosis stage, and ballooning score were all profoundly improved in comparison to the control group, strongly suggesting that increased PUFA consumption was a potential way to offset NASH progression.

**CONCLUSION:** Increased PUFA consumption is a potential promising approach for NASH prevention and reversal.

**Key words:** Poly-unsaturated fatty acid; Nonalcoholic steatohepatitis; Management

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**Core tip:** Epidemiologically, it has been reported that the prevalence of non-alcoholic fatty liver disease is increasing significantly and its associated morbidities, including non-alcoholic steatohepatitis (NASH) and hepatic failure, also impose great health and economic burdens on individuals and the whole of society. Preliminary data from our study showed that 6 mo poly-unsaturated fatty acid (PUFA) therapy improved NASH, as reflected by laboratory examination and histological evaluation. Future study is warranted to investigate whether long-term consumption of PUFA could completely reverse NASH and reduce the incidence of hepatic failure.

Li YH, Yang LH, Sha KH, Liu TG, Zhang LG, Liu XX. Efficacy of poly-unsaturated fatty acid therapy on patients with nonalcoholic steatohepatitis. *World J Gastroenterol* 2015; 21(22): 7008-7013 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/7008.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.7008>

## INTRODUCTION

Dyslipidemia, associated with increased plasma levels of triglyceride (TG) and total cholesterol (TC), is associated with a broad range of diseases such as atherosclerosis, metabolic syndrome, non-alcoholic fatty liver diseases (NAFLD), *etc*<sup>[1-3]</sup>. Epidemiologically, it has been reported that the prevalence of NAFLD has increased in the past decades, and non-alcoholic steatohepatitis (NASH) and hepatic failure induced by NAFLD impose great burdens on the patients and the whole society<sup>[1,4,5]</sup>. Previously, some studies used lipid-modified medications to evaluate whether NAFLD or NASH could be ameliorated, and the outcomes were controversial<sup>[6,7]</sup>. For example, Laurin *et al*<sup>[6]</sup> showed that clofibrate treatment was not beneficial for the improvement of liver function and histological changes in patients with hypertriglycemia and NASH. Nevertheless, data from Basaranoglu *et al*<sup>[7]</sup> suggested that gemfibrozil therapy could profoundly ameliorate liver dysfunction in patients with NASH. Similarly, the outcomes with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin) therapy in patients with NASH also produced conflicting results<sup>[8-10]</sup>.

Poly-unsaturated fatty acid (PUFA), predominantly comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been broadly used in daily life. Several sources of evidence indicate that PUFA

is capable of improving lipid disorders as well as of ameliorating systemic inflammation and oxidation<sup>[11-13]</sup>. It is well known that the pathophysiological characteristics of NAFLD and NASH include lipid-overloaded and lobular inflammation within liver tissues<sup>[14,15]</sup>. Therefore, we hypothesized that PUFA therapy might be useful and beneficial for improving NASH. In order to investigate our hypothesis, we conducted a randomized, prospective, controlled but not blinded trial. We believed that data from our trial could shed promising light for future studies in investigating the optimal therapy for NAFLD and NASH.

## MATERIALS AND METHODS

### Participants and strategies of research

All participants pathologically diagnosed with NASH according to the criteria were enrolled and written informed consents were obtained prior to randomization<sup>[16]</sup>. The current study was conducted in conformity to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics and research committees of our hospital. All participants were definitely ruled out of having secondary causes of NASH such as alcohol-induced (alcohol consumption  $\geq 20$  g/wk), medication-caused (such as tamoxifen and amiodarone), viral hepatitis and autoimmune diseases (primary biliary cirrhosis). In total, 78 participants were enrolled and randomly assigned into the control group (prescribed normal saline) and the PUFA therapy group (added 50 mL PUFA with 1:1 ratio of EPA and DHA into daily diet) for 6 mo. Additionally, both groups were advised to take modest physical exercise of 30 min at least 5 d/wk. Low-fat and low-cholesterol, and low carbohydrate diets were also recommended.

### Comparison of parameters of interest

All working staff involved in evaluating parameters were blinded to the information about both groups. A fasting venous blood sample was drawn and parameters of interest, including lipid profiles [TG; TC; low-density lipoprotein-cholesterol (LDL-C); and high-density lipoprotein-cholesterol (HDL-C)], serum levels of liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], total and direct bilirubin, fasting blood glucose (FBG) and C-reactive protein (CRP) were assessed by using the standard techniques of clinical chemistry laboratories. Serum levels of malondialdehyde (MDA), type IV collagen and pro-collagen type III pro-peptide (P-III-P) were detected in accordance to previous reports<sup>[17,18]</sup>. Body mass index (BMI), smoking status, family history of NASH and other demographic data were simultaneously collected by questionnaire. All the above parameters were evaluated at the initial visit and at 6 mo of PUFA therapy.

**Table 1** Baseline characteristics evaluation of all participants

Variables	Total	Control	PUFA	P value
<i>n</i>	78	39	39	
Age (yr)	51.9 ± 7.8	50.4 ± 7.2	52.6 ± 6.6	0.306
Male, <i>n</i> (%)	70 (89.7)	36 (92.3)	34 (87.2)	0.178
BMI (kg/m <sup>2</sup> )	27.9 ± 1.6	27.2 ± 1.3	28.0 ± 1.4	0.225
Smoking, <i>n</i> (%)	45 (57.7)	22 (56.4)	23 (59.0)	0.154
Family history, <i>n</i> (%)	3 (3.8)	1 (2.6)	2 (5.1)	0.237
ALT (U/L)	89.9 ± 10.4	91.3 ± 10.2	89.2 ± 12.4	0.313
AST (U/L)	82.4 ± 11.6	83.5 ± 8.9	82.0 ± 9.6	0.196
Bilirubin, total (md/dL)	1.2 ± 0.3	1.2 ± 0.2	1.2 ± 0.4	0.354
Bilirubin, direct (md/dL)	0.8 ± 0.2	0.7 ± 0.3	0.8 ± 0.4	0.366
FBG (mmol/L)	6.1 ± 0.3	6.1 ± 0.2	6.1 ± 0.5	0.117
TG (mmol/L)	2.6 ± 0.4	2.6 ± 0.3	2.5 ± 0.3	0.252
TC (mmol/L)	5.6 ± 0.7	5.5 ± 0.3	5.6 ± 0.4	0.408
LDL-C (mmol/L)	3.9 ± 0.7	3.9 ± 0.4	3.9 ± 0.3	0.387
HDL-C (mmol/L)	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	0.163
CRP (mg/L)	10.4 ± 1.3	10.6 ± 1.1	10.0 ± 1.5	0.204
MDA (nmol/mL)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.339
Type IV collagen (ng/mL)	4.9 ± 0.5	4.8 ± 0.6	4.9 ± 0.2	0.286
P-III-P (U/mL)	0.9 ± 0.3	0.9 ± 0.1	0.9 ± 0.4	0.350
Weekly exercise time (min)	60.3 ± 7.3	61.2 ± 5.6	60.1 ± 4.6	0.313

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; PUFA: Poly-unsaturated fatty acid; MDA: Malondialdehyde; LDL-C: Lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; CRP: C-reactive protein; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol.

### Pathological evaluation

At the initial visit and at 6 mo of PUFA therapy, liver biopsy was performed to evaluate the changes of hepatic tissues. Notably, steatosis grade, necro-inflammatory grade, fibrosis stage and ballooning score of these two groups were evaluated by two experts in pathology.

### Statistical analysis

Continuous variable was presented as mean ± SD and compared by the Student's *t*-test when data was normally distributed, otherwise compared by Wilcoxon rank-sum test. Categorical data was presented as percentage and compared by  $\chi^2$  test. Statistical analyses were performed by using SPSS software version 18.0 (SPSS, Inc., Chicago, Illinois). A value of  $P < 0.05$  was considered significant.

## RESULTS

### Baseline characteristics evaluation of all participants

As shown in Table 1, all parameters were comparable between the control and the PUFA therapy groups at the initial evaluation. Of note, more of the participants were male in this research, and most of the participants were overweight or obese. Serum levels of ALT and AST were slightly elevated in both groups. Lipid profiles revealed significantly increased serum levels of TG, TC and LDL-C. Markers of inflammation (CRP) and oxidation (MDA) were also elevated in patients with NASH. Fibrotic parameters such as type

**Table 2** Comparison of parameters 6 mo later

Variables	Controlled	PUFA	P value
<i>n</i>	39	39	
BMI (kg/m <sup>2</sup> )	26.4 ± 1.0	25.8 ± 1.2	0.065
Smoking, <i>n</i> (%)	15 (38.5)	14 (35.9)	0.278
ALT (U/L)	80.4 ± 7.6	67.8 ± 5.3	< 0.01
AST (U/L)	75.6 ± 5.8	60.3 ± 6.8	< 0.01
Bilirubin, total (md/dL)	0.8 ± 0.2	0.7 ± 0.2	0.343
Bilirubin, direct (md/dL)	0.6 ± 0.1	0.6 ± 0.2	0.338
FBG (mmol/L)	5.9 ± 0.2	5.9 ± 0.3	0.285
TG (mmol/L)	2.4 ± 0.4	1.8 ± 0.3	0.015
TC (mmol/L)	5.2 ± 0.6	4.7 ± 0.3	0.040
LDL-C (mmol/L)	3.5 ± 0.5	3.1 ± 0.6	0.062
HDL-C (mmol/L)	1.1 ± 0.3	1.3 ± 0.4	0.105
CRP (mg/L)	9.2 ± 0.8	7.6 ± 0.4	0.045
MDA (nmol/mL)	0.6 ± 0.1	0.4 ± 0.2	0.048
Type IV collagen (ng/mL)	4.6 ± 0.7	3.5 ± 0.5	0.023
P-III-P (U/mL)	0.8 ± 0.2	0.5 ± 0.3	0.039
Weekly exercise time (min)	107.6 ± 12.3	109.5 ± 10.4	0.272

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; PUFA: Poly-unsaturated fatty acid; MDA: Malondialdehyde; LDL-C: Lipoprotein-cholesterol; HDL-C: High-density lipoprotein-cholesterol; CRP: C-reactive protein; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol.

IV collagen and P-III-P were also higher than the normal range in patients with NASH.

### Comparison of parameters at 6 mo of PUFA therapy

As presented in Table 2, after 6 mo therapy, liver function was significantly improved in the PUFA group compared with the control group, as indicated by the significant reduction of ALT and AST levels. In addition, serum levels of TG and TC, CRP, MDA, type IV collagen and P-III-P were also significantly reduced in the PUFA group as compared to the control group. Of note, both the BMI and the percentage of smokers were reduced, while the exercise time per week was increased in both groups when compared to the initial assessment, and there was no significant difference in these improvements between the control and the PUFA therapy groups.

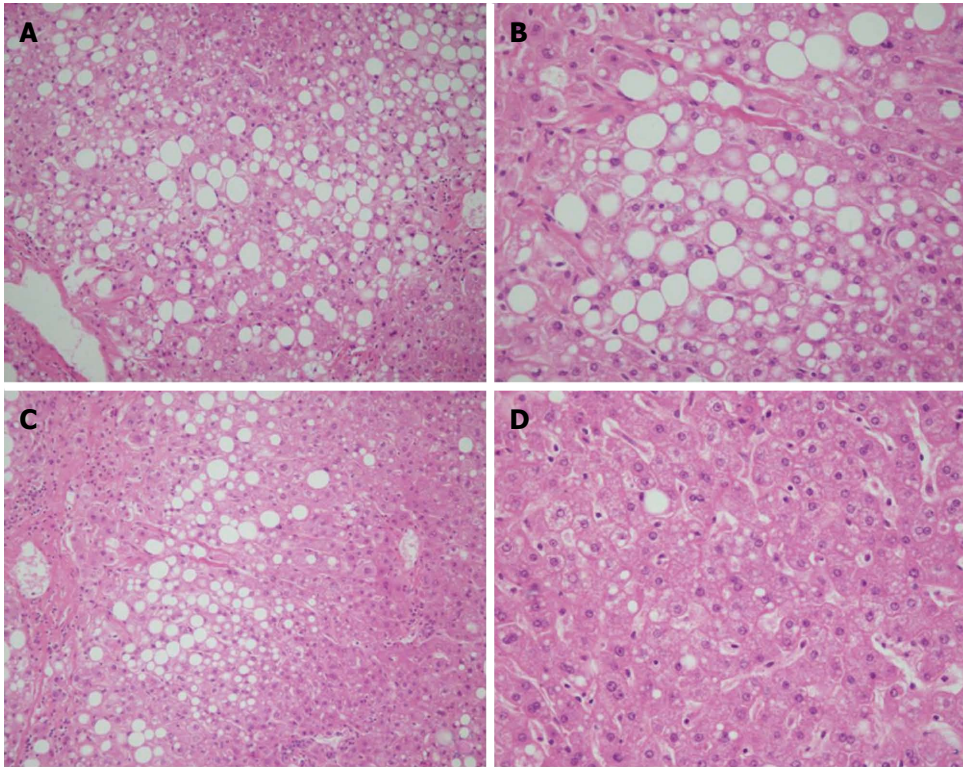
### Histological evaluation at the initial assessment and at 6 mo of PUFA therapy

Briefly, as shown in Figure 1, at the initial evaluation the parameters indicating the histological characteristics of NASH were comparable between the control and the PUFA therapy groups (as also shown in Table 3). Nevertheless, with 6 mo PUFA therapy, all parameters demonstrating the severity of NASH were significantly improved when compared to the control group.

## DISCUSSION

The prevalence of NAFLD and NASH is gradually increasing and their associated morbidity and mortality impose a great burden on the whole of society<sup>[5,19]</sup>. Currently, there are no specific and highly-





**Figure 1** Changes in pathological features before and after therapy. A and B indicate the pathological features at baseline in the control and PUFA groups, and C and D indicate the pathological features at 6 mo therapy in the control and PUFA groups. PUFA: Poly-unsaturated fatty acid.

**Table 3** Histological evaluation at initial evaluation and 6 mo later

Variables	Controlled	PUFA	P value
At the initial evaluation			
Steatosis grade	1.8 ± 0.2	1.9 ± 0.2	0.355
Necro-inflammatory grade	1.4 ± 0.2	1.4 ± 0.1	0.268
Fibrosis stage	1.7 ± 0.2	1.7 ± 0.1	0.309
Ballooning score	1.6 ± 0.3	1.6 ± 0.2	0.227
Six months later			
Steatosis grade	1.8 ± 0.1	1.4 ± 0.2	0.032
Necro-inflammatory grade	1.5 ± 0.1	1.1 ± 0.1	0.017
Fibrosis stage	1.6 ± 0.3	1.1 ± 0.2	0.020
Ballooning score	1.6 ± 0.2	1.0 ± 0.2	0.015

PUFA: Poly-unsaturated fatty acid.

effective treatments for NAFLD and NASH. Previous epidemiological studies revealed the controversial outcomes with lipid-lowering therapy in patients with NASH<sup>[6-10]</sup>. Importantly, data from our current study shows that, as compared to the control group, 6 mo of PUFA therapy significantly decreases serum levels of liver enzymes. In addition, other crucial parameters including CRP, MDA, type IV collagen and P-III-P are also profoundly reduced. Histological assessment at 6 mo further corroborates the potential benefits of PUFA therapy on NASH.

In the past decades, many risk factors associated with NAFLD and NASH development have been identified<sup>[15,20,21]</sup>. Notably, dyslipidemia, resulting from

over-consumption of cholesterol and triglycerides, is one the most critical elements for NASH development<sup>[20-22]</sup>. Knowingly<sup>[23,24]</sup>, lipid accumulating in liver tissues is the first sign of NAFLD and NASH development. Subsequently, the second sign of hepatocyte injury, inflammation, oxidation and fibrosis ensues and accelerates NASH progression. Therefore, in light of the underlying mechanisms, many medications especially lipid-lowering agents have been used for the treatment of NAFLD and NASH. In addition, other medications such as insulin-sensitizing drugs (metformin and thiazolidinedione)<sup>[25,26]</sup> and antioxidants (Vitamin C and E) have also been tested in clinical studies<sup>[27]</sup>, and disappointingly the outcomes were quite inconsistent.

Basically, PUFA is an essential nutrition for maintaining organ function properly, and previous studies also reveal that increased PUFA consumption is not only beneficial for lipid modification, but is also effective in glycemic control, hypertension management, endothelium protection and inflammation amelioration<sup>[28]</sup>. Taken together, we considered that it was reasonable to postulate that increased PUFA consumption might also be effective for NAFLD and NASH management. In our current research, we showed that with 6 mo of PUFA therapy, as compared to the control group, both the laboratory parameters of liver function and histological changes of hepatic tissues were profoundly improved, strongly suggesting that PUFA might be a potential promising candidate for NAFLD and NASH management. In



light of previous reports regarding the underlying mechanisms associated with PUFA benefits, we speculated that the following mechanisms might be responsible for our favorable findings. Firstly, as mentioned before dyslipidemia plays a continuous role on NAFLD and NASH development. While PUFA is capable of ameliorating lipid disorders<sup>[28]</sup>, and data from the present study also revealed that with 6 mo PUFA therapy serum levels of TG and TC were significantly reduced as compared to the control group. Importantly, after 6 mo of PUFA therapy, the steatosis grade was reduced, which also directly suggested that lipid accumulation in hepatic tissues could be improved with PUFA therapy. Secondly, the second sign in the process of NASH development is characterized by hepatic inflammation and oxidation. Therefore, amelioration of inflammation and oxidation might be a possible means to prevent or retard NASH progression. In our present study, we showed that after 6 mo PUFA therapy, serum levels of CRP and MDA were profoundly reduced as compared to the control group. Histological parameters, such as necro-inflammatory grade, were profoundly improved which also supported the notion that PUFA therapy was beneficial for ameliorating hepatic inflammation in patients with NASH<sup>[29]</sup>. Last but not the least, by inhibiting lipid accumulation and ameliorating inflammation, PUFA offset hepatic fibrosis as suggested by the decline of serum levels of type IV collagen and P-III-P. Histological evaluation further substantiated the fact that PUFA therapy was beneficial for improving hepatic fibrosis. Nevertheless, the mechanism associated with PUFA therapy for preventing and retarding hepatic fibrosis needs future investigation.

In conclusion, preliminary data from our study shows that 6 mo of PUFA therapy is beneficial for improving NASH, as reflected by the laboratory examination and histological evaluation. Further study is warranted to investigate whether long-term consumption of PUFA could completely reverse NASH and reduce the incidence of hepatic failure.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Poly-unsaturated fatty acid (PUFA) has been used for the therapy of dyslipidemia, which is a key risk factor for nonalcoholic steatohepatitis (NASH). Whether PUFA is beneficial for improving NASH is unknown.

### Research frontiers

Increased PUFA consumption may be beneficial for NASH improvement.

### Innovations and breakthroughs

Preliminary data from this study showed that 6 mo PUFA therapy improved NASH, as reflected by laboratory examination and histological evaluation. Further study is warranted to investigate whether long-term consumption of PUFA could completely reverse NASH and reduce the incidence of hepatic

failure.

### Applications

Current research may provide preliminary data for future studies investigating whether long-term PUFA therapy could further improve and reverse NASH.

### Terminology

Dyslipidemia, indicated by increased plasma levels of triglyceride and total cholesterol, is associated with non-alcoholic fatty liver diseases (NAFLD) and NASH. Several sources of evidence have indicated that PUFA is capable of improving lipid disorders as well as ameliorating systemic inflammation and oxidation. It is well known that the pathophysiological characteristics of NAFLD and NASH are indicated by lipid-overloaded and lobular inflammation within liver tissues. Therefore, it is reasonable to postulate that PUFA therapy may be useful and beneficial to improve NASH.

### Peer-review

The study objective is strongly justified as it is assumed that dyslipidemia resulting from over-consumption of cholesterol and triglycerides is a major risk factor for this pathology. In the future, this may have major clinical implications as potential validation of antifibrotic therapies in patients with this pathology.

## REFERENCES

- 1 **Masuoka HC**, Chalasani N. Nonalcoholic fatty liver disease: an emerging threat to obese and diabetic individuals. *Ann N Y Acad Sci* 2013; **1281**: 106-122 [PMID: 23363012 DOI: 10.1111/nyas.12016]
- 2 **DeFilippis AP**, Blaha MJ, Martin SS, Reed RM, Jones SR, Nasir K, Blumenthal RS, Budoff MJ. Nonalcoholic fatty liver disease and serum lipoproteins: the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis* 2013; **227**: 429-436 [PMID: 23419204 DOI: 10.1016/j.atherosclerosis.2013.01.022]
- 3 **Manickam P**, Sudhakar R. Dyslipidemia and non-alcoholic fatty liver disease. *Dig Dis Sci* 2013; **58**: 1435 [PMID: 23508978 DOI: 10.1007/s10620-013-2577-1]
- 4 **Ballestri S**, Lonardo A, Bonapace S, Byrne CD, Loria P, Targher G. Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 1724-1745 [PMID: 24587651 DOI: 10.3748/wjg.v20.i7.1724]
- 5 **Lazo M**, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, Koteish A, Brancati FL, Clark JM. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2013; **178**: 38-45 [PMID: 23703888 DOI: 10.1093/aje/kws448]
- 6 **Laurin J**, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. *Hepatology* 1996; **23**: 1464-1467 [PMID: 8675165 DOI: 10.1002/hep.510230624]
- 7 **Basaranoglu M**, Acbay O, Sonsuz A. A controlled trial of gemfibrozil in the treatment of patients with nonalcoholic steatohepatitis. *J Hepatol* 1999; **31**: 384 [PMID: 10453959 DOI: 10.1016/S0168-8278(99)80243-8]
- 8 **Kiyici M**, Gulten M, Gurel S, Nak SG, Dolar E, Savci G, Adim SB, Yerci O, Memik F. Ursodeoxycholic acid and atorvastatin in the treatment of nonalcoholic steatohepatitis. *Can J Gastroenterol* 2003; **17**: 713-718 [PMID: 14679419]
- 9 **Gómez-Domínguez E**, Gisbert JP, Moreno-Monteagudo JA, García-Buey L, Moreno-Otero R. A pilot study of atorvastatin treatment in dyslipemid, non-alcoholic fatty liver patients. *Aliment Pharmacol Ther* 2006; **23**: 1643-1647 [PMID: 16696815 DOI: 10.1111/j.1365-2036.2006.02926.x]
- 10 **Rallidis LS**, Drakoulis CK, Parasi AS. Pravastatin in patients with nonalcoholic steatohepatitis: results of a pilot study. *Atherosclerosis* 2004; **174**: 193-196 [PMID: 15135271 DOI: 10.1016/j.atherosclerosis.2004.01.008]
- 11 **Kazemian P**, Kazemi-Bajestani SM, Alherbish A, Steed J, Oudit GY. The use of  $\omega$ -3 poly-unsaturated fatty acids in heart failure: a preferential role in patients with diabetes. *Cardiovasc Drugs Ther* 2012; **26**: 311-320 [PMID: 22644698 DOI: 10.1007/s10557-012-

- 6397-x]
- 12 **Lluís L**, Taltavull N, Muñoz-Cortés M, Sánchez-Martos V, Romeu M, Giralt M, Molinar-Toribio E, Torres JL, Pérez-Jiménez J, Pazos M, Méndez L, Gallardo JM, Medina I, Nogués MR. Protective effect of the omega-3 polyunsaturated fatty acids: Eicosapentaenoic acid/Docosahexaenoic acid 1: 1 ratio on cardiovascular disease risk markers in rats. *Lipids Health Dis* 2013; **12**: 140 [PMID: 24083393 DOI: 10.1186/1476-511X-12-140]
  - 13 **Calzolari I**, Fumagalli S, Marchionni N, Di Bari M. Polyunsaturated fatty acids and cardiovascular disease. *Curr Pharm Des* 2009; **15**: 4094-4102 [PMID: 20041811 DOI: 10.2174/138161209789909755]
  - 14 **Castañó D**, Larequi E, Belza I, Astudillo AM, Martínez-Ansó E, Balsinde J, Argemi J, Aragon T, Moreno-Aliaga MJ, Muntane J, Prieto J, Bustos M. Cardiotrophin-1 eliminates hepatic steatosis in obese mice by mechanisms involving AMPK activation. *J Hepatol* 2014; **60**: 1017-1025 [PMID: 24362075 DOI: 10.1016/j.jhep.2013.12.012]
  - 15 **Dyson JK**, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. *Frontline Gastroenterol* 2014; **5**: 211-218 [PMID: 25018867 DOI: 10.1136/flgastro-2013-100403]
  - 16 **Tripodi A**, Fracanzani AL, Primignani M, Chantarangkul V, Clerici M, Mannucci PM, Peyvandi F, Bertelli C, Valenti L, Fargion S. Procoagulant imbalance in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **61**: 148-154 [PMID: 24657400 DOI: 10.1016/j.jhep.2014.03.013]
  - 17 **Cai F**, Dupertuis YM, Pichard C. Role of polyunsaturated fatty acids and lipid peroxidation on colorectal cancer risk and treatments. *Curr Opin Clin Nutr Metab Care* 2012; **15**: 99-106 [PMID: 22234166 DOI: 10.1097/MCO.0b013e32834feab4]
  - 18 **Karttunen T**, Sormunen R, Risteli L, Risteli J, Autio-Harmainen H. Immunoelectron microscopic localization of laminin, type IV collagen, and type III pN-collagen in reticular fibers of human lymph nodes. *J Histochem Cytochem* 1989; **37**: 279-286 [PMID: 2918219 DOI: 10.1177/37.3.2918219]
  - 19 **Perseghin G**. The role of non-alcoholic fatty liver disease in cardiovascular disease. *Dig Dis* 2010; **28**: 210-213 [PMID: 20460913 DOI: 10.1159/000282088]
  - 20 **Ogawa Y**, Imajo K, Yoneda M, Nakajima A. [Pathophysiology of Nash/NAFLD associated with high levels of serum triglycerides]. *Nihon Rinsho* 2013; **71**: 1623-1629 [PMID: 24205725]
  - 21 **Feijó SG**, Lima JM, Oliveira MA, Patrocínio RM, Moura-Junior LG, Campos AB, Lima JW, Braga LL. The spectrum of non alcoholic fatty liver disease in morbidly obese patients: prevalence and associate risk factors. *Acta Cir Bras* 2013; **28**: 788-793 [PMID: 24316747 DOI: 10.1590/S0102-86502013001100008]
  - 22 **Ioannou GN**. The natural history of NAFLD: impressively unimpressive. *Gastroenterology* 2005; **129**: 1805 [PMID: 16285987 DOI: 10.1053/j.gastro.2005.09.041]
  - 23 **Duvnjak M**, Lerotić I, Barsić N, Tomasić V, Virović Jukić L, Velagić V. Pathogenesis and management issues for non-alcoholic fatty liver disease. *World J Gastroenterol* 2007; **13**: 4539-4550 [PMID: 17729403]
  - 24 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]
  - 25 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. *Lancet* 2001; **358**: 893-894 [PMID: 11567710 DOI: 10.1016/S0140-6736(01)06042-1]
  - 26 **Buckingham RE**. Thiazolidinediones: Pleiotropic drugs with potent anti-inflammatory properties for tissue protection. *Hepatol Res* 2005; **33**: 167-170 [PMID: 16198619 DOI: 10.1016/j.hepres.2005.09.027]
  - 27 **Harrison SA**, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003; **98**: 2485-2490 [PMID: 14638353 DOI: 10.1111/j.1572-0241.2003.08699.x]
  - 28 **Mäntyselkä P**, Niskanen L, Kautiainen H, Saltevo J, Würtz P, Soininen P, Kangas AJ, Ala-Korpela M, Vanhala M. Cross-sectional and longitudinal associations of circulating omega-3 and omega-6 fatty acids with lipoprotein particle concentrations and sizes: population-based cohort study with 6-year follow-up. *Lipids Health Dis* 2014; **13**: 28 [PMID: 24507090 DOI: 10.1186/1476-511X-13-28]
  - 29 **Roncaglioni MC**, Tombesi M, Avanzini F, Barlera S, Caimi V, Longoni P, Marzona I, Milani V, Silletta MG, Tognoni G, Marchioli R. n-3 fatty acids in patients with multiple cardiovascular risk factors. *N Engl J Med* 2013; **368**: 1800-1808 [PMID: 23656645 DOI: 10.1056/NEJMoa1205409]

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## Treatment strategies for colorectal carcinoma with synchronous liver metastases: Which way to go?

Peter Ihnát, Petr Vávra, Pavel Zonča

Peter Ihnát, Petr Vávra, Pavel Zonča, Department of Surgical Studies, Faculty of Medicine, University of Ostrava, Ostrava 70300, Czech Republic

Peter Ihnát, Petr Vávra, Pavel Zonča, Department of Surgery, University Hospital Ostrava, Ostrava 70852, Czech Republic

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**Correspondence to:** Peter Ihnát, MD, PhD, MBA, Department of Surgical Studies, Faculty of Medicine, University of Ostrava, Syllabova 19, Ostrava 70300, Czech Republic. [peterihnath@yahoo.com](mailto:peterihnath@yahoo.com)  
Telephone: + 42-597-375701  
Fax: + 42-597-375054

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### Abstract

**AIM:** To offer an up-to-date review of all available

treatment strategies for patients with synchronous colorectal liver metastases (CLM).

**METHODS:** A comprehensive literature search was performed to identify articles related to the management of patients with synchronous CLM. A search of the electronic databases PubMed, MEDLINE, and Google Scholar was conducted in September 2014. The following search terms were used: synchronous colorectal liver metastases, surgery, stage IV colorectal cancer, liver-first approach, and up-front hepatectomy. These terms were employed in various combinations to maximize the search. Only articles written in English were included. Particular attention was devoted to studies and review articles that were published within the last six years (2009-2014). Additional searches of the cited references from primary articles were performed to further improve the review. The full texts of all relevant articles were accessed by two independent reviewers.

**RESULTS:** Poor long-term outcomes of patients with synchronous CLM managed by a traditional treatment strategy have led to questions about the timing and sequence of possible therapeutic interventions. Thus, alternative paradigms called reverse strategies have been proposed. Presently, there are four treatment strategies available: (1) primary first approach (or traditional approach) comprises resection of the primary colorectal tumor followed by chemotherapy; subsequent liver resection is performed 3-6 mo after colorectal resection (provided that CLM are still resectable); (2) simultaneous resection of the primary colorectal tumor and CLM during a single operation presents intriguing options for a highly select group of patients, which can be associated with significant postoperative morbidity; (3) liver-first (or chemotherapy-first) approach comprises preoperative chemotherapy (3-6 cycles) followed by liver resection, adjuvant chemotherapy, and resection of the primary colorectal tumor (it is best suited for patients with

asymptomatic primary tumors and initially unresectable or marginally resectable CLM); and (4) up-front hepatectomy (or “true” liver-first approach) includes liver resection followed by adjuvant chemotherapy, colorectal resection, and adjuvant chemotherapy (strategy can be offered to patients with asymptomatic primary tumors and initially resectable CLM).

**CONCLUSION:** None of the aforementioned strategies appears inferior. It is necessary to establish individual treatment plans in multidisciplinary team meetings through careful appraisal of all strategies.

**Key words:** Colorectal cancer; Liver-first approach; Reverse strategy; Simultaneous resection; Up-front hepatectomy

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**Core tip:** There are four treatment strategies available for synchronous liver metastases of colorectal carcinoma (CLM): (1) primary first approach comprises resection of the primary colorectal tumor followed by chemotherapy and liver resection; (2) simultaneous resection of liver and colorectal primary tumor; (3) liver-first (or chemotherapy-first) approach comprises preoperative chemotherapy, liver resection, adjuvant chemotherapy, and resection of the primary colorectal tumor (best for asymptomatic primary tumors and initially unresectable or marginally resectable CLM); and (4) up-front hepatectomy (or “true” liver-first approach) includes liver resection followed by adjuvant chemotherapy and colorectal resection (for asymptomatic primary tumors and initially resectable CLM).

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## INTRODUCTION

The liver is the most common site of colorectal cancer metastases. At the time of diagnosis, approximately 25% of patients have synchronous colorectal liver metastases (CLM)<sup>[1]</sup>. These patients are thought to have less favorable cancer biology, and are less likely to become long-term survivors compared to patients with metachronous CLM<sup>[2]</sup>. The endeavor to improve outcomes of patients with synchronous CLM led to questions about the timing and sequence of possible therapeutic interventions<sup>[3,4]</sup>. Several alternative treatment strategies have been proposed, such as simultaneous resection, the liver-first approach, and an up-front hepatectomy approach.

A search of the scientific literature shows that

there is currently no complex review available that summarizes the pros and cons of all four possible treatment strategies with respect to the management of patients with synchronous CLM. Moreover, authors usually do not clearly distinguish the up-front hepatectomy from the liver-first approach, though several principal distinctions between both strategies are evident.

The aim of the present paper is to offer an up-to-date review of all four available strategies for the treatment of patients with colorectal cancer and synchronous CLM. This article summarizes the current data concerning the rationale, benefits, and potential drawbacks of the particular strategies (primary-first, simultaneous approach, liver-first approach, and up-front hepatectomy).

## MATERIALS AND METHODS

A comprehensive literature search was performed to identify articles related to therapeutic strategies for patients with colorectal cancer and simultaneous CLM. The search combined the following terms: synchronous colorectal liver metastases, surgery, stage IV colorectal cancer, liver-first approach, and up-front hepatectomy. Sources included MEDLINE, PubMed, and Google Scholar databases. Particular attention was devoted to studies and review articles that were published within the last six years (2009-2014).

## RESULTS

### Current treatment strategies

Although there have been significant improvements in the management of stage IV colorectal cancer in the last few decades, only radical surgical resection of both the primary tumor and CLM can offer long-term survival for patients presenting with CLM<sup>[1,5,6]</sup>. Surgery is performed with the intent to achieve minimal intraoperative blood loss and low postoperative morbidity and mortality, because these factors have been shown to compromise not only short-term results, but also long-term outcomes<sup>[7-9]</sup>.

The management of patients with colorectal cancer and synchronous CLM is multimodal and comprises surgery, chemotherapy, and radiotherapy. Multimodality and the need for surgery at the two different sites (colorectal primary tumor and CLM) enable various sequences and timing for therapeutic modalities. Poor long-term outcomes of the traditional treatment strategy (primary-first approach) led to the proposal of alternative paradigms for patient management, called reverse strategies. Presently, four therapeutic strategies are available: the primary-first approach, simultaneous resection, a liver-first approach, and up-front hepatectomy.

### Primary-first approach

The primary first approach, often referred to as



the “classical” or “traditional” approach, includes resection of the primary colorectal tumor followed by chemotherapy (plus radiotherapy for rectal primaries). Liver resection is performed 3-6 mo after colorectal resection (provided CLM are still resectable).

The rationale for the primary-first approach is twofold: colorectal primary tumors are thought to be a likely source of subsequent metastases and also the source of symptoms. The main advantage of the strategy is that it avoids potential complications from the primary tumor and decreases the risk of potential progression of the primary tumor during liver surgery or initial chemotherapy<sup>[3,4]</sup>. Conversely, the main drawback of the primary-first approach is the progression of CLM beyond resectability during the primary tumor resection (especially in patients with postoperative complications after colorectal resection)<sup>[3]</sup>.

Because of frequent CLM progression beyond resectability, only few patients benefit from the traditional strategy. In 2012, analysis based on the LiverMetSurvey revealed that < 30% of patients underwent the complete treatment plan of primary-first strategy (from primary tumor resection to liver resection)<sup>[10]</sup>. Conversely, reverse strategy enables completion of the treatment plan in almost 80% of patients<sup>[3,11]</sup>.

### Simultaneous resection

Simultaneous resection of colorectal primary and synchronous CLM presents an intriguing option for many surgeons. The simultaneous resection can be employed with or without preoperative chemotherapy; adjuvant chemotherapy is applied after the surgery (plus radiotherapy for rectal primaries).

The strategy of simultaneous resection had been proposed in the effort to avoid delaying surgical resection of metastatic liver disease<sup>[12]</sup>. The main advantage of this strategy is the removal of all macroscopic cancer during a single operation followed by systemic chemotherapy with minimal delay. Conversely, the main disadvantage of this strategy is that it is associated with significantly increased postoperative morbidity and possibly mortality<sup>[12-14]</sup>. Increased risk of infectious liver complications (due to bacterial contamination from intestinal resection), increased risk of anastomotic complications (due to impaired liver function), and limited extent of feasible liver resection have been reported<sup>[13,14]</sup>. There is also some evidence that simultaneous resection may have a negative effect on progression-free survival<sup>[15]</sup>.

Several studies have demonstrated that reasonable postoperative morbidity and mortality can be achieved if colorectal resection is combined with minor hepatectomy. In recent series of simultaneous resections, postoperative morbidity in the range of 5% to 48% was reported when minor hepatectomies were performed, and from 33% to 55% when major

hepatectomies were performed simultaneously with colorectal resection<sup>[13-16]</sup>. Perioperative mortality of  $\leq$  5% was noted, but a higher number can be expected when major hepatectomies are performed.

Simultaneous resection is best suited for highly select patients; many authors recommend considering simultaneous resection only if one of the intended surgical resections is minor. It is reasonable to perform rectal resection simultaneously only with minor hepatectomy (< 3 segments), or to perform major liver resection ( $\geq$  3 segments) simultaneously with (right-sided) colon resection<sup>[14,16,17]</sup>. However, major hepatectomies should be pursued only in very carefully selected patients by an experienced hepatobiliary team. A patient's general health status and comorbidities also have to be considered.

### Liver-first (chemotherapy-first) approach

The reverse treatment strategy was first introduced by Mentha *et al*<sup>[3]</sup> in 2008. The liver first approach comprises initial preoperative chemotherapy (3-6 cycles) followed by liver resection and subsequent resection of the primary colorectal tumor. Chemotherapy (possibly with radiotherapy for rectal primaries) is administered between colorectal and liver resection.

The introduction of modern potent cytotoxic drugs (oxaliplatin-based and irinotecan-based) in combination with targeted agents (directed against epidermal growth factor receptor or vascular endothelial growth factor) resulted in improved tumor response rates (up to 60% of tumors) and prolonged survival of patients with colorectal cancer<sup>[16,18]</sup>. Effectiveness of modern chemotherapy regimens (in adjuvant settings) led to the application of chemotherapy, also in neoadjuvant settings, for patients with colorectal carcinoma and synchronous CLM. It is believed that the prognosis of patients with stage IV colorectal cancer is determined mainly by the curability of CLM and not by the primary tumor or its potential complications<sup>[3,4,14]</sup>.

As a matter of fact, preoperative chemotherapy is the initial treatment modality during the liver-first approach, which is why the term “chemotherapy-first” is suggested to be more accurate for this strategy<sup>[17]</sup>. The expression “chemotherapy-first” emphasizes the main rationale of the reverse strategy, which is to provide early systemic treatment to patients with stage IV colorectal cancer.

Benefits of the chemotherapy-first approach are: (1) early application of systemic treatment; (2) lowering the risk of CLM progression; and (3) the possibility of CLM downsizing or converting unresectable CLM to resectable.

As stage IV colorectal cancer presents as systemic disease, it seems reasonable to offer systemic chemotherapy as soon as possible after the diagnosis is established. Moreover, patients with synchronous CLM are supposed to have more aggressive tumors with less favorable cancer biology. By using preoperative

chemotherapy administration, effective systemic treatment is not delayed by colorectal surgery and its possible postoperative complications<sup>[3,4,12]</sup>.

The risk of CLM progression is significantly lower when the chemotherapy-first approach is employed, compared to the traditional strategy<sup>[3,12,17]</sup>. Furthermore, preoperative chemotherapy offers the opportunity for initial disease control and CLM downsizing. Liver metastasis shrinkage after preoperative chemotherapy enables surgeons to perform more conservative liver surgery more often and to achieve R0 resection in more patients. Preoperative chemotherapy application also allows for the assessment of tumor response to chemotherapy. In theory, another possible advantage of preoperative chemotherapy is the elimination of micrometastatic disease and the eradication of dormant cancer cells<sup>[17,19]</sup>.

Fears of complications arising from unresected primary tumors (such as bleeding, obstruction, or perforation) in the course of initial chemotherapy and liver resection represent principal arguments against the reverse strategy. Nevertheless, primary tumor complications in patients with stage IV colorectal cancer are very rare according to several studies. The vast majority (> 90%) of patients with initially asymptomatic colorectal primary tumors and synchronous CLM who receive modern chemotherapy regimens never require surgical intervention because of primary tumor-related complications<sup>[20]</sup>. Besides, Scheer *et al*<sup>[21]</sup> demonstrated that primary tumor resection provided only minimal palliative benefit to these patients. This is why systemic chemotherapy regimens are advocated as initial treatment modalities for asymptomatic primary tumors with synchronous CLM. If the tumor does not respond to preoperative chemotherapy in patients with initially unresectable CLM, useless colorectal surgery can be avoided<sup>[14,19]</sup>.

Recently, an international multidisciplinary panel generated a consensus concerning the reverse strategy<sup>[22]</sup>. The most important recommendations were as follows. First, the reverse strategy should be considered for all patients with predominant hepatic disease and asymptomatic primary tumor. Second, preoperative chemotherapy should be offered to patients with asymptomatic colorectal cancer and synchronous CLM (resectable, marginally resectable, and unresectable). Third, at least four courses of first-line chemotherapy should be given. Fourth, the use of doublet or triplex chemotherapy regimens combined with targeted therapy is recommended. Fifth, chemotherapy duration should be as short as possible and liver resection should be performed as soon as technically possible. Lastly, tumor response and patient reassessment should be performed 2 mo after starting chemotherapy.

In the last decade, many (> 400) papers focusing on liver-first strategy evaluation have been published. However, according to several recent systematic reviews<sup>[4,17,23]</sup>, scientific evidence for the justification

of the liver-first approach is very limited. For instance, there are no randomized controlled trials, and many papers have very limited scientific validity (such as reviews, case reports, letters, editorials, and abstracts). There are only four cohort retrospective studies reporting outcomes of a total of 121 patients with colorectal cancer and synchronous CLM managed by the liver-first approach<sup>[4,17,23]</sup>. In these studies, postoperative morbidity was in the range of 11% to 37%; postoperative mortality was < 4%. Disease recurrence rates were 25%-70%; three-year survival rates varied in the range of 41% to 79%, and five-year survival rates were 31%-39%<sup>[3,12,24,25]</sup>. The majority (66%-81%) of patients completed the entire liver-first strategy treatment plan (preoperative chemotherapy to colorectal resection). This is in contrast to < 30% of patients completing the primary-first strategy<sup>[3,10,12,24,25]</sup>.

The reverse strategy is best suited for patients with an asymptomatic primary tumors and advanced hepatic metastases<sup>[4,16,17,22]</sup>. There is general agreement that patients with unresectable or borderline resectable CLM should be offered aggressive doublet or triplex chemotherapy regimen combined with targeted therapy as the initial treatment modality, followed by liver resection, if technically amenable. The optimal initial treatment strategy for patients with initially resectable synchronous CLM is debatable.

### Up-front hepatectomy

Surgical resection represents the only treatment modality that can offer long-term survival to patients with synchronous resectable CLM. The limited evidence for preoperative (neoadjuvant) chemotherapy employment led to the proposal of an up-front hepatectomy strategy, which is in fact the "true" liver-first approach. The common sequence of up-front hepatectomy strategy comprises liver resection, adjuvant chemotherapy, colorectal resection, and adjuvant chemotherapy. The strategy was originally proposed by Grundmann *et al*<sup>[26]</sup> in 2008 for patients with asymptomatic colorectal carcinoma and synchronous resectable CLM.

There are several benefits to preoperative chemotherapy administration for the treatment of resectable synchronous CLM: testing tumor chemoresponsiveness, elimination of micrometastatic disease (in theory), and the possibility of tumor shrinkage enabling more conservative liver surgery in some cases; the benefits were discussed in detail in the previous section<sup>[11,14,19]</sup>.

The main drawbacks of preoperative chemotherapy include liver toxicity, missing lesions, and risk of tumor progression. Chemotherapy induces pathologic changes in the liver parenchyma, which are dependent on the number of chemotherapy cycles (such as steatosis, chemotherapy-associated steatohepatitis, and sinusoidal obstruction syndrome). In addition, chemotherapy increases the risk of systemic toxicity, postoperative bleeding, and infection (by inducing neutropenia)<sup>[19,27,28]</sup>. A recent meta-analysis demonstrated a high variability in the frequency of

chemotherapy-induced hepatotoxicity<sup>[18]</sup>. Hepatic steatosis was detected after regimens with 5-fluorouracil in 6%-76% of patients, steatohepatitis was observed after irinotecan-based regimens in 3%-8% of patients, and sinusoidal obstruction syndrome was noted after oxaliplatin-based regimens in 5%-51% of patients<sup>[18]</sup>. Chemotherapy-induced liver injury results in worse postoperative outcomes of subsequent liver resections. Increased postoperative morbidity has been demonstrated by several studies, though no impact on postoperative mortality was observed<sup>[29-33]</sup>. Especially after extended surgical resection performance, preoperative chemotherapy may contribute to the development of liver failure.

CLM that respond well to preoperative chemotherapy may no longer be visible on CT or during surgery. Tumor disappearance was noted in 2%-36% of patients after preoperative chemotherapy<sup>[28]</sup>. Problematic identification of invisible lesions during surgery is associated with a higher risk of incomplete (non-radical) resection and disease early recurrence<sup>[34]</sup>. Furthermore, lesion disappearance (on CT scans) does not mean complete pathologic response. Benoist *et al.*<sup>[19]</sup> demonstrated that > 80% of invisible metastases (invisible lesions on CT scans after chemotherapy) contained viable tumor cells at the time of resection. When the "watch and see" policy is applied (after disappearance on imaging techniques), local recurrence was reported in 38%-74% of patients<sup>[34,35]</sup>.

The risk of tumor progression in the course of preoperative chemotherapy is another drawback of its routine use in the management of patients with initially resectable CLM. According to recent systematic reviews and meta-analyses, CLM progression (changing from resectable to unresectable disease) was observed in 7%-37% of patients undergoing preoperative chemotherapy. However, some authors suggest that disease progression during preoperative chemotherapy is a consequence of highly aggressive tumor biology and may in fact prevent unnecessary postoperative surgical morbidity and mortality<sup>[36,37]</sup>.

The European Colorectal Metastases Treatment Group in its Multidisciplinary International Consensus recommends preoperative chemotherapy for patients with initially resectable synchronous CLM<sup>[22]</sup>. These recommendations are based mainly on the results of the EORTC 40983 trial, which are a slightly misleading. The EORTC trial evaluated outcomes of 364 patients with resectable CLM divided into two groups: (1) patients managed with three cycles of preoperative FOLFOX, liver resection, and three cycles of postoperative FOLFOX; and (2) patients undergoing liver resection alone without chemotherapy. In other words, the EORTC trial unfortunately did not compare the effect of preoperative chemotherapy (patients in the FOLFOX group) with patients undergoing liver resection plus adjuvant chemotherapy (the FOLFOX group was only compared with patients undergoing surgery alone). In the FOLFOX group, there was

significantly longer progression-free survival at three years (35.4% vs 28.1%), but overall survival was not increased. Moreover, higher numbers of postoperative complications were recorded in the FOLFOX group compared to patients undergoing surgery alone (25% vs 16%)<sup>[29]</sup>.

In an effort to overcome the aforementioned handicap of the EORTC trial, several studies have been executed. For instance, Adam *et al.*<sup>[38]</sup> compared 169 patients treated with preoperative chemotherapy with a retrospective group of 1302 patients who underwent surgery and adjuvant chemotherapy; postoperative complications were more frequent in the neoadjuvant group (37% vs 24%). No impact on survival or disease-free interval was found in the neoadjuvant group, but improved survival was found in patients treated with surgery and adjuvant chemotherapy. Reddy *et al.*<sup>[39]</sup> published very similar results in favor of adjuvant chemotherapy. Additional studies (with several hundreds of patients) also showed no significant differences between the outcomes of patients receiving preoperative chemotherapy compared to those without preoperative chemotherapy<sup>[40-42]</sup>.

The aforementioned pros and cons of preoperative chemotherapy administration make it difficult to determine which strategy is the best option for patients with synchronous resectable CLM. The need for prospective randomized trials of neoadjuvant vs adjuvant chemotherapy is emphasized by all authors. However, recent systematic reviews and meta-analyses focusing on the preoperative chemotherapy evaluation concluded that "routine use of neoadjuvant chemotherapy for patients with clearly resectable lesions is not recommended due to a lack of benefit on survival"<sup>[18]</sup>. Many authors share the same conviction and recommend performing up-front hepatectomy in patients with synchronous initially resectable CLM<sup>[11,14,19,27,40,42-44]</sup>.

## DISCUSSION

### **Proposal of a decision strategy**

With regard to current published data and according to all aforementioned benefits and drawbacks of particular strategies, we propose the following decision treatment scheme for patients with synchronous CLM.

Patients with colorectal cancer and synchronous CLM should undergo careful clinical examination focused on determining a patient's performance status, comorbidities, and tumor stage. It is necessary to establish an individual treatment plan for each patient in a multidisciplinary team meeting that includes experienced colorectal and hepatobiliary surgeons.

The traditional strategy (primary-first approach) is best suited for patients with symptomatic primary tumors and synchronous CLM. Assessment of the simultaneous approach execution should be conducted in patients with limited CLM extent, especially when

one of the intended surgical resections is minor (minor hepatectomy or right-sided colon resection). Patients with unresectable or marginally resectable CLM should be offered the chemotherapy-first approach. The administration of a duplex or triplex chemotherapy regimen combined with targeted therapy is recommended. Evaluation of tumor response and patient reassessment is advised after two months, followed by liver resection if technically amendable. Patients with initially resectable CLM should be offered up-front hepatectomy as a first-line treatment strategy.

The management of patients with colorectal cancer and synchronous CLM is complex and multiple factors must be considered (such as location and extent of primary tumor and CLM, presence of symptoms, patient's general health status, and comorbidities). None of the aforementioned treatment strategies (primary-first, simultaneous resection, chemotherapy-first, or up-front hepatectomy) appears inferior to the others<sup>[23]</sup>. However, the optimal treatment strategy is still unclear because of limited available evidence<sup>[4,17,23]</sup>. It is necessary to establish an individual treatment plan for each patient with synchronous CLM in multidisciplinary team meetings through careful appraisal of all strategies with the aim of avoiding unnecessary surgical complications and to achieve long-term cures.

## COMMENTS

### Background

Although there have been significant improvements in the management of stage IV colorectal cancer in the last few decades, only radical surgical resection of both the primary tumor and liver metastases can offer long-term survival. The management of patients with colorectal cancer and synchronous colorectal liver metastases (CLM) is multimodal, comprising surgery, chemotherapy, and radiotherapy. Multimodality and the need for surgery at the two different sites (primary colorectal tumor and CLM) enable various sequences and timing for therapeutic modalities.

### Research frontiers

A search of the scientific literature shows there is currently no complex review available that summarizes the pros and cons of all four possible treatment strategies with respect to the management of patients with synchronous CLM. Moreover, authors usually do not clearly distinguish the up-front hepatectomy from the liver-first approach, although several principal distinctions between both strategies are evident.

### Innovations and breakthroughs

In this paper, the authors offer a comprehensive review of all four different therapeutic strategies that are currently available for patients with synchronous CLM: primary-first approach, simultaneous resection, liver-first approach, and up-front hepatectomy. Authors summarize up-to-date data on the rationale, benefits, and potential drawbacks of the particular strategies. Based on the available published data, authors propose a decision scheme (between particular strategies) for patients with synchronous CLM.

### Applications

The present review offers comprehensive insight into current treatment options available for patients with synchronous CLM. The proposed decision scheme may work as a helpful tool for multidisciplinary teams when establishing a treatment plan for particular patients.

### Terminology

Primary-first approach comprises resection of colorectal primary followed by chemotherapy and subsequent liver resection. Simultaneous resection is resection of the primary colorectal tumor and CLM during a single

operation. Liver-first (or chemotherapy-first) approach comprises preoperative chemotherapy (3-6 cycles) followed by liver resection, adjuvant chemotherapy, and resection of the primary colorectal tumor. Up-front hepatectomy (or "true" liver-first approach) includes liver resection followed by adjuvant chemotherapy, colorectal resection, and adjuvant chemotherapy.

### Peer-review

The topic of this review is important, and the publication is well written and readable. The discussion is well organized as findings correspond with the literature.

## REFERENCES

- 1 McMillan DC, McArdle CS. Epidemiology of colorectal liver metastases. *Surg Oncol* 2007; **16**: 3-5 [PMID: 17493802]
- 2 Tsai MS, Su YH, Ho MC, Liang JT, Chen TP, Lai HS, Lee PH. Clinicopathological features and prognosis in resectable synchronous and metachronous colorectal liver metastasis. *Ann Surg Oncol* 2007; **14**: 786-794 [PMID: 17103254]
- 3 Mentha G, Roth AD, Terraz S, Giostra E, Gervaz P, Andres A, Morel P, Rubbia-Brandt L, Majno PE. 'Liver first' approach in the treatment of colorectal cancer with synchronous liver metastases. *Dig Surg* 2008; **25**: 430-435 [PMID: 19212115 DOI: 10.1159/000184734]
- 4 De Rosa A, Gomez D, Brooks A, Cameron IC. "Liver-first" approach for synchronous colorectal liver metastases: is this a justifiable approach? *J Hepatobiliary Pancreat Sci* 2013; **20**: 263-270 [PMID: 23325126 DOI: 10.1007/s00534-012-0583-x]
- 5 Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM. Epidemiology and management of liver metastases from colorectal cancer. *Ann Surg* 2006; **244**: 254-259 [PMID: 16858188]
- 6 Van den Eynde M, Hendlisz A. Treatment of colorectal liver metastases: a review. *Rev Recent Clin Trials* 2009; **4**: 56-62 [PMID: 19149763]
- 7 Giulianti F, Ardito F, Vellone M, Ranucci G, Federico B, Giovannini I, Nuzzo G. Role of the surgeon as a variable in long-term survival after liver resection for colorectal metastases. *J Surg Oncol* 2009; **100**: 538-545 [PMID: 19722234 DOI: 10.1002/jso.21393]
- 8 Ito H, Are C, Gonen M, D'Angelica M, Dematteo RP, Kemeny NE, Fong Y, Blumgart LH, Jarnagin WR. Effect of postoperative morbidity on long-term survival after hepatic resection for metastatic colorectal cancer. *Ann Surg* 2008; **247**: 994-1002 [PMID: 18520227 DOI: 10.1097/SLA.0b013e31816c405f]
- 9 Farid SG, Aldouri A, Morris-Stiff G, Khan AZ, Toogood GJ, Lodge JP, Prasad KR. Correlation between postoperative infective complications and long-term outcomes after hepatic resection for colorectal liver metastasis. *Ann Surg* 2010; **251**: 91-100 [PMID: 19858702 DOI: 10.1097/SLA.0b013e3181bfda3c]
- 10 Andres A, Toso C, Adam R, Barroso E, Hubert C, Capussotti L, Gerstel E, Roth A, Majno PE, Mentha G. A survival analysis of the liver-first reversed management of advanced simultaneous colorectal liver metastases: a LiverMetSurvey-based study. *Ann Surg* 2012; **256**: 772-778; discussion 778-779 [PMID: 23095621 DOI: 10.1097/SLA.0b013e3182734423]
- 11 Straka M, Skrovina M, Soumarova R, Kotasek R, Burda L, Vojtek C. Up front hepatectomy for metastatic rectal carcinoma - reversed, liver first approach. Early experience with 15 patients. *Neoplasma* 2014; **61**: 447-452 [PMID: 25027742]
- 12 Brouquet A, Mortenson MM, Vauthey JN, Rodriguez-Bigas MA, Overman MJ, Chang GJ, Kopetz S, Garrett C, Curley SA, Abdalla EK. Surgical strategies for synchronous colorectal liver metastases in 156 consecutive patients: classic, combined or reverse strategy? *J Am Coll Surg* 2010; **210**: 934-941 [PMID: 20510802 DOI: 10.1016/j.jamcollsurg.2010.02.039]
- 13 Fahy BN, Fischer CP. Synchronous resection of colorectal primary and hepatic metastasis. *J Gastrointest Oncol* 2012; **3**: 48-58 [PMID: 22811869 DOI: 10.3978/j.issn.2078-6891.2012.004]
- 14 Grundmann RT. Current state of surgical treatment of liver metastases from colorectal cancer. *World J Gastrointest Surg* 2011; **3**: 183-196 [PMID: 22224173 DOI: 10.4240/wjgs.v3.i12.183]



- 15 **de Haas RJ**, Adam R, Wicherts DA, Azoulay D, Bismuth H, Vibert E, Salloum C, Perdigao F, Benkabbou A, Castaing D. Comparison of simultaneous or delayed liver surgery for limited synchronous colorectal metastases. *Br J Surg* 2010; **97**: 1279-1289 [PMID: 20578183 DOI: 10.1002/bjs.7106]
- 16 **Tsoufias G**, Pramateftakis MG. Management of rectal cancer and liver metastatic disease: which comes first? *Int J Surg Oncol* 2012; **2012**: 196908 [PMID: 22778934 DOI: 10.1155/2012/196908]
- 17 **Jegatheeswaran S**, Mason JM, Hancock HC, Siriwardena AK. The liver-first approach to the management of colorectal cancer with synchronous hepatic metastases: a systematic review. *JAMA Surg* 2013; **148**: 385-391 [PMID: 23715907 DOI: 10.1001/jamasurg.2013.1216]
- 18 **Lehmann K**, Rickenbacher A, Weber A, Pestalozzi BC, Clavien PA. Chemotherapy before liver resection of colorectal metastases: friend or foe? *Ann Surg* 2012; **255**: 237-247 [PMID: 22041509 DOI: 10.1097/SLA.0b013e3182356236]
- 19 **Benoist S**, Brouquet A, Penna C, Julié C, El Hajjam M, Chagnon S, Mitry E, Rougier P, Nordlinger B. Complete response of colorectal liver metastases after chemotherapy: does it mean cure? *J Clin Oncol* 2006; **24**: 3939-3945 [PMID: 16921046]
- 20 **Poultides GA**, Servais EL, Saltz LB, Patil S, Kemeny NE, Guillem JG, Weiser M, Temple LK, Wong WD, Paty PB. Outcome of primary tumor in patients with synchronous stage IV colorectal cancer receiving combination chemotherapy without surgery as initial treatment. *J Clin Oncol* 2009; **27**: 3379-3384 [PMID: 19487380 DOI: 10.1200/JCO.2008.20.9817]
- 21 **Scheer MG**, Sloots CE, van der Wilt GJ, Ruers TJ. Management of patients with asymptomatic colorectal cancer and synchronous irresectable metastases. *Ann Oncol* 2008; **19**: 1829-1835 [PMID: 18662955 DOI: 10.1093/annonc/mdn398]
- 22 **Adam R**, De Gramont A, Figueras J, Guthrie A, Kokudo N, Kunstlinger F, Loyer E, Poston G, Rougier P, Rubbia-Brandt L, Sobrero A, Tabernero J, Teh C, Van Cutsem E. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. *Oncologist* 2012; **17**: 1225-1239 [PMID: 22962059 DOI: 10.1634/theoncologist.2012-0121]
- 23 **Lykoudis PM**, O'Reilly D, Nastos K, Fusai G. Systematic review of surgical management of synchronous colorectal liver metastases. *Br J Surg* 2014; **101**: 605-612 [PMID: 24652674 DOI: 10.1002/bjs.9449]
- 24 **Verhoef C**, van der Pool AE, Nuyttens JJ, Planting AS, Eggermont AM, de Wilt JH. The "liver-first approach" for patients with locally advanced rectal cancer and synchronous liver metastases. *Dis Colon Rectum* 2009; **52**: 23-30 [PMID: 19273952 DOI: 10.1007/D0CR.0b013e318197939a]
- 25 **de Jong MC**, van Dam RM, Maas M, Bemelmans MH, Olde Damink SW, Beets GL, Dejong CH. The liver-first approach for synchronous colorectal liver metastasis: a 5-year single-centre experience. *HPB (Oxford)* 2011; **13**: 745-752 [PMID: 21929676 DOI: 10.1111/j.1477-2574.2011.00372.x]
- 26 **Grundmann RT**, Hermanek P, Merkel S, Germer CT, Grundmann RT, Hauss J, Henne-Bruns D, Herfarth K, Hermanek P, Hopt UT, Junginger T, Klar E, Klempnauer J, Knapp WH, Kraus M, Lang H, Link KH, Löhle F, Merkel S, Oldhafer KJ, Raab HR, Rau HG, Reinacher-Schick A, Ricke J, Roder J, Schäfer AO, Schlitt HJ, Schön MR, Stippel D, Tannapfel A, Tatsch K, Vogl TJ. [Diagnosis and treatment of colorectal liver metastases - workflow]. *Zentralbl Chir* 2008; **133**: 267-284 [PMID: 18563694 DOI: 10.1055/s-2008-1076796]
- 27 **Neumann UP**, Seehofer D, Neuhaus P. The surgical treatment of hepatic metastases in colorectal carcinoma. *Dtsch Arztebl Int* 2010; **107**: 335-342 [PMID: 20532128 DOI: 10.3238/arztebl.2010.0335]
- 28 **Khan K**, Wale A, Brown G, Chau I. Colorectal cancer with liver metastases: neoadjuvant chemotherapy, surgical resection first or palliation alone? *World J Gastroenterol* 2014; **20**: 12391-12406 [PMID: 25253940 DOI: 10.3748/wjg.v20.i35.12391]
- 29 **Nordlinger B**, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, Jaecck D, Mirza D, Parks RW, Collette L, Praet M, Bethe U, Van Cutsem E, Scheithauer W, Gruenberger T. Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet* 2008; **371**: 1007-1016 [PMID: 18358928 DOI: 10.1016/S0140-6736(08)60455-9]
- 30 **Morris-Stiff G**, Tan YM, Vauthey JN. Hepatic complications following preoperative chemotherapy with oxaliplatin or irinotecan for hepatic colorectal metastases. *Eur J Surg Oncol* 2008; **34**: 609-614 [PMID: 17764887]
- 31 **Zorzi D**, Laurent A, Pawlik TM, Lauwers GY, Vauthey JN, Abdalla EK. Chemotherapy-associated hepatotoxicity and surgery for colorectal liver metastases. *Br J Surg* 2007; **94**: 274-286 [PMID: 17315288]
- 32 **Khan AZ**, Morris-Stiff G, Makuuchi M. Patterns of chemotherapy-induced hepatic injury and their implications for patients undergoing liver resection for colorectal liver metastases. *J Hepatobiliary Pancreat Surg* 2009; **16**: 137-144 [PMID: 19093069 DOI: 10.1007/s00534-008-0016-z]
- 33 **Karoui M**, Penna C, Amin-Hashem M, Mitry E, Benoist S, Franc B, Rougier P, Nordlinger B. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. *Ann Surg* 2006; **243**: 1-7 [PMID: 16371728]
- 34 **Mentha G**, Terraz S, Morel P, Andres A, Giostra E, Roth A, Rubbia-Brandt L, Majno P. Dangerous halo after neoadjuvant chemotherapy and two-step hepatectomy for colorectal liver metastases. *Br J Surg* 2009; **96**: 95-103 [PMID: 19109800 DOI: 10.1002/bjs.6436]
- 35 **Tanaka K**, Takakura H, Takeda K, Matsuo K, Nagano Y, Endo I. Importance of complete pathologic response to prehepatectomy chemotherapy in treating colorectal cancer metastases. *Ann Surg* 2009; **250**: 935-942 [PMID: 19953712]
- 36 **Adam R**, Wicherts DA, de Haas RJ, Ciacio O, Lévi F, Paule B, Ducreux M, Azoulay D, Bismuth H, Castaing D. Patients with initially unresectable colorectal liver metastases: is there a possibility of cure? *J Clin Oncol* 2009; **27**: 1829-1835 [PMID: 19273699 DOI: 10.1200/JCO.2008.19.9273]
- 37 **Stein A**, Schmoll HJ. Systemic treatment of liver metastases from colorectal cancer. *Ther Adv Med Oncol* 2013; **5**: 193-203 [PMID: 23634197 DOI: 10.1177/1758834012473347]
- 38 **Adam R**, Bhangui P, Poston G, Mirza D, Nuzzo G, Barroso E, Ijzermans J, Hubert C, Ruers T, Capussotti L, Ouellet JF, Laurent C, Cugat E, Colombo PE, Milicevic M. Is perioperative chemotherapy useful for solitary, metachronous, colorectal liver metastases? *Ann Surg* 2010; **252**: 774-787 [PMID: 21037433 DOI: 10.1097/SLA.0b013e3181fcf3e3]
- 39 **Reddy SK**, Zorzi D, Lum YW, Barbas AS, Pawlik TM, Ribero D, Abdalla EK, Choti MA, Kemp C, Vauthey JN, Morse MA, White RR, Clary BM. Timing of multimodality therapy for resectable synchronous colorectal liver metastases: a retrospective multi-institutional analysis. *Ann Surg Oncol* 2009; **16**: 1809-1819 [PMID: 18979139 DOI: 10.1245/s10434-008-0181-y]
- 40 **Lubezky N**, Geva R, Shmueli E, Nakache R, Klausner JM, Figer A, Ben-Haim M. Is there a survival benefit to neoadjuvant versus adjuvant chemotherapy, combined with surgery for resectable colorectal liver metastases? *World J Surg* 2009; **33**: 1028-1034 [PMID: 19234865 DOI: 10.1007/s00268-009-9945-1]
- 41 **Zhu D**, Zhong Y, Wei Y, Ye L, Lin Q, Ren L, Ye Q, Liu T, Xu J, Qin X. Effect of neoadjuvant chemotherapy in patients with resectable colorectal liver metastases. *PLoS One* 2014; **9**: e86543 [PMID: 24466143 DOI: 10.1371/journal.pone.0086543]
- 42 **Nanji S**, Cleary S, Ryan P, Guindi M, Selvarajah S, Al-Ali H, Grieg P, McGilvary I, Taylor B, Wei A, Moulton CA, Gallinger S. Up-front hepatic resection for metastatic colorectal cancer results in favorable long-term survival. *Ann Surg Oncol* 2013; **20**: 295-304 [PMID: 23054102 DOI: 10.1245/s10434-012-2424-1]
- 43 **Wieser M**, Sauerland S, Arnold D, Schmiegel W, Reinacher-Schick A. Peri-operative chemotherapy for the treatment of

resectable liver metastases from colorectal cancer: A systematic review and meta-analysis of randomized trials. *BMC Cancer* 2010; **10**: 309 [PMID: 20565923 DOI: 10.1186/1471-2407-10-309]

44 **Chua TC**, Saxena A, Liauw W, Kokandi A, Morris DL. Systematic

review of randomized and nonrandomized trials of the clinical response and outcomes of neoadjuvant systemic chemotherapy for resectable colorectal liver metastases. *Ann Surg Oncol* 2010; **17**: 492-501 [PMID: 19856028 DOI: 10.1245/s10434-009-0781-1]

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## Standard chemotherapy with cetuximab for treatment of colorectal cancer

Xin-Xiang Li, Lei Liang, Li-Yong Huang, San-Jun Cai

Xin-Xiang Li, Lei Liang, Li-Yong Huang, San-Jun Cai,  
Department of Colorectal Surgery, Fudan University Shanghai  
Cancer Center, Shanghai 200032, China  
Xin-Xiang Li, Lei Liang, Li-Yong Huang, San-Jun Cai,  
Department of Oncology, Shanghai Medical College, Fudan  
University, Shanghai 200032, China

**Author contributions:** Li XX participated in data collection and analysis, and wrote the manuscript; Liang L and Huang LY participated in data collection and help to perform the statistical analysis; Cai SJ conceived of the study, participated in its design and provided the critical revision; all authors read and approved the final manuscript.

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**Data sharing:** Technical appendix, statistical code, and dataset available from the corresponding author at [cai\\_sanjunsai@163.com](mailto:cai_sanjunsai@163.com).

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**Correspondence to:** San-Jun Cai, MD, Department of Colorectal Surgery, Fudan University Shanghai Cancer Center, 270 Dongan Road, Shanghai 200032, China. [cai\\_sanjunsai@163.com](mailto:cai_sanjunsai@163.com)  
Telephone: +86-21-64085875  
Fax: +86-21-64085875

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### Abstract

**AIM:** To review and assess the evidence related to cetuximab treatment in metastatic colorectal cancer (mCRC) with regard to *KRAS* status.

**METHODS:** PubMed, EMBASE, Cochrane database and American Society of Clinical Oncology meeting abstracts were searched for randomized controlled trials (RCTs) reporting the effect of *KRAS* status on efficacy of chemotherapy regimen with or without cetuximab in mCRC. Baseline information such as sex and age was summarized from the included studies. Hazard ratios of progression-free survival (PFS) and overall survival (OS) as well as objective response based on *KRAS* status were extracted for analysis.

**RESULTS:** A total of 8 RCTs with 6780 patients were included. The combined analysis showed that cetuximab failed to improve the OS and PFS in patients with mCRC. However, in subgroup analysis, the pooled data showed that addition of cetuximab to irinotecan containing chemotherapy regimen was sufficient to improve OS and PFS in wild-type *KRAS* mCRC patients, but not in patients with mutant-type *KRAS*. The addition of cetuximab increased the incidence of adverse events such as diarrhea, rash, skin toxicity/rash, and nausea and vomiting. There was no significant publication bias existing in the included studies.

**CONCLUSION:** The clinical benefit of cetuximab was only confirmed in patients with wild-type *KRAS*. *KRAS* status could be considered a biomarker of efficacy of cetuximab.

**Key words:** Cetuximab; *KRAS*; Standard chemotherapy; Metastatic colorectal cancer; Meta-analysis

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**Core tip:** The addition of cetuximab to irinotecan containing chemotherapy regimen was sufficient to improve overall survival and progression-free survival in wild-type *KRAS* metastatic colorectal cancer patients, but not in patients with mutant-type *KRAS*.

Li XX, Liang L, Huang LY, Cai SJ. Standard chemotherapy with cetuximab for treatment of colorectal cancer. *World J Gastroenterol* 2015; 21(22): 7022-7035 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/7022.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.7022>

## INTRODUCTION

Colorectal cancer remains one of the most common cancers worldwide and its incidence was about 1.2 million in 2008<sup>[1]</sup>. In the past few years, progress has been achieved in improving outcome of metastatic colorectal cancer (mCRC), and this was mainly due to the application of novel molecular targeted agents<sup>[2,3]</sup>. However, recent evidence<sup>[4-6]</sup> showed that addition of cetuximab to chemotherapy did not improve the outcome for patients with mCRC, making the anti-tumor effect of cetuximab controversial, and indicating that cetuximab should be recommended based on individual information. Therefore, it is urgent to identify patients who could benefit from cetuximab treatment most and this relies on effective biomarkers in predicting efficacy of cetuximab in the treatment of mCRC.

Cetuximab is an IgG1 monoclonal antibody to the epidermal growth factor receptor (EGFR) and it exerts clinical activity in mCRC patients who are chemotherapy-resistant<sup>[6-8]</sup>. A phase III trial in patients with oxaliplatin and fluoropyrimidines-refractory mCRC who were randomized to cetuximab plus irinotecan showed an improved outcome for the addition of cetuximab<sup>[9]</sup>. Cetuximab has been approved by United States Food and Drug Administration in 2004. However, not all the individuals are sensitive to cetuximab, and investigations about influencing factors of its effectiveness have emerged, with one of the best known being *KRAS* status<sup>[10]</sup>.

The *KRAS* protein is one of the most important downstream effectors coupling EGFR to intracellular signaling cascades, leading to cell growth, division, motility, and inhibition of apoptosis<sup>[11,12]</sup>. Single-nucleotide point mutations in the *KRAS* gene are found in approximately 40% of patients with metastatic CRC, including mutations in codons 12 and 13 of exon 2<sup>[12,13]</sup>. These mutations of *KRAS* may contribute to the lack of response to anti-EGFR monoclonal antibodies in patients with mCRC<sup>[10-12]</sup>. A meta-analysis<sup>[14]</sup> of pooled data from the CRYSTAL<sup>[15]</sup> and OPUS<sup>[16]</sup> studies confirmed that in patients with *KRAS* wild-

type tumours, adding cetuximab to chemotherapy led to a significant improvement in overall survival (OS), progression-free survival (PFS) and overall response rate (ORR). However, other trials demonstrated that *KRAS* status was not predictive of benefit when adding cetuximab to the first-line therapy<sup>[12,17,18]</sup>. Thus, it is essential to evaluate whether *KRAS* is a biomarker of effectiveness of cetuximab using pooled data.

In regard of issues mentioned above, the present meta-analysis was to investigate whether addition of cetuximab could improve treatment outcomes such as PFS and OS based on *KRAS* status in patients with mCRC, and whether *KRAS* status could be a useful indicator of benefit from cetuximab treatment.

## MATERIALS AND METHODS

### Literature search strategy

Population, intervention, control, and outcome (PICO) were defined prior to literature research. Then, electronic databases comprising PubMed, EMBASE, Cochrane, and American Society of Clinical Oncology meeting abstract (conference on colorectal cancer) were selected and used to search for randomized controlled trials (RCTs) comparing chemotherapy regimen with or without cetuximab in treatment of mCRC based on *KRAS* status. The search terms used were: ["colorectal neoplasms/therapy"(Mesh) or "carcinoma, colorectal " or "tumor, colorectal"] and ("cetuximab" or "erbitux" or "Mab C225" or "anti-EGFR agents") and ("stage III" or "stage IV" or "metasta?" or "advanced") and ("KRAS" or "K-ras"). We also used a manual reference search for relevant articles, including original articles and reviews, to identify additional studies. If more than one article was published using the same case series, only the study with the latest data was included. The search was restricted to published English language papers. The literature search was updated on December 31, 2013. The detailed information of the search strategy for the eligible studies is presented in flow diagram provided by PRISMA (Figure 1).

### Inclusion criteria

Inclusion criteria were: (1) high quality RCTs performed in mCRC patients, either in form of a full article or a meeting abstract; (2) mCRC patients treated with traditional chemotherapy regimen with or without cetuximab; (3) RCTs comparing cetuximab + chemotherapy vs chemotherapy only, with regard to *KRAS* status; and (4) primary endpoints were PFS and/or OS, and secondary endpoints were OR and toxicity information.

### Data extraction

Information in each eligible study was carefully extracted and identified by two reviewers independently (Li XX and Liang L), and classical data collection methods were applied during extraction process.



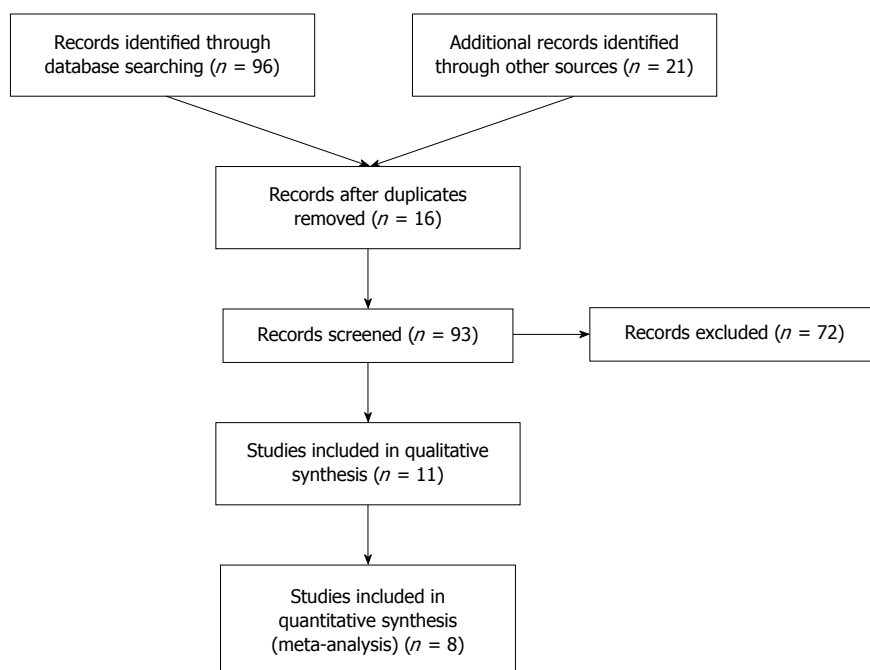


Figure 1 Flow diagram of study selection.

The following data were extracted from the included studies: numbers of patients enrolled, publication date, characteristics of patients such as age and gender, and other data such as clinical stage, method of randomization, chemotherapy regimen and details of first-line chemotherapy, doses of cetuximab, PFS, OS, and OR. If hazard ratio (HR) and its variance were not available directly from original article, the method of Parmar *et al.*<sup>[19]</sup> was introduced to establish estimates of these information. For identification of each eligible study, the first author's name and publication year were used.

### Quality control

The protocols of GRADE were used to evaluate the quality of each RCT included for this meta-analysis. Quality control was performed independently by two reviewers. If there was a disagreement about quality of a certain study, another reviewer was involved to solve it. Funnel plots were also introduced to assess the publication bias.

### Statistical analysis

RevMan 5.2 software which was provided by the Cochrane Collaboration was applied to perform all of the statistical analyses, and introduction of the Cochrane Collaboration for meta-analysis was followed to ensure the accuracy of whole analysis process. We assessed the between-study heterogeneity by Cochran's  $Q$  test and quantified by  $I^2$  (a significance level of  $P < 0.10$  and/or  $I^2 \geq 50\%$ ). If the  $P$ -value of the  $Q$  test is  $> 0.05$ , the summary OR estimate of each study was calculated using the fixed-effect model. Otherwise, the random-effect model was used. A funnel plot and

Egger's linear regression test were used to investigate any possible publication bias<sup>[20]</sup>. For all analyses, a two-sided  $P$ -value less than 0.05 was considered to be statistically significant.

The statistical methods of this study were reviewed by San-Jun Cai from Department of Colorectal Surgery, Fudan University Shanghai Cancer Center.

## RESULTS

### Characteristics of included studies

A total of eight RCTs<sup>[17,21-27]</sup> were included for this meta-analysis involving a number of 6780 mCRC patients. Among these eligible trials, full articles are available from databases. The baseline information and adverse events of these studies are shown in Tables 1 and 2. Five of them<sup>[17,22,25-27]</sup> assessed oxaliplatin based chemotherapy regimen plus cetuximab in the first-line treatment of metastatic or advanced CRC, while two studies<sup>[21,24]</sup> evaluated the effect of cetuximab in combination with the FOLFIRI regimen on outcome of metastatic or advanced CRC patients, and only one trial<sup>[23]</sup> involved both FOLFIRI or oxaliplatin based regimen. All studies<sup>[17,21-26]</sup> reported the status of *KRAS* in mCRC except the study of Borner *et al.*<sup>[27]</sup>. Data from these RCTs were sufficient to support the statistically pooled analysis of PFS and OS.

### Analysis of OS

**OS regardless of *KRAS* status:** Five RCTs<sup>[21,22,24,25,27]</sup> were included for the analysis of whether addition of cetuximab to standard chemotherapy could improve OS than chemotherapy alone. The result showed that the application of cetuximab failed to provide a

**Table 1** Included studies on efficacy of cetuximab plus chemotherapy *vs* chemotherapy alone in patients with metastatic or advanced colorectal cancer

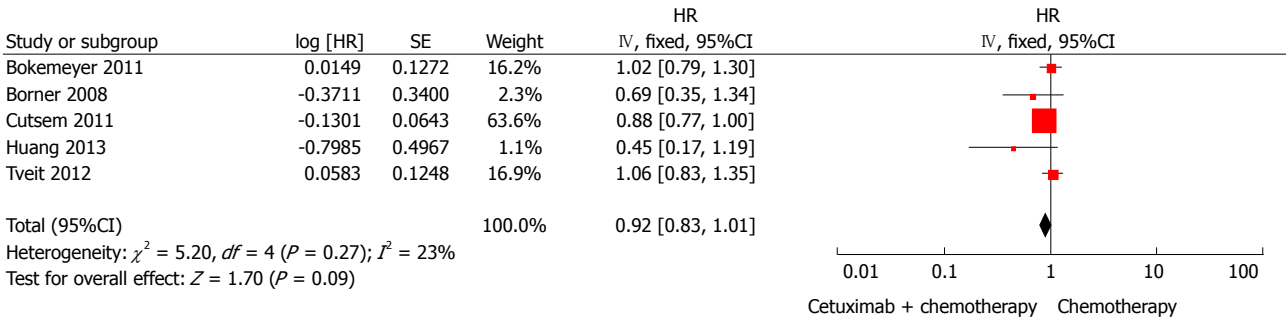
Study	Type of article	Patients	Intervention		Main endpoints (HR, 95%CI)	Mutation status reported		Quality control
			Cetuximab + chemotherapy	Chemotherapy		KRAS	BRAF	
Borner <i>et al</i> <sup>[27]</sup>	Full manuscript	74	Cetuximab + XELOX	XELOX	PFS: NR; OS: NR	No	No	Moderate
Bokemeyer <i>et al</i> <sup>[25]</sup>	Full manuscript	337	Cetuximab + FOLFOX-4	FOLFOX-4	PFS: 0.931, 0.705-1.230; OS: 1.015, 0.791-1.303	Yes	Yes	Good
Van Cutsem <i>et al</i> <sup>[21]</sup>	Full manuscript	1198	Cetuximab + FOLFIRI	FOLFIRI	PFS: 0.851, 0.726-0.998; OS: 0.878, 0.774-0.995	Yes	Yes	Good
Maughan <i>et al</i> <sup>[17]</sup>	Full manuscript	1630	Cetuximab + oxaliplatin + fluoropyrimidine	Oxaliplatin + fluoropyrimidine	PFS: 0.96, 0.82-1.12; OS: 1.04, 0.87-1.23	Yes	Yes	Good
Tveit <i>et al</i> <sup>[22]</sup>	Full manuscript	571	Cetuximab + FLOX	FLOX	PFS: NR; OS: NR	Yes	Yes	Good
Alberts <i>et al</i> <sup>[26]</sup>	Full manuscript	2686	Cetuximab + mFOLFOX6	mFOLFOX6	PFS: NR; OS: NR	Yes	Yes	Good
Huang <i>et al</i> <sup>[24]</sup>	Full manuscript	146	Cetuximab + FOLFIRI	FOLFIRI	PFS: 0.53, 0.26-1.10; OS: 0.45, 0.2-1.2	Yes	Yes	Good
Ye <i>et al</i> <sup>[23]</sup>	Full manuscript	138	Cetuximab + mFOLFOX6/ FOLFIRI	mFOLFOX6/ FOLFIRI	PFS: 0.60, 0.41-0.87; OS: 0.54, 0.33-0.89	Yes	Yes	Good

XELOX: Capecitabine and oxaliplatin regimen; FOLFOX-4: Oxaliplatin and folinic acid and 5-fluorouracil regimen; FOLFIRI: Fluorouracil and leucovorin and irinotecan regimen; FLOX: Fluorouracil/folinic acid and oxaliplatin regimen; mFOLFOX6: Oxaliplatin and leucovorin and fluorouracil regimen; PFS: Progression free survival; OS: Overall survival; NR: Not reported.

**Table 2** Adverse events (grade 3 and 4)

Study	Neutropenia		Nausea and vomiting		Skin toxicity/rash		Rash		Diarrhea	
	Control group	Cetuximab group	Control group	Cetuximab group	Control group	Cetuximab group	Control group	Cetuximab group	Control group	Cetuximab group
Borner <i>et al</i> <sup>[27]</sup>	3	0	6	10	0	16	0	8	16	22
Bokemeyer <i>et al</i> <sup>[25]</sup>	57	51	NA	NA	1	30	1	19	12	14
Van Cutsem <i>et al</i> <sup>[21]</sup>	150	169	30	28	1	117	0	49	63	94
Maughan <i>et al</i> <sup>[17]</sup>	NA	NA	NA	NA	14	114	NA	NA	NA	NA
Tveit <i>et al</i> <sup>[22]</sup>	47	95	3	14	1	51	1	51	10	33
Alberts <i>et al</i> <sup>[26]</sup>	89	110	59	70	NA	NA	3	186	83	148
Huang <i>et al</i> <sup>[24]</sup>	15	3	0	18	NA	NA	0	11	15	6
Ye <i>et al</i> <sup>[23]</sup>	6	8	3	3	1	2	2	9	3	4

GI: Gastrointestinal toxic effects; NA: Not available.



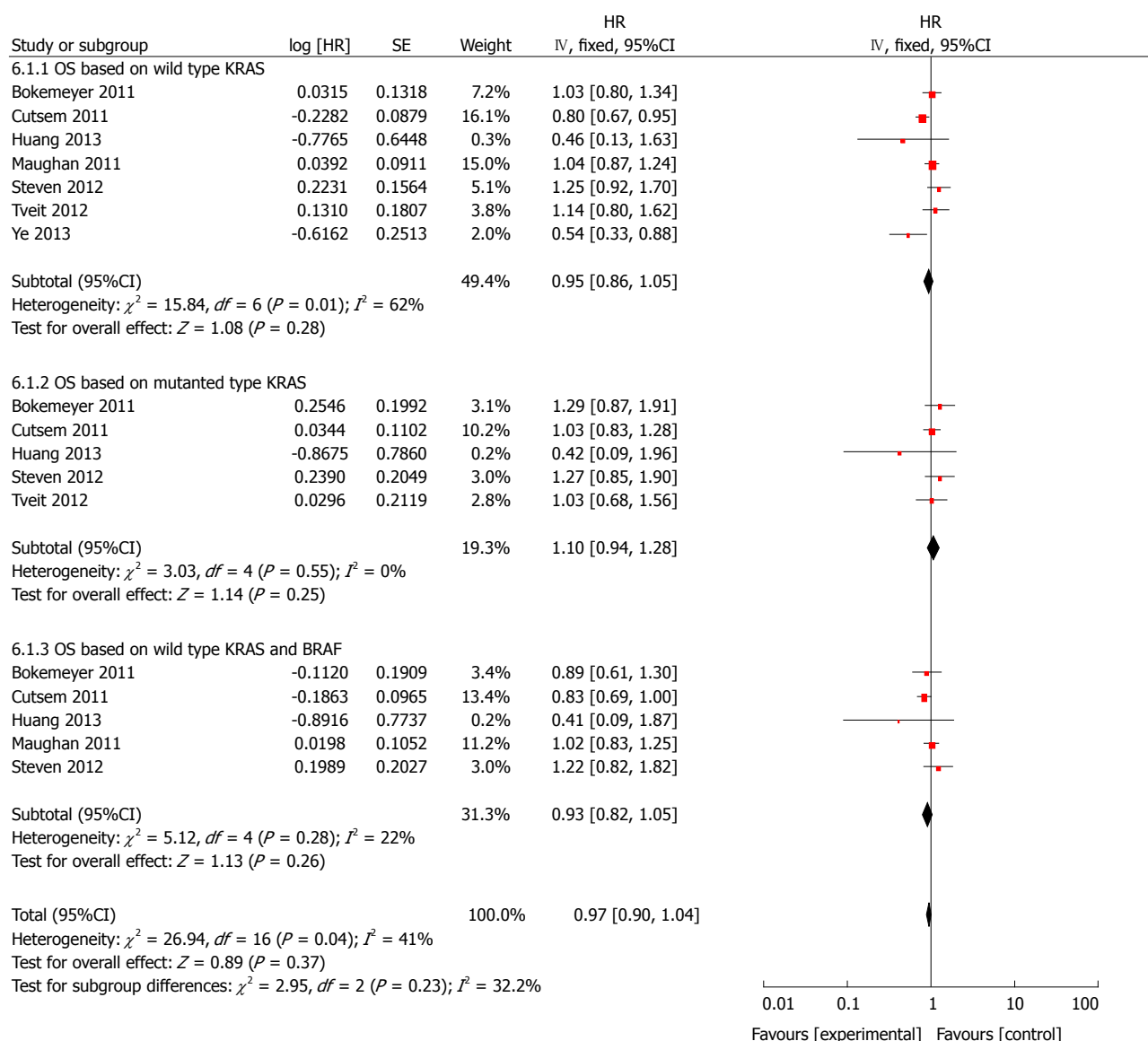
**Figure 2** Meta-analysis of effect of cetuximab plus chemotherapy on overall survival regardless of *KRAS* status.

significant improvement of OS regardless of *KRAS* status (HR = 0.92, 95%CI: 0.83-1.01;  $P > 0.05$ ; Figure 2).

**OS based on wild-type *KRAS*:** To evaluate whether cetuximab plus chemotherapy could benefit OS in population harboring wild-type *KRAS*, seven studies were included<sup>[17,21-26]</sup>. As shown in Figure 3, though cetuximab plus chemotherapy seemed to provide a

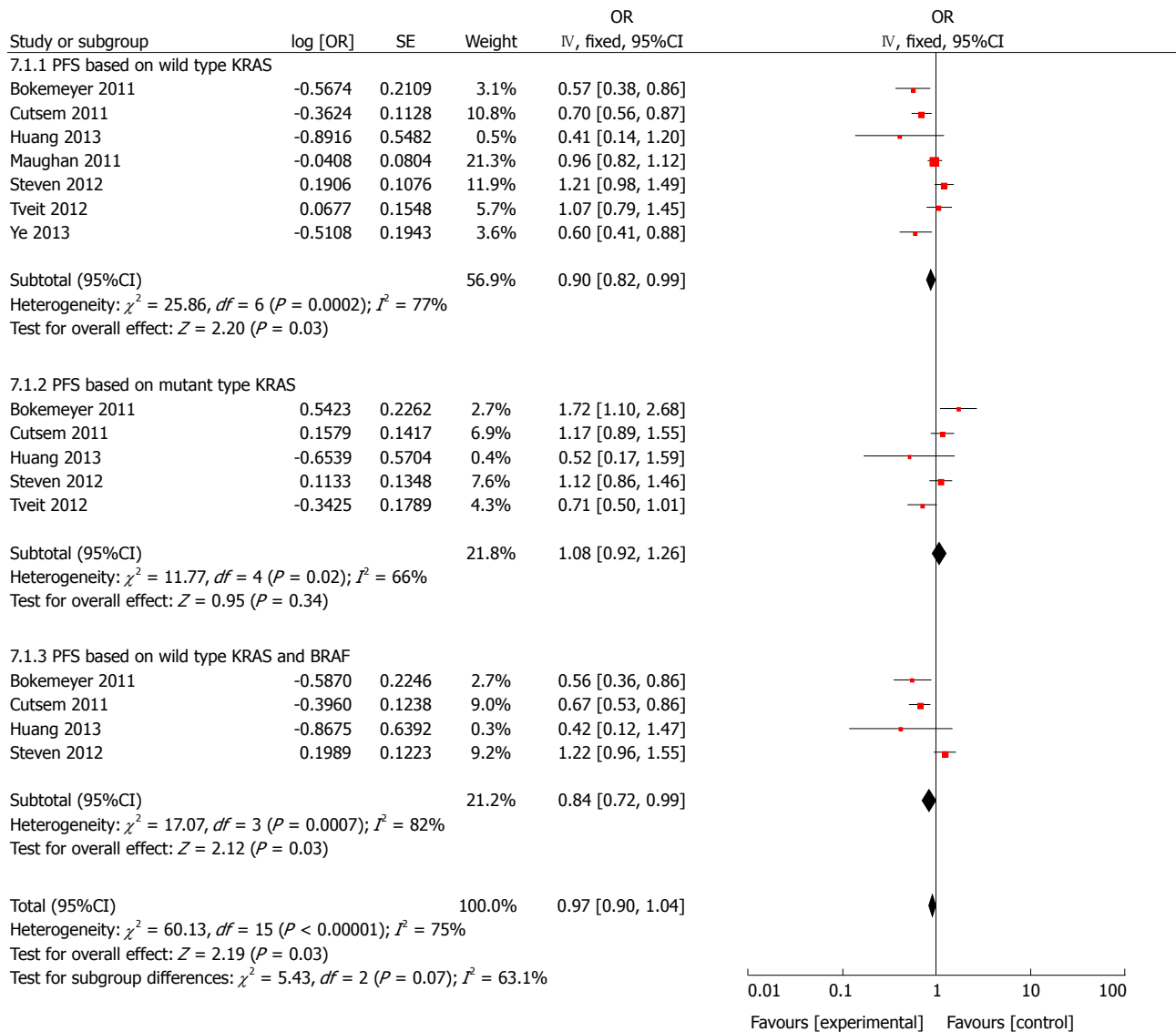
benefit in prolonging OS, there was no statistically significance (HR = 0.95, 95%CI: 0.86-1.05;  $P > 0.05$ ).

**OS based on mutated *KRAS*:** There was five studies<sup>[21,22,24-26]</sup> involved in the analysis of OS based on mutant *KRAS* in patients who received cetuximab combined with chemotherapy. A significant difference was not observed from the pooled analysis (HR = 1.10, 95%CI: 0.94-1.28;  $P > 0.05$ ; Figure 3).

Figure 3 Meta-analysis of effect of cetuximab plus chemotherapy on overall survival based on status of *KRAS*.Figure 4 Meta-analysis of effect of cetuximab plus chemotherapy on progression-free survival regardless of *KRAS* status.

**OS based on wild-type *KRAS* and *BRAF*:** We also analyzed the effect of cetuximab on OS in patients with both wild-type *KRAS* and *BRAF* by using five

studies<sup>[21,22,24-26]</sup>. Still, it showed that there was no significant improvement on OS (HR = 0.93, 95%CI: 0.82-1.05;  $P > 0.05$ ; Figure 3), though in the setting

Figure 5 Meta-analysis of effect of cetuximab plus chemotherapy on progression-free survival based on status of *KRAS*.

of wild-type targeted genes.

### Analysis of PFS

**PFS regardless of *KRAS* status:** Five trials<sup>[21,22,24,25,27]</sup> were used to evaluate the improvement in PFS with cetuximab combined with chemotherapy vs chemotherapy alone. Compared with chemotherapy, there was a significantly prolonged PFS in patients treated with cetuximab (HR = 0.87, 95%CI: 0.77-0.97;  $P < 0.05$ ; Figure 4).

**PFS based on wild-type *KRAS*:** We further performed a sub-group analysis of cetuximab combined with chemotherapy vs chemotherapy in patients having wild-type *KRAS*. As shown in Figure 5, cetuximab succeeded to provide a significant improvement in PFS (HR = 0.90, 95%CI: 0.82-0.99;  $P < 0.05$ ).

**PFS based on mutated *KRAS*:** A total of five

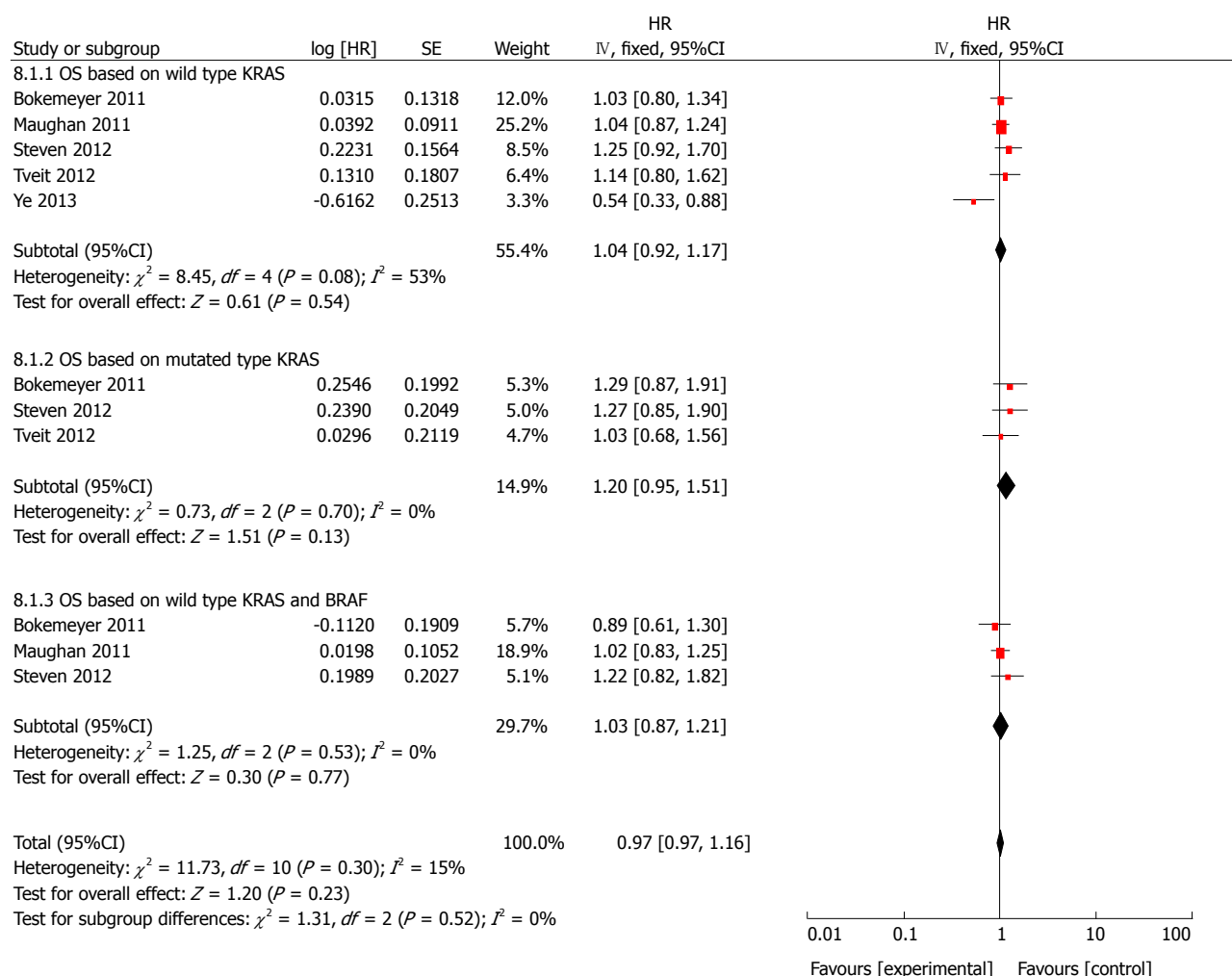
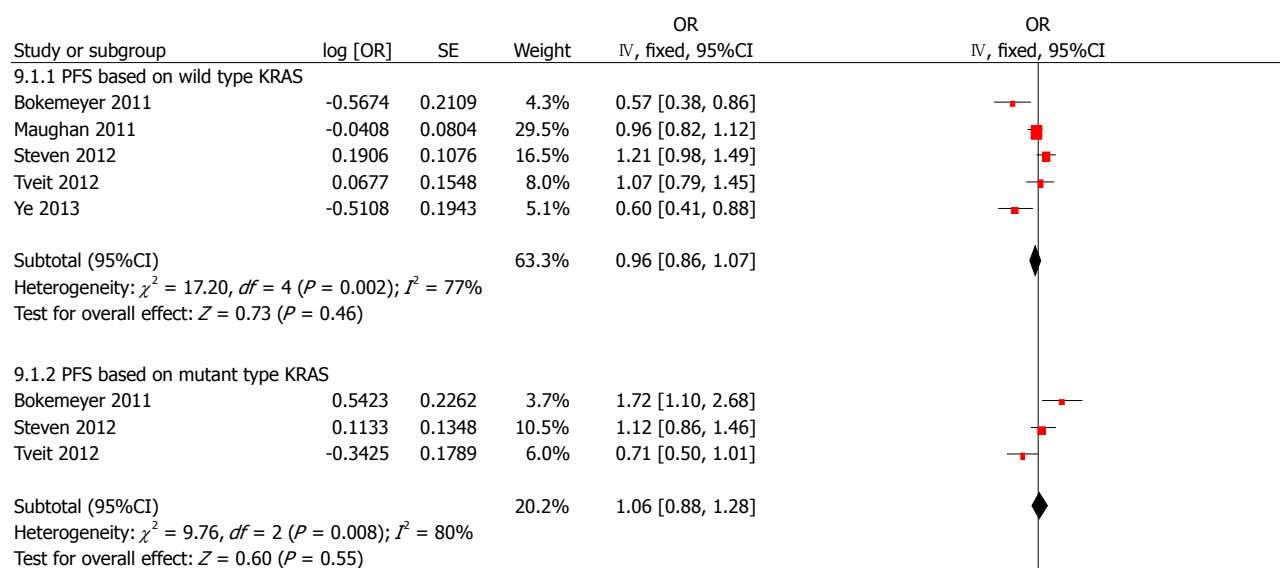
studies<sup>[21,22,24-26]</sup> were selected for the analysis of PFS based on mutant *KRAS* in patients who received cetuximab combined with chemotherapy. A significant difference was not observed from the result (HR = 1.08, 95%CI: 0.92-1.26;  $P > 0.05$ , Figure 5).

**PFS based on wild-type *KRAS* and *BRAF*:** Analysis of cetuximab plus chemotherapy vs chemotherapy was performed using data extracted from four RCTs<sup>[21,24-26]</sup>. A positive result was obtained and it presented that cetuximab therapy benefited PFS significantly in patients with wild-type *KRAS* and *BRAF* (HR = 0.84, 95%CI: 0.72-0.99;  $P < 0.05$ ; Figure 5).

### Analysis of PFS and OS based on chemotherapy regimen

We performed another combined analysis based on different chemotherapy regimens. Whether the regimen contained irinotecan was used as the standard to group the included studies. Cetuximab added to irinotecan-



Figure 6 Meta-analysis of effect of cetuximab plus irinotecan-free chemotherapy on overall survival based on status of *KRAS*.

9.1.3 PFS based on wild type *KRAS* and *BRAF*

Bokemeyer 2011	-0.5870	0.2246	3.8%	0.56 [0.36, 0.86]
Steven 2012	0.1989	0.1223	12.8%	1.22 [0.96, 1.55]

Subtotal (95%CI) 16.5% 1.02 [0.83, 1.26]

Heterogeneity:  $\chi^2 = 9.44$ ,  $df = 1$  ( $P = 0.002$ );  $I^2 = 89\%$

Test for overall effect:  $Z = 0.18$  ( $P = 0.86$ )

Total (95%CI) 100.0% 0.99 [0.91, 1.08]

Heterogeneity:  $\chi^2 = 37.27$ ,  $df = 9$  ( $P < 0.0001$ );  $I^2 = 76\%$

Test for overall effect:  $Z = 0.24$  ( $P = 0.81$ )

Test for subgroup differences:  $\chi^2 = 0.86$ ,  $df = 2$  ( $P = 0.65$ );  $I^2 = 0\%$

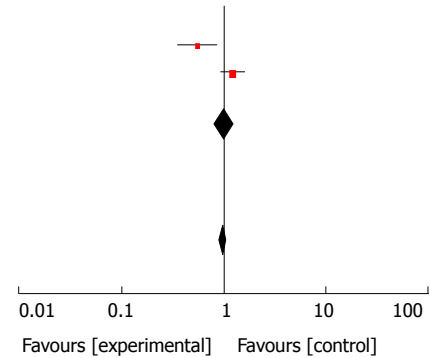


Figure 7 Meta-analysis of effect of cetuximab plus irinotecan-free chemotherapy on progression-free survival based on status of *KRAS*.

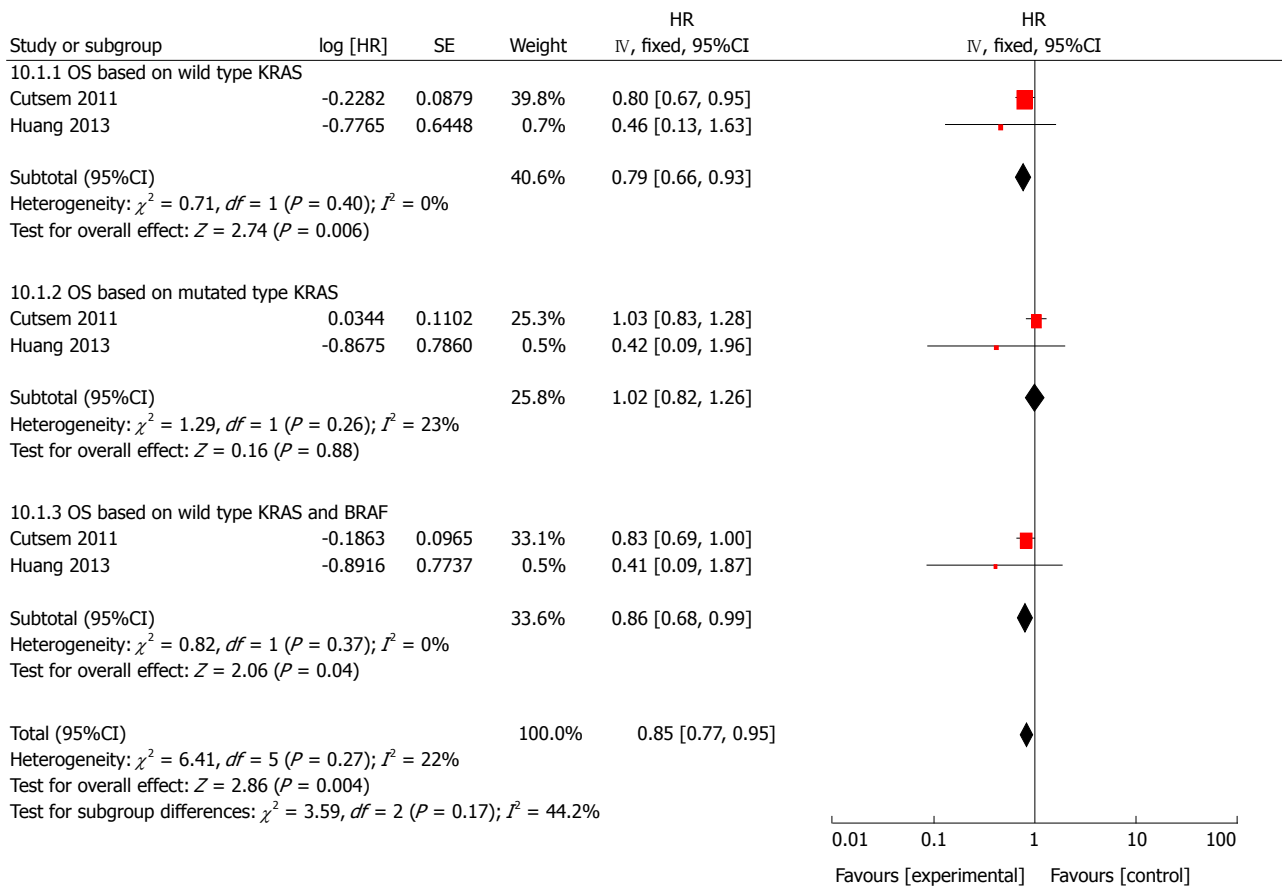
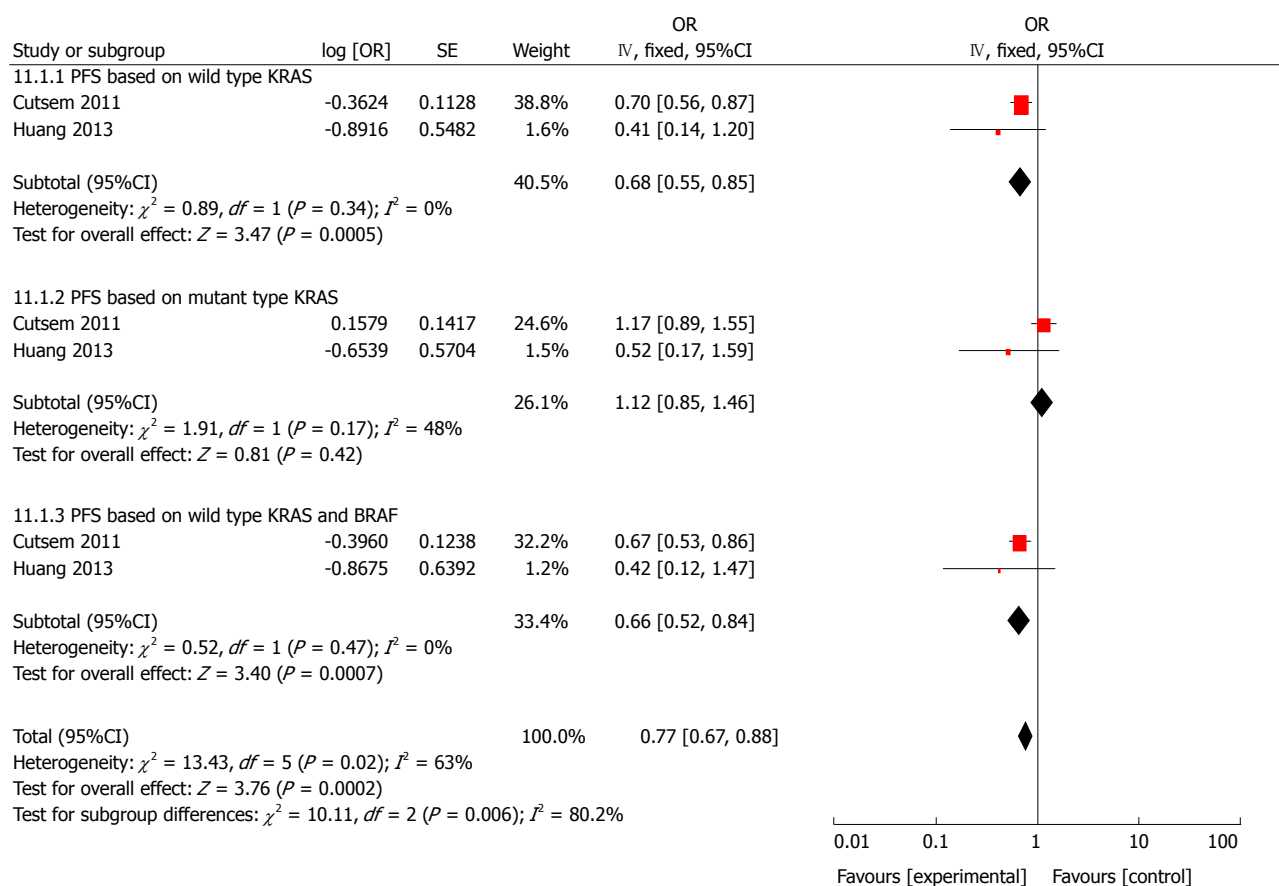
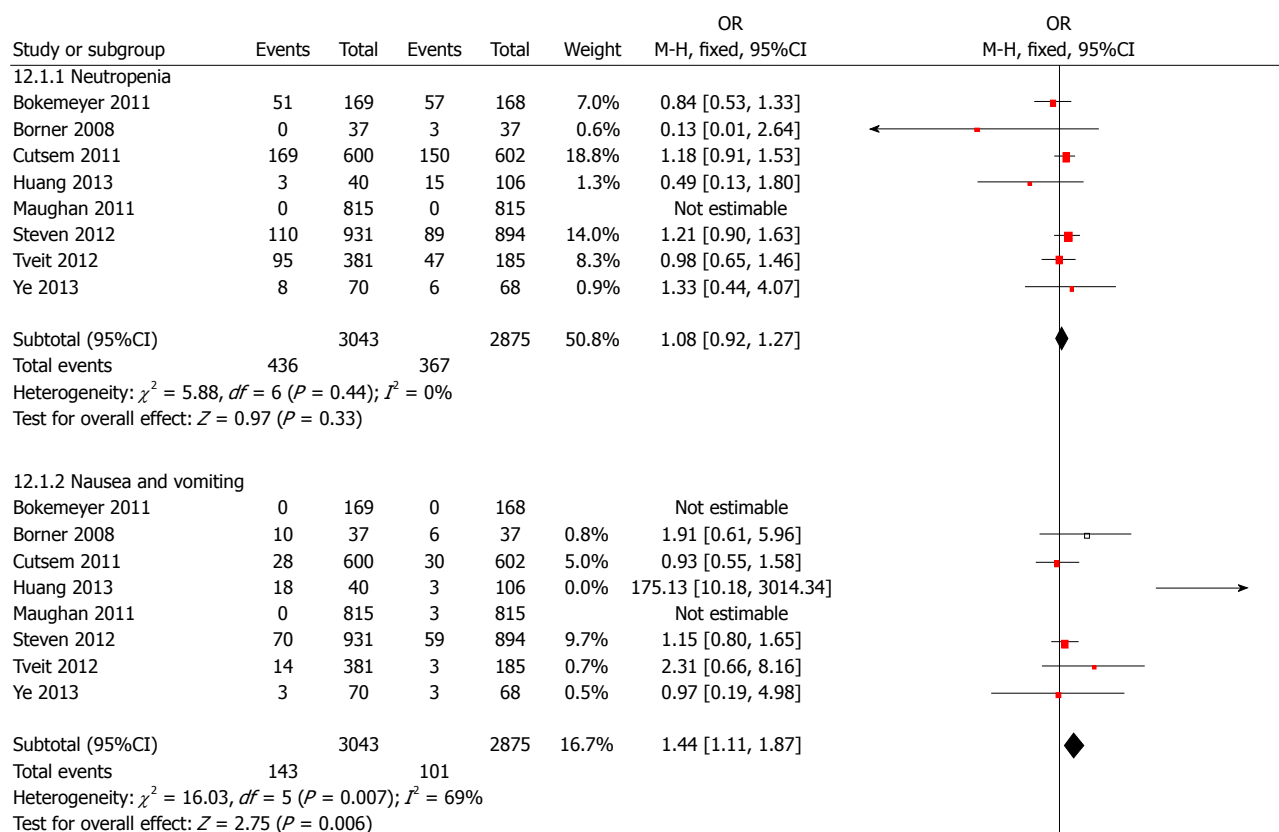


Figure 8 Meta-analysis of effect of cetuximab plus irinotecan containing chemotherapy on overall survival based on status of *KRAS*.

free regimen did not significantly improve the OS (HR = 1.06, 95%CI: 0.97-1.16;  $P = 0.23$ ; Figure 6) or PFS (HR = 0.99, 95%CI: 0.91-1.08;  $P = 0.81$ ; Figure 7) in patients with mCRC, regardless of status of *KRAS* and/or *BRAF*. Next, we compared the outcomes of patients receiving cetuximab and irinotecan, and the weighted results showed that cetuximab and irinotecan significantly improved OS (HR = 0.85, 95%CI: 0.77-0.95;  $P = 0.004$ ; Figure 8) and PFS (HR = 0.77, 95%CI: 0.67-0.88;  $P = 0.0002$ ; Figure 9) in mCRC patients with wild-type *KRAS*, but not in patients with mutant *KRAS*.

### Analysis of adverse events

As cetuximab is a targeted agent, we examined the effect of cetuximab on adverse events. The results determined that patients who received cetuximab suffered from more adverse events such as skin toxicity/rash (HR = 18.35, 95%CI: 11.28-29.86;  $P = 0.008$ , Figure 10), rash (HR = 43.27, 95%CI: 21.73-86.17;  $P = 0.0002$ ; Figure 10), diarrhea (HR = 1.66, 95%CI: 1.37-2.02;  $P < 0.001$ ; Figure 10), and nausea and vomiting (HR = 1.44, 95%CI: 1.11-1.87;  $P = 0.007$ ; Figure 10), indicating that the application of cetuximab should be carefully considered not only

Figure 9 Meta-analysis of effect of cetuximab plus irinotecan containing chemotherapy on progression-free survival based on status of *KRAS*.

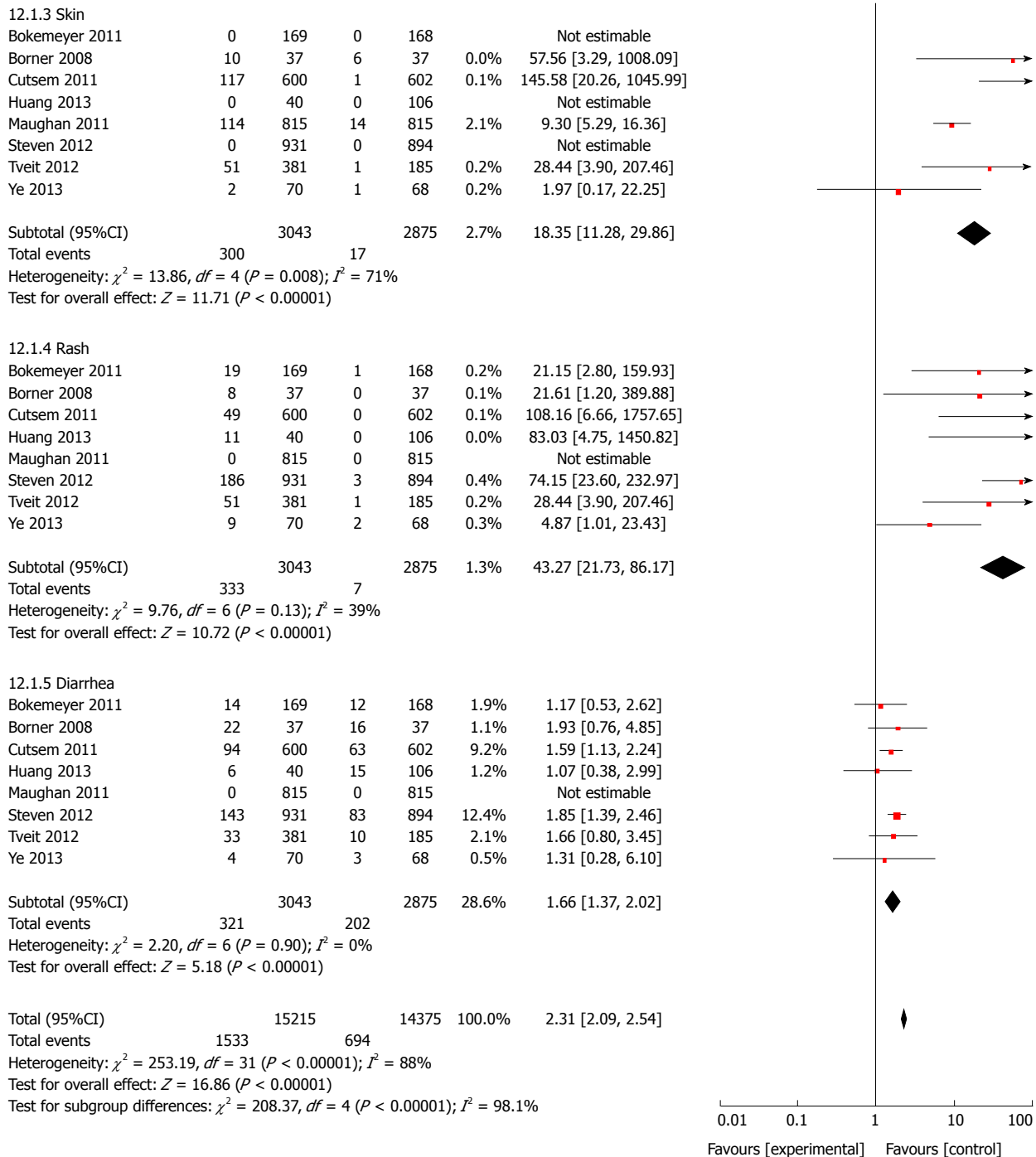


Figure 10 Meta-analysis of adverse events in patients receiving cetuximab or not.

based on status of targeted genes, but also the quality of life and safety.

### Analysis of publication bias

To evaluate the publication bias, we performed the Egger's test and funnel plot. As illustrated by Figure 11, only 2 studies exceeded the confidence interval and the Egger's test showed that there was no significant publication bias within the included studies ( $P < 0.05$ ).

## DISCUSSION

Cetuximab, in combination with chemotherapy, has been approved for the treatment of mCRC patients<sup>[28]</sup>. Many clinical trials<sup>[14-18,28]</sup> have been published to evaluate the efficacy of cetuximab in mCRC, especially based on *KRAS* status. However, the outcomes from these studies were not consistent. Thus, it is essential to provide clinical evidence relating to the application of



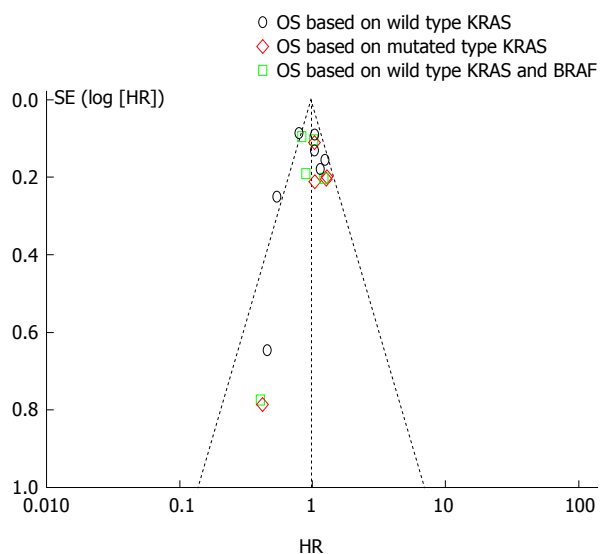


Figure 11 Funnel plot for detecting publication bias.

cetuximab in mCRC treatment. Indeed, several meta-analyses<sup>[29-33]</sup> have been published in recent years, but the arguments about whether cetuximab could benefit outcomes of mCRC patients with different *KRAS* status still exist. The present meta-analysis was performed to address the issues mentioned above, and to increase the statistical power of efficacy analysis of cetuximab in mCRC patients and further to identify what kind of population could benefit from treatment of cetuximab most.

The present meta-analysis confirms that adding anti-EGFR therapy to standard chemotherapy could result in clinical benefits in the treatment of mCRC containing wild-type *KRAS*, with a significantly prolonged PFS. For patients harboring wild-type *KRAS*, a statistically significant longer PFS was found when using cetuximab with traditional chemotherapy regimens. However, cetuximab treatment was associated with an impaired improvement in OS, and no statistical significance was achieved. Compared with the clinical benefit of addition of cetuximab to chemotherapy regimens in wild-type *KRAS* patients, mutation of *KRAS* is a predictor of less sensitivity to cetuximab in mCRC patients with regard to PFS and OS. The subgroup analysis demonstrated that cetuximab in combination with irinotecan containing regimen could improve OS and PFS in patients with wild-type *KRAS* and/or *BRAF*, but not in patients with mutant *KRAS*. These benefits were not observed in patients treated with irinotecan-free chemotherapy. Notably, the incidence of adverse events in the cetuximab group was much higher than that in patients without cetuximab treatment.

The results of this meta-analysis is in accordance with those of other meta-analyses<sup>[34]</sup>. In the study performed by Qiu *et al.*<sup>[31]</sup>, they compared the efficacy of cetuximab combined with chemotherapy vs chemotherapy for patients with mCRC, as well as the

influence of *KRAS* mutation status on the outcomes, and the results showed that in wild-type *KRAS* patients, cetuximab plus chemotherapy significantly improved PFS when compared with chemotherapy alone, but not for OS, whereas in mutant *KRAS* patients, there was no significant benefit between those treated with cetuximab plus chemotherapy and those with chemotherapy alone regarding PFS and OS. In addition, Bokemeyer *et al.*<sup>[14]</sup> enrolled CRYSTAL and OPUS studies for meta-analysis and by analyzing pooled data from the CRYSTAL and OPUS studies, they confirmed that adding cetuximab to first-line chemotherapy in patients with *KRAS* wild-type mCRC could obtain benefit in all efficacy end-points. Barni *et al.*<sup>[32]</sup> conducted a meta-analysis evaluating the efficacy of cetuximab in patients with wild-type *KRAS* as second- or further-line therapy, and they demonstrated that treatment with cetuximab plus chemotherapy in mCRC patients with wild-type *KRAS* pretreated with one or more lines of therapy could improve survival outcomes, however, this meta-analysis did not include patients treated with chemotherapy alone and patients with mutant *KRAS* status.

In contrast to the results mentioned above, the meta-analysis of Zhou *et al.*<sup>[29]</sup> showed that the addition of cetuximab or panitumumab to oxaliplatin-based chemotherapy in first-line treatment of mCRC in wild-type *KRAS* population did not improve survival benefit or response rate. They explained that the nature and interaction of drugs used in combination may be responsible for this observation<sup>[29]</sup>. Indeed, constitutive activation of the intracellular signaling pathway downstream of EGFR would counteract the effects of anti-EGFR agents<sup>[12]</sup>, though it is in the setting of *KRAS* mutation status. In addition, another meta-analysis demonstrated that efficacy of cetuximab could be influenced by drugs used in combination<sup>[34]</sup>. Indeed, irinotecan has been widely used in the treatment of mCRC, but not all of the patients were treated with this agent. Our study proved that efficacy of cetuximab combined with irinotecan chemotherapy was much better those without irinotecan. These all demonstrated the complexity of tumor pathology and capacity of response to different chemotherapy. More elegant trials considering suitable drugs used in combination treatment are needed.

It seems that *KRAS* status is a good predictor of sensitivity to cetuximab treatment, making it reasonable to detect the exact mutation location in *KRAS* gene. In our study, we observed that patients with wild-type *KRAS* could benefit a lot from cetuximab treatment, while this did not happen in patients with mutant *KRAS*. However, several studies<sup>[30,35,36]</sup> reported that certain specific mutations in *KRAS* could gain a greater clinical response to anti-EGFR treatment than patients with other *KRAS* mutations. This again demonstrated the complexity in the treatment of mCRC patients regarding *KRAS* status.

There are a few limitations in the present meta-analysis. First, the randomization is not appropriately applied in some of the studies, and heterogeneity in trial protocols, age, sex, and endpoint variables is inevitable. Second, information about PFS and OS is not directly available from each included study. Finally, only a subset of specimens were available from all participants, despite that the initial trials were carefully designed. However, these limitations could be attenuated partly by using random effect model analysis. More elegant RCTs assessing efficacy of cetuximab in the treatment of mCRC patients with different *KRAS* status are warranted.

In conclusion, the results from this meta-analysis strength the evidence supporting the use of cetuximab treatment in combination with traditional chemotherapy in mCRC patients with wild-type *KRAS*. *KRAS* status should be explored prior to the initiation of adding cetuximab to treatment of mCRC patients in order to avoid ineffective and toxic therapies. For patients with unclear status of *KRAS* and/or *BRAF*, cetuximab should be initially considered. More challenges emerged in the search of better biomarkers of cetuximab in mCRC, in the setting that certain *KRAS* mutational status is also associated with an favorable outcome when encountering with other mutational status. It is expected to find that judicious application of biomarkers will provide more chances to optimize the use of cetuximab.

## COMMENTS

### Background

The application of novel molecular targeted agents prolongs the survival of patients with metastatic colorectal cancer (mCRC). However, some research showed that addition of cetuximab to chemotherapy did not improve the outcome of mCRC patients, making the anti-tumor effect of cetuximab controversial, and indicating that cetuximab should be recommended based on individual information. Therefore, it is urgent to identify patients who could benefit from cetuximab treatment most and this relies on effective biomarkers in predicting efficacy of cetuximab in the treatment of mCRC.

### Research frontiers

The *KRAS* protein is one of the most important downstream effectors coupling EGFR to intracellular signaling cascades. The mutations of *KRAS* may contribute to the lack of response to anti-EGFR monoclonal antibodies in patients with mCRC. Thus, it is essential to evaluate whether *KRAS* is a biomarker of effectiveness of cetuximab using pooled data.

### Innovations and breakthroughs

Pooled data from the CRYSTAL and OPUS studies confirmed that in patients with *KRAS* wild-type tumors, adding cetuximab to chemotherapy led to a significant improvement in overall survival (OS), progression-free survival (PFS) and overall response rate. However, other trials demonstrated that *KRAS* status was not predictive of benefit when adding cetuximab to the first-line therapy.

### Applications

The results from this meta-analysis strength the evidence supporting the use of cetuximab treatment in combination with traditional chemotherapy in mCRC patients with wild-type *KRAS*. *KRAS* status should be explored prior to the initiation of adding cetuximab to treatment of mCRC patients in order to avoid ineffective and toxic therapies. For patients with unclear status of *KRAS* and/or *BRAF*, cetuximab should be initially considered.

### Terminology

In this systematic review, the authors performed a series of subgroup analysis regarding not only PFS or OS of the patients, but also the mutation status of

*KRAS* and/or *BRAF*, to compare the efficacy and safety of additional cetuximab to irinotecan containing chemotherapy under these different circumstances.

### Peer-review

This is a good systematic review in which the authors analyzed the cetuximab treatment in mCRC with regard to *KRAS* status. The results are clear and interesting and suggest that *KRAS* is a biomarker of effectiveness of cetuximab, and *KRAS* status should be explored prior to the initiation of adding cetuximab to treatment of mCRC patients in order to avoid ineffective and toxic therapies.

## REFERENCES

- 1 **World Health Organisation.** Globocan. 2008
- 2 **Sargent DJ,** Wieand HS, Haller DG, Gray R, Benedetti JK, Buyse M, Labianca R, Seitz JF, O'Callaghan CJ, Francini G, Grothey A, O'Connell M, Catalano PJ, Blanke CD, Kerr D, Green E, Wolmark N, Andre T, Goldberg RM, De Gramont A. Disease-free survival versus overall survival as a primary end point for adjuvant colon cancer studies: individual patient data from 20,898 patients on 18 randomized trials. *J Clin Oncol* 2005; **23**: 8664-8670 [PMID: 16260700 DOI: 10.1200/JCO.2005.01.6071]
- 3 **Arnold D,** Stein A. New developments in the second-line treatment of metastatic colorectal cancer: potential place in therapy. *Drugs* 2013; **73**: 883-891 [PMID: 23743737 DOI: 10.1007/s40265-013-0076-5]
- 4 **Woo J,** Palmisiano N, Tester W, Leighton JC. Controversies in antiepidermal growth factor receptor therapy in metastatic colorectal cancer. *Cancer* 2013; **119**: 1941-1950 [PMID: 23504768 DOI: 10.1002/cncr.27994]
- 5 **Tol J,** Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, Erdkamp FL, Vos AH, van Groenigen CJ, Sinnige HA, Richel DJ, Voest EE, Dijkstra JR, Vink-Börger ME, Antonini NF, Mol L, van Krieken JH, Dalesio O, Punt CJ. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 563-572 [PMID: 19196673 DOI: 10.1056/NEJMoa0808268]
- 6 **Troiani T,** Zappavigna S, Martinelli E, Addeo SR, Stiuso P, Ciardiello F, Caraglia M. Optimizing treatment of metastatic colorectal cancer patients with anti-EGFR antibodies: overcoming the mechanisms of cancer cell resistance. *Expert Opin Biol Ther* 2013; **13**: 241-255 [PMID: 23281932 DOI: 10.1517/14712598.2012.756469]
- 7 **Cunningham D,** Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313 DOI: 10.1056/NEJMoa033025]
- 8 **Lenz HJ,** Van Cutsem E, Khambata-Ford S, Mayer RJ, Gold P, Stella P, Mirtsching B, Cohn AL, Pippas AW, Azarnia N, Tsuchihashi Z, Mauro DJ, Rowinsky EK. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. *J Clin Oncol* 2006; **24**: 4914-4921 [PMID: 17050875 DOI: 10.1200/JCO.2006.06.7595]
- 9 **Sobrero AF,** Maurel J, Fehrenbacher L, Scheithauer W, Abubakr YA, Lutz MP, Vega-Villegas ME, Eng C, Steinhauer EU, Prausova J, Lenz HJ, Borg C, Middleton G, Kröning H, Luppi G, Kisker O, Zube A, Langer C, Kopit J, Burris HA. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 2311-2319 [PMID: 18390971 DOI: 10.1200/JCO.2007.13.1193]
- 10 **Oyan B.** Why do targeted agents not work in the adjuvant setting in colon cancer? *Expert Rev Anticancer Ther* 2012; **12**: 1337-1345 [PMID: 23176621 DOI: 10.1586/era.12.111]
- 11 **Schubbert S,** Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. *Nat Rev Cancer* 2007; **7**: 295-308 [PMID: 17384584 DOI: 10.1038/nrc2109]
- 12 **Patel GS,** Karapetis CS. Personalized treatment for advanced

- colorectal cancer: *KRAS* and beyond. *Cancer Manag Res* 2013; **5**: 387-400 [PMID: 24294007 DOI: 10.2147/CMAR.S35025]
- 13 **Bos JL**. ras oncogenes in human cancer: a review. *Cancer Res* 1989; **49**: 4682-4689 [PMID: 2547513]
  - 14 **Bokemeyer C**, Van Cutsem E, Rougier P, Ciardiello F, Heeger S, Schlichting M, Celik I, Köhne CH. Addition of cetuximab to chemotherapy as first-line treatment for *KRAS* wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. *Eur J Cancer* 2012; **48**: 1466-1475 [PMID: 22446022 DOI: 10.1016/j.ejca.2012.02.057]
  - 15 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]
  - 16 **Bokemeyer C**, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donea S, Ludwig H, Schuch G, Stroh C, Loos AH, Zubel A, Koralewski P. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 663-671 [PMID: 19114683 DOI: 10.1200/JCO.2008.20.8397]
  - 17 **Maughan TS**, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, Idziaszczyk S, Harris R, Fisher D, Kenny SL, Kay E, Mitchell JK, Madi A, Jasani B, James MD, Bridgewater J, Kennedy MJ, Claes B, Lambrechts D, Kaplan R, Cheadle JP. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011; **377**: 2103-2114 [PMID: 21641636 DOI: 10.1016/S0140-6736(11)60613-2]
  - 18 **Tveit K**, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, Kure E, Ikdahl T, Skovlund E, Christoffersen T. Randomized phase III study of 5-fluorouracil/folinic acid/oxaliplatin given continuously or intermittently with or without cetuximab, as first-line treatment of metastatic colorectal cancer: The NORDIC VII study (NCT00145314), by the Nordic Colorectal Cancer Biomodulation Group. *Ann Oncol* 2010; **21** Suppl 8: viii9
  - 19 **Parmar MK**, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med* 1998; **17**: 2815-2834 [PMID: 9921604]
  - 20 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563]
  - 21 **Van Cutsem E**, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor *KRAS* and *BRAF* mutation status. *J Clin Oncol* 2011; **29**: 2011-2019 [PMID: 21502544 DOI: 10.1200/JCO.2010.33.5091]
  - 22 **Tveit KM**, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, Sigurdsson F, Kure E, Ikdahl T, Skovlund E, Fokstuen T, Hansen F, Hofslø E, Birkemeyer E, Johnsson A, Starkhammar H, Yilmaz MK, Keldsen N, Erdal AB, Dajani O, Dahl O, Christoffersen T. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. *J Clin Oncol* 2012; **30**: 1755-1762 [PMID: 22473155 DOI: 10.1200/JCO.2011.38.0915]
  - 23 **Ye LC**, Liu TS, Ren L, Wei Y, Zhu DX, Zai SY, Ye QH, Yu Y, Xu B, Qin XY, Xu J. Randomized controlled trial of cetuximab plus chemotherapy for patients with *KRAS* wild-type unresectable colorectal liver-limited metastases. *J Clin Oncol* 2013; **31**: 1931-1938 [PMID: 23569301 DOI: 10.1200/JCO.2012.44.8308]
  - 24 **Huang J**, Nair SG, Mahoney MR, Nelson GD, Shields AF, Chan E, Goldberg RM, Gill S, Kahlenberg MS, Quesenberry JT, Thibodeau SN, Smyrk TC, Grothey A, Sinicrope FA, Webb TA, Farr GH, Pockaj BA, Berenberg JL, Mooney M, Sargent DJ, Alberts SR. Comparison of FOLFIRI with or without cetuximab in patients with resected stage III colon cancer; NCCTG (Alliance) intergroup trial N0147. *Clin Colorectal Cancer* 2014; **13**: 100-109 [PMID: 24512953 DOI: 10.1016/j.clcc.2013.12.002]
  - 25 **Bokemeyer C**, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, Celik I, Schlichting M, Koralewski P. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 2011; **22**: 1535-1546 [PMID: 21228335 DOI: 10.1093/annonc/mdq632]
  - 26 **Alberts SR**, Sargent DJ, Nair S, Mahoney MR, Mooney M, Thibodeau SN, Smyrk TC, Sinicrope FA, Chan E, Gill S, Kahlenberg MS, Shields AF, Quesenberry JT, Webb TA, Farr GH, Pockaj BA, Grothey A, Goldberg RM. Effect of oxaliplatin, fluorouracil, and leucovorin with or without cetuximab on survival among patients with resected stage III colon cancer: a randomized trial. *JAMA* 2012; **307**: 1383-1393 [PMID: 22474202 DOI: 10.1001/jama.2012.385]
  - 27 **Borner M**, Koeberle D, Von Moos R, Saletti P, Rauch D, Hess V, Trojan A, Helbling D, Pestalozzi B, Caspar C, Ruhstaller T, Roth A, Kappeler A, Dietrich D, Lanz D, Mingrone W. Adding cetuximab to capecitabine plus oxaliplatin (XELOX) in first-line treatment of metastatic colorectal cancer: a randomized phase II trial of the Swiss Group for Clinical Cancer Research SAKK. *Ann Oncol* 2008; **19**: 1288-1292 [PMID: 18349029 DOI: 10.1093/annonc/mdn058]
  - 28 **Chen MC**, Chiang FF, Wang HM. Cetuximab plus chemotherapy as first-line treatment for metastatic colorectal cancer: effect of *KRAS* mutation on treatment efficacy in Taiwanese patients. *Neoplasma* 2013; **60**: 561-567 [PMID: 23790176 DOI: 10.4149/neo\_2013\_073]
  - 29 **Zhou SW**, Huang YY, Wei Y, Jiang ZM, Zhang YD, Yang Q, Xie DR. No survival benefit from adding cetuximab or panitumumab to oxaliplatin-based chemotherapy in the first-line treatment of metastatic colorectal cancer in *KRAS* wild type patients: a meta-analysis. *PLoS One* 2012; **7**: e50925 [PMID: 23226426 DOI: 10.1371/journal.pone.0050925]
  - 30 **Mao C**, Huang YF, Yang ZY, Zheng DY, Chen JZ, Tang JL. *KRAS* p.G13D mutation and codon 12 mutations are not created equal in predicting clinical outcomes of cetuximab in metastatic colorectal cancer: a systematic review and meta-analysis. *Cancer* 2013; **119**: 714-721 [PMID: 22972628 DOI: 10.1002/cncr.27804]
  - 31 **Qiu LX**, Mao C, Zhang J, Zhu XD, Liao RY, Xue K, Li J, Chen Q. Predictive and prognostic value of *KRAS* mutations in metastatic colorectal cancer patients treated with cetuximab: a meta-analysis of 22 studies. *Eur J Cancer* 2010; **46**: 2781-2787 [PMID: 20580219 DOI: 10.1016/j.ejca.2010.05.022]
  - 32 **Barni S**, Ghilardi M, Borgonovo K, Cabiddu M, Zaniboni A, Petrelli F. Cetuximab/irinotecan-chemotherapy in *KRAS* wild-type pretreated metastatic colorectal cancer: a pooled analysis and review of literature. *Rev Recent Clin Trials* 2013; **8**: 101-109 [PMID: 23859115]
  - 33 **Hoyle M**, Crathorne L, Peters J, Jones-Hughes T, Cooper C, Napier M, Tappenden P, Hyde C. The clinical effectiveness and cost-effectiveness of cetuximab (mono- or combination chemotherapy), bevacizumab (combination with non-oxaliplatin chemotherapy) and panitumumab (monotherapy) for the treatment of metastatic colorectal cancer after first-line chemotherapy (review of technology appraisal No.150 and part review of technology appraisal No. 118): a systematic review and economic model. *Health Technol Assess* 2013; **17**: 1-237 [PMID: 23547747 DOI: 10.3310/hta17140]
  - 34 **Ku GY**, Haaland BA, de Lima Lopes G. Cetuximab in the first-line treatment of *K-ras* wild-type metastatic colorectal cancer: the choice and schedule of fluoropyrimidine matters. *Cancer Chemother Pharmacol* 2012; **70**: 231-238 [PMID: 22699811 DOI: 10.1007/s00280-012-1898-7]
  - 35 **Chen J**, Ye Y, Sun H, Shi G. Association between *KRAS* codon 13 mutations and clinical response to anti-EGFR treatment in

patients with metastatic colorectal cancer: results from a meta-analysis. *Cancer Chemother Pharmacol* 2013; **71**: 265-272 [PMID: 23090619 DOI: 10.1007/s00280-012-2005-9]

- 36 **Modest DP**, Brodowicz T, Stintzing S, Jung A, Neumann J, Laubender RP, Ocvirk J, Kurteva G, Papai Z, Knittelfelder R,

Kirchner T, Heinemann V, Zielinski CC. Impact of the specific mutation in *KRAS* codon 12 mutated tumors on treatment efficacy in patients with metastatic colorectal cancer receiving cetuximab-based first-line therapy: a pooled analysis of three trials. *Oncology* 2012; **83**: 241-247 [PMID: 22948721 DOI: 10.1159/000339534]

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## Sequential vs simultaneous revascularization in patients undergoing liver transplantation: A meta-analysis

Jia-Zhong Wang, Yang Liu, Jin-Long Wang, Le Lu, Ya-Fei Zhang, Hong-Wei Lu, Yi-Ming Li

Jia-Zhong Wang, Yang Liu, Jin-Long Wang, Le Lu, Ya-Fei Zhang, Hong-Wei Lu, Yi-Ming Li, Department of General Surgery, Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine, Xi'an 710004, Shaanxi Province, China

**Author contributions:** Li YM and Wang JZ conceived this meta-analysis, wrote the initial manuscript, critically reviewed and revised the manuscript; Wang JZ, Wang JL and Lu L acquired data, performed the initial data analysis; Lu HW critically supervised data acquisition and analysis; and Li YM approved the final version of the manuscript; all authors contributed to this paper.

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**Correspondence to:** Yi-Ming Li, Professor, Department of General Surgery, Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine, 76 Yanta West Road, Xi'an 710004, Shaanxi Province, China. [wjz05202156@stu.xjtu.edu.cn](mailto:wjz05202156@stu.xjtu.edu.cn)  
Telephone: +86-29-87679746  
Fax: +86-29-87679746

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### Abstract

**AIM:** We undertook this meta-analysis to investigate the relationship between revascularization and outcomes after liver transplantation.

**METHODS:** A literature search was performed using MeSH and key words. The quality of the included studies was assessed using the Jadad Score and the Newcastle-Ottawa Scale. Heterogeneity was evaluated by the  $\chi^2$  and  $I^2$  tests. The risk of publication bias was assessed using a funnel plot and Egger's test, and the risk of bias was assessed using a domain-based assessment tool. A sensitivity analysis was conducted by reanalyzing the data using different statistical approaches.

**RESULTS:** Six studies with a total of 467 patients were included. Ischemic-type biliary lesions were significantly reduced in the simultaneous revascularization group compared with the sequential revascularization group (OR = 4.97, 95%CI: 2.45-10.07;  $P < 0.00001$ ), and intensive care unit (ICU) days were decreased (MD = 2.00, 95%CI: 0.55-3.45;  $P = 0.007$ ) in the simultaneous revascularization group. Although warm ischemia time was prolonged in simultaneous revascularization group (MD = -25.84, 95%CI: -29.28-22.40;  $P < 0.00001$ ), there were no significant differences in other outcomes between sequential and simultaneous revascularization groups. Assessment of the risk of bias showed that the methods of random sequence generation and blinding might have been a source of bias. The sensitivity analysis strengthened the reliability of the results of this meta-analysis.

**CONCLUSION:** The results of this study indicate that simultaneous revascularization in liver transplantation may reduce the incidence of ischemic-type biliary lesions and length of stay of patients in the ICU.

**Key words:** Revascularization; Liver transplantation; Outcomes; Biliary complications; Meta-analysis

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**Core tip:** The current methods of revascularization in liver transplantation can be divided into two main groups. We carried out this meta-analysis in order to study the relationship between revascularization and outcomes after liver transplantation. Ischemic-type biliary lesions were significantly reduced in the simultaneous revascularization group compared with the sequential revascularization group ( $P < 0.00001$ ), and intensive care unit days were decreased ( $P = 0.007$ ) in the simultaneous revascularization group. There were no significant differences in other outcomes between sequential and simultaneous revascularization groups, such as blood transfusions, hospital days, graft failure and mortality in one month and one year, operation time.

Wang JZ, Liu Y, Wang JL, Lu L, Zhang YF, Lu HW, Li YM. Sequential vs simultaneous revascularization in patients undergoing liver transplantation: A meta-analysis. *World J Gastroenterol* 2015; 21(22): 7036-7046 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/7036.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.7036>

## INTRODUCTION

Sequential portal and arterial revascularization (SeqR) and simultaneous portal and arterial revascularization (SimR) have been advocated to improve outcomes after liver transplantation<sup>[1-5]</sup>. In SeqR, the liver graft is sequentially reperfused by portal and arterial reperfusion. By contrast, in SimR, the liver graft is simultaneously reperfused by the portal vein and the hepatic artery. Because the portal vein contributes approximately three fourths of the blood supply to the liver and is easily anastomosed to shorten the warm ischemia time (WIT) and the anhepatic phase during the operation, SeqR is the more widely performed sequence of revascularization<sup>[6]</sup>. However, the primary disadvantage of SeqR is the potential increased risk of arterial ischemic injury to the bile ducts, which depend solely on the arterial blood supply<sup>[7]</sup>. Therefore, some authors have recommended the use of SimR for its reduction of the risk of arterial ischemic damage to biliary epithelial cells, which are more susceptible to ischemia-reperfusion injury than hepatocytes<sup>[8,9]</sup>. However, the disadvantage of SimR is that it prolongs the WIT and the anhepatic phase, which can be detrimental to patient mortality and morbidity related to the graft<sup>[10]</sup>. The better method of revascularization in liver transplantation remains controversial.

Although some meta-analyses have been conducted to compare the incidence of total biliary complications between SimR and SeqR in liver transplantation<sup>[1,2]</sup>, the method that results in a greater reduction in the

incidence of ischemic-type biliary lesions (ITBLs) and other outcomes remains unclear. The primary purpose of this meta-analysis was to investigate the relationship between revascularization and ITBLs. In addition, we also evaluated other outcomes, such as blood transfusions, hospital days, graft failure and mortality in one month and one year, operation time.

## MATERIALS AND METHODS

This systematic review and meta-analysis were conducted according to the PRISMA statement<sup>[11]</sup>.

### Literature search

To identify relevant studies, a search of the literature was performed in MEDLINE, the Cochrane database, the Science Citation Index (SCI), PLOS ONE, Wiley Online Library, Springer, and China National Knowledge Infrastructure (CNKI) without restrictions on the year or language. We performed a systematic search using both "MeSH" and "key words" protocols. More specifically, the following terms retrieved from the MeSH browser provided by PubMed were utilized: ["liver" (All Fields) OR "hepatic" (All Fields)] AND "transplantation" (All Fields) AND "revascularization" (All Fields) OR "reperfusion" (MeSH). A multiple "key words" search was performed with the terms "liver transplantation" AND "revascularization".

### Eligibility criteria

**Types of studies:** Clinical studies conducted comparing SimR and SeqR in liver transplantation were included regardless of blinding, publication status, or sample size. Further, these clinical studies had to contain sufficient data about outcomes after liver transplantation. Literature on animal experiments, reviews, letters to the editor and clinical studies conducted without control groups were excluded.

**Types of participants:** Patients undergoing SeqR or SimR in liver transplantation were included regardless of age, gender, nationality, or reason for liver transplantation.

**Types of interventions:** Studies with comparisons between SeqR and SimR were included regardless of whether piggy-back or conventional orthotopic liver transplantation was performed.

**Types of outcomes:** (1) ITBLs; (2) blood transfusions (units of blood and plasma); (3) Hospital days [intensive care unit (ICU) and total hospital days]; (4) graft failure and mortality in one month and one year; and (5) Operation time (total operation and WIT).

### Literature selection and data extraction

Two independent reviewers (Wang JL, Lu L) evaluated each title, abstract, citation, and selected relevant studies according to the eligibility criteria. Disagreements

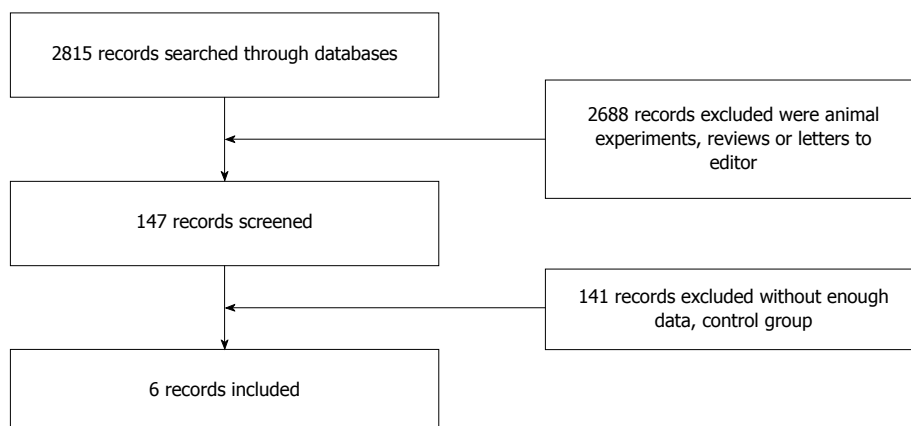


Figure 1 Flow diagram of the included studies.

were resolved by another reviewer (Lu HW). Data were extracted separately from the included studies, and a reviewer (Lu HW) reviewed and confirmed the accuracy of the extracted data. Data from the included studies were recorded as follows: (1) authors, country, year; (2) publication; (3) number of participants in the SimR and SeqR groups; and (4) outcomes after liver transplantation.

### Quality assessment of studies

The methodological quality of each randomized controlled trials (RCTs) was independently assessed using the Jadad score. This five-point quality scale includes points for randomization (0-2 points), double blinding (0-2 points), and withdrawals and dropouts (0-1 points). Total scores of 0-2 were considered indicative of low quality, whereas studies with total scores  $\geq 3$  were defined as high quality. The Newcastle-Ottawa Scale (NOS) was used to assess the quality of each retrospective study. The NOS contains 8 items, which are divided into three categories: selection (4 items), comparability (1 item), and exposure for case-control studies (3 items). A star system was used to allow for a semi-quantitative assessment of study quality. A study can be awarded a maximum of one star for each item within the selection and exposure categories. A maximum of two stars can be given for comparability. Studies were defined as high quality if they had more than seven stars; as medium quality if they had between four and six stars; and as poor quality if they had fewer than four stars.

### Statistical analysis

The statistical methods of this study were reviewed by Deyu Meng from the Faculty of Mathematics and Statistics, Xi'an Jiaotong University. Data were analyzed by the statistical software Review Manager 5.2 (The Cochrane Collaboration) using a fixed effects model. The results of continuous and discontinuous data were reported as MD with 95%CI and as OR with 95%CI, respectively.  $P < 0.05$  was considered to indicate a statistically significant difference. The

heterogeneity of the included studies was evaluated by the  $\chi^2$  and  $I^2$  tests (Review Manager 5.2), and  $P < 0.10$  or  $I^2 > 25\%$  was defined as indicating heterogeneity. A funnel plot (Review Manager 5.2) and Egger's test (Stata 10.0) were used to assess the risk of publication bias, and  $P < 0.10$  indicated statistical publication bias. The risk of bias in the studies was assessed by two independent reviewers (Wang JZ, Li YM) using a domain-based assessment tool recommended by the Cochrane collaboration. Two methods of sensitivity analysis were conducted by comparing the effect size between a fixed effects model and a random effects model; the fixed effect model was applied after excluding the greatest weight study.

## RESULTS

### Flow diagram of the included studies

We screened the title and abstract of 2815 studies after performing the database search. Then, 2688 studies were excluded because they were animal experiments, reviews, or letters to the editor, and 141 clinical studies did not meet our criteria due to their lack of a control group or insufficient data on outcomes after liver transplantation. A total of six studies were included<sup>[12-17]</sup>, comprising a total of 467 patients (Figure 1).

### Study characteristics

Six studies with a total of 467 patients were included. Two studies were RCTs<sup>[16,17]</sup>, with a total of 120 patients; the other studies were retrospective, with a total of 347 patients<sup>[13-17]</sup>. The included studies were conducted in five countries: two in Italy, one in China, one in the United States, one in Brazil, and one in the Netherlands. None of the six studies was considered low quality (Table 1).

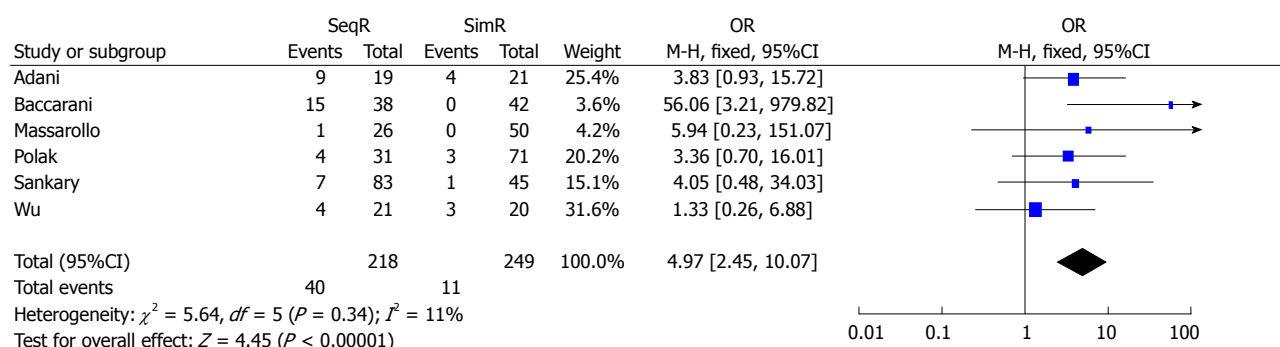
### Synthesis of outcomes and assessments of heterogeneity

**Simultaneous revascularization reduced the incidence of ITBLs:** The incidence of ITBLs was significantly reduced in the SimR group compared to

**Table 1** Characteristics of the included studies

Study	Year	Country	No. of SeqR/ SimR	Type of study	Jadad score	NOS
Sankary <i>et al</i> <sup>[12]</sup>	1995	United States	83/45	Retrospective study	-	6
Massarollo <i>et al</i> <sup>[13]</sup>	1998	Brazil	26/50	Retrospective study	-	7
Polak <i>et al</i> <sup>[14]</sup>	2005	the Netherlands	71/31	Retrospective study	-	7
Wu <i>et al</i> <sup>[15]</sup>	2006	China	21/20	Retrospective study	-	6
Adani <i>et al</i> <sup>[16]</sup>	2011	Italy	19/21	RCT	3	-
Baccarani <i>et al</i> <sup>[17]</sup>	2012	Italy	38/42	RCT	3	-

RCT: Randomized controlled trial; NOS: Newcastle-Ottawa scale.



**Figure 2** Meta-analysis results of incidence of ischemic-type biliary lesions. The occurrence of incidence of ischemic-type biliary lesions was significantly reduced in the SimR group over the SeqR group (OR = 4.97, 95%CI: 2.45-10.07,  $P < 0.00001$ ). SeqR: Sequential revascularization; SimR: Simultaneous revascularization.

the SeqR group according to this meta-analysis (OR = 4.97, 95%CI: 2.45-10.07;  $P < 0.00001$ ). Furthermore, the results of the  $\chi^2$  and  $I^2$  tests did not indicate heterogeneity ( $P = 0.34$ ,  $I^2 = 11\%$ ) (Figure 2).

**Simultaneous revascularization did not increase blood transfusions:** Two and three studies, respectively, were conducted to compare units of blood cell and plasma transfusions between SeqR and SimR. There were no significant differences in units of blood cell transfusions (MD = 0.55, 95%CI: -0.84-1.94;  $P = 0.44$ ) and plasma transfusions (MD = -416.71, 95%CI: -997.01-163.59;  $P = 0.16$ ). The results of the  $\chi^2$  and  $I^2$  tests were  $P = 0.21$  ( $I^2 = 37\%$ ) and  $P = 0.08$  ( $I^2 = 66\%$ ), respectively (Figure 3).

**Simultaneous revascularization decreased ICU days:** Two studies were conducted to compare ICU and total hospital days. SimR significantly decreased ICU days (MD = 2.00, 95%CI: 0.55-3.45;  $P = 0.007$ ), while no significant difference was shown in total hospital days (MD = 0.46, 95%CI: -1.99-2.90;  $P = 0.71$ ). The results of the  $\chi^2$  and  $I^2$  tests did not indicate heterogeneity ( $P = 1.00$ ,  $I^2 = 0\%$  and  $P = 0.23$ ,  $I^2 = 31\%$ ), respectively (Figure 4).

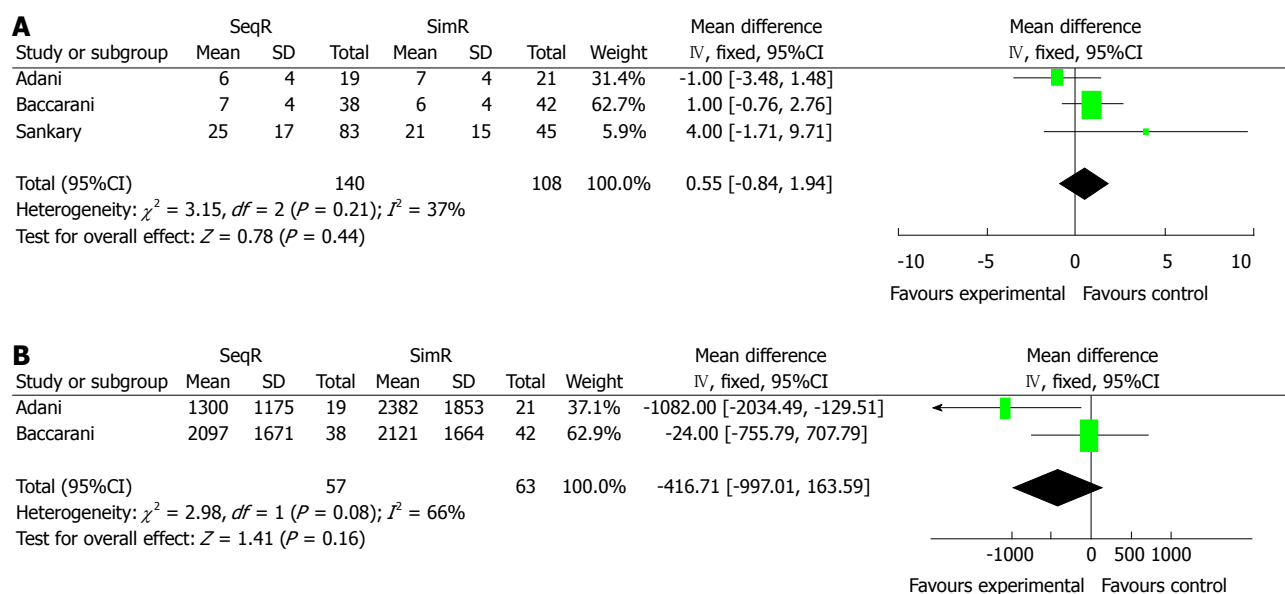
**Simultaneous revascularization increased WIT:** Although there was no significant difference in total operation time (MD = 22.59, 95%CI: -4.79-49.96;  $P = 0.11$ ), SimR significantly prolonged WIT (MD = -25.84,

95%CI: -29.28-22.40;  $P < 0.00001$ ). However, the results of  $\chi^2$  and  $I^2$  tests in WIT showed heterogeneity ( $P = 0.05$ ,  $I^2 = 75\%$ ), which was not indicated in total operation time ( $P = 0.86$ ,  $I^2 = 0\%$ ) (Figure 5).

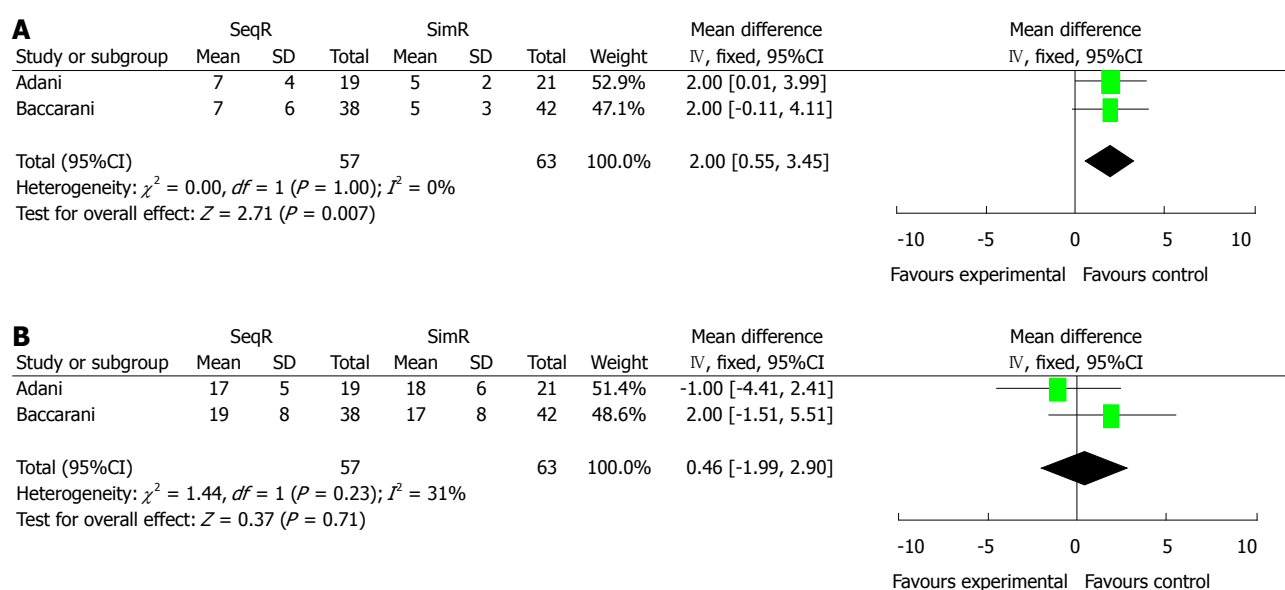
**Simultaneous revascularization did not increase patient mortality and graft failure in one month and one year:** There were two and three studies comparing graft failure and mortality in one month and one year between SeqR and SimR, respectively. There were no significant differences in patient graft failure and mortality in one month (OR = 1.19, 95%CI: 0.50-2.81;  $P = 0.70$ ; OR = 1.44, 95%CI: 0.57-3.68,  $P = 0.44$ ), or in one year (OR = 1.31, 95%CI: 0.57-3.04,  $P = 0.53$ ; OR = 0.83, 95%CI: 0.39-1.78,  $P = 0.64$ ). All results of  $\chi^2$  and  $I^2$  tests did not indicate heterogeneity ( $P = 0.59$ ,  $I^2 = 0\%$ ;  $P = 0.23$ ,  $I^2 = 30\%$ ;  $P = 0.60$ ,  $I^2 = 0\%$ ;  $P = 0.67$ ,  $I^2 = 0\%$ ) (Figure 6).

**Publication bias and risk of bias and sensitivity analyses** Although the funnel plot was not strictly symmetrical, Egger's test did not show publication bias ( $P = 0.136$ ) (Figure 7). The analyses indicated that random sequence generation and blinding, which were not properly described in these studies, might have been a source of bias (Figure 8). The sensitivity analysis showed the same effect sizes among a fixed effect model ( $P < 0.00001$ ), a random effect model ( $P = 0.002$ ) and a fixed effect model after excluding the largest weight study ( $P < 0.00001$ ) (Figure 9).





**Figure 3 Meta-analysis results of blood cell and plasma transfusions.** A: Three studies were conducted to compare units of blood cell (MD = 0.55, 95%CI: -0.84-1.94,  $P = 0.44$ ); B: Two studies were conducted to compare plasma transfusions (MD = -416.71, 95%CI: -997.01-163.59,  $P = 0.16$ ).

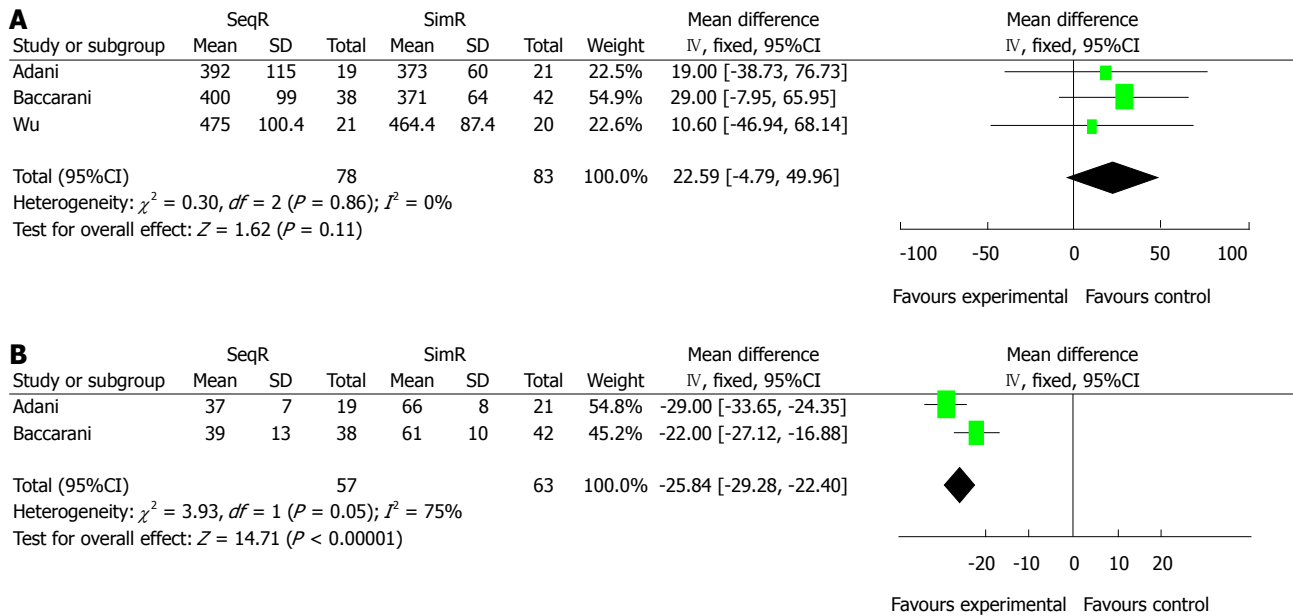


**Figure 4 Meta-analysis results of intensive care unit and total hospital days.** A: SimR significantly decreased intensive care unit days (MD = 2.00, 95%CI: 0.55-3.45,  $P = 0.007$ ); B: No significant difference was shown in total hospital days (MD = 0.46, 95%CI: -1.99-2.90,  $P = 0.71$ ).

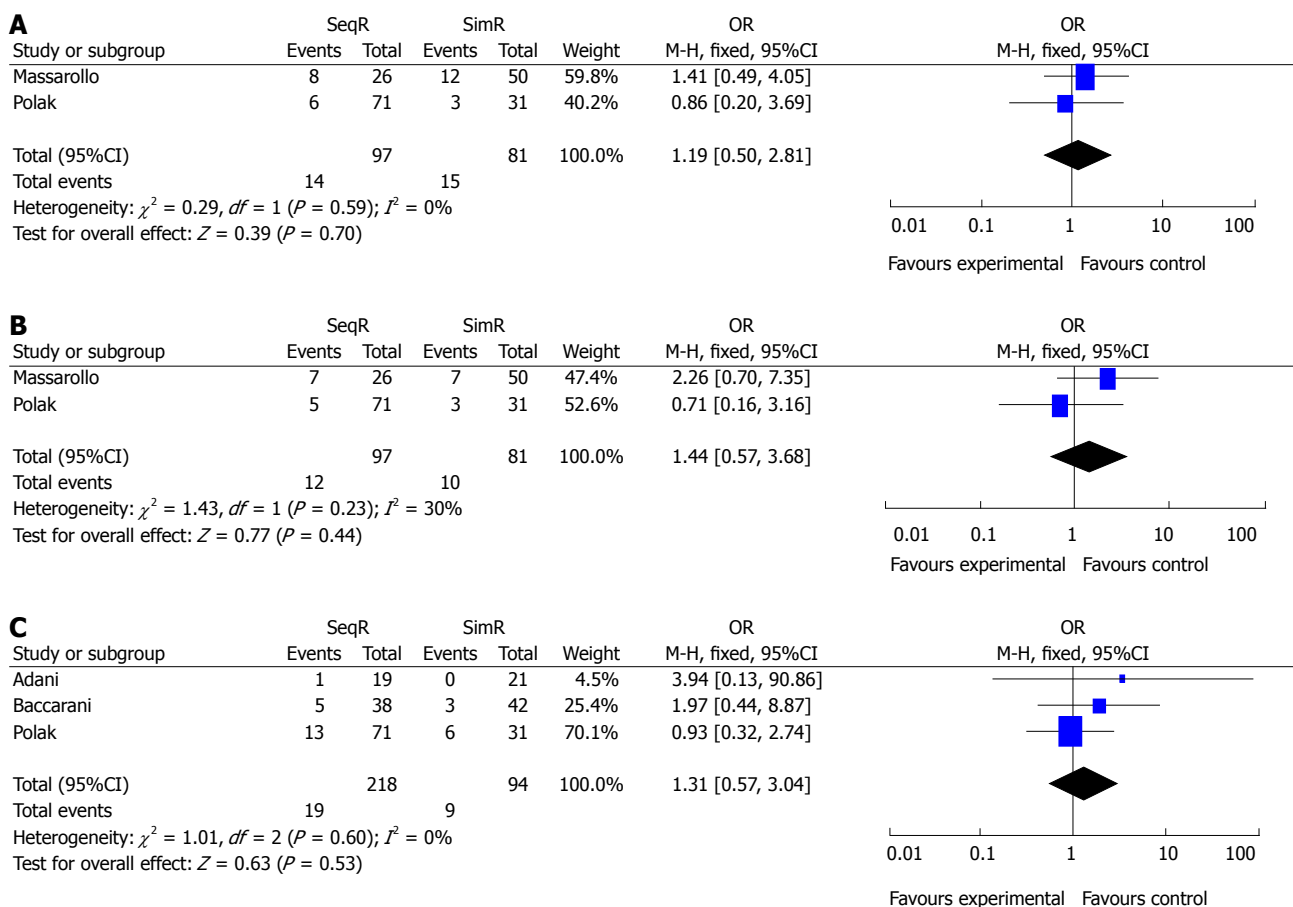
## DISCUSSION

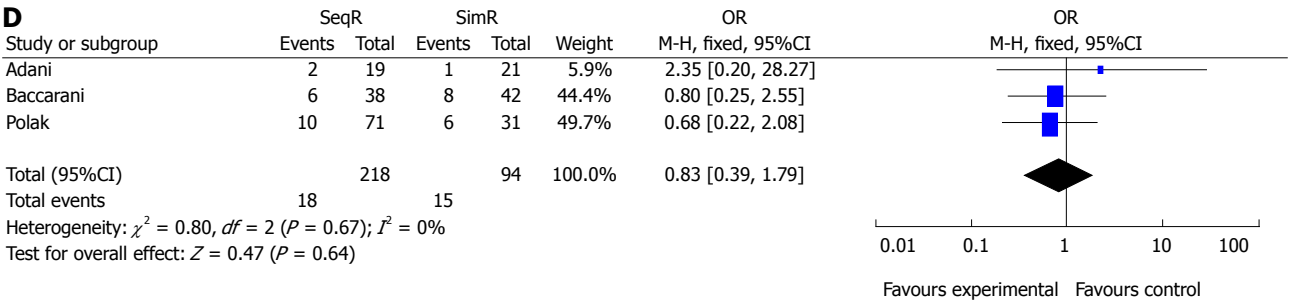
The current methods of revascularization in liver transplantation can be divided into two main groups according to whether the liver graft is reperfused sequentially or simultaneously. In SeqR cases, the first reconstructed vessel of the liver graft is one of the portal vein, the hepatic artery, or the inferior vena cava, followed by subsequent revascularization of the remaining vessels. By contrast, in SimR cases, the graft is simultaneously reperfused *via* the portal vein and the hepatic artery<sup>[3]</sup>. Although the best method of revascularization in liver transplantation remains

debatable<sup>[18]</sup>, the most commonly used procedure for the revascularization of liver grafts is SeqR<sup>[19]</sup>. Theoretically, there are some potential advantages to SeqR: (1) the portal vein contributes approximately 3/4 of the blood supply and 1/2 of the oxygen supply to the liver; (2) the portal vein is easily anastomized to shorten the WIT and anhepatic phase during the operation; and (3) the arterial anastomosis is performed under better technical conditions, without retrograde bleeding from the graft hepatic artery and in a surgical field free of hemorrhages<sup>[20]</sup>. However, SeqR increases warm ischemic injury to the bile ducts, which depend on the hepatic artery. Because ischemic

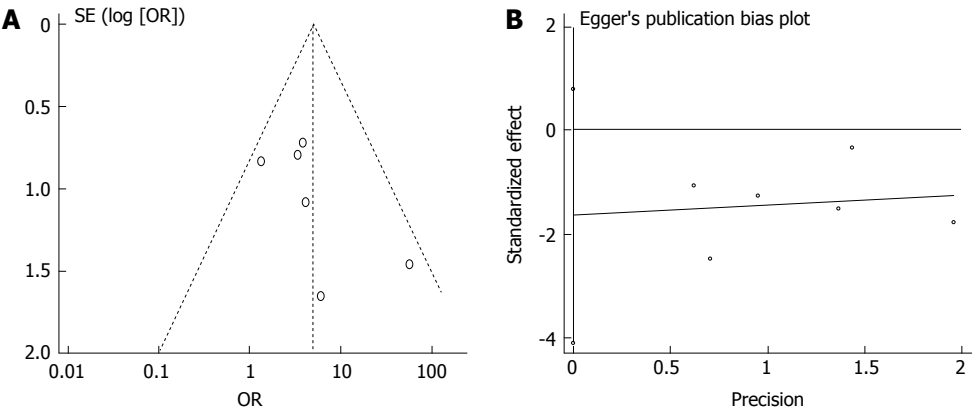


**Figure 5 Meta-analysis results of total operation time and warm ischemia time.** A: There was no significant difference in total operation time (MD = 22.59, 95%CI: -4.79-49.96,  $P = 0.11$ ); B: SimR significantly prolonged warm ischemia time (WIT) (MD = -25.84, 95%CI: -29.28-22.40,  $P < 0.00001$ ). However, the results of  $\chi^2$  and  $I^2$  tests in WIT showed heterogeneity ( $P = 0.05$ ,  $I^2 = 75\%$ ).

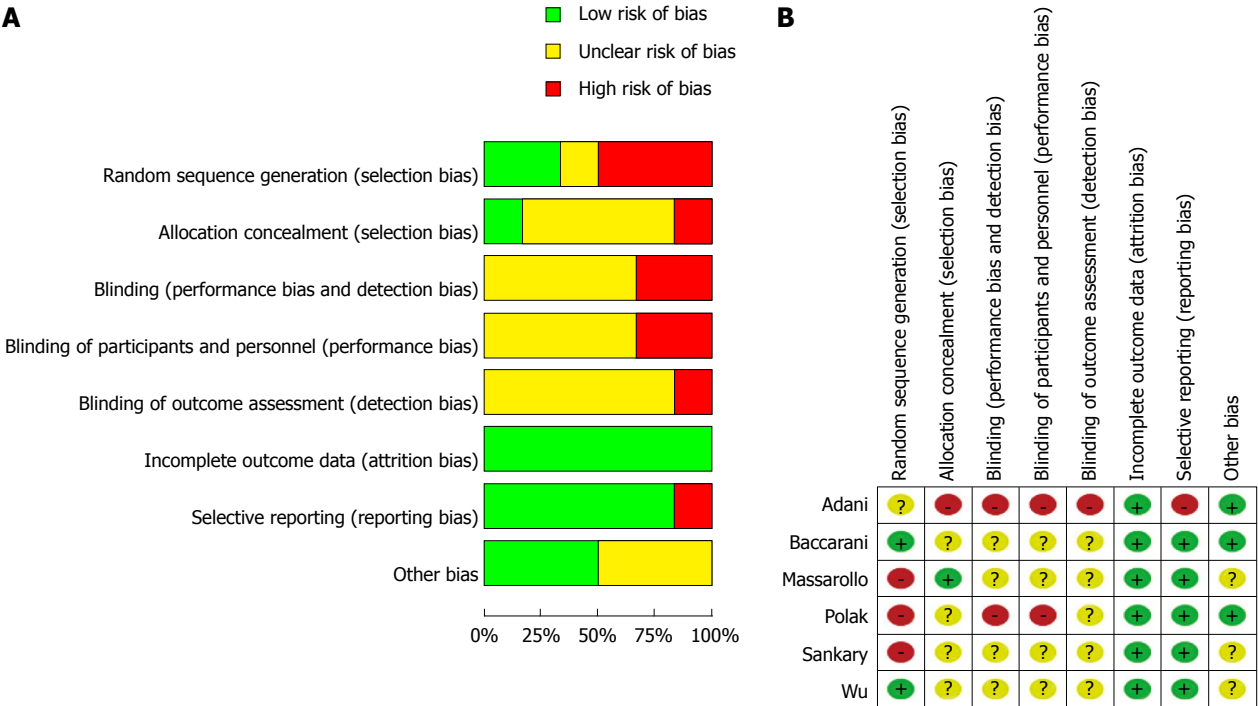




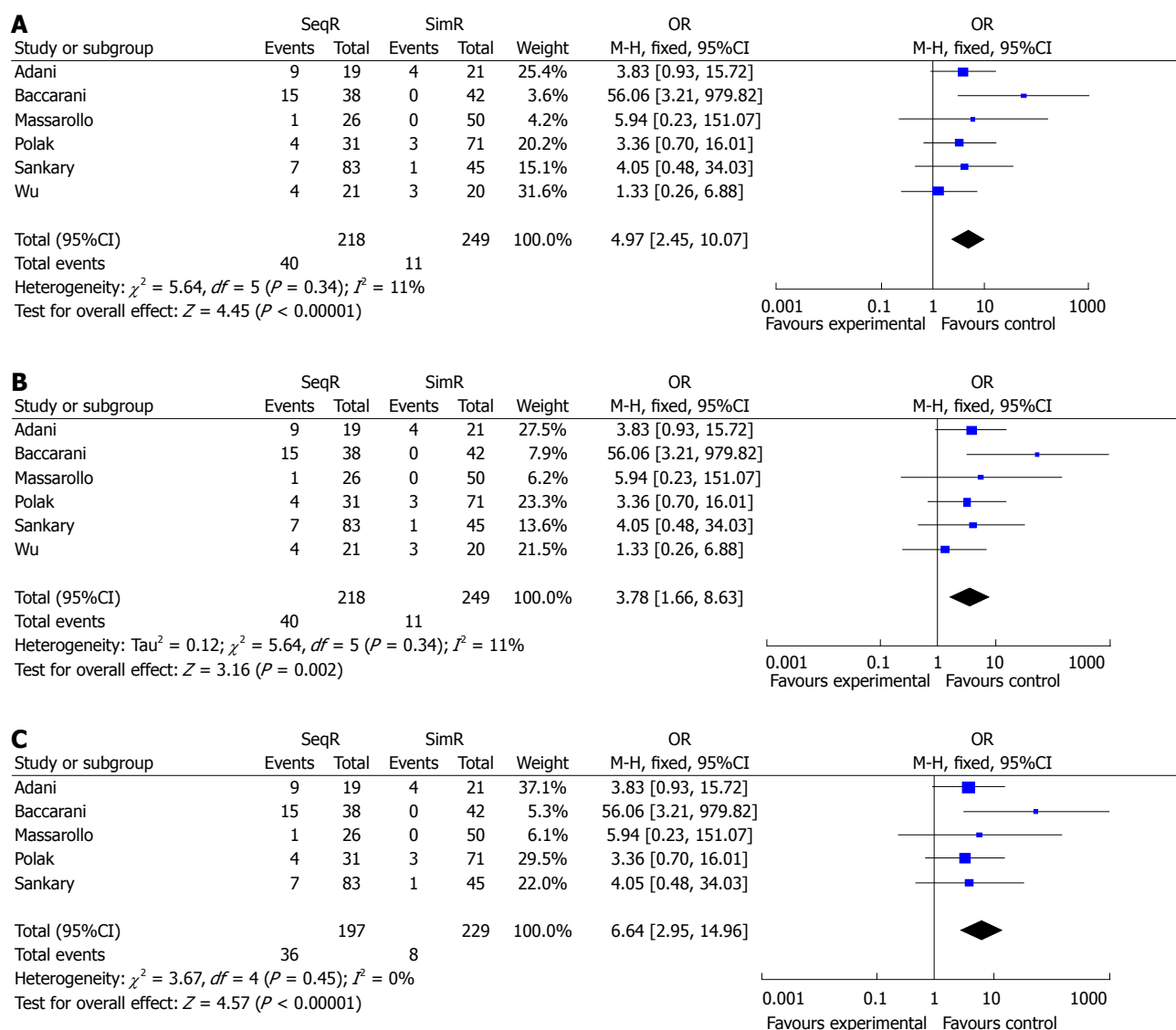
**Figure 6** Meta-analysis results of graft failure and mortality in one month and one year. A: There were no significant differences in graft failure in one month (OR = 1.19, 95%CI: 0.50-2.81,  $P = 0.70$ ); B: There were no significant differences in mortality in one month (OR = 1.44, 95%CI: 0.57-3.68,  $P = 0.44$ ); C: There were no significant differences in graft failure in one year (OR = 1.31, 95%CI: 0.57-3.04,  $P = 0.53$ ); D: There were no significant differences in mortality in one year (OR = 0.83, 95%CI: 0.39-1.78,  $P = 0.64$ ).



**Figure 7** Funnel plot and Egger's test of studies on incidence of ischemic-type biliary lesions. A: Funnel plot was not strictly symmetrical; B: Egger's test did not show publication bias ( $P = 0.136$ ).



**Figure 8** Risk of bias graph: review authors' judgments regarding each risk of bias item for each included study.



**Figure 9 Sensitivity analysis of this meta-analysis.** The sensitivity analysis showed the same effect sizes among different models. A: A fixed effects model ( $P < 0.00001$ ); B: A random effects model ( $P = 0.002$ ); C: A fixed effects model after excluding the greatest weight study ( $P < 0.00001$ ).

injuries to the bile ducts account for a majority of cases of morbidity, mortality, and graft failure in patients after liver transplantation<sup>[21-25]</sup>, some researchers have advocated the use of SimR. Although some meta-analyses have demonstrated that SeqR increases total biliary complications<sup>[1,2]</sup>, including anastomotic biliary complications and nonanastomotic biliary complications (*i.e.*, ITBLs)<sup>[26,27]</sup>, little is known about the relationship between revascularization and ITBLs.

We comprehensively reviewed the literature on the relationship between revascularization and ITBLs. The results of this meta-analysis on ITBLs imply that SimR reduces the incidence of ITBLs after liver transplantation, which is in accordance with previous clinical studies<sup>[12,13]</sup>. In addition, the heterogeneity tests did not show heterogeneity, and the sensitivity analysis showed the same effect sizes, which strengthened the confidence that can be placed in these results.

Although the specific mechanisms of SimR

that reduce the incidence of ITBLs remain unclear, reduced arterial ischemic injury to the bile ducts may be the main reason. The peribiliary vascular plexus is composed of branches arising directly from the right and left hepatic arteries (and accessory hepatic arteries when present) and from their segmental branches as well as branches arising indirectly from the gastroduodenal artery *via* the arteries supplying the common bile duct<sup>[28]</sup>. In SeqR cases, the graft is exclusively perfused through the portal vein for at least 10 min until the completion of hepatic arterial anastomosis<sup>[29,30]</sup>. The delay of rearterialization in SeqR is associated with more pronounced microvascular disturbances and subsequent graft dysfunction, including ischemic injury to the bile ducts. In a rat model of liver transplantation, SimR resulted in the best microcirculatory perfusion of the graft. In addition, the authors found less leukocyte accumulation in the sinusoids and more abundant bile flow after



simultaneous reperfusion compared to SeqR<sup>[31]</sup>. Moreover, SimR also elicits a remarkable improvement in oxygen tension and the maintenance of tissue ATP compared to SeqR, which may be helpful for reducing the incidence of ITBLs<sup>[32]</sup>. Although there are some potential benefits of SimR, the main disadvantages of SimR are the prolonged WIT and anhepatic phase, which can be detrimental to postoperative graft function, survival, and morbidity<sup>[3]</sup>. However, Adani *et al.*<sup>[16]</sup> reported that delayed graft function was diagnosed in 10% vs 9% in SeqR and SimR, while Polak *et al.*<sup>[14]</sup> reported that primary nonfunction was diagnosed in 3% (SeqR) vs 1% (SimR) and the rate of retransplantation was 9% in SeqR and 7% in SimR, respectively. Vascular complications were absent except for one case of hepatic artery thrombosis (HAT) leading to retransplantation in SeqR<sup>[16]</sup>. Liver ischemia-reperfusion injury, resulting in ITBLs during liver transplantation, triggers a cascade of events leading to biliary apoptosis, necrosis, and cholangitis which may even lead to graft failure. The main cause of prolonged WIT in SimR may be the simultaneous reconstruction of the portal vein and hepatic artery, which is inevitable due to this particular surgical technique. However, interestingly, SimR did not significantly prolong the total operation time or increase the incidence of blood transfusions, graft failure or mortality (one month and one year) in this study. Therefore, this is a burning issue to find the answer in the worlds of liver transplantation.

Besides different kinds of revascularization, some donor factors affect ITBLs, such as ABO incompatibility, gender, cytomegalovirus (CMV) infection and metalloproteinase-2 (MMP-2) polymorphism. The incidence of ITBLs after ABO-incompatible liver transplantation in adults is much higher than in ABO-compatible liver transplantation<sup>[33]</sup>. MMP-2 genotype in both donor and recipient is strongly and independently related to the development of ITBLs within 4 years after liver transplantation<sup>[34]</sup>.

Although this systematic review and meta-analysis imply that SimR reduces the incidence of ITBLs after liver transplantation, there are undoubtedly some limitations: (1) the number of patients in the included studies ranged from 19 to 83; (2) although we do believe that our search strategy was sufficient, only six studies were included in this meta-analysis of ITBLs, which may lead to inaccurate conclusions<sup>[35,36]</sup>; and (3) assessment of risk of bias showed that the methods of random sequence generation and blinding, which were not properly described in these studies, might have been a source of bias, which may have led to an overstatement of the treatment effects of SimR<sup>[37]</sup>.

In conclusion, the present meta-analysis included all current relevant clinical studies from various countries through July 2014, and the findings indicate that SimR reduces the incidence of ITBLs and decreases ICU days. Additional randomized and blinded clinical trials with a sufficient number of participants are needed to adequately compare SimR and SeqR in liver

transplantation.

## COMMENTS

### Background

Sequential portal and arterial revascularization (SeqR) and simultaneous portal and arterial revascularization (SimR) have been advocated to improve outcomes after liver transplantation. However, little is known about the relationship between revascularization and ischemic-type biliary lesions (ITBLs). The authors undertook this meta-analysis to investigate the relationship between revascularization and outcomes after liver transplantation.

### Research frontiers

There are two main methods for revascularization of the liver graft: SimR and SeqR. The sequence of graft reperfusion may be relevant for the development of ITBLs. In some retrospective studies, the incidence of ITBLs in patients who underwent SimR of the graft was lower compared to patients who had SeqR. Some clinical studies are conducted to validate whether SimR is better than SeqR.

### Innovations and breakthroughs

Although some meta-analyses have been conducted to compare the incidence of total biliary complications between SimR and SeqR in liver transplantation, the method that results in a greater reduction in the incidence of ITBLs and other outcomes remains unclear. The current study demonstrated that ITBLs and ICU days were significantly reduced in the SimR group compared with the SeqR group.

### Applications

The present meta-analysis indicates that SimR reduces the incidence of ITBLs and may be more suitable to protect the integrity of the intrahepatic biliary tree.

### Terminology

In SimR, the liver graft is simultaneously reperfused by the portal vein and the hepatic artery. Some scientists have recommended the use of SimR for its reduction of the risk of arterial ischemic damage to biliary epithelial cells, which are more susceptible to ischemia-reperfusion injury than hepatocytes.

### Peer-review

This is a well written paper analyzing a hot topic in liver transplantation technique and its outcomes. This work is a meta-analysis concerning the aspect of sequential vs simultaneous portal and arterial reperfusion in liver transplantation. The authors performed a structured literature review with a final analysis of six studies including 467 patients overall. As expected, in patients with simultaneous reperfusion a significantly longer warm ischemic time was found. In contrast, ischemic-type biliary lesions were significant reduced in the group of patients with simultaneous reperfusion. Graft failure and mortality were not different between both groups at one month and one year after liver transplantation.

## REFERENCES

- 1 **Gurusamy KS**, Naik P, Abu-Amara M, Fuller B, Davidson BR. Techniques of flushing and reperfusion for liver transplantation. *Cochrane Database Syst Rev* 2012; **3**: CD007512 [PMID: 22419324 DOI: 10.1002/14651858.CD007512.pub2]
- 2 **Manzini G**, Kremer M, Houben P, Gondan M, Bechstein WO, Becker T, Berlakovich GA, Friess H, Guba M, Hohenberger W, Ijzermans JN, Jonas S, Kalff JC, Klar E, Klempnauer J, Lerut J, Lippert H, Lorf T, Nadalin S, Nashan B, Otto G, Paul A, Pirenne J, Pratschke J, Ringers J, Rogiers X, Schilling MK, Seehofer D, Senninger N, Settmacher U, Stippel DL, Tscheliessnigg K, Ysebaert D, Binder H, Schemmer P. Reperfusion of liver graft during transplantation: techniques used in transplant centres within Eurotransplant and meta-analysis of the literature. *Transpl Int* 2013; **26**: 508-516 [PMID: 23517278 DOI: 10.1111/tri.12083]
- 3 **Polak WG**, Porte RJ. The sequence of revascularization in liver transplantation: it does make a difference. *Liver Transpl* 2006; **12**: 1566-1570 [PMID: 17058245 DOI: 10.1002/lt.20797]
- 4 **Sanchez-Perez B**, Aranda-Narvaez JM, Suarez-Munoz MA, Fernandez-Aguilar JL, Perez-Daga JA, Gonzalez-Sanchez AJ, Montiel-Casado C, Becerra-Ortiz RM, Pulido-Roa I, Santoyo J. Simultaneous (Arterial/Portal) or Sequential (Portal) Revascularization of the Liver

- Graft: Does It Make Any Difference? *Transpl Int* 2011; **17**: S236-S236
- 5 **Brockmann JG**, August C, Wolters HH, Hömme R, Palmes D, Baba H, Spiegel HU, Dietl KH. Sequence of reperfusion influences ischemia/reperfusion injury and primary graft function following porcine liver transplantation. *Liver Transpl* 2005; **11**: 1214-1222 [PMID: 16184569 DOI: 10.1002/lt.20480]
  - 6 **Puhl G**, Schaser KD, Pust D, Köhler K, Vollmar B, Menger MD, Neuhaus P, Settmacher U. The delay of rearterialization after initial portal reperfusion in living donor liver transplantation significantly determines the development of microvascular graft dysfunction. *J Hepatol* 2004; **41**: 299-306 [PMID: 15288480 DOI: 10.1016/j.jhep.2004.04.017]
  - 7 **Moreno C**, Sabaté A, Figueras J, Camprubí I, Dalmau A, Fabregat J, Koo M, Ramos E, Lladó L, Rafecas A. Hemodynamic profile and tissular oxygenation in orthotopic liver transplantation: Influence of hepatic artery or portal vein revascularization of the graft. *Liver Transpl* 2006; **12**: 1607-1614 [PMID: 16724337 DOI: 10.1002/lt.20794]
  - 8 **Cursio R**, Gugenheim J. Ischemia-Reperfusion Injury and Ischemic-Type Biliary Lesions following Liver Transplantation. *J Transplant* 2012; **2012**: 164329 [PMID: 22530107 DOI: 10.1155/2012/164329]
  - 9 **Ayanoglu HO**, Ulukaya S, Tokat Y. Causes of postreperfusion syndrome in living or cadaveric donor liver transplantations. *Transplant Proc* 2003; **35**: 1442-1444 [PMID: 12826185 DOI: 10.1016/S0041-1345(03)00483-4]
  - 10 **Guichelaar MM**, Benson JT, Malinchoc M, Krom RA, Wiesner RH, Charlton MR. Risk factors for and clinical course of non-anastomotic biliary strictures after liver transplantation. *Am J Transplant* 2003; **3**: 885-890 [PMID: 12814481 DOI: 10.1034/j.1600-6143.2003.00165.x]
  - 11 **Moher D**, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010; **8**: 336-341 [PMID: 20171303 DOI: 10.1016/j.ijsu.2010.02.007]
  - 12 **Sankary HN**, McChesney L, Frye E, Cohn S, Foster P, Williams J. A simple modification in operative technique can reduce the incidence of nonanastomotic biliary strictures after orthotopic liver transplantation. *Hepatology* 1995; **21**: 63-69 [PMID: 7806170 DOI: 10.1002/hep.1840210112]
  - 13 **Massarollo PC**, Mies S, Raia S. Simultaneous arterial and portal revascularization in liver transplantation. *Transplant Proc* 1998; **30**: 2883-2884 [PMID: 9745610 DOI: 10.1016/S0041-1345(98)00854-9]
  - 14 **Polak WG**, Miyamoto S, Nemes BA, Peeters PM, de Jong KP, Porte RJ, Slooff MJ. Sequential and simultaneous revascularization in adult orthotopic piggyback liver transplantation. *Liver Transpl* 2005; **11**: 934-940 [PMID: 16035059 DOI: 10.1002/lt.20513]
  - 15 **Wu D**, Zheng S, Dong J, Wang S, Bie P, Yang Z. Comparative study of two intraoperative reperfusion modes affecting liver function at the early stage after orthotopic liver transplantation. *J Digest Surg* 2006; **3**: 175-178
  - 16 **Adani GL**, Rossetto A, Bresadola V, Lorenzin D, Baccarani U, De Anna D. Contemporaneous Portal-Arterial Reperfusion during Liver Transplantation: Preliminary Results. *J Transplant* 2011; **2011**: 251656 [PMID: 21559253 DOI: 10.1155/2011/251656]
  - 17 **Baccarani U**, Rossetto A, Lorenzin D, Bidinost S, Lugano M, Bresadola V, De Anna D, Della Rocca G, Adani GL. Protection of the Intrahepatic Biliary Tree by Contemporaneous Portal and Arterial Reperfusion during Liver Transplantation: Results of a Prospective Randomized Trial. *Liver Transpl* 2012; **18**: S83-S83
  - 18 **Walsh TS**, Garden OJ, Lee A. Metabolic, cardiovascular, and acid-base status after hepatic artery or portal vein reperfusion during orthotopic liver transplantation. *Liver Transpl* 2002; **8**: 537-544 [PMID: 12037785 DOI: 10.1053/jlts.2002.33481]
  - 19 **Sadler KM**, Walsh TS, Garden OJ, Lee A. Comparison of hepatic artery and portal vein reperfusion during orthotopic liver transplantation. *Transplantation* 2001; **72**: 1680-1684 [PMID: 11726832 DOI: 10.1097/00007890-200111270-00019]
  - 20 **Adani GL**, Rossetto A, Lorenzin D, Lugano M, De Anna D, Della Rocca G, Donini A, Bresadola V, Risaliti A, Baccarani U. Sequential versus contemporaneous portal and arterial reperfusion during liver transplantation. *Transplant Proc* 2011; **43**: 1107-1109 [PMID: 21620064 DOI: 10.1016/j.transproceed.2011.01.123]
  - 21 **Stahl JE**, Kreke JE, Malek FA, Schaefer AJ, Vacanti J. Consequences of cold-ischemia time on primary nonfunction and patient and graft survival in liver transplantation: a meta-analysis. *PLoS One* 2008; **3**: e2468 [PMID: 18575623 DOI: 10.1371/journal.pone.0002468]
  - 22 **Buis CI**, Verdonk RC, Van der Jagt EJ, van der Hilst CS, Slooff MJ, Haagsma EB, Porte RJ. Nonanastomotic biliary strictures after liver transplantation, part 1: Radiological features and risk factors for early vs. late presentation. *Liver Transpl* 2007; **13**: 708-718 [PMID: 17457932 DOI: 10.1002/lt.21166]
  - 23 **Verdonk RC**, Buis CI, van der Jagt EJ, Gouw AS, Limburg AJ, Slooff MJ, Kleibeuker JH, Porte RJ, Haagsma EB. Nonanastomotic biliary strictures after liver transplantation, part 2: Management, outcome, and risk factors for disease progression. *Liver Transpl* 2007; **13**: 725-732 [PMID: 17457935 DOI: 10.1002/lt.21165]
  - 24 **Heidenhain C**, Pratschke J, Puhl G, Neumann U, Pascher A, Veltzke-Schlieker W, Neuhaus P. Incidence of and risk factors for ischemic-type biliary lesions following orthotopic liver transplantation. *Transpl Int* 2010; **23**: 14-22 [PMID: 19691661 DOI: 10.1111/j.1432-2277.2009.00947.x]
  - 25 **Wang B**, Zhang Q, Zhu B, Cui Z, Zhou J. Protective effect of gadolinium chloride on early warm ischemia/reperfusion injury in rat bile duct during liver transplantation. *PLoS One* 2013; **8**: e52743 [PMID: 23341905 DOI: 10.1371/journal.pone.0052743]
  - 26 **Esmat MEE**, Buckels JA, Mirza DF, Mayer DA, Gunson BG, Yen CY, Hegab BH, Bramhall SR. Biliary complications after orthotopic liver transplantation; Ten years experience. *Transpl Int* 2007; **20**: 258-259
  - 27 **Gastaca M**, Matarraz A, Muñoz F, Valdivieso A, Aguinaga A, Testillano M, Bustamante J, Terreros I, Suarez MJ, Montejo M, Ortiz de Urbina J. Biliary complications in orthotopic liver transplantation using choledochcholedochostomy with a T-tube. *Transplant Proc* 2012; **44**: 1554-1556 [PMID: 22841211 DOI: 10.1016/j.transproceed.2012.05.008]
  - 28 **Vellar ID**. Preliminary study of the anatomy of the venous drainage of the intrahepatic and extrahepatic bile ducts and its relevance to the practice of hepatobiliary surgery. *ANZ J Surg* 2001; **71**: 418-422 [PMID: 11450918 DOI: 10.1046/j.1440-1622.2001.02150.x]
  - 29 **Baccarani U**, Isola M, Adani GL, Avellini C, Lorenzin D, Rossetto A, Currò G, Comuzzi C, Toniutto P, Risaliti A, Soldano F, Bresadola V, De Anna D, Bresadola F. Steatosis of the hepatic graft as a risk factor for post-transplant biliary complications. *Clin Transplant* 2010; **24**: 631-635 [PMID: 19878512 DOI: 10.1111/j.1399-0012.2009.01128.x]
  - 30 **Adani GL**, Lorenzin D, Avellini C, Isola M, Rossetto A, Toniutto P, Bresadola V, Risaliti A, Baccarani U. Steatosis of the Hepatic Graft as a Risk Factor for Post-Transplant Biliary Complications. *Liver Transpl* 2009; **15**: S156-S156
  - 31 **Post S**, Palma P, Gonzalez AP, Rentsch M, Menger MD. Timing of arterialization in liver transplantation. *Ann Surg* 1994; **220**: 691-698 [PMID: 7979619 DOI: 10.1097/00000658-199411000-00014]
  - 32 **Reck T**, Steinbauer F, Steinbauer M, Schwille PO, Wittekind C, Hohenberger W, Köckerling F. Impact of arterialization on hepatic oxygen supply, tissue energy phosphates, and outcome after liver transplantation in the rat. *Transplantation* 1996; **62**: 582-587 [PMID: 8830819 DOI: 10.1097/00007890-199609150-00007]
  - 33 **Wu J**, Ye S, Xu X, Xie H, Zhou L, Zheng S. Recipient outcomes after ABO-incompatible liver transplantation: a systematic review and meta-analysis. *PLoS One* 2011; **6**: e16521 [PMID: 21283553 DOI: 10.1371/journal.pone.0016521]
  - 34 **Ten Hove WR**, Korkmaz KS, op den Dries S, de Rooij BJ, van Hoek B, Porte RJ, van der Reijden JJ, Coenraad MJ, Dubbeld J, Hommes DW, Verspaget HW. Matrix metalloproteinase 2 genotype is associated with nonanastomotic biliary strictures after orthotopic liver transplantation. *Liver Int* 2011; **31**: 1110-1117 [PMID: 21745270 DOI: 10.1111/j.1478-3231.2011.02459.x]
  - 35 **Tseng TY**, Dahm P, Poolman RW, Preminger GM, Canales BJ,

- Montori VM. How to use a systematic literature review and meta-analysis. *J Urol* 2008; **180**: 1249-1256 [PMID: 18707741 DOI: 10.1016/j.juro.2008.06.046]
- 36 **Bhandari M**, Devereaux PJ, Montori V, Cinà C, Tandan V, Guyatt GH. Users' guide to the surgical literature: how to use a systematic literature review and meta-analysis. *Can J Surg* 2004; **47**: 60-67 [PMID: 14997929]
- 37 **Moher D**, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomised controlled trials: the QUOROM statement. QUOROM Group. *Br J Surg* 2000; **87**: 1448-1454 [PMID: 11091231 DOI: 10.1046/j.1365-2168.2000.01610.x]

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## Laparoscopic fenestration of pancreatic serous cystadenoma: Minimally invasive approach for symptomatic benign disease

Safi Dokmak, Béatrice Aussilhou, Fanjandrairy Rasoaherinomenjanahary, Alain Sauvanet,  
Marie-Pierre Vullierme, Vinciane Rebours, Philippe Lévy

Safi Dokmak, Béatrice Aussilhou, Fanjandrairy Rasoaherinomenjanahary, Alain Sauvanet, Department of HPB Surgery and Liver Transplantation, Beaujon Hospital, 92210 Clichy, France

Marie-Pierre Vullierme, Department of Radiology, Beaujon Hospital, 92210 Clichy, France

Vinciane Rebours, Philippe Lévy, Department of Gastroenterology, Beaujon Hospital, 92210 Clichy, France

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**Correspondence to:** Safi Dokmak, MD, Department of HPB Surgery and Liver Transplantation, Beaujon Hospital, 92210 Clichy, France. [safi.dokmak@bjn.aphp.fr](mailto:safi.dokmak@bjn.aphp.fr)  
Telephone: +33-1-40875795  
Fax: +33-1-40870926

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### Abstract

Serous cystadenoma (SC) is a benign pancreatic cystic tumor. Surgical resection is recommended for symptomatic forms, but laparoscopic fenestration of large symptomatic macrocystic SC was not yet described in the literature. In this study, 3 female patients underwent laparoscopic fenestration for macrocystic SC (12-14 cm). Diagnosis was established *via* magnetic resonance imaging and endoscopic ultrasound, with intra-cystic dosage of tumors markers (ACE and CA19-9) in 2 patients. All patients were symptomatic and operated on 15-60 mo after diagnosis. Radiological evaluation showed constant cyst growth. Patients were informed about this new surgical modality that can avoid pancreatic resection. The mean operative time was 103 min (70-150 min) with one conversion. The post-operative course was marked by a grade A pancreatic fistula in one patient and was uneventful in the other two. The hospital stay was 3, 10, and 18 d, respectively. The diagnosis of macrocystic SC was histologically-confirmed in all cases. At the last follow-up (13-26 mo), all patients were symptom-free, and radiological evaluation showed complete disappearance of the cyst. Laparoscopic fenestration, as opposed to resection, should be considered for large symptomatic macrocystic SC, thereby avoiding pancreatic resection morbidity and mortality.

**Key words:** Pancreatic serous cystadenoma; Laparoscopic fenestration; Symptomatic

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**Core tip:** Although surgical resection is the classical modality for treating symptomatic serous cystadenoma (SC), laparoscopic fenestration for large macrocystic SC was not yet described in the literature. In this study, 3 female patients underwent laparoscopic fenestration of symptomatic macrocystic SC. Conversion was needed in one patient for bile duct injury, while another patient developed a grade A pancreatic fistula. Histology confirmed the diagnosis in all patients and, after a follow-up of 13-26 mo, all patients are asymptomatic. Radiological evaluation showed complete disappearance of SC. This mini-invasive approach avoids the high mortality and morbidity encountered with pancreatic resection.

Dokmak S, Aussilhou B, Rasoaherinomenjanahary F, Sauvanet A, Vullierme MP, Rebours V, Lévy P. Laparoscopic fenestration of pancreatic serous cystadenoma: Minimally invasive approach for symptomatic benign disease. *World J Gastroenterol* 2015; 21(22): 7047-7051 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/7047.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.7047>

## INTRODUCTION

With the widespread use of cross-sectional imaging studies, pancreatic cysts are more frequently discovered, and a 2.6%-20% prevalence has been reported in patients undergoing computed tomography (CT) scans or magnetic resonance imaging (MRI) for non-related pancreatic disorders<sup>[1]</sup>. These lesions are represented mainly by intraductal papillary mucinous neoplasia (IPMN), mucinous cystadenoma (MC), and serous cystadenoma (SC)<sup>[2]</sup>. SC is mainly observed in females with a mean age of 60 years. Overall tumor size ranges from less than 1 cm to more than 10 cm. The diameter of cystic components inside the tumor may also vary from a few micrometers to several centimeters<sup>[3,4]</sup>, and can be unilocular or pseudo-solid<sup>[4,5]</sup>. The diagnosis of SC can be accurately established on a CT scan<sup>[6]</sup>, MRI<sup>[7]</sup>, and coupled, if needed, with endoscopic ultrasound plus fine needle aspiration and intra-cystic dosage of tumors markers<sup>[8-11]</sup>. SC is a benign pancreatic lesion with a slow rate of size increase, a very low risk of complications, and malignant evolution. When the diagnosis is certain, no treatment is required in asymptomatic patients<sup>[12]</sup>. Although the main indications of surgical resection are symptomatic SC, there are doubtful cases that cannot be differentiated from malignant or low potential malignant diseases<sup>[13-15]</sup>. Many cases are operated because they are falsely considered to be symptomatic (*i.e.*, non-specific abdominal pain and irritable bowel syndrome) or are doubtful cases (neuro-endocrine tumor, MC, and IPMN) after an incomplete diagnostic evaluation. With the increased prevalence of this disease and unclear data about management, recent

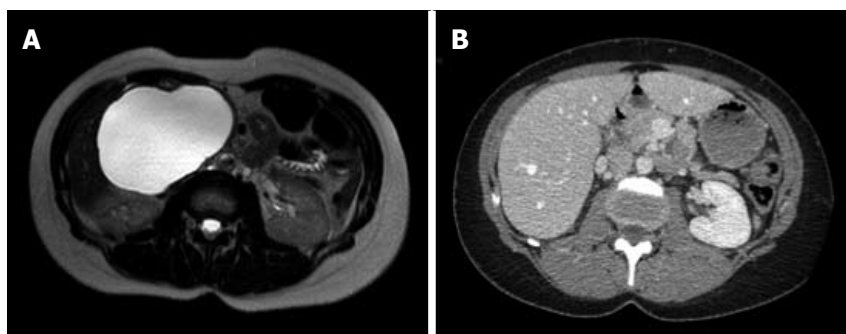
studies have focused mainly on the long-term risk of growth in order to select the subgroup of patients who might benefit from surgical resection<sup>[16]</sup>. Symptomatic or misdiagnosed lesions are usually treated by open or laparoscopic pancreatic resection, with the inherent risk of morbidity, mortality, and long-term pancreatic insufficiency related to these procedures<sup>[17]</sup>. The aim of this short series is to show that some symptomatic SC can also be treated by a much more conservative surgical approach consisting only of cyst fenestration, without any pancreatic resection. We describe 3 cases of large symptomatic SC of the pancreatic head treated by laparoscopic fenestration.

## CASE REPORT

Between September 2012 and June 2013, three female patients (33-66 years old) were operated for large symptomatic SC in the pancreatic head. SC had been diagnosed 15, 40, and 60 mo before surgery. The main symptoms were pain and fullness in the right subcostal area ( $n = 3$ ), palpable mass ( $n = 3$ ), signs of gastric outlet obstruction ( $n = 1$ ), and moderate cholestasis without jaundice ( $n = 1$ ). All patients underwent MRI, with the aspect of the cyst being typical in 2. Endoscopic ultrasound with intra-cystic ACE level measurement was performed in 2 patients, and showed 2 and 0.2  $\mu\text{g/L}$ , respectively. One patient had multiple SC, and the diagnosis of Von Hippel Lindau disease was ruled out by genetic screening. Cyst growth was observed in all patients (Figures 1, 2 and 3), and the size increased from 4, 9, and 10 cm at diagnosis to 12, 13, and 14 cm just before surgery, respectively.

### Surgical technique

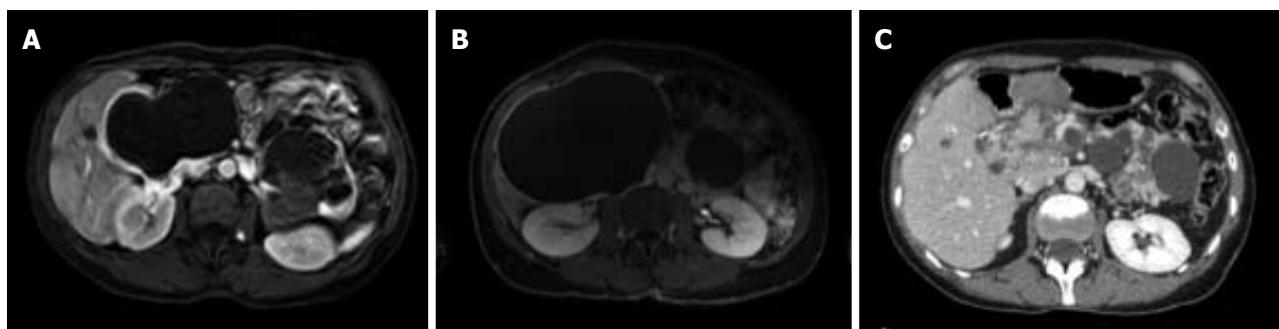
The patient was installed in the supine position under general anesthesia, with legs spread apart and the monitor to the patient's left. Open coelioscopy was performed through the umbilicus; a total of 4 trocars were necessary for this procedure. The trocar placement was done in order to avoid crossing the hands of the surgeon and assistant. The 30-degree optic trocar was installed in the right hypochondrium, the 10 mm operator trocar in the umbilicus, and another two 5 mm trocars in the left hypochondrium and right subcostal area for apprehension. Harmonic shears (Harmonic; Ethicon, Issy les Moulineaux, France) and a bipolar cautery coagulation device were needed. Once exploration was complete, the right gastrocolic ligament was largely divided in order to expose the anterior surface of the pancreatic gland and the area of the cyst to be opened. The cyst was freed from some collateral circulation that can be encountered related to venous compression. The cyst was opened, the content was aspirated, liquid sampling for tumors markers dosages was taken, and as large as possible (5-10 cm) fenestration was performed (Figure 4). The specimen was removed in a surgical bag by the trocar incision for



**Figure 1** Magnetic resonance imaging and post-operative computed tomography scan of patient 1. Magnetic resonance imaging shows a large serous cystadenoma in the pancreatic head measured at 13 cm (A), with post-operative computed tomography scan showing complete disappearance after laparoscopic fenestration (B).



**Figure 2** Computed tomography scan and post-operative magnetic resonance imaging of patient 2. Computed tomography scan showed a serous cystadenoma in the pancreatic head measured at 4 cm at diagnosis (A) and 12 cm (B) before surgery; post-operative magnetic resonance imaging showing complete cyst disappearance (C).



**Figure 3** Magnetic resonance imaging and post-operative computed tomography scan of patient 3. Magnetic resonance imaging showing multiple serous cystadenoma, with a large one in the pancreatic head measuring at 10 cm at diagnosis (A) and 14 cm before surgery (B); post-operative computed tomography scan showed complete disappearance (C).

pathological examination. A small suction drain was left in the cyst cavity.

The mean operative time was 103 min (70-150 min). Conversion was needed in one patient for bile duct injury, which was treated by end-to-end biliary anastomosis with a biliary drain. SC was fenestrated anteriorly behind the anterior aspect of the pancreatic head ( $n = 2$ ) and laterally posterior to the duodenum and hepatic pedicle in one patient. One patient needed pancreatectomy in order to have access to the cyst. In one patient, the great omentum was inserted in the cystic cavity.

The post-operative course was marked by a grade A

pancreatic fistula in the one patient who had undergone pancreatectomy, while the other two had an uneventful post-operative course. Hospital stay was 3, 10, and 18 d, respectively. The definitive diagnosis of macrocystic SC was confirmed on histology in all patients, who showed a glycogen-rich epithelium, sometimes with abrasion, without any mucinous secretion or ovarian stroma.

#### Long-term follow-up

At the last follow-up (13, 21, and 26 mo), all 3 patients were symptom-free, and radiological evaluation showed complete disappearance of the cyst (Figures 1, 2 and 3).

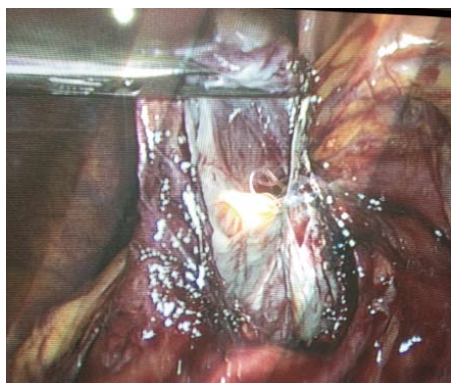


Figure 4 Intraoperative view, showing large fenestration of a large serous cystadenoma in the pancreatic head.

## DISCUSSION

To the best of our knowledge, laparoscopic fenestration of pancreatic SC was not yet described in the literature. Only one case using an open approach of fenestration indicated for jaundice was published, and had an excellent long-term result<sup>[18]</sup>.

This small series suggests that laparoscopic fenestration of large symptomatic SC is safe and effective. However, a larger series with longer post-operative follow-up is warranted to ascertain the fate of SC after such treatment.

Some cases of malignant transformation (< 1%) to lymph node invasion or liver metastases<sup>[19,20]</sup> had been described. However, this risk appears extremely low, if it exists at all, and has to be compared to the mortality risk of surgical pancreatic resection. Therefore, surgical treatment should be indicated only for symptoms clearly related to SC, and for cases remaining doubtful after a complete workup. Small SC without any local compression are less likely to be responsible for any symptoms. On the other hand, large SC might be symptomatic, and compression on the nearby structures might be observed either clinically (pain, abdominal mass, jaundice, or gastric outlet obstruction), radiologically (dilated main pancreatic or bile ducts) or biochemically (cholestasis). The impact of the observed symptoms on patient quality of life should be balanced against the risk of pancreatic surgical resection and its sequelae.

Some authors have described very large SC with local invasion of vessels, bile duct, stomach, and duodenum. In a recent study on 257 resected SC, it was shown that local invasion was mainly observed in large lesions (> 10 cm) located in the pancreatic head<sup>[3]</sup>. Two of our patients had gastric outlet obstruction and bile duct dilatation with mild cholestasis, respectively. Slow growth rate is observed in some SC. In a recent study on 145 patients who underwent MRI, it was shown that the growth rate of SC was 0.1 cm/year in the first 7 years, and 0.6 cm/year between 7 and 10 years from the baseline evaluation. This growth was mainly observed in oligocystic lesions, in patients with other

malignant history, and in those of an advanced age<sup>[4]</sup>. Of course, the progression in size should not be an indication for surgery *per se*. Regular radiological follow-up should be the main option in asymptomatic patients, regardless of the overall size of the SC.

Pancreatic resection is considered the only surgical modality for symptomatic lesions. However, pancreatic resection, even for benign disease, is still associated with very high morbidity and long-term endocrine and exocrine insufficiency risks<sup>[21]</sup>. This mortality, even reduced to its minimum rate, should be considered very poor for such a benign lesion. Even if the risk of pancreatic insufficiency is much lower after atypical pancreatic resections (enucleation or central pancreatectomy), the risk of surgical morbidity or mortality remains high. To avoid this morbidity, indications for surgical resection should be refined, and other treatment modalities should be discussed. In our opinion, the subgroup of patients with large symptomatic SC might safely benefit from cyst fenestration. A large cyst can modify the anatomy of the pancreatic head, and the area to be fenestrated should be selected on the pre-operative CT scan in order to avoid injury of surrounded structures. All our patients were operated by laparoscopic approach, but even the open approach can be considered for anatomical reasons. We should mention that fenestration with incomplete resection for SC is justified by the fact that the risk of malignant transformation is extremely low, and was probably not encountered with these SC due to their being purely cystic without any solid component. Histology of the cyst wall should be obtained to rule out malignancy.

Theoretically, there is a risk of recurrence, since a great part of the cyst and its epithelium are not resected or destroyed. This risk needs to be evaluated by a longer follow-up of our patients. In another paper, we described a case of calcic involution even after endoscopic ultrasound and complete aspiration of SC<sup>[22]</sup>.

In conclusion, laparoscopic fenestration of large pancreatic macrocystic SC appears to be safe and very effective. It can be added to the less aggressive surgical tools for symptomatic SC.

## COMMENTS

### Case characteristics

Three female patients presented with epigastric and right hypochondrium pain.

### Clinical diagnosis

An abdominal mass was noted on the clinical exam.

### Differential diagnosis

The differential diagnosis was mainly serous cystadenoma, mucinous cystadenoma, and pseudocyst.

### Laboratory diagnosis

Intracystic ACE level was low, which was suggestive of a diagnosis of serous cystadenoma.

### Imaging diagnosis

Magnetic resonance imaging showed large macrocystic lesions in the pancreatic head compatible with the diagnosis of serous cystadenoma. Multiple



cysts were present in one patient.

### Pathological diagnosis

After partial resection, the diagnosis of macrocystic serous cystadenoma was confirmed in all patients.

### Treatment

Laparoscopic fenestration was performed in all 3 patients.

### Related reports

This treatment was not yet described in the literature, and can be a method of avoiding classical treatment by pancreatic resection.

### Term explanation

Fenestration is incomplete resection, with only a part of the cyst being removed.

### Experiences and lessons

Laparoscopic fenestration, as opposed to resection, should be offered to any patient with symptomatic large macrocystic serous cystadenoma.

### Peer-review

This treatment can be proposed because the risk of malignancy is exceptional. Histology should be obtained during fenestration.

## REFERENCES

- Laffan TA, Horton KM, Klein AP, Berlanstein B, Siegelman SS, Kawamoto S, Johnson PT, Fishman EK, Hruban RH. Prevalence of unsuspected pancreatic cysts on MDCT. *AJR Am J Roentgenol* 2008; **191**: 802-807 [PMID: 18716113 DOI: 10.2214/AJR.07.3340]
- Kim SY, Lee JM, Kim SH, Shin KS, Kim YJ, An SK, Han CJ, Han JK, Choi BI. Macrocystic neoplasms of the pancreas: CT differentiation of serous oligocystic adenoma from mucinous cystadenoma and intraductal papillary mucinous tumor. *AJR Am J Roentgenol* 2006; **187**: 1192-1198 [PMID: 17056905 DOI: 10.2214/AJR.05.0337]
- Khashab MA, Shin EJ, Amateau S, Canto MI, Hruban RH, Fishman EK, Cameron JL, Edil BH, Wolfgang CL, Schulick RD, Giday S. Tumor size and location correlate with behavior of pancreatic serous cystic neoplasms. *Am J Gastroenterol* 2011; **106**: 1521-1526 [PMID: 21468008 DOI: 10.1038/ajg.2011.117]
- Malleo G, Bassi C, Rossini R, Manfredi R, Butturini G, Massignani M, Painsi M, Pederzoli P, Salvia R. Growth pattern of serous cystic neoplasms of the pancreas: observational study with long-term magnetic resonance surveillance and recommendations for treatment. *Gut* 2012; **61**: 746-751 [PMID: 21940725 DOI: 10.1136/gutjnl-2011-300297]
- Chatelain D, Hammel P, O'Toole D, Terris B, Vilgrain V, Palazzo L, Belghiti J, Lévy P, Ruszniewski P, Fléjou JF. Macrocystic form of serous pancreatic cystadenoma. *Am J Gastroenterol* 2002; **97**: 2566-2571 [PMID: 12385440 DOI: 10.1111/j.1572-0241.2002.06024.x]
- Lee JH, Kim JK, Kim TH, Park MS, Yu JS, Choi JY, Kim JH, Kim YB, Kim KW. MRI features of serous oligocystic adenoma of the pancreas: differentiation from mucinous cystic neoplasm of the pancreas. *Br J Radiol* 2012; **85**: 571-576 [PMID: 21304008 DOI: 10.1259/bjr/42007785]
- Lee SE, Kwon Y, Jang JY, Kim YH, Hwang DW, Kim MA, Kim SH, Kim SW. The morphological classification of a serous cystic tumor (SCT) of the pancreas and evaluation of the preoperative diagnostic accuracy of computed tomography. *Ann Surg Oncol* 2008; **15**: 2089-2095 [PMID: 18478300 DOI: 10.1245/s10434-008-9959-1]
- Hammel P, Levy P, Voitot H, Levy M, Vilgrain V, Zins M, Flejou JF, Molas G, Ruszniewski P, Bernades P. Preoperative cyst fluid analysis is useful for the differential diagnosis of cystic lesions of the pancreas. *Gastroenterology* 1995; **108**: 1230-1235 [PMID: 7535275 DOI: 10.1016/0016-5085(95)90224-4]
- O'Toole D, Palazzo L, Hammel P, Ben Yaghlene L, Couvelard A, Felce-Dachez M, Fabre M, Dancour A, Aubert A, Sauvanet A, Maire F, Lévy P, Ruszniewski P. Macrocystic pancreatic cystadenoma: The role of EUS and cyst fluid analysis in distinguishing mucinous and serous lesions. *Gastrointest Endosc* 2004; **59**: 823-829 [PMID: 15173795 DOI: 10.1016/S0016-5107(04)00346-3]
- Linder JD, Geenen JE, Catalano MF. Cyst fluid analysis obtained by EUS-guided FNA in the evaluation of discrete cystic neoplasms of the pancreas: a prospective single-center experience. *Gastrointest Endosc* 2006; **64**: 697-702 [PMID: 17055859 DOI: 10.1016/j.gie.2006.01.070]
- Allen PJ, Qin LX, Tang L, Klimstra D, Brennan MF, Lokshin A. Pancreatic cyst fluid protein expression profiling for discriminating between serous cystadenoma and intraductal papillary mucinous neoplasm. *Ann Surg* 2009; **250**: 754-760 [PMID: 19806054 DOI: 10.1097/SLA.0b013e3181bd7f20]
- Tseng JF, Warshaw AL, Sahani DV, Lauwers GY, Rattner DW, Fernandez-del Castillo C. Serous cystadenoma of the pancreas: tumor growth rates and recommendations for treatment. *Ann Surg* 2005; **242**: 413-419; discussion 419-421 [PMID: 16135927]
- Galanis C, Zamani A, Cameron JL, Campbell KA, Lillemoe KD, Caparrelli D, Chang D, Hruban RH, Yeo CJ. Resected serous cystic neoplasms of the pancreas: a review of 158 patients with recommendations for treatment. *J Gastrointest Surg* 2007; **11**: 820-826 [PMID: 17440789 DOI: 10.1007/s11605-007-0157-4]
- Kimura W, Moriya T, Hirai I, Hanada K, Abe H, Yanagisawa A, Fukushima N, Ohike N, Shimizu M, Hatori T, Fujita N, Maguchi H, Shimizu Y, Yamao K, Sasaki T, Naito Y, Tanno S, Tobita K, Tanaka M. Multicenter study of serous cystic neoplasm of the Japan pancreas society. *Pancreas* 2012; **41**: 380-387 [PMID: 22415666 DOI: 10.1097/MPA.0b013e31822a27db]
- Valsangkar NP, Morales-Oyarvide V, Thayer SP, Ferrone CR, Wargo JA, Warshaw AL, Fernández-del Castillo C. 851 resected cystic tumors of the pancreas: a 33-year experience at the Massachusetts General Hospital. *Surgery* 2012; **152**: S4-12 [PMID: 22770958 DOI: 10.1016/j.surg.2012.05.033]
- El-Hayek KM, Brown N, O'Rourke C, Falk G, Morris-Stiff G, Walsh RM. Rate of growth of pancreatic serous cystadenoma as an indication for resection. *Surgery* 2013; **154**: 794-800; discussion 800-802 [PMID: 24074417 DOI: 10.1016/j.surg.2013.07.005]
- Hwang HK, Kim H, Kang CM, Lee WJ. Serous cyst adenoma of the pancreas: appraisal of active surgical strategy before it causes problems. *Surg Endosc* 2012; **26**: 1560-1565 [PMID: 22179458 DOI: 10.1007/s00464-011-2070-x]
- Watanabe H, Ohtsubo K, Yamaguchi Y, Mouri H, Motoo Y, Noto M, Kitagawa H, Kayahara M, Ohta T, Gabata T, Sakamoto S, Sawabu N. Successful cystic fenestration for a macrocystic serous cystadenoma of the pancreas causing obstructive jaundice: report of a case. *Surg Today* 2006; **36**: 89-93 [PMID: 16378203 DOI: 10.1007/s00595-005-3079-3]
- King JC, Ng TT, White SC, Cortina G, Reber HA, Hines OJ. Pancreatic serous cystadenocarcinoma: a case report and review of the literature. *J Gastrointest Surg* 2009; **13**: 1864-1868 [PMID: 19459016 DOI: 10.1007/s11605-009-0926-3]
- Strobel O, Z'graggen K, Schmitz-Winnenthal FH, Friess H, Kappeler A, Zimmermann A, Uhl W, Büchler MW. Risk of malignancy in serous cystic neoplasms of the pancreas. *Digestion* 2003; **68**: 24-33 [PMID: 12949436 DOI: 10.1159/000073222]
- Falconi M, Mantovani W, Crippa S, Mascetta G, Salvia R, Pederzoli P. Pancreatic insufficiency after different resections for benign tumours. *Br J Surg* 2008; **95**: 85-91 [PMID: 18041022 DOI: 10.1002/bjs.5652]
- Charpignon C, Corcos O, Vullierme MP, Hammel P, Ruszniewski P, Lévy P. [Calcic involution of a serous cystadenoma]. *Gastroenterol Clin Biol* 2006; **30**: 923-924 [PMID: 16885885 DOI: 10.1016/S0399-8320(06)73348-6]

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## Diagnostic utility of endoscopic ultrasound-guided fine-needle aspiration biopsy for glomus tumor of the stomach

Shin Kato, Kaoru Kikuchi, Kenji Chinen, Takahiro Murakami, Fumihito Kunishima

Shin Kato, Kaoru Kikuchi, Kenji Chinen, Department of Gastroenterology, Okinawa Prefectural Chubu Hospital, Okinawa 904-2293, Japan

Takahiro Murakami, Department of Surgery, Okinawa Prefectural Chubu Hospital, Okinawa 904-2293, Japan

Fumihito Kunishima, Department of Pathology, Okinawa prefectural Chubu Hospital, Okinawa 904-2293, Japan

**Author contributions:** Kato S wrote the manuscript; Kato S, Chinen K and Murakami T managed the patient and performed the procedure; Kunishima F provided the figures and discussion of the pathology; Kikuchi K supervised the research; all authors approved the final manuscript for publication.

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**Informed consent:** The patient of this case provided informed consent prior to the procedure. The patient also approved to publish the case.

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**Correspondence to:** Shin Kato, MD, Department of Gastroenterology, Okinawa Prefectural Chubu Hospital, Miyazato 281, Uruma, Okinawa 904-2293, Japan. [shinchan1231@gmail.com](mailto:shinchan1231@gmail.com)  
 Telephone: +81-98-9734111  
 Fax: +81-98-9732703

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### Abstract

A 52-year-old man was referred for further investigation of a gastric submucosal tumor on the greater curvature of the antrum. Endoscopic ultrasonography demonstrated a hypoechoic solid mass, which was primarily connected to the muscular layer of the stomach. We performed endoscopic ultrasound-guided fine-needle aspiration biopsy. The pathological examination showed proliferation of oval-shaped cells with nest formation, which stained strongly positive for muscle actin, and negative for c-kit, CD34, CD56, desmin, S-100, chromogranin, and neuron-specific enolase. Therefore, we performed laparoscopy and endoscopy cooperative surgery based on the preoperative diagnosis of glomus tumor of the stomach. The final histological diagnosis confirmed the preoperative diagnosis. Although preoperative diagnosis of glomus tumor of the stomach is difficult with conventional images and endoscopic biopsy, endoscopic ultrasound-guided fine-needle aspiration biopsy is an essential tool to gain histological evidence of glomus tumor of the stomach for early diagnosis.

**Key words:** Glomus tumor; Endoscopic ultrasound-guided fine-needle aspiration biopsy; Stomach; Preoperative diagnosis

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**Core tip:** Preoperative diagnosis of glomus tumor of the stomach is difficult as its exhibits a similar clinical appearance on conventional images (computed tomography, magnetic resonance imaging, endoscopic ultrasonography) to other submucosal tumors of the stomach. Furthermore, pathological evidence for diagnosis is difficult to obtain by conventional endoscopic biopsy. Endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) is an essential and useful diagnostic tool for glomus tumor of the stomach to obtain pathological evidence including

immunohistochemical staining which is critically important to diagnose pathologically. There are only eight literatures of gastric glomus tumors which were diagnosed by FNA. This is a first report to review these literatures.

Kato S, Kikuchi K, Chinen K, Murakami T, Kunishima F. Diagnostic utility of endoscopic ultrasound-guided fine-needle aspiration biopsy for glomus tumor of the stomach. *World J Gastroenterol* 2015; 21(22): 7052-7058 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/7052.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.7052>

## INTRODUCTION

Glomus tumor is a rare benign neoplastic proliferation of modified smooth muscle cells arising from the neuroarterial structure called glomus body<sup>[1]</sup>. These tumors generally appear as skin lesions. Although glomus tumors are generally benign, several malignant cases have been reported<sup>[2-5]</sup>.

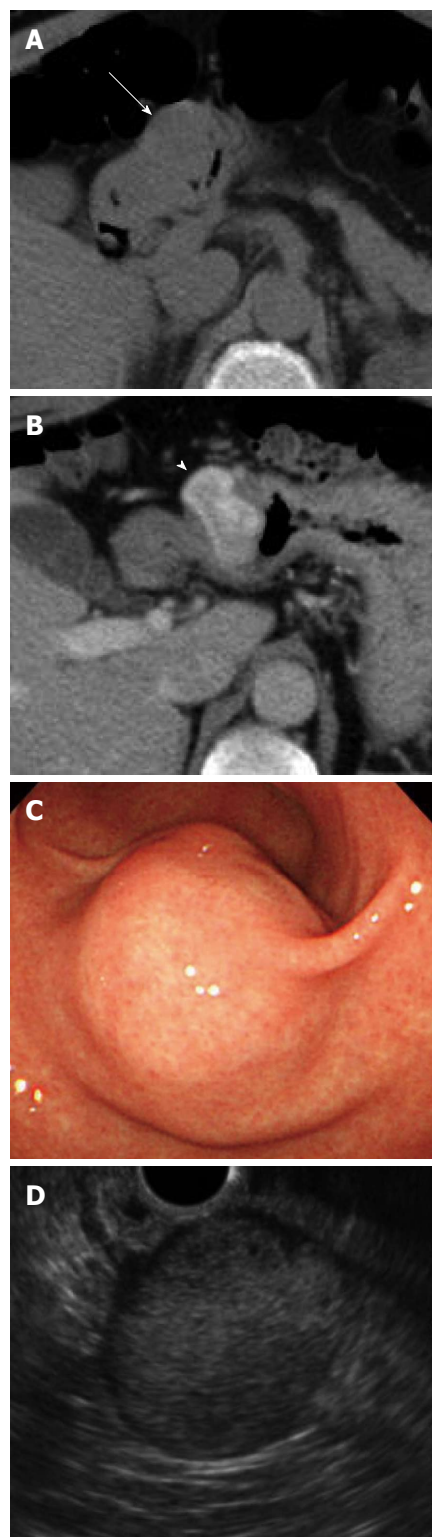
Preoperative diagnosis of glomus tumor of the stomach is difficult, since glomus tumors and other typical submucosal tumors of the stomach, such as gastrointestinal stromal tumors (GISTs) and leiomyomas, exhibit a similar clinical appearance on conventional images. Furthermore, pathological evidence for diagnosis is difficult to obtain by conventional endoscopic biopsy as these tumors originate from submucosal lesions. Therefore, almost all reported cases of glomus tumor of the stomach are diagnosed from resected specimens.

Herein, we report a case of glomus tumor of the stomach that was preoperatively diagnosed by endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) and resected.

## CASE REPORT

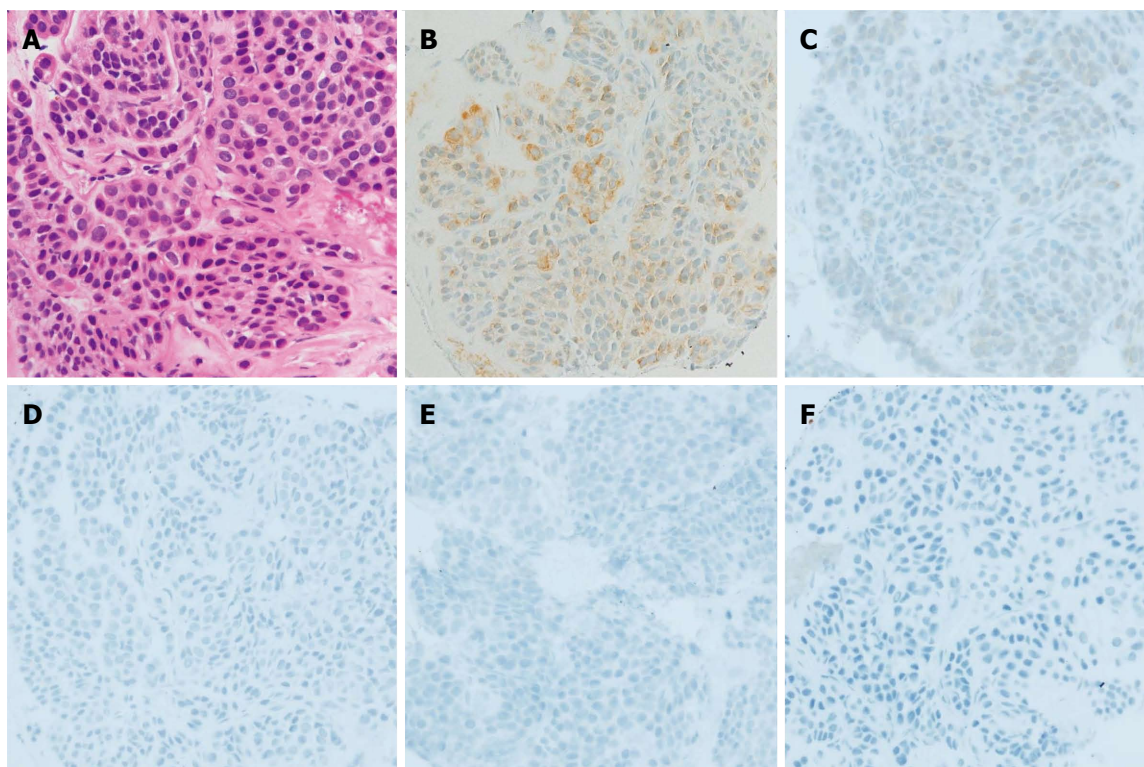
A 52-year-old asymptomatic Japanese male with a past medical history of polycystic kidney was referred to our hospital for further investigation of a gastric submucosal tumor (SMT) on the greater curvature of the antrum, which was detected at an annual health check. There was no significant finding on physical examination. Initial laboratory data were within the normal range including the tumor markers, carcinoembryonic antigen, and carbohydrate antigen 19-9.

Plain computed tomography (CT) showed an approximately 3.0 cm round shaped mass lesion on the gastric antrum. A contrast-enhanced CT identified an enhanced mass without cystic change or calcification (Figure 1A and B). Esophagogastroduodenoscopy (EGD) revealed a 30 mm SMT on the greater curvature of the antrum without cushion sign or dell (Figure 1C). Endoscopic ultrasonography (EUS) showed a

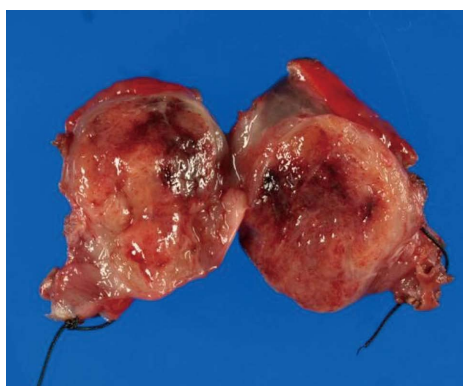


**Figure 1** Images of the submucosal mass on the greater curvature of the antrum. A: Plain computed tomography showed a round shaped mass lesion on the gastric antrum (arrow); B: The tumor showed mainly peripheral, not homogeneous, enhancement (arrowhead); C: Esophagogastroduodenoscopy revealed a submucosal tumor on the greater curvature of the antrum with bridging fold; D: Endoscopic ultrasonography showed that the tumor was located on the muscle layer.

hypoechoic lesion with a small anechoic component, which was primarily connected to the muscular layer



**Figure 2** Endoscopic ultrasound-guided fine-needle aspiration biopsy pathology. A: Proliferating oval-shaped cells in a small nest formation and a high nuclear cytoplasmic ratio were observed (hematoxylin and eosin stain,  $\times 400$  magnification). IHC staining was positive for B: muscle actin, and slightly positive for C: synaptophysin, but negative for D: chromogranin, E: c-kit, F: desmin ( $\times 400$ ).



**Figure 3** Gross description. The tumor was mainly homogeneous without necrosis or cystic change and measured 3.5 cm at the largest dimension.

of the stomach (Figure 1D).

We performed EUS-FNA (UCT-240; Olympus Medical Systems, Tokyo, Japan) using a 22-gauge needle (Echotip; Wilson-Cook, NC, United States) while taking care to avoid needle penetration and puncture of the anechoic component of the mass to prevent tumor seeding. The obtained specimen revealed the proliferation of oval-shaped cells with a small nest formation and high nucleo-cytoplasmic ratio (Figure 2).

Immunohistochemical (IHC) staining revealed that the tumor cells were strongly and focally positive for muscle actin, slightly positive for synaptophysin, and negative for c-kit, CD34, CD56, desmin, S-100,

chromogranin, and neuron-specific enolase (Figure 2).

The patients underwent laparoscopy and endoscopy cooperative surgery based on the preoperative diagnosis of glomus tumor.

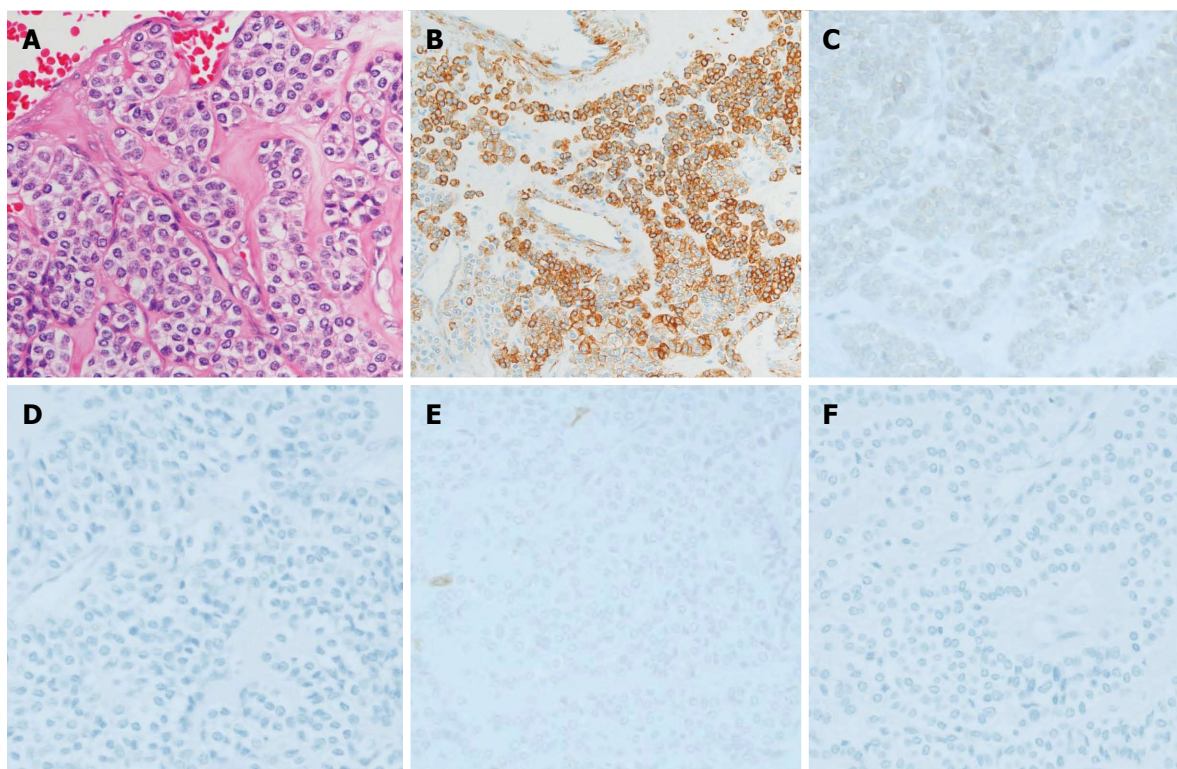
The surgical specimen showed a well-demarcated solid mass lesion located on the resected wall of the gastric antrum, measuring up to 3.5 cm in greatest diameter. The mass was homogeneous without necrosis or cystic change (Figure 3). Histologically, oval-shaped cell with a high nucleo-cytoplasmic ratio proliferated on the proper muscle layer forming small solid nests. IHC analysis was consistent with the EUS-FNA pathology results (Figure 4). Mitotic activity was absent. This patient did not receive any adjuvant therapy after surgery, as no evidence of malignancy was found in the resected specimen. Regular clinical follow up with EGD was performed, and the patient shows no signs of recurrence at 36 mo after surgery.

## DISCUSSION

Glomus tumor is generally a benign neoplasm. These tumors commonly appear under the fingernails and arise from the arterial portion of the glomus body<sup>[1]</sup>. Glomus tumors of the stomach, which were first reported by De Bussacher in 1948<sup>[6]</sup>, are extremely rare, accounting for 1% of the occurrence of GISTs<sup>[7]</sup>.

Based on the histological characteristics, glomus tumors are now considered mesenchymal tumors





**Figure 4** Histology and Immunohistochemical analysis of resected specimen was consistent with the endoscopic ultrasound-guided fine-needle aspiration biopsy pathology results. A: Hematoxylin and eosin stain ( $\times 400$ ); B: Muscle actin; C: Synaptophysin; D: Chromogranin; E: c-kit; F: Desmin ( $\times 400$ ).

with malignant potential. Although almost all glomus tumors are benign, some malignant cases have been reported<sup>[2-5]</sup>. Folpe *et al*<sup>[8]</sup> proposed the following criteria for malignant glomus tumor: deep location,  $\geq 2$  cm in size, atypical mitotic figures, moderate to high nuclear grade, and  $\geq 5$  mitotic figures/50 high-power fields. They found that metastasis was observed in 38% of glomus tumors fulfilling the criteria for malignancy. Therefore, complete resection based on the collect preoperative diagnosis is necessary. In our case, the EUS image showed that the tumor was located on the muscle layer and was over 2 cm in size. Although mitosis was not seen, the possibility of malignancy could not be ruled out in the preoperative diagnosis.

Preoperative diagnosis of glomus tumor of the stomach is difficult with conventional images, such as CT and magnetic resonance imaging (MRI). The typical CT image shows dense homogeneous enhancement in the arterial phase and continuous enhancement in the delayed phase<sup>[9-12]</sup>. These findings are key in distinguishing glomus tumors from other hypovascular submucosal lesions, such as leiomyomas, lipomas and ectopic pancreas. However, glomus tumors, GISTs, and neuro-endocrine tumors exhibit similar findings on CT, making differential diagnosis difficult. Moreover, some cases of glomus tumor of the stomach do not demonstrate the typical dense homogeneous and continuous enhancement pattern. In our case, the tumor showed mainly peripheral, not homogeneous,

enhancement. Therefore, no conventional images, including CT and MRI, have the ability to replace histological diagnosis.

On EUS, gastric glomus tumors appear as circumscribed low echoic masses in the third and/or fourth layer. The mass components are mostly homogeneous, but sometimes described as heterogeneous echo mixed with high echo spot<sup>[13]</sup>. These findings are similar to those of GISTs or other gastrointestinal mesenchymal tumors<sup>[14-16]</sup>. Thus, it is rather difficult to distinguish glomus tumors from other mesenchymal tumors by EUS images.

EUS-FNA is an effective method to obtain pathological specimens of gastric submucosal neoplasms. Mekky *et al*<sup>[17]</sup> reported that adequate specimens were obtained in 83% of gastric submucosal neoplasm cases by EUS-FNA. Furthermore, the diagnostic accuracy rate was 95.6%. In cases of small SMT, it is rather difficult to obtain specimens by EUS-FNA, and the procedure carries the risks of needle penetration and malignant cells seeding. However, Akahoshi *et al*<sup>[18]</sup> reported the safety of EUS-FNA for gastric SMTs smaller than 2 cm after performing EUS-FNA in 90 cases without complication. EUS-FNA for small SMT ( $< 2.0$  cm) should be performed carefully to prevent needle penetration and seeding, especially in cases exhibiting some malignant characteristics (*e.g.*, necrotic change in the tumor, and rapid growth).

Pathological diagnosis of glomus tumor of the stomach using only hematoxylin and eosin (HE)



Table 1 Cases of gastric glomus tumor with preoperative fine-needle aspiration biopsy

	Vinette-Leduc <i>et al.</i> <sup>[19]</sup>	Gu <i>et al.</i> <sup>[16]</sup>	Debol <i>et al.</i> <sup>[15]</sup>	Jones <i>et al.</i> <sup>[20]</sup>	Minoda <i>et al.</i> <sup>[21]</sup>	Mohanty <i>et al.</i> <sup>[22]</sup>	Matevosian <i>et al.</i> <sup>[23]</sup>	Alahosh <i>et al.</i> <sup>[18]</sup>	Our case
Age and sex	72, female	32, female	62, female	47, female	50, female	51, male	44	N.D.	52, male
Tumor location	Antrum	Body (LC)	Antrum	ND	Angle	Antrum	Antrum	Body	Antrum
size (cm)	2	2.3 × 1.6	2.8 × 2.5 × 1.7	2.5 × 1.5	1.5	2.4 × 2.0	5	1.2	3
Enhanced CT	ND	ND	ND	ND	ND	ND	ND	N.D.	Enhanced in peripheral lesion, not homogeneously.
EUS	ND	Irregular shaped heterogeneous tumor arising from muscularis propria	Hypochoic mass arise from the muscularis propria	Heterogeneous rounded lesion arising from muscularis propria	Homogeneous, hypochoic tumor with continuity to the muscle layer	Hypochoic submucosal lesion	Poorly reflective, non-homogeneous submucosal, solid tumor.	N.D.	Hypochoic mass primarily connected to muscular layer
Diagnosis by images	ND	GIST	ND	GIST	GIMT including GIST	ND	GIST	N.D.	GIMT including GIST
FNA-procedure	Percutaneous FNA	EUS-FNA	EUS-FNA	EUS-FNA	EUS-FNA (25-gauge needle)	EUS-FNA	EUS-FNA (19-gauge needle)	EUS-FNA (22 or 25-gauge)	EUS-FNA (22-gauge needle)
FNA cytology (HE)	Well-demarcated nests of small, round to polygonal cells.	Small, uniform, round, epithelioid cells with round nuclei and scanty, amphophilic cytoplasm	Well differentiated small blue cell neoplasm like carcinoid tumor.	Epithelioid tumor cells.	Proliferation of oval shaped cells with eosinophilic cytoplasm arrange in nests.	Uniform round cells with ill-defined cytoplasmic borders and scanty amphophilic cytoplasm.	Hemorrhagic biopsy sample without representative cells.	N.D.	proliferation of oval-shaped cell with small nest formation.
FNA cytology (IHC staining)	Not performed.	c-kit, CD34, desmin, chromogranin, synaptophysin, desmin (-), SMA, vimentin (+)	CD34, synaptophysin, chromogranin, desmin, s-100, Desmin, CD117 (-), SMA, vimentin (+)	CD34, 56, c-kit, desmin, S-100, chromogranin, synaptophysin, SMA (+)	CD34, 56, c-kit, desmin, S-100, chromogranin, synaptophysin, SMA, vimentin (+)	CD34, 117, c-kit, desmin, chromogranin, synaptophysin, Pancytokeratin (-), SMA, vimentin (+)	Not performed	N.D.	CD34, 56, c-kit, desmin, S-100, chromogranin(-), synaptophysin (±), SMA (+)
Preoperative diagnosis	Neuroendocrine tumor	Glomus tumor	Glomus tumor	Glomus tumor	Glomus tumor	Glomus tumor	GIST	Glomus tumor	Glomus tumor
Pathology in resected specimen (HE)	Highly vascular, tumor nests were separated by fascicles of smooth muscle. Uniform, small and round tumor cells.	Non encapsulated, with convoluted boundaries, confined to muscularis propria. Round and uniform tumor cells	Circumscribed, highly vascular, and contained nests of monomorphic, polygonal cells.	Confirmed the preoperative diagnosis of a glomus tumor.	Same as FNA pathology.	The pathological diagnosis was confirmed on resection	ND	N.D.	Oval shaped cells with high N/C ratio proliferated on proper muscle layer performing solid small nests.
IHC staining in resected specimen	Desmin, chromogranin (-), SMA, Vimentin (+)	Similar to those performed FNA	CD34, CD117, chromogranin (-), SMA, Vimentin (+)	ND	Same as FNA staining.	The pathological diagnosis was confirmed on resection	CD117 (-), Vimentin/actin (+)	N.D.	Same as FNA staining

ND: No description; GIMT: Gastrointestinal mesenchymal tumor; HE: Hematoxylin-eosin; IHC: Immunohistochemical; LC: Lesser curvature.

staining is difficult because of the similarities between glomus tumors and neuroendocrine tumors. EUS-FNA allows for the collection of sufficient specimen not only for HE staining, but also for IHC analysis of SMT lesions. Therefore, it is now considered an essential tool for the preoperative diagnosis of glomus tumor of the stomach.

In the eight reported cases of gastric glomus tumor, FNA (EUS-FNA in seven cases, percutaneous FNA in one case) was performed preoperatively for pathological diagnosis<sup>[15,16,18-23]</sup>. Table 1 shows the clinical characteristics of these eight cases as well as our case. In seven cases a correct preoperative diagnosis was achieved from EUS-FNA specimens, whereas two cases were misdiagnosed as a neuroendocrine tumor or GIST. In the misdiagnosed cases, FNA specimens were not subjected to IHC analysis<sup>[19,23]</sup>. All the cases that performed IHC analysis were able to achieve correct preoperative diagnosis. It is important to perform IHC analysis to ensure an accurate preoperative diagnosis. No case was able to attain a preoperative diagnosis using only conventional images (CT, EUS). The IHC analysis of our case revealed positivity for both muscle actin and synaptophysin. Synaptophysin positivity is occasionally found in specimens from glomus tumors of the stomach, whereas other neuroendocrine markers, including chromogranin A, are generally negative<sup>[24]</sup>. Therefore, we consider these IHC results to be consistent with a glomus tumor.

It remains controversial whether a 22G or 25G needle can adequately obtain a specimen from SMT lesions. Although we selected a 22G needle in this case, 25G needles were used to obtain sufficient specimens in other reported cases. Further analysis regarding needle gauge selection is expected to resolve this issue.

EUS-FNA is an essential and useful tool for the preoperative diagnosis of glomus tumor of the stomach. Preoperative diagnosis by EUS-FNA allows for early and minimal resection.

## COMMENTS

### Case characteristics

A 52-year-old man was referred for further investigation of a gastric submucosal tumor on the greater curvature of the antrum without particular symptoms.

### Clinical diagnosis

There was no significant finding on physical examination which led to the clinical diagnosis.

### Differential diagnosis

Gastrointestinal mesenchymal tumor, such as gastrointestinal stromal tumors, leiomyoma.

### Laboratory diagnosis

This patient had no remarkable findings for the laboratory tests including tumor markers.

### Imaging diagnosis

Plain computed tomography showed a round shaped mass lesion on the gastric antrum. Esophagogastroduodenoscopy revealed a 30 mm submucosal tumor without cushion sign on the antrum. Endoscopic ultrasonography (EUS) showed a hypoechoic lesion with a small anechoic component, which was primarily

connected to the muscular layer of the stomach.

### Pathological diagnosis

The specimen obtained by endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) revealed the proliferation of oval-shaped cells with a small nest formation and high nucleo-cytoplasmic ratio. Immunohistochemical staining revealed that the tumor cells were strongly and focally positive for muscle actin, and negative for c-kit, CD34, CD56, desmin, S-100, chromogranin, and neuron-specific enolase. These results were compatible with glomus tumor.

### Treatment

The patients underwent laparoscopy and endoscopy cooperative surgery based on the preoperative diagnosis of glomus tumor of the stomach.

### Related reports

In the only eight reported cases of gastric glomus tumor, FNA was performed preoperatively for pathological diagnosis. Six cases of them were diagnosed correctly by immunohistochemical staining. In the two misdiagnosed cases, FNA specimens were not subjected to immunohistochemical analysis.

### Term explanation

Glomus tumor is now considered mesenchymal tumors with malignant potential. Glomus tumors of the stomach are extremely rare, accounting for 1% of the occurrence of GIMTs.

### Experiences and lessons

Preoperative pathological diagnosis of glomus tumor of the stomach is difficult with conventional endoscopic biopsy. Therefore, EUS-FNA is an essential tool to gain histological evidence of glomus tumor of the stomach. It allows for the collection of sufficient specimen not only for HE staining, but also for immunohistochemical analysis which is necessary for correct diagnosis of glomus tumor.

### Peer-review

The authors have described a case of glomus tumor of the stomach which was correctly preoperatively diagnosed by EUS-FNA biopsy. The authors reviewed eight former reports of gastric glomus tumor and suggested the utility of EUS-FNA for diagnosis. The article provided a quite useful method for early preoperative diagnosis of glomus tumor of the stomach.

## REFERENCES

- 1 **Enzinger F**, Weiss S. Soft Tissue Tumors. 3rd ed. St Louis: Mosby, 1995
- 2 **Aiba M**, Hirayama A, Kuramochi S. Glomangiosarcoma in a glomus tumor. An immunohistochemical and ultrastructural study. *Cancer* 1988; **61**: 1467-1471 [PMID: 2449949 DOI: 10.1002/1097-0142(19880401)61]
- 3 **Brathwaite CD**, Poppiti RJ. Malignant glomus tumor. A case report of widespread metastases in a patient with multiple glomus body hamartomas. *Am J Surg Pathol* 1996; **20**: 233-238 [PMID: 8554113 DOI: 10.1097/00000478-199602000-00012]
- 4 **Gould EW**, Manivel JC, Albores-Saavedra J, Monforte H. Locally infiltrative glomus tumors and glomangiosarcomas. A clinical, ultrastructural, and immunohistochemical study. *Cancer* 1990; **65**: 310-318 [PMID: 2153045 DOI: 10.1002/1097-0142(19900115)65]
- 5 **Hiruta N**, Kameda N, Tokudome T, Tsuchiya K, Nonaka H, Hatori T, Akima M, Miura M. Malignant glomus tumor: a case report and review of the literature. *Am J Surg Pathol* 1997; **21**: 1096-1103 [PMID: 9298887 DOI: 10.1097/00000478-199709000-00015]
- 6 **DeBusscher G**. Etude morphologique et consideration physiologique sur la vascularization de l'estomac. *Acta Gastroenterol Belg* 1948; **11**: 333-351
- 7 **Miettinen M**, Paal E, Lasota J, Sobin LH. Gastrointestinal glomus tumors: a clinicopathologic, immunohistochemical, and molecular genetic study of 32 cases. *Am J Surg Pathol* 2002; **26**: 301-311 [PMID: 11859201 DOI: 10.1097/00000478]
- 8 **Folpe AL**, Fanburg-Smith JC, Miettinen M, Weiss SW. Atypical and malignant glomus tumors: analysis of 52 cases, with a proposal for the reclassification of glomus tumors. *Am J Surg Pathol* 2001; **25**: 1-12 [PMID: 11145243 DOI: 10.1097/00000478]
- 9 **Cha SH**, Cho SB, Kim YW, Park CM. Helical CT appearance of glomus tumor of the stomach. *Eur Radiol* 2000; **10**: 671-673

- [PMID: 10795552 DOI: 10.1007/s003300050981]
- 10 **Kim JK**, Won JH, Cho YK, Kim MW, Joo HJ, Suh JH. Glomus tumor of the stomach: CT findings. *Abdom Imaging* 2011; **26**: 303-305 [PMID: 11429959 DOI: 10.1007/s002610000209]
  - 11 **Tang M**, Hou J, Wu D, Han XY, Zeng MS, Yao XZ. Glomus tumor in the stomach: computed tomography and endoscopic ultrasound findings. *World J Gastroenterol* 2013; **19**: 1327-1329 [PMID: 23482388 DOI: 10.3748/wjg.v19.i8.1327]
  - 12 **Patel TH**, Horton KM, Hruban RH, Fishman EK. Glomus Tumor of the Stomach: Depiction by Multidetector CT and Three-Dimensional Volume Rendering Imaging. *Case Rep Med* 2010; **2010**: 126095 [PMID: 20204127 DOI: 10.1155/2010/126095]
  - 13 **Imamura A**, Tochihiro M, Natsui K, Murashima Y, Suga T, Yaosaka T, Fujinaga A, Koito K, Miyakawa H, Higashino K. Glomus tumor of the stomach: endoscopic ultrasonographic findings. *Am J Gastroenterol* 1994; **89**: 271-272 [PMID: 8304316]
  - 14 **Akahoshi K**, Sumida Y, Matsui N, Oya M, Akinaga R, Kubokawa M, Motomura Y, Honda K, Watanabe M, Nagaie T. Preoperative diagnosis of gastrointestinal stromal tumor by endoscopic ultrasound-guided fine needle aspiration. *World J Gastroenterol* 2007; **13**: 2077-2082 [PMID: 17465451]
  - 15 **Debol SM**, Stanley MW, Mallery S, Sawinski E, Bardales RH. Glomus tumor of the stomach: cytologic diagnosis by endoscopic ultrasound-guided fine-needle aspiration. *Diagn Cytopathol* 2003; **28**: 316-321 [PMID: 12768637 DOI: 10.1002/dc.10294]
  - 16 **Gu M**, Nguyen PT, Cao S, Lin F. Diagnosis of gastric glomus tumor by endoscopic ultrasound-guided fine needle aspiration biopsy. A case report with cytologic, histologic and immunohistochemical studies. *Acta Cytol* 2002; **46**: 560-566 [PMID: 12040654 DOI: 10.1159/000326878]
  - 17 **Mekky MA**, Yamao K, Sawaki A, Mizuno N, Hara K, Nafeh MA, Osman AM, Koshikawa T, Yatabe Y, Bhatia V. Diagnostic utility of EUS-guided FNA in patients with gastric submucosal tumors. *Gastrointest Endosc* 2010; **71**: 913-919 [PMID: 20226456 DOI: 10.1016/j.gie.2009.11.044]
  - 18 **Akahoshi K**, Oya M, Koga T, Koga H, Motomura Y, Kubokawa M, Gibo J, Nakamura K. Clinical usefulness of endoscopic ultrasound-guided fine needle aspiration for gastric subepithelial lesions smaller than 2 cm. *J Gastrointest Liver Dis* 2014; **23**: 405-412 [PMID: 25531999 DOI: 10.15403/jgld.2014.1121.234.eug]
  - 19 **Vinette-Leduc D**, Yazdi HM. Fine-needle aspiration biopsy of a glomus tumor of the stomach. *Diagn Cytopathol* 2001; **24**: 340-342 [PMID: 11335965]
  - 20 **Jones J**, Cichowitz A, Crosthwaite GL. Endoscopic ultrasound-guided fine needle aspiration as a diagnostic tool for gastric glomus tumours. *ANZ J Surg* 2012; **82**: 94 [PMID: 22507517 DOI: 10.1111/j.1445-2197.2011.05960.x]
  - 21 **Minoda Y**, Akahoshi K, Oya M, Kubokawa M, Motomura Y, Nakamura K. Gastric glomus tumor diagnosed by endoscopic ultrasound-guided fine-needle aspiration biopsy: report of a case. *Fukuoka Igaku Zasshi* 2014; **105**: 105-109 [PMID: 25076782]
  - 22 **Mohanty SK**, Pradhan D, Stavropoulos S, Donovan V, Gupta M. Diagnosis of gastric glomus tumour by endoscopic ultrasound-guided fine needle aspiration cytology: a case report. *Cytopathology* 2014; **25**: 205-207 [PMID: 23635014 DOI: 10.1111/cyt.12068]
  - 23 **Matevossian E**, Brücher BL, Nährig J, Feußner H, Hüser N. Glomus tumor of the stomach simulating a gastrointestinal stromal tumor: a case report and review of literature. *Case Rep Gastroenterol* 2008; **2**: 1-5 [PMID: 21490829 DOI: 10.1159/000112862]
  - 24 **Wang ZB**, Yuan J, Shi HY. Features of gastric glomus tumor: a clinicopathologic, immunohistochemical and molecular retrospective study. *Int J Clin Exp Pathol* 2014; **7**: 1438-1448 [PMID: 24817939]

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## Abnormal layering of muscularis propria as a cause of chronic intestinal pseudo-obstruction: A case report and literature review

Napat Angkathunyakul, Suporn Treepongkaruna, Sani Molagool, Nichanan Ruangwattanapaisarn

Napat Angkathunyakul, Department of Pathology, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand  
Suporn Treepongkaruna, Department of Pediatrics, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand  
Sani Molagool, Department of Surgery, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand  
Nichanan Ruangwattanapaisarn, Department of Diagnostic and Therapeutic Radiology, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Fax: +66-2-3547266

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**Correspondence to:** Napat Angkathunyakul, MD, Pathologist, Department of Pathology, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand. [napat.ang@mahidol.ac.th](mailto:napat.ang@mahidol.ac.th)  
Telephone: +66-2-2011667

### Abstract

Visceral myopathy is one of the causes of chronic intestinal pseudo-obstruction. Most cases pathologically reveal degenerative changes of myocytes or muscularis propria atrophy and fibrosis. Abnormal layering of muscularis propria is extremely rare. We report a case of a 9-mo-old Thai male baby who presented with chronic intestinal pseudo-obstruction. Histologic findings showed abnormal layering of small intestinal muscularis propria with an additional oblique layer and aberrant muscularization in serosa. The patient also had a short small bowel without malrotation, brachydactyly, and absence of the 2<sup>nd</sup> to 4<sup>th</sup> middle phalanges of both hands. The patient was treated with cisapride and combined parenteral and enteral nutritional support. He had gradual clinical improvement and gained body weight. Subsequently, the parenteral nutrition was discontinued. The previously reported cases are reviewed and discussed.

**Key words:** Abnormal layering of muscularis propria; Brachydactyly; Chronic intestinal pseudo-obstruction; Serosal muscularization; Short small bowel; Visceral myopathy

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**Core tip:** This report describes the case of a 9-month-old boy who presented with chronic intestinal pseudo-obstruction. Full-thickness small bowel biopsy showed abnormal layering of muscularis propria (additional oblique layer) and serosal aberrant muscularization. There have been only eight previously reported abnormal layering cases and only one case with an additional oblique layer. The patient also had a short small bowel without malrotation, brachydactyly, and absence of the 2<sup>nd</sup> to 4<sup>th</sup> middle phalanges of both hands. The patient showed clinical improvement with medical treatment and nutritional support.

Angkathunyakul N, Treepongkaruna S, Molagool S, Ruangwattanapaisarn N. Abnormal layering of muscularis propria as a cause of chronic intestinal pseudo-obstruction: A case report and literature review. *World J Gastroenterol* 2015; 21(22): 7059-7064 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v21/i22/7059.htm> DOI: <http://dx.doi.org/10.3748/wjg.v21.i22.7059>

## INTRODUCTION

Chronic intestinal pseudo-obstruction (CIPO) was first described by Dudley in 1958<sup>[1]</sup>. It is a rare, severe gastrointestinal disorder in which intestinal motility is impaired. CIPO can be congenital or acquired (primary or secondary)<sup>[2]</sup>, and is characterized by recurrent signs and symptoms of intestinal obstruction in the absence of true mechanical obstruction<sup>[3]</sup>. It can affect both adults and children. Most pediatric patients manifest at birth or in early infancy<sup>[4]</sup>. The two main pathophysiologic types of this motility disorder are myopathic and neuropathic<sup>[5]</sup>. This report describes a pediatric case of CIPO due to visceral myopathy with rare histology (abnormal layering muscularis propria and serosal aberrant muscularization) along with a review of the literature.

## CASE REPORT

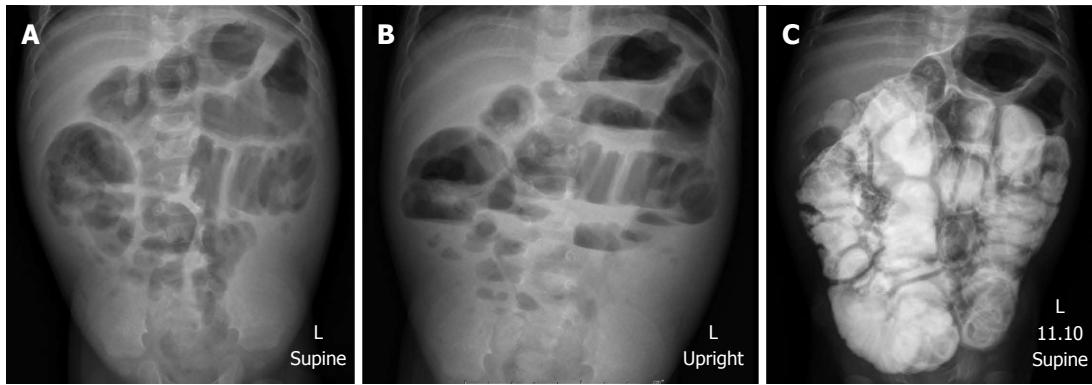
A 9-month-old Thai male baby presented with abdominal distention and bilious vomiting that had begun at 2 wk of age. He could be fed orally and defecate daily. He was the first child with an uneventful history of prenatal, perinatal, and neonatal periods, and his birth weight was 3065 g. No family member had similar symptoms. He had visited another hospital at the age of 3 mo, and upper gastrointestinal studies were conducted revealing no intestinal malrotation or other gastrointestinal obstruction. His abdominal distention and bilious vomiting progressed and he had poor weight gain.

Upon presentation to our hospital, a physical examination showed a body weight of 5500 g (below the 3<sup>rd</sup> percentile), length 64 cm (10<sup>th</sup> percentile), no

dysmorphic features, marked abdominal distention, hyperactive bowel sounds, visible peristalsis, and bilateral indirect inguinal hernia. He also had brachydactyly on both hands. X-ray film demonstrated an absence of middle phalanges of the 2<sup>nd</sup> to 4<sup>th</sup> fingers. The other systems were unremarkable. The plain abdominal radiographs showed diffuse small bowel dilatation (Figure 1). Barium enema showed no transitional zone or signs of Hirschsprung disease, but irregular mucosa of nearly his entire colon was noted. Small bowel follow-through showed dilatation with thickening fold of almost the entire small bowel from duodenum to ileum with hyperperistalsis, and partial distal small bowel obstruction was suspected (Figure 1). A bilateral inguinal hernia was suspected as the cause of partial gastrointestinal obstruction, and bilateral herniorrhaphy was performed. Unfortunately, his symptoms did not improve. Upper endoscopy and colonoscopy were performed to exclude mucosal diseases, and were unremarkable. The histology of biopsies from esophageal, duodenal, gastric, and colonic mucosa showed no significant pathologic findings or tissue eosinophilia. His clinical symptoms were worse with progressive bilious vomiting, abdominal distension, and poor weight gain. As intestinal obstruction could not be excluded, exploratory laparotomy was performed at the age of 9 mo. Intraoperative findings showed normal size of the colon, but thickening of the short small bowel, which measured 86 cm from the duodenojejunal junction to the ileocecal valve. A pale thickened and inflamed Tenia coli-like line was noted on the antimesenteric side from his duodenojejunal junction to 15 cm above the ileocecal valve (Figure 2). Appendectomy and full-thickness biopsy of the distal ileum were performed and sent for intraoperative consultation. The specimen was fixed in formalin afterward.

## Histopathology

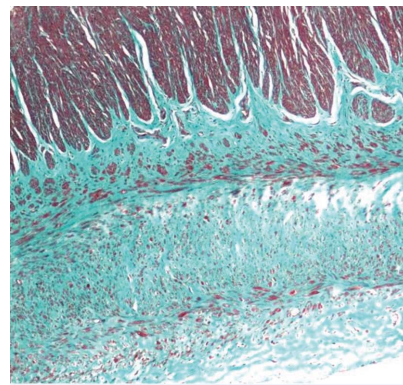
Full-thickness biopsy of the terminal ileum revealed unremarkable mucosa with no significant tissue eosinophilia. The submucosa showed hyalinization and fibrosis. Unremarkable ganglion cells in the submucosa (Henle's) and deep submucosa (Meissner's) were identified. Muscularis propria was markedly thickened and revealed abnormal layering into three layers; (1) inner circular; (2) additional oblique; and (3) outer longitudinal layer (Figure 3A). No degenerative change of myocyte (e.g., cytoplasmic vacuolation, variation in muscle fiber size, nuclear pleomorphism) or increased mitosis was observed. Periodic acid-Schiff staining revealed no intracytoplasmic inclusion. Diffuse delicate interstitial fibrosis in all muscular layers highlighted by Masson's trichrome stain was noted (Figure 3B). Meissner's plexuses were located between the inner circular and additional oblique layers. There was no inflammation in muscular layers or around ganglion cells or neural plexuses. The serosa showed three



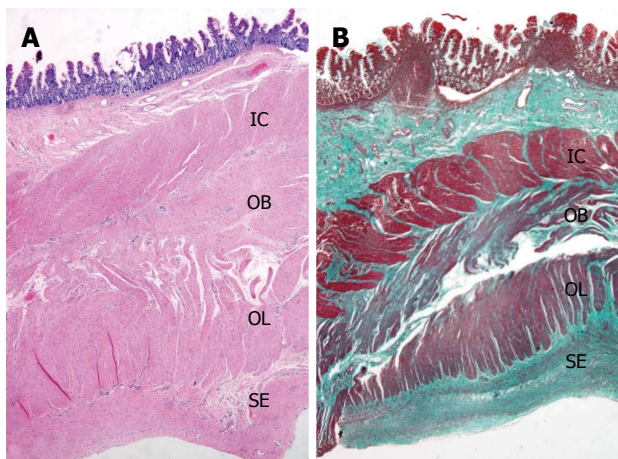
**Figure 1** Plain abdominal radiographs showed diffused dilatation of small bowel loops. A: Supine; B: Upright; C: Small bowel follow-through showed dilatation with thickening fold of almost the entire small bowel, from duodenum to ileum.



**Figure 2** Exploratory laparotomy revealed thickening of the short small bowel. The bowel was pale, thickened, and an inflamed Tenia coli-like line was noted on the antimesenteric side from the duodenojejunal junction to 15 cm above the ileocecal valve.



**Figure 4** Serosal aberrant muscularization into three bizarre layers of smooth muscle (Masson's trichrome, 100x).



**Figure 3** Full-thickness biopsy from distal ileum. A: (HE, 20x) Hypertrophic muscularis propria with abnormal layering into 3 layers (IC: Inner circular; OB: Additional oblique; OL: Outer longitudinal); B: Delicate interstitial fibrosis and serosal muscularization (SE) are highlighted by Masson's trichrome staining (20x).

bizarre layers of aberrant muscularization and diffuse interstitial fibrosis into smooth muscles, grossly forming a Tenia coli-like line. Congo red stain excluded amyloidosis (Figure 4).

Sections of vermiform appendix showed unremarkable mucosal, mural, and serosal layers.

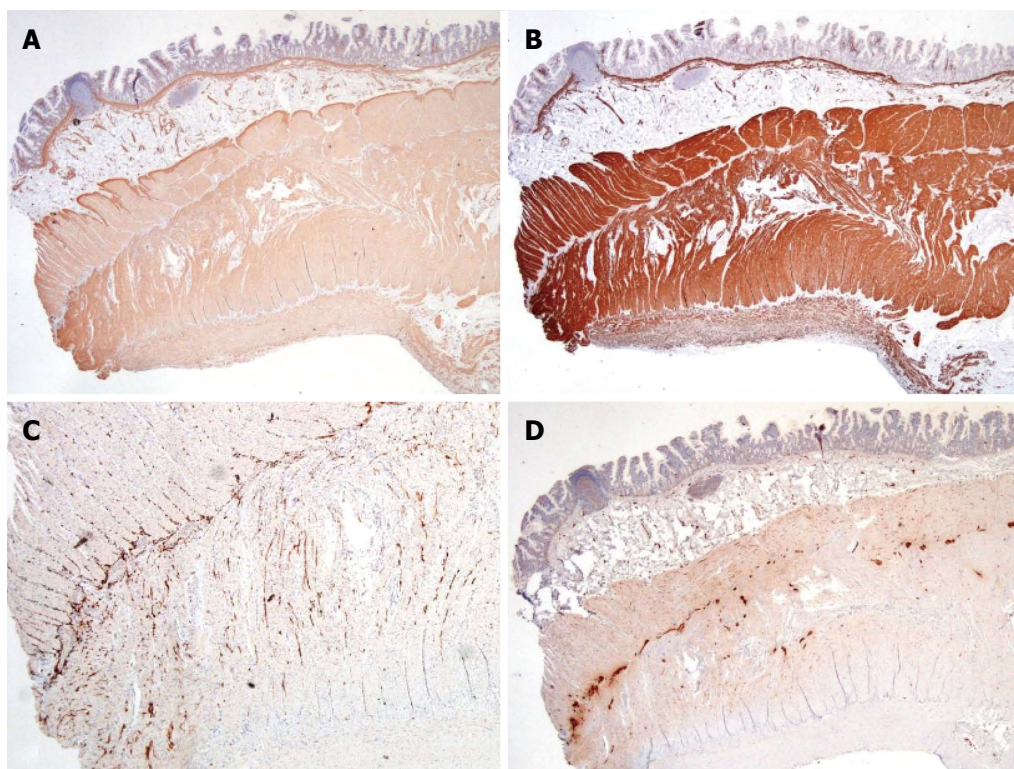
#### Immunohistochemical study

Muscularis mucosae, all muscular layers in muscularis propria, and serosa showed diffusely strong expression of smooth-muscle actin, desmin, and muscle actin. (Figure 5). S100 highlighted normal distribution of Henle's, Meissner's, and Auerbach's neural plexuses with unremarkable ganglion cells. Normal expression of Bcl-2 was observed in all plexuses. Immunohistochemistry for CD117 revealed normal interstitial cells of Cajal networks around Auerbach's plexus and extending to inner circular and outer longitudinal muscular layers. Thus, neuropathic and interstitial cells of Cajal abnormalities were excluded.

#### DISCUSSION

This report describes a pediatric case of primary CIPD due to visceral myopathy from abnormal layering of muscularis propria. Clinical presentations were similar to most cases of CIPD. The symptoms begin at birth in 50% of patients, and by age one year in 75%. Bilious vomiting, abdominal distension, and obstipation are almost universal presentations<sup>[6]</sup>. The majority





**Figure 5 Immunohistochemical study.** A: Smooth muscle  $\alpha$ -actin; B: Desmin were strongly expressed in all layers of smooth muscle; C: CD117 showed interstitial cells of Cajal network; D: S100 highlighted Auerbach's neural plexuses; (20 $\times$ ).

of the pediatric cases are primary or idiopathic CIPO. Antonucci *et al*<sup>[7]</sup> identified secondary causes, such as organic, systemic, or metabolic causes, in only 4/77 CIPO patients. Antroduodenal manometry is useful for differentiating between the neuropathy and myopathy type<sup>[8]</sup>. Unfortunately, this investigation is not available in our institute. Definite diagnosis of CIPO requires full-thickness biopsies for histopathology.

Visceral myopathy can be divided into two groups. The first group is comprised of intrinsic myocyte defects with characteristic findings of vacuolar degeneration and fibrosis in inner circular and/or outer longitudinal muscular layers<sup>[9-12]</sup>. The other changes include nuclear atypia, increased mitotic activity, periodic acid-Schiff-positive intracytoplasmic inclusions<sup>[13]</sup>, and absence or decreased smooth-muscle  $\alpha$ -actin immunostaining in the circular muscle layer<sup>[14]</sup>. The second group comprises morphogenic abnormalities of muscularis propria. Most cases are of an atrophic pattern<sup>[8,13,15]</sup>. A few cases of a hypertrophic pattern with hypertrophy of one or both layers have been reported<sup>[8,16-18]</sup>. The abnormal layering of muscularis propria is exceedingly rare, and only eight cases have been reported (Table 1).

Most cases with abnormally layered muscularis propria are male. The clinical symptoms appear early in life and depend on the site of involvement. Patients with colonic involvement present with constipation, whereas small intestine involvement manifests as vomiting and abdominal distension. Additional circular muscle coats in various locations are the most

common feature. Only one case with an additional oblique layer similar to our case was reported by Yamagiwa *et al*<sup>[19]</sup>. To our knowledge, serosal Tenia coli-like aberrant muscularization has never been described in the literature.

Common associated abnormalities are a short small intestine (mostly with malrotation)<sup>[4,20]</sup>, urinary involvement, including megacystis and megaureter<sup>[21]</sup>, and skeletal deformity (spine and extremities). To our knowledge, there has been no report of association with brachydactyly and absence of the middle phalangeal bones in visceral myopathy cases. Smith *et al*<sup>[9]</sup> suggested an X-linked mode of inheritance in three related boys with abnormal layered muscularis propria of small and large intestines with megacystis. DNA analysis was not performed in our case due to unavailability in our institute.

The impact of abnormal layering of muscularis propria on the natural history or prognosis remains unknown as the numbers of cases are small. Among the eight reported cases, clinical outcome could be identified in only two cases. The first case had clinical improvement of constipation and grew into a healthy young man after partial resection of a dilated sigmoid colon and rectum. However, the second case died due to multiple organ anomalies<sup>[22]</sup>.

There is no specific treatment for CIPO from visceral myopathy, and nutritional support is the mainstay of management in these children. Pharmacotherapy, including cisapride, erythromycin,

**Table 1** Previously reported cases of visceral myopathy due to abnormal layering of muscularis propria

Case	Sex/age	Presenting symptom	Site	Muscularis propria pathology		Other GI abnormality	Other findings	Remark	Ref.
				Additional layer	Location				
1	M/11 d	CIPO	S	Oblique	External to OL	Short small intestine + malrotation	Sclerocornea, cryptorchidism, scoliosis, deformity of extremities		[19]
2	M	CIPO	S and L	Circular	Between IC and OL	Short intestine + malrotation	Megacystis	X-linked	[9]
3	M	CIPO	S and L	Circular	Between IC and OL	Short intestine + malrotation	Megacystis	X-linked	[9]
4	M	CIPO	S and L	Circular	Between IC and OL	Short intestine + malrotation	Megacystis	X-linked	[9]
5	F	Constipation	L	Circular	Internal to IC	-	-		[9]
6	F	Constipation	L	Circular	Internal to IC	-	Megaureter		[9]
7	M/16 yr	Constipation	L	Circular	Internal to IC	-	Dysmorphic facies and toes, seizures, leukoencephalopathy, cataract	Clinically improved after surgery	[22]
8	M/birth	Respiratory problem	S	Circular	External to OL	Short small intestine + malrotation	Diaphragmatic defect, High-arched palate, ASD, ventriculomegaly, Subependymal heterotopias, arachnoid cyst, spina bifida, proximally placed thumbs	Dead	[22]
9	M/9 mo	CIPO	S	Oblique	Between IC and OL	Short small intestine	Brachydactyly, absent 2 <sup>nd</sup> to 4 <sup>th</sup> middle phalanges of both hands	Clinically improved by medication	Present case

CIPO: Chronic intestinal pseudo-obstruction; GI: Gastrointestinal; S: Small intestine; L: Large intestine; IC: Inner circular layer; OL: Outer longitudinal layer.

and octreotide, to stimulate intestinal contractions, may be useful in some select cases<sup>[23,24]</sup>. Small bowel transplantation has a potential role for those who have irreversible intestinal failure and permanent dependence on parenteral nutrition<sup>[25,26]</sup>. Prognosis is fair to poor and a requirement for long-term parenteral nutrition is common in these patients<sup>[27]</sup>. A mortality rate of 25% has been reported in a large cohort study, and common causes of death are parenteral-related complications<sup>[21]</sup>. Our patient also had a short bowel accompanying the CIPO. Management modalities of short-bowel syndrome include enteral and parenteral nutritional support, treating small bowel bacterial overgrowth, and fish-oil-based lipid emulsions<sup>[28]</sup>. Currently, teduglutide, a recombinant analog of human glucagon-like peptide-2, is an emerging treatment for those with intestinal failure<sup>[29]</sup>. Our patient was treated with cisapride and combined parenteral and enteral nutritional support. He had gradual clinical improvement and gained body weight, and parenteral nutrition was subsequently discontinued. At the time of the writing of this report, he is 3 years-old and has normal growth, with a body weight of 15 kg (65<sup>th</sup> percentile) and height of 95 cm (55<sup>th</sup> percentile).

## COMMENTS

### Case characteristics

A 9-mo-old Thai male baby presented with abdominal distention that had been present since birth and bilious vomiting since 2 wk of age.

### Clinical diagnosis

Chronic intestinal pseudo-obstruction.

### Differential diagnosis

Hirschsprung's disease.

### Laboratory diagnosis

Unremarkable findings for the laboratory tests.

### Imaging diagnosis

Abdominal X-ray showed diffused dilatation of small bowel loops. Small bowel follow-through showed generalized bowel dilatation with thickening fold of entire small bowel loops from duodenum to ileum.

### Pathological diagnosis

Full-thickness biopsy of ileum revealed abnormal layering of muscularis propria (additional oblique layer between inner circular and outer longitudinal layers) and serosal aberrant muscularization.

### Treatment

Medication (cisapride) and combined parenteral and enteral nutritional support.

### Related reports

There have been only eight previously reported abnormal layering cases, and only one case showed an additional oblique layer. The patient also had aberrant serosal muscularization, brachydactyly, and absence of 2<sup>nd</sup> to 4<sup>th</sup> middle phalanges in both hands, which have never been reported.

### Term explanation

Abnormal layering of muscularis propria is an exceedingly rare condition of abnormal morphogenesis of muscularis propria (visceral myopathy), which causes chronic intestinal pseudo-obstruction.

### Experiences and lessons

The case report presents the unique abnormal layering of muscularis propria and aberrant serosal muscularization in a case of chronic intestinal pseudo-obstruction and different associated anomalies from previously reported cases. We learned that medical treatment and combined parenteral and enteral nutritional support improved clinical outcome.

### Peer-review

The authors report an interesting case of a very rare situation presenting with intestinal pseudo-obstruction linked to a short small bowel and brachydactyly. In the gut, it was associated with an additional oblique muscle layer and with muscularization of the serosa. Although descriptive, this study also reviews the few cases described in the literature exhibiting visceral myopathy due to



abnormal layering of the muscularis propria. It shows that the current case corresponds to a situation never previously reported.

## REFERENCES

- Dudley HA, Sinclair IS, McLaren IF, McNair TJ, Newsam JE. Intestinal pseudo-obstruction. *J R Coll Surg Edinb* 1958; **3**: 206-217 [PMID: 13514744]
- Rudolph CD, Hyman PE, Altschuler SM, Christensen J, Colletti RB, Cucchiara S, Di Lorenzo C, Flores AF, Hillemeier AC, McCallum RW, Vanderhoof JA. Diagnosis and treatment of chronic intestinal pseudo-obstruction in children: report of consensus workshop. *J Pediatr Gastroenterol Nutr* 1997; **24**: 102-112 [PMID: 9093995 DOI: 10.1097/00005176-199701000-00021]
- Cucchiara S, Borrelli O, Salvia G, Iula VD, Fecarotta S, Gaudiello G, Boccia G, Annese V. A normal gastrointestinal motility excludes chronic intestinal pseudoobstruction in children. *Dig Dis Sci* 2000; **45**: 258-264 [PMID: 10711435 DOI: 10.1023/A:1005491921972]
- Heneyke S, Smith VV, Spitz L, Milla PJ. Chronic intestinal pseudo-obstruction: treatment and long term follow up of 44 patients. *Arch Dis Child* 1999; **81**: 21-27 [PMID: 10373127 DOI: 10.1136/adc.81.1.21]
- Georgescu EF, Vasile I, Ionescu R. Intestinal pseudo-obstruction: an uncommon condition with heterogeneous etiology and unpredictable outcome. *World J Gastroenterol* 2008; **14**: 954-959 [PMID: 18240359 DOI: 10.3748/wjg.14.954]
- Kocoshis SA, Reyes J, Todo S, Starzl TE. Small intestinal transplantation for irreversible intestinal failure in children. *Dig Dis Sci* 1997; **42**: 1997-2008 [PMID: 9365126]
- Antonucci A, Fronzoni L, Cogliandro L, Cogliandro RF, Caputo C, De Giorgio R, Pallotti F, Barbara G, Corinaldesi R, Stanghellini V. Chronic intestinal pseudo-obstruction. *World J Gastroenterol* 2008; **14**: 2953-2961 [PMID: 18494042 DOI: 10.3748/wjg.14.2953]
- Connor FL, Di Lorenzo C. Chronic intestinal pseudo-obstruction: assessment and management. *Gastroenterology* 2006; **130**: S29-S36 [PMID: 16473068 DOI: 10.1053/j.gastro.2005.06.081]
- Smith VV, Milla PJ. Histological phenotypes of enteric smooth muscle disease causing functional intestinal obstruction in childhood. *Histopathology* 1997; **31**: 112-122 [PMID: 9279561 DOI: 10.1046/j.1365-2559.1997.2250839.x]
- Moore SW, Schneider JW, Kaschula RD. Non-familial visceral myopathy: clinical and pathologic features of degenerative leiomyopathy. *Pediatr Surg Int* 2002; **18**: 6-12 [PMID: 11793055 DOI: 10.1007/s003830200002]
- Smith VV, Gregson N, Foggensteiner L, Neale G, Milla PJ. Acquired intestinal aganglionosis and circulating autoantibodies without neoplasia or other neural involvement. *Gastroenterology* 1997; **112**: 1366-1371 [PMID: 9098023 DOI: 10.1016/S0016-5085(97)70151-3]
- De Giorgio R, Guerrini S, Barbara G, Stanghellini V, De Ponti F, Corinaldesi R, Moses PL, Sharkey KA, Mawe GM. Inflammatory neuropathies of the enteric nervous system. *Gastroenterology* 2004; **126**: 1872-1883 [PMID: 15188182 DOI: 10.1053/j.gastro.2004.02.024]
- Fogel SP, DeTar MW, Shimada H, Chandrasoma PT. Sporadic visceral myopathy with inclusion bodies. A light-microscopic and ultrastructural study. *Am J Surg Pathol* 1993; **17**: 473-481 [PMID: 8385883 DOI: 10.1097/00000478-199305000-00006]
- Knowles CH, Silk DB, Darzi A, Veress B, Feakins R, Raimundo AH, Crompton T, Browning EC, Lindberg G, Martin JE. Deranged smooth muscle alpha-actin as a biomarker of intestinal pseudo-obstruction: a controlled multinational case series. *Gut* 2004; **53**: 1583-1589 [PMID: 15479676 DOI: 10.1136/gut.2003.037275]
- De Giorgio R, Sarnelli G, Corinaldesi R, Stanghellini V. Advances in our understanding of the pathology of chronic intestinal pseudo-obstruction. *Gut* 2004; **53**: 1549-1552 [PMID: 15479666 DOI: 10.1136/gut.2004.043968]
- McClelland HA, Lewis MJ, Naish JM. Idiopathic steatorrhea with intestinal pseudo-obstruction. *Gut* 1962; **3**: 142-145 [PMID: 18668747 DOI: 10.1136/gut.3.2.142]
- Naish JM, Capper WM, Brown NJ. Intestinal pseudoobstruction with steatorrhea. *Gut* 1960; **1**: 62-66 [PMID: 14425851 DOI: 10.1136/gut.1.1.62]
- Koh S, Bradley RF, French SW, Farmer DG, Cortina G. Congenital visceral myopathy with a predominantly hypertrophic pattern treated by multivisceral transplantation. *Hum Pathol* 2008; **39**: 970-974 [PMID: 18329691 DOI: 10.1016/j.humpath.2007.10.016]
- Yamagiwa I, Ohta M, Obata K, Washio M. Intestinal pseudo-obstruction in a neonate caused by idiopathic muscular hypertrophy of the entire small intestine. *J Pediatr Surg* 1988; **23**: 866-869 [PMID: 3183906]
- Tanner MS, Smith B, Lloyd JK. Functional intestinal obstruction due to deficiency of argyrophil neurones in the myenteric plexus. Familial syndrome presenting with short small bowel, malrotation, and pyloric hypertrophy. *Arch Dis Child* 1976; **51**: 837-841 [PMID: 1008589 DOI: 10.1136/adc.51.11.837]
- Mousa H, Hyman PE, Cocjin J, Flores AF, Di Lorenzo C. Long-term outcome of congenital intestinal pseudoobstruction. *Dig Dis Sci* 2002; **47**: 2298-2305 [PMID: 12395903 DOI: 10.1023/A:1020199614102]
- Kapur RP, Correa H. Architectural malformation of the muscularis propria as a cause for intestinal pseudo-obstruction: two cases and a review of the literature. *Pediatr Dev Pathol* 2009; **12**: 156-164 [PMID: 18788889 DOI: 10.2350/08-07-0495.1]
- Di Lorenzo C, Reddy SN, Villanueva-Meyer J, Mena I, Martin S, Hyman PE. Cisapride in children with chronic intestinal pseudoobstruction. An acute, double-blind, crossover, placebo-controlled trial. *Gastroenterology* 1991; **101**: 1564-1570 [PMID: 1955122]
- Di Lorenzo C, Lucanto C, Flores AF, Idries S, Hyman PE. Effect of sequential erythromycin and octreotide on antroduodenal manometry. *J Pediatr Gastroenterol Nutr* 1999; **29**: 293-296 [PMID: 10467994 DOI: 10.1097/00005176-199909000-00010]
- Bond GJ, Reyes JD. Intestinal transplantation for total/near-total aganglionosis and intestinal pseudo-obstruction. *Semin Pediatr Surg* 2004; **13**: 286-292 [PMID: 15660322 DOI: 10.1053/j.semped surg.2004.10.016]
- Gambarara M, Ferretti F, Diamanti A, Papadatou B, D'Orto F, Sabbati T, Castro M. Parenteral nutrition dependence in pediatric patients: an indication for small bowel transplantation. *Transplant Proc* 2002; **34**: 882-883 [PMID: 12034220 DOI: 10.1016/S0041-1345(02)02684-2]
- Ueno T, Wada M, Hoshino K, Sakamoto S, Furukawa H, Fukuzawa M. A national survey of patients with intestinal motility disorders who are potential candidates for intestinal transplantation in Japan. *Transplant Proc* 2013; **45**: 2029-2031 [PMID: 23769101 DOI: 10.1016/j.transproceed.2013.01.092]
- Uko V, Radhakrishnan K, Alkhouri N. Short bowel syndrome in children: current and potential therapies. *Paediatr Drugs* 2012; **14**: 179-188 [PMID: 22452596 DOI: 10.2165/11594880-000000000-00000]
- Wilhelm SM, Lipari M, Kulik JK, Kale-Pradhan PB. Teduglutide for the Treatment of Short Bowel Syndrome. *Ann Pharmacother* 2014; **48**: 1209-1213 [PMID: 24871569 DOI: 10.1177/1060028014537468]

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