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Contents

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EDITORIAL

Terlipressin and hepatorenal syndrome: What is important for nephrologists and hepatologists

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Abstract

Hepatorenal syndrome (HRS) is a reversible form of functional renal failure that occurs with advanced hepatic cirrhosis and liver failure. Despite mounting research in HRS, its etiology and medical therapy has not been resolved. HRS encompasses 2 distinct types. Type 1 is characterized by the rapid development of renal failure that occurs within 2 wk and involves a doubling of initial serum creatinine. Type 2 has a more insidious onset and is often associated with ascites. Animal studies have shown that both forms, in particular type 1 HRS, are often precipitated by bacterial infections and circulatory changes. The prognosis for HRS remains very poor. Type 1 and 2 both have an expected survival time of 2 wk and 6 mo, respectively. Progression of liver cirrhosis and the resultant portal hypertension leads to the pooling of blood in the splanchnic vascular bed. The ensuing hyperdynamic circulation causes an ineffective circulatory volume which subsequently activates neurohormonal systems. Primarily the sympathetic nervous system and the renin angiotensin system are activated, which, in the early stages of HRS, maintain adequate circulation. Both advanced cirrhosis and prolonged activation of neurohormonal mechanisms result in fatal complications. Locally produced nitric oxide may have the potential to induce a deleterious vasodilatory effect on the splanchnic circulation. Currently medical therapy is aimed at reducing splanchnic vasodilation to resolve the ineffective circulation and maintain good renal perfusion pressure. Terlipressin, a vasopressin analogue, has shown potential benefit in the treatment of HRS. It prolongs both survival time and has the ability to reverse HRS in the majority of patients. In this review we aim to focus on the pathogenesis of HRS and its treatment with terlipressin *vs* other drugs.

Key words: Heptorenal syndrome; Terlipressin; Kidney; Liver

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INTRODUCTION

Many studies have been carried out on hepatorenal syndrome (HRS); the pathophysiology and its management

however have not been completely resolved. HRS is a reversible form of functional renal failure that occurs predominantly with advanced liver disease arising from hepatic cirrhosis or severe liver injury from any condition such as severe alcoholic hepatitis or metastatic tumors[1]. The important features of HRS are characterized by peripheral vasodilation with subsequent profound intrarenal vasoconstriction, leading to decreased glomerular filtration rate (GFR)[2,3].

Currently, HRS encompasses 2 distinct types. Type 1 HRS often manifests itself rapidly; without appropriate treatment the mean survival time is approximately 2 wk[4]. The distinguishing feature of type 1 HRS is rapid progressive renal failure that occurs within 2 wk and is associated with doubling of baseline serum creatinine or a 50% reduction in creatinine clearance[5]. In more than 70% of cases there is an identifiable trigger for type 1 HRS[6-9]. A large number of studies have shown that type 1 HRS can be precipitated by preceding spontaneous bacterial peritonitis infections, gastrointestinal bleeding and large-volume abdominal paracentesis without albumin replacement[6,8]. Furthermore, type 2 HRS has a gradual onset with a steady decline in renal function. Interestingly, the hallmark for type 2 HRS is refractory ascites and often has no precipitating factors[4]. The survival time is better in type 2 HRS at approximately 6 mo[1,5]. Some would consider injudicious use of diuretics as a precipitating factor.

Importantly, the core feature of pathogenesis of HRS is peripheral arterial vasodilation, in particular in the splanchnic vasculature[10]. This develops with advanced liver cirrhosis, which causes increased resistance to blood flow with high portal pressure. In turn, to ease the pressure within the hepatic portal system, locally acting vasoactive substances are released that cause vasodilation of the splanchnic vasculature[10]. The overall resultant effect is circulatory dysfunction arising from a depleted intravascular volume that ultimately leads to poor renal perfusion and activation of compensatory mechanisms (renin angiotensin aldosterone system, sympathetic nervous system and vasopressin). These compensatory mechanisms with time become detrimental and result in sustained severe intrarenal arterial vasoconstriction with progressive physiological renal failure[2]. The pooling of blood in the splanchnic vascular bed with the associated hypoperfusion of the kidneys and the ensuing intrarenal arterial vasoconstriction forms the basis for the development of HRS.

HRS has very poor prognosis with spontaneous recovery being unlikely[2]. Treatment of HRS can be divided into medical and surgical, the latter being more beneficial. Current treatment modalities are used as a bridge to surgical intervention (liver transplant), although most patients do not survive long enough to receive a liver transplant[2]. Pharmacotherapy is the initial treatment which buys time for a liver transplant but unfortunately there is no universally agreed first-line therapy. There are a number of pharmacological agents that have been investigated in the management of HRS and thus far most drugs aim to reverse the peripheral and splanchnic vasodilation. Usually treatment is a combined therapy of vasoconstrictors with albumin to augment their efficacy[11].

Vasoconstrictive drugs such as vasopressin analogues (ornipressin, terlipressin), octreotide and noradrenaline have been used in attempts to reduce the pooling of blood in the splanchnic vasculature and the peripheral arterial vasodilation[12]. A few studies have investigated ornipressin combined with albumin or dopamine and they have been shown to reverse HRS[13-15]. Globally the use of ornipressin has been abandoned in HRS because of the high risk of an adverse event, in particular ischemic events. Other studies have shown that with the use of potent vasoconstrictors such as ornipressin, the result can be ischemic mesenteric mucosa, myocardial ischemia, and associated ventricular arrhythmias[16]. A safe alternative treatment is terlipressin (vasopressin analogue) which so far has shown promising results.

Several studies have confirmed that terlipressin combined with albumin achieves acceptable GFR and it almost normalizes the plasma creatinine levels in 42% to 77% of cases[17-20]. The aim of the current review is to evaluate the recent developments made in the pathogenesis of HRS and the role of terlipressin, including its possible mechanism of it action.

PATHOGENSIS OF HRS

The etiopathogenesis of HRS has not been fully resolved and there are possible theories to explain it at a cellular and molecular level. The defining feature of HRS is profound vasoconstriction of the renal vasculature due to inadequate blood flow to the kidneys[21-24]. The culmination of several factors leads to the development of HRS: (1) portal hypertension (PHT); (2) altered peripheral blood circulation; (3) activation of the sympathetic nervous system; and (4) the release of chemical mediators.

PHT AND NITRIC OXIDE

Over time, liver cirrhosis leads to structural changes at both a microscopic and macroscopic level within the hepatocytes. The pressure within the hepatic microcirculation becomes raised and the so-called sinusoidal PHT occurs[25]. Furthermore, this is complemented by the ongoing changes taking place within the myofibroblasts, stellate cells and portal venules which all contribute towards the development of increased resistance to portal blood flow[26,27]. Most research on animals has suggested 2 possible theories to explain the development of PHT: (1) "forward theory" - this theory puts forward that PHT arises as a direct consequence of increased resistance to portal inflow; and (2) "backward theory" which proposes that PHT occurs due to high portal blood inflow because of a hyperdynamic circulation[28,29]. Furthermore, it is insinuated that this abnormally high portal blood inflow sustains the PHT[30].

In spite of the etiology of PHT, some of the portal venous blood gets redirected *via* collaterals vessels and this is partially to take pressure off the portal system[31]. Gradu-

ally, with persistent PHT, local and systemic changes occur; neurohormonal systems are activated and locally produced vasoactive substances such as nitric oxide are released[32]. Other locally acting vasodilatory substances released include carbon monoxide and prostacyclin[33]. Nitric oxide however is widely believed to be one of the main culprits for initiating the splanchnic arterial vasodilation[34].

In animal models nitric oxide has been shown to play an important role in vascular tone and splanchnic vasodilation[35,36]. In other animal studies, it is postulated that the production of nitric oxide may be related to bacteria stimulating macrophages which in turn induce nitric oxide synthase (NOS)[37-39]. NOS is an enzyme that forms nitric oxide from L-arginine, which is found throughout the body in numerous different types of cells. In addition, NOS has been shown to have 3 isoforms which are NOS I - neuronal NOS (nNOS), NOS Ⅱ - inducible NOS (iNOS) and NOS Ⅲ - endothelial NOS (eNOS)[40-42].

Isoform nNOS is primarily found in the central nervous system and it has been shown to have a key role in controlling blood pressure. Several studies on rats have demonstrated that by inhibiting this isoenzyme it generates increased sympathetic activity with ensuing tachycardia, and hypertension[43-45]. Conversely, iNOS is present in humans in several tissues including hepatocytes and alveolar macrophages; its release is induced by several cytokines including interleukin 1, interferon γ, tumor necrosis factor and lipopolysaccharides[46,47]. Finally eNOS, as the name suggests, is predominantly found in endothelial cells in humans in both arterial and venous vessels[48,49]. In the literature, eNOS has been shown to be involved in the peripheral arterial vasodilation that occurs in HRS and there are raised levels of eNOS in the circulation[48]. Overall eNOS has an important role in maintaining sympathetic vascular tone and can be synthesized within the endothelium in response to stimuli.

HYPERKINETIC CIRCULATION AND COMPENSATORY MECHANISM

The hemodynamic changes that develop in cirrhosis in the splanchnic circulation have been studied extensively, and only slow progress has been made in determining its pathophysiology. A number of plausible theories have been postulated in the last 2 decades based on both *in vitro* and *in vivo* studies. Hyperdyanamic circulation is a phenomena that happens over a period of time as a direct consequence of long standing PHT (Figure 1)[50]. The hallmarks of this circulatory dysfunction are tachycardia, increased cardiac output and abnormally low peripheral vascular resistance with decreased arterial blood pressure[31].

Hyperkinetic circulation develops in several steps: (1) splanchnic and peripheral vasodilation; (2) an increase in total blood volume with inadequate circulating volume; (3) increased cardiac output; and (4) the activation of a compensatory mechanism. Initially there is pooling of blood in the splanchnic vasculature due to PHT and this causes decreased circulatory volume.

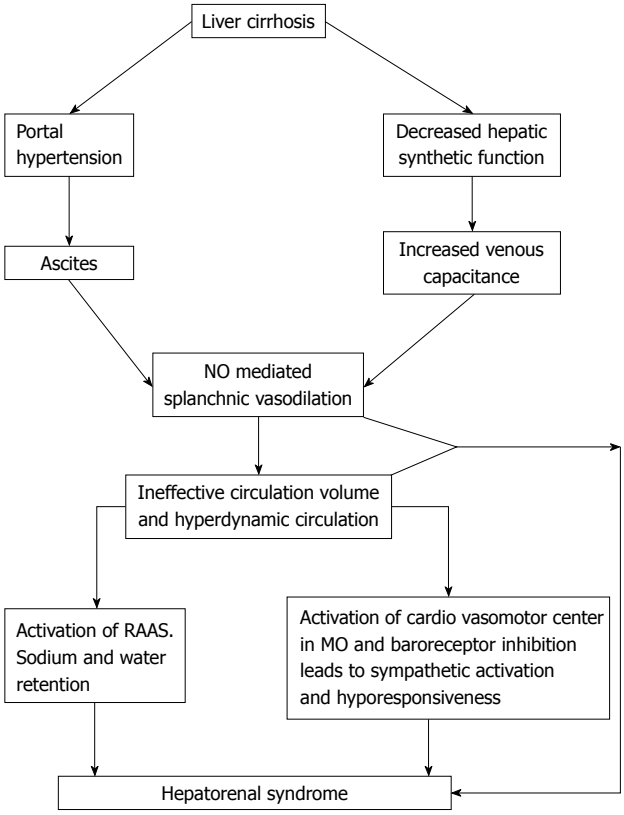

Figure 1 Flow chart showing the vicious cycle that develops with decompensated liver cirrhosis and the serious of events that lead to hepatorenal syndrome. NO: Nitric oxide; RAAS: Renin angiotensinogen aldosterone system; MO: Medulla oblongata.

One of the earliest indicators of a hyperdynamic circulation is the redistribution of blood volume into the splanchnic circulation. This event has been demonstrated in Doppler ultrasonography studies in which patients with cirrhosis had remarkably high splanchnic blood flow when compared to normal subjects[51]. The development of increased blood flow to splanchnic circulation and the pooling of blood produce a decreased circulating volume that triggers neurohormonal responses.

SYMPATHETIC NERVOUS SYSTEM, CARDIAC OUTPUT AND THE BARORECEPTORS REFLEX

A low circulating volume (low blood pressure) is detected by the baroreceptors or pressoreceptors located mainly in the internal carotid artery (carotid sinus) and ascending aorta. They are also found in small quantities in the wall of almost every large artery in the neck and thorax[1]. These are pressure sensitive receptors which are physiologically inactivated when the aforementioned arteries become less stretched as a result of low blood pressure (Figure 1)[52]. Consequently, the carotid and aortic baroreceptors signal conduction to the cardio and vasomotor regulatory centers in the medulla oblongata, *via* the glossopharyngeal and vagus nerves, respectively, is subdued. Thus, the cardio-

vasomotor regulatory centers become more active and sequentially induce the sympathetic nervous system to become active while suppressing parasympathetic (vagus nerve) stimulation to the heart. The sympathetic nervous system through the cardiac accelerator nerve increases the heart rate and cardiac output. Furthermore, the adrenal medulla, under the influence of the sympathetic nervous system, releases both adrenaline and noradrenaline. This eventually leads to an increase in mean arterial pressure through increased cardiac output and peripheral vascular resistance by acting on adrenergic receptors. The sympathetic response is further augmented by the activation of the RAAS and the release of vasopressin[53]. Unfortunately, those complex neurohormonal compensatory responses to low blood pressure are temporary and the whole system that comprises the baroreceptors, RAAS and vasopressin becomes adapted and mal-responsive to the low circulating volume stimulus within 48-72 h[4]. Consequently all the above outlined responses are reversed resulting in profound hypotension, renal hypoperfusion and worsening renal failure.

RENIN ANGIOTENSIN ALDOSTERONE SYSTEM

Arterial hypotension and reduced blood flow to the kidney results in decreased sodium delivery to the macula densa which in turn causes the release of renin from the juxtaglomerular apparatus[54]. The renin release is the rate determining step for the activation of the RAAS. The activation of RAAS causes sodium and water reabsorption and vasoconstriction of the renal arteries (Figure 1)[55]. These compensatory mechanisms maintain effective circulation in the early stages of the disease (compensated) but with time their effects eventually become deleterious and lead to the development of complications. These include ascites, hyperkinetic circulation, nitric oxide release, renal failure, and increased venous capacitance with decreased venous compliance. Essentially all these culminate to form the bases of HRS[55].

Liver cirrhosis has been shown to be associated with activation of RAAS but also an increase in vasopressin secretion[56]. The activation of RAAS may contribute to decreased renal perfusion[56]. Importantly, albumin administration has been shown to be associated with a decrease in plasma renin level[57]. In contrast, administration of vasopressin does not substantially change renal perfusion, though it induces splanchnic vasoconstriction[57]. This may explain the potential benefit of administration of vasopressin and its analogues in HRS. The progression of PHT is associated with an increase in vasodilation of the splanchnic circulation and marked resistant to vasopressin. It is likely that further research is needed to address the interaction between RAAS and vasopressin.

In addition, the activation of the RAAS has an important role in hemodynamic regulation in the liver as well as proliferation of vascular smooth muscle cells and fibrosis through 2 well coordinated complementary pathways: (1) vasoconstriction/proliferative pathway, incorporating the angiotensin converting enzyme (ACE), angiotensin (Ang) Ⅱ-Ang Ⅱ type 1 (AT1) receptor; and (2) a counter- regulatory vasodilatation/antiproliferative pathway, involving ACE2-Ang-(1-7)-Mas receptor[58]. It is important to point out that the ACE, vasoconstriction/proliferation pathway induces contraction and proliferation of hepatic stellate cells, which lead to fibrosis[58]. In contrast, activation of ACE2-Ang-(1-7) is not only associated with liver cirrhosis-induced splanchnic and systemic vasodilatation but also anti-fibrotic effects[58].

Vilas-Boas *et al*[59] suggested that the administration of a combination of propranolol and ACE inhibitor or AT1 receptor blocker may have potential benefit in cirrhotic patients. Furthermore, administration of propranolol *per se* has been shown to be associated with unfavorable consequences on the 2 main RAAS components, Ang Ⅱ and Ang(1-7), in the splanchnic and peripheral circulation[59]. In contrast, inhibition of RAAS by ACE inhibitors and AT1 receptor blockers has been shown to be associated with potential benefit in slowing progression of liver fibrosis and even cardiac and renal fibrosis[60].

Interestingly, it has been hypothesized that the effect of Ang Ⅱ dominates in advanced liver disease while the effect of Ang(1-7) dominates in moderate liver disease[61]. Therefore, the RAAS can be viewed as a dual system that leads to vasoconstriction, fibrosis, vasodilatation and antifibrosis. However, further research is urgently needed to establish a therapeutic benefit of the dual function of the RAAS in targeting liver disease and preventing fibrosis in humans and, in particular, in the management of HRS.

DETRIMENTAL EFFECTS OF THE SYMPATHETIC NERVOUS SYSTEM AND RAAS

These wonderful compensatory mechanisms with prolonged activation result in even more increased sodium and water retention with a subsequent increase in total circulatory volume. RAAS, with its overall effect of salt and fluid retention contributes to the development of ascites usually in the presence of PHT. In addition, splanchnic vasodilation appears to be one of main culprits in the formation of ascites[50]. Ascites develops when there is increased sinusoidal pressure which forces fluid to leak into the abdominal cavity[1]. Ascites further fuels the constant activation of the RAAS and the sympathetic nervous system which fail to maintain effective circulating volume.

The lack of response to neurohormonal mechanisms in the latter stages of cirrhosis may be due to several factors: (1) nitric oxide-mediated pooling of blood in splanchnic vascular bed; (2) hyporesponsiveness of splanchnic vasodilation to neurohormonal mechanism; (3) ascites; and (4) downregulation of receptors.

Eventually the pooled blood in the splanchnic circulation cannot be utilized fully in the presence of ineffective

circulation. This is on account of the fact that the reservoir of blood in the splanchnic circulation continues to increase and is mediated by locally-produced nitric oxide. A recent study by Li *et al*[62] demonstrated that nitric oxide caused changes in mesenteric venous capacitance and increased pooling of blood in rats with liver cirrhosis. In this study it was shown that the cirrhotic rats had a nitric oxide-mediated increase in venous capacitance and decrease in compliance. Nitric oxide is produced locally by eNOS which is activated by the high shear stress in the splanchnic vascular endothelium, which is caused by the increased splanchnic blood flow. In both animal and human studies, nitric oxide appears to be the main orchestrator of the splanchnic vasodilation that facilitates the pooling of blood[63,64], *via* its direct action on the vascular smooth muscles.

Additionally, it is widely accepted that nitric oxide antagonizes the sympathetic and RAAS-driven vasoconstriction thus inducing vasodilatation of the splanchnic vascular bed. Accordingly, ineffective circulation continues to trigger neurohormonal responses, though in the latter stages of the disease there is hyporesponsiveness to these compensatory mechanisms. In the early stages of compensated liver cirrhosis, the neurohormonal activation is able to overcome the splanchnic vasodilation and maintain an acceptable circulating volume. However with decompensated liver cirrhosis; and increased PHT, the hyporesponsiveness is often an indicator of progression towards the end stage.

It is suggested that the hyporesponsiveness may be due to desensitization and downregulation of adrenergic receptors. In cirrhotic rats there appears to be β-adrenergic receptor hyporesponsiveness to catecholamines[65]. This concept is not new; previous studies looking at heart failure have shown that prolonged activation of the neurohormonal response leads to a downregulation of β-adrenergic receptors[66]. The hyporesponsiveness of the myocardium to catecholamine stimulation that is seen in cirrhosis is termed cirrhotic cardiomyopathy[67].

FUNCTIONAL RENAL FAILURE

The renal system, through autoregulation, maintains a physiologically acceptable GFR over a range of blood pressures. Autoregulation consists of myogenic and neurohormonal responses. The development of a hyperkinetic circulation results in renal hypoperfusion despite increased total circulating volume. Initially with low blood pressure, the kidneys respond with smooth muscle contraction in the vessels (myogenic response), which helps to maintain the perfusion pressure[1]. This response alone is not adequate, therefore the sympathetic nervous system and RAAS are activated and subsequently lead to renal vasoconstriction. The peripheral vasodilatation that occurs is perceived by the kidneys as a hypovolemia, that continues to promote renal vasoconstriction. The resultant effect is reduced GFR, oligo-anuria, raised plasma creatinine and the development of hepatorenal failure[2].

Renal biopsies in HRS patients have shown remarkably normal renal histology architecture despite the dismal renal function. The findings have led to the term "reversible functional renal failure" being coined[22]. The primary problem causing renal failure is liver cirrhosis and this functional renal failure can be reversed with liver transplantation. Kidney transplants from palliated patients with HRS into those with intrinsic renal failure, have remarkably been show to have reversed to normal renal function[68,69]. This further supports the concept of reversibility of functional renal failure in patients with HRS. The definitive treatment for HRS is liver transplantation; however with increasing shortages of organ donation and with long waiting lists, patients are more often than not succumbing to the dismal prognosis of HRS. Nevertheless treatment with vasopressin analogues, in particular terlipressin, has been shown to reverse the renal failure and can be used as a bridge to definitive treatment (liver transplant).

TERLIPRESSIN AS POTENTIAL TREATMENT FOR HRS

Terlipressin, an analogue of vasopressin, is used as potential treatment of HRS. In this review we focus on clinical trials and their strengths and weaknesses. It is worth mentioning that in the majority of these studies, terlipressin was used in combination with albumin. Furthermore, other trials compared the effect of terlipressin with noradrenaline. We included clinical trials with evidence-based medicine. Hence, the subsequent discussion will focus on the impact of terlipressin *vs* placebo and terlipressin *vs* noradrenaline with and without albumin.

Terlipressin and clinical trials

Terlipressin (without albumin) *vs* placebo: Hadengue *et al*[70] carried out a double-blind, crossover, randomized study in 9 patients with type 1 HRS. The patients received terlipressin (2 mg/d for 2 d) and a placebo for 2 d in a randomized order. Terlipressin administration significantly increased creatinine clearance and urine output, but did not significantly change urinary sodium concentration. Urinary sodium excretion was not significantly different after placebo administration or terlipressin administration. Terlipressin administration significantly decreased plasma concentrations of renin and aldosterone but not atrial natriuretic peptide levels, and these biochemical changes were not seen in the placebo group. The study by Solanki *et al*[19] was a randomized, controlled, single-blind trial. They assigned 24 consecutive patients with HRS to treatment with terlipressin 1 mg iv at 12 h intervals (group A, $n = 12$) or placebo at 12 h intervals (group B, $n = 12$). The end-point of the study was improvement in renal function defined as reversal of HRS and survival at 15 d. Terlipressin administration was shown to be associated with an improvement in parameters of renal function, mean arterial blood pressure and importantly reversal of HRS in 5 of the 12 patients in group A.

Table 1 Summary of effect of terlipressin associated with albumin on hepatorenal syndrome

Study	Main outcome
Sanyal *et al*[72], 2008	Terlipressin administration with albumin shown to be associated with improvement in renal function and appeared superior to placebo in reversing type 1 HRS
Martín-Llahí *et al*[73], 2008	Terlipressin administration with albumin shown to be associated with improvement in renal function in patient with liver cirrhosis and type 1 HRS, without significant impact on 3-mo survival
Neri *et al*[74], 2008	Terlipressin administration with albumin shown to be associated with improvement in renal function in patients with type 1 HRS and also a high probability of survival
Uriz *et al*[17], 2000	Terlipressin associated with albumin appeared to be a safe and effective treatment of HRS and decreased the frequent ischemic complications associated with terlipressin treatment alone. Terlipressin associated with albumin therapy was associated with marked improvement in renal function, reversal of HRS and improvement in circulatory function with an increase in mean arterial blood pressure

HRS: Hepatorenal syndrome.

Interestingly, Testro *et al*[71] reviewed outcomes of 69 patients treated with terlipressin between 2001 and 2005. Their findings showed that 49 episodes (71%) of HRS were type 1, and 20 episodes (29%) were type 2. Forty-one (59.4%) patients responded to terlipressin. Twenty-one (30.4%) patients survived; 17 (81%) had type 1 HRS while 4 (19%) had type 2 HRS ($P = 0.27$). The only factor predicting transplant-free survival was type 1 HRS. No patients with type 2 HRS survived without transplantation ($P = 0.02$). These trials clearly showed the potential benefit of administration of terlipressin in individuals with HRS. However, Terlipressin administration was associated with minimal reversible ischemic events e.g. crampy abdominal pain and cardiac arrhythmias. We suggest that randomized clinical trials are now warranted.

Terlipressin (with albumin) *vs* placebo: Interestingly, concomitant administration of terlipressin and albumin is shown to be associated with better clinical outcomes. A summary of studies that used terlipressin (with albumin) *vs* placebo is provided in Table 1.

Terlipressin *vs* noradrenaline: The use of noradrenaline, a cheap and widely available drug, in the management of HRS was shown to be as effective as terlipressin but associated with increased risk of ischemic events. Data from an unblinded, pilot study suggested that noradrenaline was as effective and safe as terlipressin in patients with HRS. Twenty-two consecutive cirrhotic patients with HRS (9 with type 1 HRS; 13 with type 2 HRS) were randomly assigned to treatment with noradrenaline (0.1-0.7 μg/kg per minute) and albumin (10 patients) or with terlipressin (1-2 mg/4 h) and albumin (12 patients). Treatment was administered until HRS reversal or for a maximum of 2 wk. Reversal of HRS was observed in 7 of the 10 patients (70%) treated with noradrenaline and in 10 of the 12 patients (83%) treated with terlipressin. Treatment led, in both groups, to a significant improvement in renal and circulatory function; no patient developed signs of myocardial ischemia[75]. Sharma *et al*[76] reported similar beneficial results in treating HRS with noradrenaline, however, they also reported that 2 patients had ventricular ectopies with noradrenaline. We suggest that further studies are urgently needed to evaluate the use of noradrenaline as potential treatment for HRS.

Terlipressin and meta-analyses

Several meta-analyses have been conducted to determine the effect of terlipressin in HRS with regard to the duration of treatment, infusion of albumin and comparison with noradrenaline. Dobre *et al*[77] concluded in their meta-analysis that terlipressin administration was associated with improvement in HRS reversal and that noradrenaline has the same effect as terlipressin in improving surrogate markers of HRS. Sagi *et al*[11] showed that the risk ratio for reversal in type 1 HRS with terlipressin therapy was 3.66 [95% confidence interval (CI): 2.15-6.23]. Recurrence of HRS was low (8%). Serious side effects requiring discontinuation of therapy were seen only in 6.8% of patients on terlipressin therapy. There was a trend towards improved transplant-free survival at 90 d in the Terlipressin group (relative risk 1.86, 95% CI: 1.0-3.4, $P = 0.05$). The conclusion of their meta-analysis was that terlipressin is effective in reversing HRS type 1 and recurrence of HRS is rare with at least 14 d of therapy and associated with an increased survival. Importantly, Fabrizi *et al*[78] showed in their meta-analysis that discontinuation of terlipressin therapy was associated with a significant increase in the number of relapses. Furthermore, Fabrizi *et al*[79], in another meta-analysis, showed that terlipressin was more effective in reversing HRS than placebo without apparent impact of terlipressin on survival in HRS patients. This may again suggest the need for large clinical trials addressing the impact of terlipressin in HRS patient survival. Interestingly, administration of albumin with terlipressin showed a reduction in mortality in type 1 HRS[10]. Therefore, the current evidence suggests that terlipressin can have a potential benefit in treating HRS and that an improvement in survival can be achieved with its concomitant administration with albumin.

CONCLUSION

HRS continues to be a challenging task to manage following chronic liver cirrhosis. The grave prognosis and the short survival times have fuelled great interest in clinical

trials; its reversibility creates scope for prolonging both survival and quality of life. The current literature reviewed has further re-enforced terlipressin as a potential first-line treatment in HRS**.** Terlipressin has so far shown to increase survival rates and reverse functional renal failure. The increased neurohormonal response, especially of the RAAS, has been decreased with the administration of terlipressin. This subsequently improves circulatory dysfunction and lowers plasma creatinine levels near to baseline values. The effects of nitric oxide, which is a factor in the deleterious neurohormonal response, appears to be overcome by the administration of terlipressin through unknown mechanisms.

In addition, terlipressin has few adverse side effects, which allows patients to continue with treatment in order to achieve desirable effects. At present however, we acknowledge that there are a limited number of randomized, controlled studies carried out on terlipressin and therefore there is a real need for large multi-centered trials to be carried out. We recommend that terlipressin, with concomitant administration of albumin, may be the first line treatment in the management of HRS.

REFERENCES

1 **Arroyo V**, Ginès P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, Reynolds TB, Ring-Larsen H, Schölmerich J. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. *Hepatology* 1996; **23**: 164-176

2 **Wadei HM**, Mai ML, Ahsan N, Gonwa TA. Hepatorenal syndrome: pathophysiology and management. *Clin J Am Soc Nephrol* 2006; **1**: 1066-1079

3 **Salerno F**, Gerbes A, Ginès P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Gut* 2007; **56**: 1310-8

4 **Ginès P**, Guevara M, Arroyo V, Rodés J. Hepatorenal syndrome. *Lancet* 2003; **362**: 1819-1827

5 **Simonson MS**. Endothelins: multifunctional renal peptides. *Physiol Rev* 1993; **73**: 375-411

6 **Colle I**, Durand F, Pessione F, Rassiat E, Bernuau J, Barrière E, Lebrec D, Valla DC, Moreau R. Clinical course, predictive factors and prognosis in patients with cirrhosis and type 1 hepatorenal syndrome treated with Terlipressin: a retrospective analysis. *J Gastroenterol Hepatol* 2002; **17**: 882-888

7 **Watt K**, Uhanova J, Minuk GY. Hepatorenal syndrome: diagnostic accuracy, clinical features, and outcome in a tertiary care center. *Am J Gastroenterol* 2002; **97**: 2046-2050

8 **Wong F**, Pantea L, Sniderman K. Midodrine, octreotide, albumin, and TIPS in selected patients with cirrhosis and type 1 hepatorenal syndrome. *Hepatology* 2004; **40**: 55-64

9 **Péron JM**, Bureau C, Gonzalez L, Garcia-Ricard F, de Soyres O, Dupuis E, Alric L, Pourrat J, Vinel JP. Treatment of hepatorenal syndrome as defined by the international ascites club by albumin and furosemide infusion according to the central venous pressure: a prospective pilot study. *Am J Gastroenterol* 2005; **100**: 2702-2707

10 **Gluud LL**, Christensen K, Christensen E, Krag A. Systematic review of randomized trials on vasoconstrictor drugs for hepatorenal syndrome. *Hepatology* 2010; **51**: 576-584

11 **Sagi SV**, Mittal S, Kasturi KS, Sood GK. Terlipressin therapy for reversal of type 1 hepatorenal syndrome: a meta-analysis of randomized controlled trials. *J Gastroenterol Hepatol* 2010; **25**: 880-885

12 **Ortega R**, Ginès P, Uriz J, Cárdenas A, Calahorra B, De Las Heras D, Guevara M, Bataller R, Jiménez W, Arroyo V, Rodés J. Terlipressin therapy with and without albumin for patients with hepatorenal syndrome: results of a prospective, nonrandomized study. *Hepatology* 2002; **36**: 941-948

13 **Gülberg V**, Bilzer M, Gerbes AL. Long-term therapy and retreatment of hepatorenal syndrome type 1 with ornipressin and dopamine. *Hepatology* 1999; **30**: 870-875

14 **Guevara M**, Ginès P, Fernández-Esparrach G, Sort P, Salmerón JM, Jiménez W, Arroyo V, Rodés J. Reversibility of hepatorenal syndrome by prolonged administration of ornipressin and plasma volume expansion. *Hepatology* 1998; **27**: 35-41

15 **Lenz K**, Hörtnagl H, Druml W, Grimm G, Laggner A, Schneeweisz B, Kleinberger G. Beneficial effect of 8-ornithin vasopressin on renal dysfunction in decompensated cirrhosis. *Gut* 1989; **30**: 90-96

16 **Obritsch MD**, Bestul DJ, Jung R, Fish DN, MacLaren R. The role of vasopressin in vasodilatory septic shock. *Pharmacotherapy* 2004; **24**: 1050-1063

17 **Uriz J**, Ginès P, Cárdenas A, Sort P, Jiménez W, Salmerón JM, Bataller R, Mas A, Navasa M, Arroyo V, Rodés J. Terlipressin plus albumin infusion: an effective and safe therapy of hepatorenal syndrome. *J Hepatol* 2000; **33**: 43-48

18 **Alessandria C**, Venon WD, Marzano A, Barletti C, Fadda M, Rizzetto M. Renal failure in cirrhotic patients: role of terlipressin in clinical approach to hepatorenal syndrome type 2. *Eur J Gastroenterol Hepatol* 2002; **14**: 1363-1368

19 **Solanki P**, Chawla A, Garg R, Gupta R, Jain M, Sarin SK. Beneficial effects of terlipressin in hepatorenal syndrome: a prospective, randomized placebo-controlled clinical trial. *J Gastroenterol Hepatol* 2003; **18**: 152-156

20 **Moreau R**, Durand F, Poynard T, Duhamel C, Cervoni JP, Ichaï P, Abergel A, Halimi C, Pauwels M, Bronowicki JP, Giostra E, Fleurot C, Gurnot D, Nouel O, Renard P, Rivoal M, Blanc P, Coumaros D, Ducloux S, Levy S, Pariente A, Perarnau JM, Roche J, Scribe-Outtas M, Valla D, Bernard B, Samuel D, Butel J, Hadengue A, Platek A, Lebrec D, Cadranel JF. Terlipressin in patients with cirrhosis and type 1 hepatorenal syndrome: a retrospective multicenter study. *Gastroenterology* 2002; **122**: 923-930

21 **Papper S**. Hepatorenal syndrome. *Contrib Nephrol* 1980; **23**: 55-74

22 **Hecker R**, Sherlock S. Electrolyte and circulatory changes in terminal liver failure. *Lancet* 1956; **271**: 1121-1125

23 **Epstein M**, Berk DP, Hollenberg NK, Adams DF, Chalmers TC, Abrams HL, Merrill JP. Renal failure in the patient with cirrhosis. The role of active vasoconstriction. *Am J Med* 1970; **49**: 175-185

24 **Platt JF**, Ellis JH, Rubin JM, Merion RM, Lucey MR. Renal duplex Doppler ultrasonography: a noninvasive predictor of kidney dysfunction and hepatorenal failure in liver disease. *Hepatology* 1994; **20**: 362-369

25 **Bosch J**, Abraldes JG, Groszmann R. Current management of portal hypertension. *J Hepatol* 2003; **38** Suppl 1: S54-S68

26 **Wiest R**, Groszmann RJ. The paradox of nitric oxide in cirrhosis and portal hypertension: too much, not enough. *Hepatology* 2002; **35**: 478-491

27 **Pinzani M**, Gentilini P. Biology of hepatic stellate cells and their possible relevance in the pathogenesis of portal hypertension in cirrhosis. *Semin Liver Dis* 1999; **19**: 397-410

28 **Benoit JN**, Womack WA, Hernandez L, Granger DN. "Forward" and "backward" flow mechanisms of portal hypertension. Relative contributions in the rat model of portal vein stenosis. *Gastroenterology* 1985; **89**: 1092-1096

29 **Vorobioff J**, Bredfeldt JE, Groszmann RJ. Increased blood flow through the portal system in cirrhotic rats. *Gastroenterology* 1984; **87**: 1120-1126

30 **Vorobioff J**, Bredfeldt JE, Groszmann RJ. Hyperdynamic circulation in portal-hypertensive rat model: a primary factor for maintenance of chronic portal hypertension. *Am J Physiol* 1983; **244**: G52-G57

31 **Kim MY**, Baik SK. [Hyperdynamic circulation in patients with liver cirrhosis and portal hypertension] *Korean J Gastroenterol* 2009; **54**: 143-148
32 **Knotek M**, Rogachev B, Schrier RW. Update on peripheral arterial vasodilation, ascites and hepatorenal syndrome in cirrhosis. *Can J Gastroenterol* 2000; **14** Suppl D: 112D-121D
33 **Moreau R**, Lebrec D. Endogenous factors involved in the control of arterial tone in cirrhosis. *J Hepatol* 1995; **22**: 370-376
34 **Martin PY**, Ginès P, Schrier RW. Nitric oxide as a mediator of hemodynamic abnormalities and sodium and water retention in cirrhosis. *N Engl J Med* 1998; **339**: 533-541
35 **Palmer RM**, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; **333**: 664-666
36 **Ros J**, Clària J, Jiménez W, Bosch-Marcé M, Angeli P, Arroyo V, Rivera F, Rodés J. Role of nitric oxide and prostacyclin in the control of renal perfusion in experimental cirrhosis. *Hepatology* 1995; **22**: 915-920
37 **Adams LB**, Hibbs JB Jr, Taintor RR, Krahenbuhl JL. Microbiostatic effect of murine-activated macrophages for Toxoplasma gondii. Role for synthesis of inorganic nitrogen oxides from L-arginine. *J Immunol* 1990; **144**: 2725-2729
38 **Moncada S**, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; **43**: 109-142
39 **Stuehr DJ**, Marletta MA. Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide. *Proc Natl Acad Sci USA* 1985; **82**: 7738-7742
40 **Zhou L**, Zhu DY. Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. *Nitric Oxide* 2009; **20**: 223-230
41 **Ni J**, McLoughlin RM, Brodovitch A, Moulin P, Brouckaert P, Casadei B, Feron O, Topley N, Balligand JL, Devuyst O. Nitric oxide synthase isoforms play distinct roles during acute peritonitis. *Nephrol Dial Transplant* 2010; **25**: 86-96
42 **Förstermann U**, Boissel JP, Kleinert H. Expressional control of the 'constitutive' isoforms of nitric oxide synthase (NOS I and NOS III). *FASEB J* 1998; **12**: 773-790
43 **Togashi H**, Sakuma I, Yoshioka M, Kobayashi T, Yasuda H, Kitabatake A, Saito H, Gross SS, Levi R. A central nervous system action of nitric oxide in blood pressure regulation. *J Pharmacol Exp Ther* 1992; **262**: 343-347
44 **Sakuma I**, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Levi R. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? *Circ Res* 1992; **70**: 607-611
45 **el Karib AO**, Sheng J, Betz AL, Malvin RL. The central effects of a nitric oxide synthase inhibitor (N omega-nitro-L-arginine) on blood pressure and plasma renin. *Clin Exp Hypertens* 1993; **15**: 819-832
46 **Geller DA**, Lowenstein CJ, Shapiro RA, Nussler AK, Di Silvio M, Wang SC, Nakayama DK, Simmons RL, Snyder SH, Billiar TR. Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes. *Proc Natl Acad Sci USA* 1993; **90**: 3491-3495
47 **Kobzik L**, Bredt DS, Lowenstein CJ, Drazen J, Gaston B, Sugarbaker D, Stamler JS. Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localization. *Am J Respir Cell Mol Biol* 1993; **9**: 371-377
48 **Pollock JS**, Nakane M, Buttery LD, Martinez A, Springall D, Polak JM, Förstermann U, Murad F. Characterization and localization of endothelial nitric oxide synthase using specific monoclonal antibodies. *Am J Physiol* 1993; **265**: C1379-C1387
49 **Wu KK**. Regulation of endothelial nitric oxide synthase activity and gene expression. *Ann N Y Acad Sci* 2002; **962**: 122-130
50 **Schrier RW**, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; **8**: 1151-1157
51 **Iwao T**, Oho K, Sakai T, Tayama C, Sato M, Nakano R, Yamawaki M, Toyonaga A, Tanikawa K. Splanchnic and extrasplanchnic arterial hemodynamics in patients with cirrhosis. *J Hepatol* 1997; **27**: 817-823
52 **Møller S**, Henriksen JH. Circulatory abnormalities in cirrhosis with focus on neurohumoral aspects. *Semin Nephrol* 1997; **17**: 505-519
53 **Arroyo V**, Colmenero J. Ascites and hepatorenal syndrome in cirrhosis: pathophysiological basis of therapy and current management. *J Hepatol* 2003; **38** Suppl 1: S69-S89
54 **Peti-Peterdi J**, Harris RC. Macula densa sensing and signaling mechanisms of renin release. *J Am Soc Nephrol* 2010; **21**: 1093-1096
55 **Kashani A**, Landaverde C, Medici V, Rossaro L. Fluid retention in cirrhosis: pathophysiology and management. *QJM* 2008; **101**: 71-85
56 **Henriksen JH**, Moller S. Liver cirrhosis and arterial hypertension. *World J Gastroenterol* 2006; **12**: 678-685
57 **Schrier RW**. Renin-angiotensin in preascitic cirrhosis: evidence for primary peripheral arterial vasodilation. *Gastroenterology* 1998; **115**: 489-491
58 **Pereira RM**, dos Santos RA, da Costa Dias FL, Teixeira MM, Simões e Silva AC. Renin-angiotensin system in the pathogenesis of liver fibrosis. *World J Gastroenterol* 2009; **15**: 2579-2586
59 **Vilas-Boas WW**, Ribeiro-Oliveira A Jr, Ribeiro Rda C, Vieira RL, Almeida J, Nadu AP, Simões e Silva AC, Santos RA. Effect of propranolol on the splanchnic and peripheral renin angiotensin system in cirrhotic patients. *World J Gastroenterol* 2008; **14**: 6824-6830
60 **Jonsson JR**, Clouston AD, Ando Y, Kelemen LI, Horn MJ, Adamson MD, Purdie DM, Powell EE. Angiotensin-converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. *Gastroenterology* 2001; **121**: 148-155
61 **Vilas-Boas WW**, Ribeiro-Oliveira A Jr, Pereira RM, Ribeiro Rda C, Almeida J, Nadu AP, Simões e Silva AC, dos Santos RA. Relationship between angiotensin-(1-7) and angiotensin II correlates with hemodynamic changes in human liver cirrhosis. *World J Gastroenterol* 2009; **15**: 2512-2519
62 **Li Y**, Liu H, Gaskari SA, Tyberg JV, Lee SS. Altered mesenteric venous capacitance and volume pooling in cirrhotic rats are mediated by nitric oxide. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G252-G259
63 **Ginès P**, Cárdenas A, Arroyo V, Rodés J. Management of cirrhosis and ascites. *N Engl J Med* 2004; **350**: 1646-1654
64 **Hernández-Guerra M**, García-Pagán JC, Bosch J. Increased hepatic resistance: a new target in the pharmacologic therapy of portal hypertension. *J Clin Gastroenterol* 2005; **39**: S131-S137
65 **Lee SS**, Marty J, Mantz J, Samain E, Braillon A, Lebrec D. Desensitization of myocardial beta-adrenergic receptors in cirrhotic rats. *Hepatology* 1990; **12**: 481-485
66 **Ruffolo RR Jr**, Kopia GA. Importance of receptor regulation in the pathophysiology and therapy of congestive heart failure. *Am J Med* 1986**;** **80**: 67-72
67 **Liu H**, Gaskari SA, Lee SS. Cardiac and vascular changes in cirrhosis: pathogenic mechanisms. *World J Gastroenterol* 2006; **12**: 837-842
68 **Koppel MH**, Coburn JW, Mims MM, Goldstein H, Boyle JD, Rubini ME. Transplantation of cadaveric kidneys from patients with hepatorenal syndrome. Evidence for the functionalnature of renal failure in advanced liver disease. *N Engl J Med* 1969; **280**: 1367-1371
69 **Iwatsuki S**, Popovtzer MM, Corman JL, Ishikawa M, Putnam CW, Katz FH, Starzl TE. Recovery from "hepatorenal syndrome" after orthotopic liver transplantation. *N Engl J Med* 1973; **289**: 1155-1159
70 **Hadengue A**, Gadano A, Moreau R, Giostra E, Durand F, Valla D, Erlinger S, Lebrec D. Beneficial effects of the 2-day

administration of terlipressin in patients with cirrhosis and hepatorenal syndrome. *J Hepatol* 1998; **29**: 565-570
71 **Testro AG**, Wongseelashote S, Angus PW, Gow PJ. Long-term outcome of patients treated with terlipressin for types 1 and 2 hepatorenal syndrome. *J Gastroenterol Hepatol* 2008; **23**: 1535-1540
72 **Sanyal AJ**, Boyer T, Garcia-Tsao G, Regenstein F, Rossaro L, Appenrodt B, Blei A, Gülberg V, Sigal S, Teuber P. A randomized, prospective, double-blind, placebo-controlled trial of terlipressin for type 1 hepatorenal syndrome. *Gastroenterology* 2008; **134**: 1360-1368
73 **Martín-Llahí M**, Pépin MN, Guevara M, Díaz F, Torre A, Monescillo A, Soriano G, Terra C, Fábrega E, Arroyo V, Rodés J, Ginès P. Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. *Gastroenterology* 2008; **134**: 1352-1359
74 **Neri S**, Pulvirenti D, Malaguarnera M, Cosimo BM, Bertino G, Ignaccolo L, Siringo S, Castellino P. Terlipressin and albumin in patients with cirrhosis and type I hepatorenal syndrome. *Dig Dis Sci* 2008; **53**: 830-835
75 **Alessandria C**, Ottobrelli A, Debernardi-Venon W, Todros L, Cerenzia MT, Martini S, Balzola F, Morgando A, Rizzetto M, Marzano A. Noradrenalin vs terlipressin in patients with hepatorenal syndrome: a prospective, randomized, unblinded, pilot study. *J Hepatol* 2007; **47**: 499-505
76 **Sharma P**, Kumar A, Shrama BC, Sarin SK. An open label, pilot, randomized controlled trial of noradrenaline versus terlipressin in the treatment of type 1 hepatorenal syndrome and predictors of response. *Am J Gastroenterol* 2008; **103**: 1689-1697
77 **Dobre M**, Demirjian S, Sehgal AR, Navaneethan SD. Terlipressin in hepatorenal syndrome: a systematic review and meta-analysis. *Int Urol Nephrol* 2010; Epub ahead of print
78 **Fabrizi F**, Dixit V, Martin P. Meta-analysis: terlipressin therapy for the hepatorenal syndrome. *Aliment Pharmacol Ther* 2006; **24**: 935-944
79 **Fabrizi F**, Dixit V, Messa P, Martin P. Terlipressin for hepatorenal syndrome: A meta-analysis of randomized trials. *Int J Artif Organs* 2009; **32**: 133-140

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EDITORIAL

Growth factor- and cytokine-driven pathways governing liver stemness and differentiation

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Abstract

Liver is unique in its capacity to regenerate in response to injury or tissue loss. Hepatocytes and other liver cells are able to proliferate and repopulate the liver. However, when this response is impaired, the contribution of hepatic progenitors becomes very relevant. Here, we present an update of recent studies on growth factors and cytokine-driven intracellular pathways that govern liver stem/progenitor cell expansion and differentiation, and the relevance of these signals in liver development, regeneration and carcinogenesis. Tyrosine kinase receptor signaling, in particular, c-Met, epidermal growth factor receptors or fibroblast growth factor receptors, contribute to proliferation, survival and differentiation of liver stem/progenitor cells. Different evidence suggests a dual role for the transforming growth factor (TGF)-β signaling pathway in liver stemness and differentiation. On the one hand, TGF-β mediates progression of differentiation from a progenitor stage, but on the other hand, it contributes to the expansion of liver stem cells. Hedgehog family ligands are necessary to promote hepatoblast proliferation but need to be shut off to permit subsequent hepatoblast differentiation. In the same line, the Wnt family and β-catenin/T-cell factor pathway is clearly involved in the maintenance of liver stemness phenotype, and its repression is necessary for liver differentiation during development. Collectively, data indicate that liver stem/progenitor cells follow their own rules and regulations. The same signals that are essential for their activation, expansion and differentiation are good candidates to contribute, under adequate conditions, to the paradigm of transformation from a pro-regenerative to a pro-tumorigenic role. From a clinical perspective, this is a fundamental issue for liver stem/progenitor cell-based therapies.

Key words: Hepatocyte growth factor; Epidermal growth factor; Fibroblast growth factor; Transforming growth factor-β; Hedgehog and β-catenin; Liver; Stem cell

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INTRODUCTION

Liver is unique in its capacity to regenerate in response

to injury or tissue loss. Following two-thirds partial hepatectomy, hepatocytes exit the G0 phase of the cell cycle and synchronously re-enter the cell cycle to regenerate the liver mass completely in 6-7 d in rodents, or 3-4 mo in humans[1-4]. Liver stem/progenitor cells do not seem to be required for this process. However, when this response is impaired, as in the case of a hepatocyte-selective proliferative defect or acute liver failure, the contribution of liver progenitors becomes much more relevant[5,6]. A population of small cells with a low cytoplasmic/nuclear ratio that arose from a small number of portal cells, not from hepatocytes, were first observed in rodents and became known as "oval cells"[7,8]. Although several lines of evidence suggest that oval cells derive from the biliary compartment, other origins have been also suggested[4].

Significant work in the past few years has focused on the signaling pathways, as well as cell-cell interactions, that control the initial proliferation/expansion and the terminal differentiation of liver stem/progenitor cells. Oval cells express MET, the receptor of the hepatocyte growth factor (HGF). The superabundance of HGF-producing cells in the immediate vicinity of oval cell proliferation and differentiation suggests that this growth factor is involved in all aspects of stem cell behavior, proliferation, migration, and differentiation, through a paracrine mechanism[9]. Oval cells also express receptors for epidermal growth factor (EGF)-like ligands, and *in vivo* infusion of a combination of HGF and EGF enhance the mitogenic response of oval cells after administration of 2-acetylaminofluorene[10], which reveals the relevance of both growth factors in liver stem/progenitor cell biology. Additionally, different studies have revealed that oval cells also respond to other growth factors in an autocrine/paracrine manner[11]. The transforming growth factor (TGF)-β family of cytokines play a relevant role in the maintenance of embryonic stem cell identity, and it has been shown that the specification of pancreas and liver progenitors is restricted by the TGF-β pathway[12]. All these growth factors and cytokines might modulate not only proliferation of liver stem/progenitor cells, but also cell death, as well as contributing to their terminal differentiation.

This review gives an update on recent relevant studies of the growth factors and cytokine-driven intracellular pathways that govern liver stem/progenitor cell expansion and differentiation, and the relevance of these signals in liver development, regeneration and carcinogenesis.

TYROSINE KINASE RECEPTOR-MEDIATED SIGNALING PATHWAYS

HGF

HGF was firstly identified in the 1980s as a potent mitogen for hepatocytes[13-15]. A factor secreted by fibroblasts and smooth muscle cells was discover separately, which promoted epithelial cell scattering[16]. Later studies unraveled that HGF and scatter factor were indistinguishable[17,18]. HGF is a growth factor that induces a wide range of biological activities, including stimulation of proliferation, migration, morphogenesis, and survival of a variety of cell types[19-24], which plays a major role in tissue formation and homeostasis. HGF acts through binding to its tyrosine kinase receptor, Met. Ligand-receptor binding results in autophosphorylation of the receptor in specific tyrosine residues located in the C-terminal domain, and subsequent phosphorylation/activation of multiple adapter and signal transducing proteins, such as growth factor receptor-bound protein 2 (Grb2)/Sos, Ras-mitogen-activated protein kinase, Grb2-associated binding protein 1 (Gab1), phosphoinositide 3-kinase (PI3K), phospholipase C-γ, p38, and signal transducer and activator of transcription (STAT)-3, among others, which mediate the biological activities of HGF/c-Met[25,26]. For decades, HGF has been recognized as a growth factor involved in the hepatocyte proliferative response during liver regeneration (a recent review on the role of HGF in liver regeneration can be found in[3]), but an unequivocal demonstration of an essential role of the HGF/c-Met signaling in liver regeneration has only been provided recently. Thus, liver specific c-Met and HGF conditional knock-out mice show an impairment of the regenerative response[27-29]. Hepatocytes that lack a functional c-Met display reduced basal survival and a higher sensitivity to Fas-induced liver damage, both *in vivo* and *in vitro*. Moreover, after toxic liver injury induced by exposure to CCl_4, c-met$^{-/-}$ livers showed delayed healing from necrotic injury[28]. Complete abolition of the cell cycle has also been demonstrated when c-Met is deleted after partial hepatectomy, by using RNA interference techniques[30]. In addition to the effects on proliferation, these authors have described an alteration in expression of apoptosis-related genes, particularly, increased expression of pro-apoptotic genes, and decreased expression of anti-apoptotic genes, and enhanced activation of caspase 3. Altogether, these studies provide clear evidences of a role for this ligand/receptor system in promoting hepatocyte proliferation, survival, and tissue remodeling during liver regeneration.

HGF/c-Met signaling is also essential during fetal liver development. Both HGF- and c-Met-deficient embryos show abnormally small livers and liver-to-body weight ratios and massive hepatocyte apoptosis[31-33]. Recent studies from Dr. Maina's laboratory have demonstrated HGF/c-Met survival properties in primary embryonic hepatocytes, as shown by the ability of HGF to impair Fas-induced apoptosis by acting through PI3K and AKT to prevent FLICE inhibitory protein degradation[34]. Additionally, in the context of the adult liver, numerous works have reported an important contribution of the pro-survival activity of HGF in protecting liver during fibrosis and other hepatic dysfunction[35-40], thus expanding the scenarios in which the anti-apoptotic activity of HGF plays an active role.

Although hepatocytes have long been considered the prime target of the actions of HGF, there is accumulating evidence of an important role for HGF/c-Met signaling on liver stem/progenitor cell function and behavior. Liver stem/progenitor cells express c-met[41]. Furthermore,

during oval cell activation induced by N-acetyl-2-aminofluorene/partial hepatectomy (AAF/PH) in rats, HGF expression increases coincidentally with oval cell proliferation, mainly on the periportal regions where oval cells are located[9,41]. These data suggest that the HGF/c-Met system regulates some aspects of liver stem/progenitor cell biology. In support of this, *in vivo* infusion of HGF during AAF/PH-induced liver regeneration stimulates oval cell expansion into the liver lobules[10]. Similar results have been obtained by *in vivo* transfer of HGF cDNA into liver subjected to the Solt-Farber regime[42]. HGF-dependent mitogenic activity has also been shown in rat and mouse oval cell lines *in vitro* by either adenovirus-mediated transfer of the HGF gene or addition of exogenous HGF[43-46]. The molecular mechanisms that mediate the mitogenic effects of HGF in liver progenitors appear to be cell-type specific, because PI3K/AKT activation[43] and nuclear factor-κB activation, downstream of p38 and extracellular signal-regulated kinase (ERK) MAPKs[44], are involved. Additionally, bi-potential hepatoblast cell lines (precursors of both hepatocytes and cholangiocytes) have been established from transgenic animals expressing a constitutively active human Met[47]. However, HGF is much more than a mitogen for liver stem/progenitor cells. HGF effectively protects WB-F344 cell from apoptosis induced by tumor necrosis factor-α in a dose-dependent manner[44]. More recently, using a novel *in vitro* model of genetically modified oval cells that harbor an inactivated Met tyrosine kinase, we have demonstrated that loss of Met increases sensitivity to apoptosis caused by serum deprivation or treatment with TGF-β[46]. By virtue of these results, we hypothesize that Met-driven anti-apoptotic activity plays an important role supporting the expansion of liver progenitors following liver injury, by helping them to overcome the local tissue injuries and inhibitory signals. Therefore, the HGF/c-Met signaling pathway might be a major survival pathway in liver that operates during liver development, homeostasis, and regeneration (both hepatocyte and oval cell-mediated).

The role of HGF as a morphogen is already established[48]. Morphogenesis is a complex invasive growth program that plays a fundamental role in normal development[49,50]. Not surprisingly, morphogenic properties of HGF are exhibited in embryonic and postnatal mouse non-parenchymal epithelial cell-derived cell lines. In these cells, HGF induces a morphogenic response that includes cell scattering and ductal branching in collagen gels. These changes are not observed by treatment with other liver growth factors including EGF, TGF-β, acidic fibroblast growth factor (aFGF) and insulin, and are not inhibited by TGF-β[51]. A similar response has also been described in pancreatic oval cells *in vitro*; a cell population closely related to its hepatic counterpart[52]. Recently, HGF has been directly involved in promoting motility and invasiveness of human liver progenitor cells through Matrigel; an effect that is partially mediated by matrix-metalloproteinase-mediated extracellular matrix (ECM) proteolytic degradation and activation of the MAPK/ERK pathway[53].

Another important biological activity of HGF in liver progenitor cells that cannot be overlooked is its modulatory effect on the differentiation capacity of these cells. HGF has been reported to be required not only for an efficient proliferation and survival, but for hepatocytic differentiation of embryonic hepatic stem cells *in vitro*[54-57]. Furthermore, virtually all the strategies for *in vitro* differentiation of embryonic and adult stem/progenitor cells of different origin into hepatocytes include HGF as a hepatic-inducing factor (a thorough compilation of *in vitro* differentiation methods is found in[58]). Successful differentiation is generally achieved by step by step addition of growth factors, cytokines, and hormones, trying to emulate the sequence of events taking place during *in vivo* hepatogenesis. The requirement of HGF in this process relies on the specific role played by this growth factor at different liver developmental stages. During the commitment phase, HGF might antagonize differentiation of bipotential hepatoblasts along the cholangiocytic lineage, which results in support of growth and differentiation of fetal hepatocytes[59]. In fact, results from Suzuki *et al*[57] have demonstrated that HGF can initiate differentiation of albumin-negative liver stem cells into albumin-positive hepatic precursors at the same time that allows their expansion and decreases expression of cholangiocyte-lineage markers, such as CK19 or γ-glutamyl transferase, but it cannot induce latter markers of hepatocyte differentiation, such as glucose-6-phosphatase or tryptophan-2,3-dioxygenase.

Later, after birth, the expression of both HGF and c-Met significantly increases in the liver, and activation of this pathway crucially assists during complete functional hepatic maturation[41,60]. We have also described that HGF combined with TGF-β helps to maintain the expression of hepatocyte differentiation markers in rat fetal hepatocytes in culture[61,62]. In rat primary neonatal hepatocytes, HGF promotes scattering, but this effect is not associated with a dedifferentiation process, as shown by an increase in the expression of hepatic differentiation markers using HGF treatment[63,64]. Although a role for HGF in hepatocyte differentiation seems to be beyond reasonable doubt, it should be pointed out that HGF has also been directly implicated in transdifferentiation of hepatocytes to biliary epithelial cells[65]. These data highlight the enormous complexity in terms of signaling networks and molecular mechanisms associated with regulation of phenotypic transitions in liver epithelial cells.

All these results provide sufficient evidence to support a crucial role for an HGF/c-Met-induced signaling pathway in liver stem/progenitor cell biology. What it is not totally clear, however, is its mode of action. HGF is mainly produced by mesenchymal cells, and c-Met is expressed in epithelial cells, therefore, this ligand-receptor system is generally considered to act in a paracrine fashion[66]. Consistent with this, the main producers of HGF in the liver are the stellate cells[67,68], although sinusoidal endothelial cells also express HGF[69]. c-Met is expressed in hepatocytes, biliary epithelial cells, and liver progenitor cells[41,54,70,71]. As men-

tioned above, in rat models of oval cell activation *in vivo*, HGF mRNA has been identified in the desmin-positive stellate cells that surround the ductal structures of oval cells, or interspersed with the oval cells, whereas c-Met expression is detected in oval cells[9,41]. These results have prompted the conclusion that the HGF/c-Met system operates in oval cells *via* a paracrine mechanism. However, *in vitro* studies carried out in our laboratory in oval cell lines have shown an autocrine regulatory mechanism for HGF/c-Met involved in protection against apoptosis[46]. Whether the autocrine regulation plays a role *in vivo* has not been explored, but these data suggest that oval cells might respond to both autocrine and paracrine Met signaling in a context-dependent fashion.

EGF receptor ligands

Another tyrosine kinase receptor family that regulates liver pathophysiology is the EGF receptor (EGFR) family. This receptor is part of a complex signaling system that includes multiple ligands, namely TGF-α, EGF, heparin-binding EGF, amphiregulin, betacellulin, epiregulin, epigen, and crypto; and four transmembrane receptors: EGFR (Her1/ErbB-1), ErbB-2 (Her2/neu), ErbB-3 (Her3), and ErbB-4 (Her4). Complexity of this pathway relies on differential ligand binding affinity to the receptors, as well as formation of receptor homo- and heterodimers, all of which leads to activation of distinct intracellular signaling cascade and diverse biological activities. Among all the ligands, TGF-α and EGF are the most widely studied. Both TGF-α and EGF bind and activate the same receptor, EGFR. Ligand binding results in dimerization and autophosphorylation of EGFR in tyrosine residues of the cytoplasmic domain. This leads to recruitment of adapter proteins, such as Grb2 and Shc, and subsequent activation of multiple downstream pathways, including PI3K, Ras-MAPK, c-Jun N-terminal kinase, p38, protein kinase C and STAT-3, which mediate cell proliferation, migration, differentiation and evasion from apoptosis[72-76]. TGF-α and EGF are well-known regulators of hepatocyte proliferation. Infusion of either one of the two factors initiates DNA synthesis in liver of adult rats[77,78], and when added exogenously, they stimulate growth of primary hepatocytes at all developmental stages: fetal, neonatal, and adult[79-82]. Together with HGF, EGFR ligands represent the only complete mitogens in adult hepatocytes in serum-free medium, and the most important growth factors involved in the proliferative response during liver regeneration[3]. Demonstration of a critical role for EGFR in hepatocyte proliferation during the initial phases of liver regeneration has recently been provided by generating mice with a liver-specific EGFR deficiency[83]. In addition to the regulatory role in proliferation, and similarly to Met, EGFR is a major survival pathway in the liver[84]. EGF-mediated EGFR activation is able to abolish completely the apoptotic response induced by TGF-β in fetal rat hepatocytes[62] or by Fas receptor stimulation in mouse hepatocytes[85]. Furthermore, EGFR ligands appear to be important modulators of hepatocyte differentiation as well. Morphological and gene expression studies from our laboratory and collaborators have shown that EGF, acting in cooperation with other cytokines and hormones, maintains primary fetal and neonatal hepatocyte differentiation[64,86,87].

Expression studies have suggested that ErbB1-triggered signaling also plays a role in stem/progenitor-cell-mediated liver regeneration. Indeed, both TGF-α and EGFR are transcriptionally upregulated during the period of active proliferation and differentiation of progenitor cells in the rat liver subjected to the 2-AAF/PH protocol[88], and they appear to drive the early proliferation of the progenitor cell compartment[89,10]. Although both HGF and EGF promote the expansion of oval cells *in vivo*, some differences are observed. HGF increased number of both ductal and Ito cells at a similar rate, whereas infusion of EGF mostly increases ductal cells. Our laboratory and others have also shown that EGF and TGF-α are mitogens in mouse and rat oval cells *in vitro*[11,46,90].

The effects of this signaling pathway on liver stem/progenitor cells are not restricted to mitogenesis. *In vivo* infusion with either EGF or HGF not only amplifies liver progenitor expansion following liver injury but also decreases apoptosis[10]. We also have evidence of an important role for EGFR-mediated signaling in regulating oval cell survival *in vitro*. Thus, inhibition of EGFR *via* treatment with a synthetic inhibitor increases basal apoptosis (in the absence of serum and exogenous stimuli) and strongly amplifies TGF-β-induced apoptosis (our unpublished results). EGFR-mediated motogenic, morphogenic, and differentiation activities have also been reported. EGF, combined with TGF-β, triggers a scattering response in mouse oval cell lines *in vitro*[11]. In addition to this, EGF is often included as a hepatogenic factor in strategies to induce *in vitro* differentiation of stem/progenitor cells into hepatocyte-like cells[58]. Consistently, TβT-FH cell lines - a cell population of fetal hepatocytes that has suffered an epithelial mesenchymal transition (EMT) and a dedifferentiation process after TGF-β treatment - recover the original epithelial phenotype and gain cytokeratin-19 expression by treatment with EGF and DMSO, which suggests conversion to hepatoblast-like cells[91].

It should be noted that we have seen autocrine regulation for EGFR-dependent signaling in oval cell lines (our unpublished results). Different to the HGF/c-Met system, an EGFR-ligand-mediated autocrine mechanism has been suggested, based on the detection of EGFR ligand transcripts in oval cells during liver regeneration[88]. *In vivo* and *in vitro* studies have shown that choline-deficient ethionine-supplemented diet-treated mouse livers express cytokines such as lymphotoxin-β, interferon-γ, and interleukin (IL)-6[92,93]. Collectively, these data strongly support that autocrine regulatory mechanisms are important in liver progenitor cells. The significance of the c-Met and EGFR autocrine loops in oval cells is not yet totally understood. Certainly, autocrine signaling has been mostly associated with malignancy. This seems to apply to stem/progenitor

cells as well, because tumorigenic conversion of mouse oval cell has been associated with growth factor production and alteration in growth factor responsiveness[11]. More specifically, an HGF/c-Met autocrine loop has been identified in spontaneously transformed WB-F344 rat liver stem-like cells, which contributes to drive autonomous cell proliferation[94]. Spontaneously transformed oval cells express TGF-α. These cells form tumors when injected into nude mice, a capacity that is significantly reduced by transfection with TGF-α antisense gene[95]. A cross-talk between Met and EGFR has also been proposed. Thus, rat liver-epithelial-cell-derived tumor cell lines that constitutively express TGF-α display increased levels of both c-met gene and protein, as well as an amplified response to HGF[96]. However, 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-treated mouse liver-derived oval cell lines, which show autocrine activation of Met and EGFR-dependent pathways, do not show any sign of transformation[46] (and our unpublished results). Whether this is due to a matter of time, dose, or lack of critical partners for the neoplastic transformation, it is not yet known. For now, awaiting further conclusive experiments, a direct link between the establishment of functional c-Met and EGFR-dependent autocrine loops and neoplastic transformation cannot be firmly established in liver progenitor cells.

All the data summarized above highlight not only a relevant role of Met and EGFR-triggered signaling pathways in regulating the liver progenitor cell compartment, but a striking parallelism between the two pathways, both of which mediate growth, survival, migration and hepatocytic differentiation (Figure 1). Further studies will clarify which of these biological activities are totally overlapping and which are not. In this sense, Met and EGFR mutant mice and cell models should provide very useful and invaluable tools in this regard.

FGFs

The FGFs are a family of growth factors with high affinity for heparan sulfate proteoglycans, which bind to transmembrane tyrosine kinase FGF receptors (FGFRs). The role of these growth factors in hepatic fate specification of pre-hepatic endoderm cells during liver development is well known. There are a number of excellent reviews on this subject, some of which are cited here[97-99]. Activation of adult stem/progenitor cells is believed to use similar, if not identical, genetic programs as the embryonic progenitors, therefore, FGF is among the main targets to be analyzed in rodent models of oval cell expansion. Indeed, aFGF (also known as FGF-1) is upregulated at the stage of oval cell progression[89,100], being expressed by both oval cells and Ito cells. FGFR1 and FGFR2 are also expressed at high levels during the period of active proliferation and differentiation of oval cells, but exhibit a different pattern. Although FGFR1 is mainly expressed in oval cells, FGFR2 is expressed in both oval and Ito cells[101]. These results suggest a differential role for these two receptors during liver stem/progenitor-cell-mediated regeneration, as well as the establishment of both autocrine and paracrine signaling. In addition to these *in vivo* observations, *in vitro* studies have shown that aFGF is able to push Met murine hepatocytes-bi-potential precursors isolated from transgenic livers, which express a constitutively active human Met - to progress from a very early state of differentiation to a more mature state, associated with the expression of liver functions[47]. Moreover, FGF1-pretreated cells are resistant to the TGF-β dedifferentiation effect[102]. Essentially, FGFs have proved to be effective in mediating early hepatic differentiation, and therefore are included in most *in vitro* differentiation protocols[58].

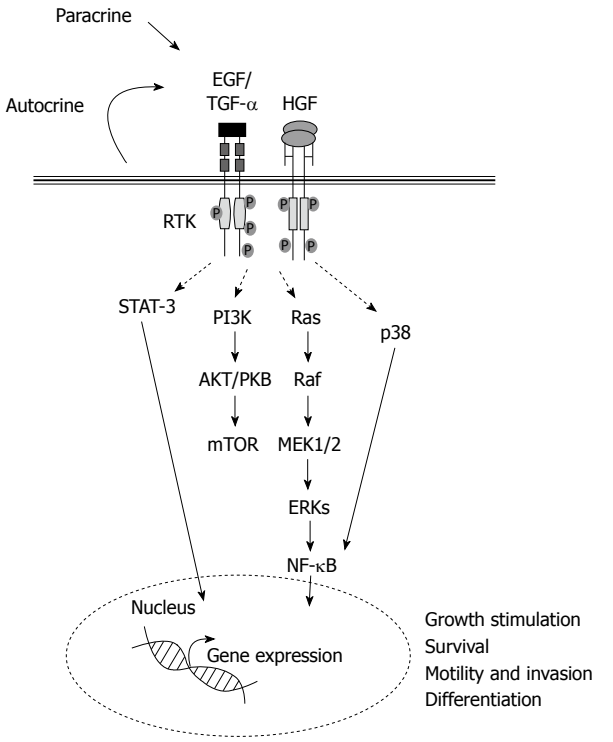

Figure 1 Schematic illustration of the major signaling pathways and biological activities induced by hepatocyte growth factor and epidermal growth factor receptor ligands in liver progenitor cells. HGF: Hepatocyte growth factor; EGF: Epidermal growth factor; TGF: Transforming growth factor; STAT: Signal transducer and activator of transcription; PI3K: Phosphoinositide 3-kinase; NF-κB: Nuclear factor κB; mTOR: Mammalian target of rapamycin; MEK: Mitogen-activated protein kinase kinase; ERKs: Extracellular-signal-regulated kinases.

TGF-β FAMILY

The TGF-β family of cytokines regulates hepatocyte proliferation and death, and plays relevant roles during liver regeneration[103]. However, TGF-β and other pro-inflammatory cytokines are important inducers of fibrocarcinogenesis, due to their ability to induce myofibroblast differentiation and ECM deposition[104,105]. Recent evidence has shown that TGF-β might also regulate liver stemness and phenotype[106].

TGF-β active form acts as a dimer, and signals by bringing together two receptors with serine-threonine kinase activity, which are known as type I and type II receptors (Figure 2). After TGF-β binding, the type II receptor

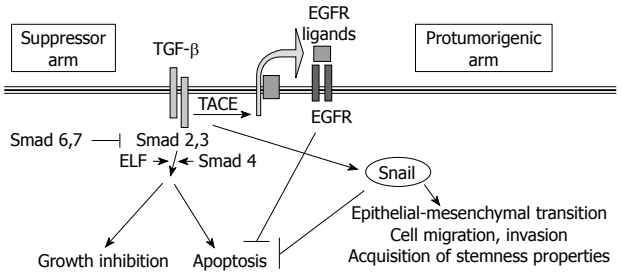

Figure 2 Overview of the major signaling pathways, and their cellular effects, induced by transforming growth factor-β in liver cells. TGF: Transforming growth factor; EGFR: Epidermal growth factor receptor; ELF: Embryonic liver fodrin; TACE: Tumor necrosis factor-converting enzyme.

phosphorylates and activates the type I receptor, which is responsible for phosphorylation of the receptor-regulated Smad family of transcription factors (R-Smads), i.e. Smad 2 and 3 in the case of TGF-β1, β2 and β3[107]. After phosphorylation, Smad 2 or 3 forms a complex with Smad 4 and shuttles to the nucleus, where it possesses DNA-binding activity, although it must associate with other DNA-binding co-factors to achieve high-affinity binding. Indeed, through combination with different transcription factors, the same TGF-β stimulus can induce or repress many different target genes. Activation of the Smad transcriptional activity can be counteracted by the presence of other members of the Smad family that play inhibitory effects (inhibitory Smads), such as Smad 6 and Smad 7, which compete with Smad 2 and 3 for binding either to the receptor complex or to Smad 4.

The most relevant Smad-mediated cell responses to TGF-β are growth inhibition and apoptosis. TGF-β inhibits proliferation in adult[108,109], as well as fetal or regenerating hepatocytes[82,109], and it is a well-known inducer of hepatocyte cell death[110,111]. The inhibitory effects of TGF-β on liver stem/progenitor cell growth and survival are somewhat unclear. TGF-β overexpression results in impairment of oval cell expansion induced by DDC[112]. Consistently, we have shown that treatment with TGF-β inhibits growth and induces apoptosis in DDC-derived oval cell lines *in vitro*[46]. However, when compared to adult hepatocytes, other authors have reported that the majority of the adult liver progenitor cells are more resistant to endogenously produced TGF-β anti-proliferative and pro-apoptotic effects *in vitro*[113], which indicates a differential sensitivity to TGF-β between liver progenitors and mature hepatocytes. Indeed, several mechanisms have been proposed that would explain this differential response. On the one hand, the ratio between R-Smads and inhibitory Smads might be different in oval cells. In this sense, Nguyen *et al*[114] have recently reported that oval cell lines show higher levels of Smad 6 than do adult hepatocytes. On the other hand, oval cells might show over-activation of survival signals, such as the MAPK/ERK pathway[115] that might be related to the autocrine production of growth/survival factors, such as HGF[46]. It is worth noting that, when combined with growth factors such as EGF or HGF, TGF-β might contribute to cellular scattering and morphological differentiation of hepatoblasts and liver stem cells[87,116,117]. In this same line of evidence, hepatocytes are undifferentiated and liver architecture is lost in Smad 2+/- and Smad 3+/- mice, as well as in ELF (a β-spectrin that is crucial for the propagation of the TGF-β signal) mutants[118-121]. Defects in Smad 2+/- and Smad 3+/- mutants are restored by HGF treatment. Although mechanisms by which TGF-β signals can be supplanted by HGF are unknown, data seem to support cooperation between two independent pathways, that converge on β1-integrin expression, rather than direct pathway crosstalk[119]. Emerging new data have reinforced the idea that TGF-β is important for stem cell transitioning to a progenitor cell phenotype, and ultimately, its conversion to a fully differentiated phenotype. Indeed, TGF-β signaling, and particularly ELF, appears to play a crucial role in hepatocyte proliferation and transitional phenotype during human liver regeneration, and its loss is associated with activation of liver progenitor cells[106]. It has also been shown recently that TGF-β might be a determinant in the appearance of progenitor/stem cells in hepatocarcinogenesis. The examination of human hepatocellular carcinoma (HCC) has revealed that cells that are labeled with stem-cell markers have unexpectedly lost the TGF-β receptor Ⅱ and ELF, and show marked activation of the IL-6 pathway, a major stem-cell signaling pathway[121,122]. These data support that absence of TGF-β-driven epithelial differentiation favors carcinogenesis.

However, TGF-β is a pleiotropic cytokine inducing several, and sometimes contradictory, signals in epithelial cells. Indeed, in addition to the well-known Smad-mediated transcriptional responses that predominantly address tumor suppressor actions, TGF-β induces other Smad-dependent or independent effects that contribute to tumor progression[107]. Among these, related to the topic of this review, one of the more relevant is the capacity to induce EMT processes. EMT is a physiological process during embryogenesis, in which an epithelial cell loses expression of adhesion molecules, such as E-cadherin, and other components responsible for cell polarity. Instead, they express mesenchymal components of the cytoskeleton and acquire motility and scattering properties[123]. A closely related phenotypic conversion is also detected in fibrosis and neoplasia and is associated with disease progression[124]. Members of the TGF-β family can initiate and maintain EMT in a variety of biological systems and pathophysiological situations, through activation of major signaling pathways and transcriptional regulators integrated in extensive signaling networks[125,126]. In culture, hepatocytes and hepatoma cells undergo EMT in response to TGF-β[127-130], which support a potentially crucial role for TGF-β in the development and progression of hepatic fibrogenesis and cancer. The mechanisms that allow cells to escape from the apoptotic effects of TGF-β and undergo EMT are not completely understood. However, recent results have indicated that, in some epithelial cells, including fetal rat hepatocytes and hepatoma cells,

TGF-β might induce both pro- and anti-apoptotic signals and their balance defines the cell fate[129,131-133]. In fact, certain evidence indicates a crosstalk between the genetic programs that control TGF-β-induced growth arrest/apoptosis and those that regulate EMT, because once the cell has adopted a mesenchymal phenotype, it does not respond to TGF-β suppressor effects[128,134,135]. Thus, TGF-β might regulate its own signaling to switch from tumor suppression to tumor progression. Indeed, it is interesting to point out that transcription factors of the Snail family, repressors of the E-cadherin gene, are required for cell survival during EMT[136,137].

In recent works from our group, we have demonstrated that transdifferentiation of liver cells from an epithelial to a mesenchymal phenotype, such as that mediated by TGF-β, induces dedifferentiation and allows enrichment in a population of cells with putative liver progenitor properties[91,128,138]. The phenotypic characteristics observed (elongated phenotype, strong expression of Thy-1, vimentin or α-smooth muscle actin) are also highly reminiscent of myofibroblasts[139]. These finding are in agreement with the recently proven fact that EMT not only endows cells with migratory and invasive properties, but also induces stem cell properties[123]. Furthermore, additional works have proved the capacity of TGF-β to induce/maintain a stemness phenotype in other tissues[140]. Preliminary results have indicated that human fetal hepatocytes respond to TGF-β downregulation of E-cadherin and upregulation of Snail, thus acquiring a fibroblastic-like morphology and a liver progenitor cell phenotype (unpublished observations from our group in collaboration with Dr. N. Fausto, University of Washington, Seattle, USA).

These findings might have implications for regenerative biology of the liver and open new perspectives for the *in vitro* isolation of putative liver stem/progenitor cells to be used in basic and translational research in the liver. Moreover, a recent study has also suggested that TGF-β treatment of HCC cells might induce the selection of cells expressing high levels of CD133, a putative stem-cell marker in diverse hematopoietic and non-hematopoietic tissues and cancers[141], through a mechanism partially dependent on the Smads pathway, and that involves epigenetic regulation of the CD133 promoter. These results indicate that TGF-β might also play a role in transdifferentiating liver tumor cells to liver cancer stem cells.

In the embryo, progenitors to pancreatic β cells and hepatocyte lineages arise from neighboring domains of ventral foregut endoderm. It has been recently found that the specification of pancreas and liver progenitors is restricted by the TGF-β pathway[12]. It has been speculated that TGF-β signaling restrains lateral endoderm specification until the cells move sufficiently far from the heart and into a bone morphogenetic protein signaling domain, with the latter then becoming the dominant Smad 4-dependent pathway, which leads to pancreatic induction. TGF-β signaling appears to be strongly inhibitory to the pancreatic lineage and modestly inhibitory to the liver lineage, which suggests that TGF-β also plays a relevant role in maintaining the stemness phenotype of endodermal precursors during liver development.

All these results suggest a dual role for the TGF-β signaling pathway in liver stemness and differentiation. On the one hand, TGF-β mediates progression of differentiation from a stem or progenitor stage, but on the other hand, it contributes to the expansion of liver stem cells. Further work is necessary to understand better the relevance of both effects in liver development, regeneration and carcinogenesis.

HEDGEHOG FAMILY LIGANDS

Hedgehog (Hh) family ligands are widely acknowledged morphogens that regulate tissue remodeling during embryogenesis[142,143]. In particular, Indian hedgehog and Sonic hedgehog ligands, and their receptor, Patched, are expressed at different stages of liver organogenesis, and available data point to dynamic signal activation and temporarily restricted effects on hepatoblast regulation. Thus, Hh signaling is necessary to promote hepatoblast proliferation but it needs to be shut off to permit subsequent hepatoblast differentiation[143-148]. Hh signaling is also involved in the maintenance of a hepatic progenitor reservoir throughout life, both in humans and mice. Blockade of Hh activity in hepatic progenitors *in vitro* decreases survival, whereas stimulation of Hh activity inhibits endogenous apoptosis. Both autocrine and paracrine modes of action are suggested, based on simultaneous expression of Hh ligands and receptors in liver progenitor cells[149]. Additional observations suggest that Hh pathway activation is a common feature of various types of chronic liver injury associated with mobilization of hepatic progenitor populations. Participation of the Hh pathway has been demonstrated in the ductular reaction that is elicited by chronic alcohol-induced liver injury in mice and humans[150]; non-alcoholic steatohepatitis in humans[151]; methionine choline-deficient ethionine-supplemented diet[152] and fatty liver damage[153] in mice. Hh ligands are released by hepatocytes and myofibroblasts, and lead to enhanced viability and proliferation of bile ductular cells and hepatic progenitors, thus promoting the ductular response and fibrogenesis. Myofibroblasts, but not hepatocytes, also respond to Hh ligands with an increase in viability[152,154-157]. Either Hh paracrine signaling between myofibroblast and hepatic progenitors or autocrine signaling, or both, promote EMT in hepatic progenitors, which ultimately contributes to liver fibrosis[157-159].

The way in which Hh signaling interacts with other pathways to control the fate of hepatic progenitor populations during liver repair after damage or neoplasia is still not well understood. Nonetheless, some interesting pieces of information are emerging. Similar to that which has been described in embryogenesis[160], a functional crosstalk between the TGF-β and Hh signaling pathways has been proposed in the context of adult liver progenitor expansion during liver injury. In ethanol-fed mice, TGF-β induces production of Hh ligands in hepatocytes,

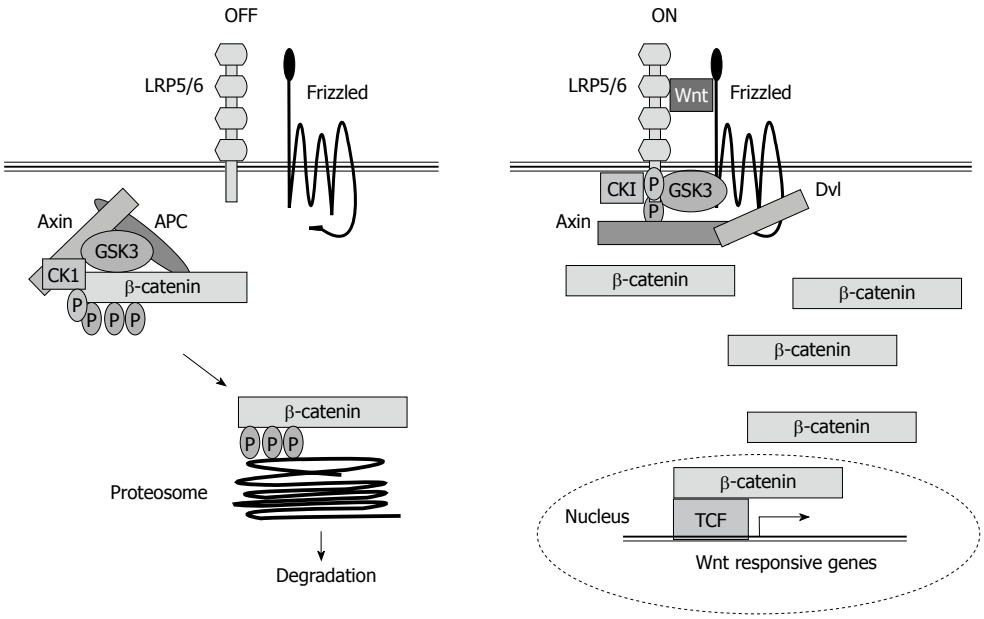

Figure 3 Wnt/β-catenin signaling. Left figure shows how β-catenin is pushed to degradation in the absence of Wnt ligand. Right figure shows how in the presence of Wnt, β-catenin regulates gene expression. TCF: T-cell factor; LRP: Low density lipoprotein receptor-related protein; APC: Adenomatous polyposis coli; CK1: Casein kinase 1; GSK: Glycogen synthase kinase.

which subsequently promotes survival and expansion of ductular/oval cell populations. This supports the concept that enhanced exposure to TGF-β contributes to the accumulation of cells with a ductular phenotype, which are protected from TGF-β-mediated apoptosis[155,161]. Reciprocally, Hh-induced EMT might depend, at least partially, on induction of TGF-β[159]. Further research is required to delineate the Hh-TGF-β interaction.

These findings suggest a role for the Hh pathway as a major mediator in the communication network established between myofibroblast and liver progenitors, which promotes progenitor accumulation and contributes to hepatic fibrosis pathogenesis through EMT induction.

WINGLESS/β-CATENIN PATHWAY

Proteins of the wingless (Wnt) family are secreted signaling molecules that regulate multiple processes in animal development and control tissue homeostasis. The Wnt signaling pathway is highly conserved throughout evolution and plays essential roles in controlling cell proliferation, cell to cell adhesion, and motility[162]. In the liver, it plays many crucial roles during hepatic development and regeneration, and its dysregulation is evident in aberrant growth during hepatocarcinogenesis[163]. Wnt signaling is itself inherently complex[164]. On the one hand, both the ligands and receptors involved in Wnt signal transduction belong to large multi-gene families, which allows for a large number of possible ligand-receptor interactions. On the other hand, Wnt-receptor interactions can elicit a variety of intracellular responses, the best-known of which results in the activation of β-catenin/T-cell factor (TCF) transcriptional complexes. In the absence of signals, β-catenin accumulates in adherens junctions and free β-catenin levels are very low because the tumor suppressor adenomatous polyposis coli forms a trimer complex with glycogen synthase kinase-3-β and axin/conductin, which interacts with and phosphorylates β-catenin, thus targeting it for degradation to the proteasome (Figure 3). Wnts bind a two-part receptor: a seven transmembrane Frizzled and low-density lipoprotein-related protein (LRP)5/6, both being required for canonical signaling. Ligand binding mediates phosphorylation of the cytoplasmic tail of LRP5/6, which creates an axin-binding site. Axin recruitment inactivates the destruction complex. This stabilizes β-catenin, which enters into the nuclei where it displaces Groucho from TCF, nucleating formation of a multiprotein activator complex, and activating Wnt target genes[165] (Figure 3). Furthermore, β-catenin is at the crossroads of growth factor and cytokine signaling. Indeed, certain evidence indicates that TGF-β might induce nuclear β-catenin accumulation, through induction of platelet-derived growth factor signaling[166]. β-catenin expression leads to elevated EGFR levels in hepatocytes and immunohistological analysis shows high correlation between the expression of nuclear/cytoplasmic β-catenin and EGFR in most hepatoblastomas[167]. β-catenin also participates in homotypic cell-cell interactions through its association with E-cadherin. Thus, nuclear β-catenin accumulation in HCC cells might contribute to impaired E-cadherin expression, which mediates the EMT process, migration and survival. It is also known that β-catenin and Met form a complex on hepatocyte membranes. Upon HGF stimulation, the complex is dissociated, in a β-catenin tyrosine phosphorylation-dependent but Wnt-independent manner, which results in β-catenin nuclear translocation[168]. In addition, a role for β-catenin as a downstream effector of HGF in HGF-induced hepatomegaly has been demonstrated[169].

It has been recently suggested that oval cells respond to Wnt ligands *in vitro* with an increase in amino-terminus dephosphorylated β-catenin and cell cycle entry[170], and that canonical Wnt/β-catenin/TCF signaling plays a key role in the normal activation and proliferation of adult hepatic stem cells[171,172]. In this same line of evidence, repression of Wnt/β-catenin signaling in the anterior endoderm is essential for liver and pancreas specification[173], which indicates that turning Wnt signaling off is essential for liver differentiation. An opposite situation might be found during hepatocarcinogenesis, where reactivation of the Wnt/β-catenin pathway, or accumulation of nuclear β-catenin due to other alterations might contribute to the expansion of liver tumor stem cells. Indeed, recent reports have highlighted the relevance of Wnt/β-catenin signaling in the activation of tumorigenic liver progenitor cells[174] and the acquisition of hepatic stem-cell markers[175] in HCC. Accumulation of nuclear β-catenin has been shown to induce an early liver progenitor phenotype in HCC, which correlates with tumor recurrence[176].

In summary, Wnt family and β-catenin/TCF pathways are clearly involved in the maintenance of the liver stemness phenotype, and its repression is necessary for liver differentiation during development. Its overactivation during liver tumorigenesis might contribute to the acquisition of a liver cancer stem cell phenotype.

CONCLUSION

For the past 10-15 years, significant progress has been made in elucidating the cellular and molecular mechanisms that contribute to control of liver stem/progenitor cell behavior. Its complexity and multifaceted nature is evident. Among the diverse signaling pathways at play, the major growth factors, cytokines, and other ligand/receptor systems known to play an important role in liver development, homeostasis and regeneration, certainly occupy a central position. Many of the classical responses elicited by these factors are conserved. However, differences in the downstream mechanisms following ligand-receptor engagement, or signal interaction and crosstalk, as well as specific alterations in intracellular mediators, are being identified as contributing factors to the differential response of liver stem/progenitor cells compared with their mature counterparts. In addition, besides delivery of signals by the surrounding microenvironment, accumulating observations are pointing to co-existence of paracrine and autocrine modes of response as general regulatory mechanisms on this cell population. Collectively, these data indicate that liver stem/progenitor cells follow their own rules and regulations. It is also clear that the same signals that are essential for their activation, expansion, and differentiation, are good candidates to contribute, under adequate conditions, to the paradigm of transformation from a pro-regenerative to a pro-tumorigenic role. Unquestionably, from a clinical perspective, this is a fundamental issue for liver/stem progenitor cell-based therapies. Despite the fact that additional efforts are required to delineate perfectly how the functional switch takes place, the studies summarized here are steps in the right direction.

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REFERENCES

1 **Taub R**. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004; **5**: 836-847
2 **Fausto N**, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006; **43**: S45-S53
3 **Michalopoulos GK**. Liver regeneration. *J Cell Physiol* 2007; **213**: 286-300
4 **Michalopoulos GK**. Liver regeneration: Alternative epithelial pathways. *Int J Biochem Cell Biol* 2009; Epub ahead of print
5 **Fausto N**. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* 2004; **39**: 1477-1487
6 **Duncan AW**, Dorrell C, Grompe M. Stem cells and liver regeneration. *Gastroenterology* 2009; **137**: 466-481
7 **Grisham JW**, Hartroft WS. Morphologic identification by electron microscopy of "oval" cells in experimental hepatic degeneration. *Lab Invest* 1961; **10**: 317-332
8 **Sell S**, Osborn K, Leffert HL. Autoradiography of "oval cells" appearing rapidly in the livers of rats fed N-2-fluorenylacetamide in a choline devoid diet. *Carcinogenesis* 1981; **2**: 7-14
9 **Alison MR**, Poulsom R, Jeffery R, Anilkumar TV, Jagoe R, Sarraf CE. Expression of hepatocyte growth factor mRNA during oval cell activation in the rat liver. *J Pathol* 1993; **171**: 291-299
10 **Nagy P**, Bisgaard HC, Santoni-Rugiu E, Thorgeirsson SS. In vivo infusion of growth factors enhances the mitogenic response of rat hepatic ductal (oval) cells after administration of 2-acetylaminofluorene. *Hepatology* 1996; **23**: 71-79
11 **Isfort RJ**, Cody DB, Richards WG, Yoder BK, Wilkinson JE, Woychik RP. Characterization of growth factor responsiveness and alterations in growth factor homeostasis involved in the tumorigenic conversion of mouse oval cells. *Growth Factors* 1998; **15**: 81-94
12 **Wandzioch E**, Zaret KS. Dynamic signaling network for the specification of embryonic pancreas and liver progenitors. *Science* 2009; **324**: 1707-1710
13 **Michalopoulos G**, Houck KA, Dolan ML, Leutteke NC. Control of hepatocyte replication by two serum factors. *Cancer Res* 1984; **44**: 4414-4419
14 **Nakamura T**, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. *Biochem Biophys Res Commun* 1984; **122**: 1450-1459
15 **Russell WE**, McGowan JA, Bucher NL. Partial characterization of a hepatocyte growth factor from rat platelets. *J Cell Physiol* 1984; **119**: 183-192
16 **Stoker M**, Gherardi E, Perryman M, Gray J. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature* 1987; **327**: 239-242
17 **Naldini L**, Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, Narsimhan RP, Hartmann G, Zarnegar R, Michalopoulos GK. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *EMBO J* 1991; **10**: 2867-2878
18 **Weidner KM**, Arakaki N, Hartmann G, Vandekerckhove J, Weingart S, Rieder H, Fonatsch C, Tsubouchi H, Hishida T, Daikuhara Y. Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc Natl Acad Sci USA* 1991; **88**: 7001-7005
19 **Zarnegar R**, Michalopoulos GK. The many faces of hepato-

cyte growth factor: from hepatopoiesis to hematopoiesis. *J Cell Biol* 1995; **129**: 1177-1180
20 **Matsumoto K**, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. *J Biochem* 1996; **119**: 591-600
21 **Birchmeier C**, Gherardi E. Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. *Trends Cell Biol* 1998; **8**: 404-410
22 **Birchmeier C**, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003; **4**: 915-925
23 **Zhang YW**, Vande Woude GF. HGF/SF-met signaling in the control of branching morphogenesis and invasion. *J Cell Biochem* 2003; **88**: 408-417
24 **Tulasne D**, Foveau B. The shadow of death on the MET tyrosine kinase receptor. *Cell Death Differ* 2008; **15**: 427-434
25 **Furge KA**, Zhang YW, Vande Woude GF. Met receptor tyrosine kinase: enhanced signaling through adapter proteins. *Oncogene* 2000; **19**: 5582-5589
26 **Stuart KA**, Riordan SM, Lidder S, Crostella L, Williams R, Skouteris GG. Hepatocyte growth factor/scatter factor-induced intracellular signalling. *Int J Exp Pathol* 2000; **81**: 17-30
27 **Borowiak M**, Garratt AN, Wüstefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. *Proc Natl Acad Sci USA* 2004; **101**: 10608-10613
28 **Huh CG**, Factor VM, Sánchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci USA* 2004; **101**: 4477-4482
29 **Phaneuf D**, Moscioni AD, LeClair C, Raper SE, Wilson JM. Generation of a mouse expressing a conditional knockout of the hepatocyte growth factor gene: demonstration of impaired liver regeneration. *DNA Cell Biol* 2004; **23**: 592-603
30 **Paranjpe S**, Bowen WC, Bell AW, Nejak-Bowen K, Luo JH, Michalopoulos GK. Cell cycle effects resulting from inhibition of hepatocyte growth factor and its receptor c-Met in regenerating rat livers by RNA interference. *Hepatology* 2007; **45**: 1471-1477
31 **Bladt F**, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 1995; **376**: 768-771
32 **Schmidt C**, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, Gherardi E, Birchmeier C. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* 1995; **373**: 699-702
33 **Uehara Y**, Minowa O, Mori C, Shiota K, Kuno J, Noda T, Kitamura N. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* 1995; **373**: 702-705
34 **Moumen A**, Ieraci A, Patané S, Solé C, Comella JX, Dono R, Maina F. Met signals hepatocyte survival by preventing Fas-triggered FLIP degradation in a PI3k-Akt-dependent manner. *Hepatology* 2007; **45**: 1210-1217
35 **Ogasawara J**, Watanabe-Fukunaga R, Adachi M, Matsuzawa A, Kasugai T, Kitamura Y, Itoh N, Suda T, Nagata S. Lethal effect of the anti-Fas antibody in mice. *Nature* 1993; **364**: 806-809
36 **Kosai KI**, Finegold MJ, Thi-Huynh BT, Tewson M, Ou CN, Bowles N, Woo SL, Schwall RH, Darlington GJ. Retrovirus-mediated in vivo gene transfer in the replicating liver using recombinant hepatocyte growth factor without liver injury or partial hepatectomy. *Hum Gene Ther* 1998; **9**: 1293-1301
37 **Kosai K**, Matsumoto K, Funakoshi H, Nakamura T. Hepatocyte growth factor prevents endotoxin-induced lethal hepatic failure in mice. *Hepatology* 1999; **30**: 151-159
38 **Ueki T**, Kaneda Y, Tsutsui H, Nakanishi K, Sawa Y, Morishita R, Matsumoto K, Nakamura T, Takahashi H, Okamoto E, Fujimoto J. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat Med* 1999; **5:** 226-230
39 **Phaneuf D**, Chen SJ, Wilson JM. Intravenous injection of an adenovirus encoding hepatocyte growth factor results in liver growth and has a protective effect against apoptosis. *Mol Med* 2000; **6**: 96-103
40 **Xue F**, Takahara T, Yata Y, Kuwabara Y, Shinno E, Nonome K, Minemura M, Takahara S, Li X, Yamato E, Watanabe A. Hepatocyte growth factor gene therapy accelerates regeneration in cirrhotic mouse livers after hepatectomy. *Gut* 2003; **52**: 694-700
41 **Hu Z**, Evarts RP, Fujio K, Marsden ER, Thorgeirsson SS. Expression of hepatocyte growth factor and c-met genes during hepatic differentiation and liver development in the rat. *Am J Pathol* 1993; **142**: 1823-1830
42 **Shiota G**, Kunisada T, Oyama K, Udagawa A, Nomi T, Tanaka K, Tsutsumi A, Isono M, Nakamura T, Hamada H, Sakatani T, Sell S, Sato K, Ito H, Kawasaki H. In vivo transfer of hepatocyte growth factor gene accelerates proliferation of hepatic oval cells in a 2-acetylaminofluorene/partial hepatectomy model in rats. *FEBS Lett* 2000; **470**: 325-330
43 **Okano J**, Shiota G, Matsumoto K, Yasui S, Kurimasa A, Hisatome I, Steinberg P, Murawaki Y. Hepatocyte growth factor exerts a proliferative effect on oval cells through the PI3K/AKT signaling pathway. *Biochem Biophys Res Commun* 2003; **309**: 298-304
44 **Yao P**, Zhan Y, Xu W, Li C, Yue P, Xu C, Hu D, Qu CK, Yang X. Hepatocyte growth factor-induced proliferation of hepatic stem-like cells depends on activation of NF-kappaB. *J Hepatol* 2004; **40**: 391-398
45 **Kato H**, Shimomura T, Murai R, Gonda K, Ishii K, Yoshida Y, Kanbe T, Hashiguchi K, Sakabe T, Takubo K, Okano J, Tsuchiya H, Hoshikawa Y, Kurimasa A, Shiota G. Regulation of hepatic oval cell proliferation by adenoviral mediated hepatocyte growth factor gene transfer and signal transduction inhibitors. *Hepatogastroenterology* 2007; **54**: 821-825
46 **del Castillo G**, Factor VM, Fernández M, Alvarez-Barrientos A, Fabregat I, Thorgeirsson SS, Sánchez A. Deletion of the Met tyrosine kinase in liver progenitor oval cells increases sensitivity to apoptosis in vitro. *Am J Pathol* 2008; **172**: 1238-1247
47 **Spagnoli FM**, Amicone L, Tripodi M, Weiss MC. Identification of a bipotential precursor cell in hepatic cell lines derived from transgenic mice expressing cyto-Met in the liver. *J Cell Biol* 1998; **143**: 1101-1112
48 **Montesano R**, Matsumoto K, Nakamura T, Orci L. Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell* 1991; **67**: 901-908
49 **Comoglio PM**, Trusolino L. Invasive growth: from development to metastasis. *J Clin Invest* 2002; **109**: 857-862
50 **Rosário M**, Birchmeier W. How to make tubes: signaling by the Met receptor tyrosine kinase. *Trends Cell Biol* 2003; **13**: 328-335
51 **Johnson M**, Koukoulis G, Matsumoto K, Nakamura T, Iyer A. Hepatocyte growth factor induces proliferation and morphogenesis in nonparenchymal epithelial liver cells. *Hepatology* 1993; **17**: 1052-1061
52 **Jeffers M**, Rao MS, Rulong S, Reddy JK, Subbarao V, Hudson E, Vande Woude GF, Resau JH. Hepatocyte growth factor/scatter factor-Met signaling induces proliferation, migration, and morphogenesis of pancreatic oval cells. *Cell Growth Differ* 1996; **7**: 1805-1813
53 **Delgado JP**, Vanneaux V, Branger J, Touboul T, Sentilhes L, Mainot S, Lainas P, Leclerc P, Uzan G, Mahieu-Caputo D, Weber A. The role of HGF on invasive properties and repopulation potential of human fetal hepatic progenitor cells. *Exp Cell Res* 2009; **315**: 3396-3405
54 **Suzuki A**, Zheng Y, Kondo R, Kusakabe M, Takada Y, Fukao K, Nakauchi H, Taniguchi H. Flow-cytometric separation and enrichment of hepatic progenitor cells in the developing mouse liver. *Hepatology* 2000; **32**: 1230-1239
55 **Minguet S**, Cortegano I, Gonzalo P, Martínez-Marin JA, de Andrés B, Salas C, Melero D, Gaspar ML, Marcos MA. A population of c-Kit(low)(CD45/TER119)- hepatic cell progenitors

of 11-day postcoitus mouse embryo liver reconstitutes cell-depleted liver organoids. *J Clin Invest* 2003; **112**: 1152-1163
56 **Suzuki A**, Zheng YW, Kaneko S, Onodera M, Fukao K, Nakauchi H, Taniguchi H. Clonal identification and characterization of self-renewing pluripotent stem cells in the developing liver. *J Cell Biol* 2002; **156**: 173-184
57 **Suzuki A**, Iwama A, Miyashita H, Nakauchi H, Taniguchi H. Role for growth factors and extracellular matrix in controlling differentiation of prospectively isolated hepatic stem cells. *Development* 2003; **130**: 2513-2524
58 **Snykers S**, De Kock J, Rogiers V, Vanhaecke T. In vitro differentiation of embryonic and adult stem cells into hepatocytes: state of the art. *Stem Cells* 2009; **27**: 577-605
59 **Kinoshita T**, Miyajima A. Cytokine regulation of liver development. *Biochim Biophys Acta* 2002; **1592**: 303-312
60 **Kamiya A**, Kinoshita T, Miyajima A. Oncostatin M and hepatocyte growth factor induce hepatic maturation via distinct signaling pathways. *FEBS Lett* 2001; **492**: 90-94
61 **Sanchez A**, Alvarez AM, Benito M, Fabregat I. Transforming growth factor beta modulates growth and differentiation of fetal hepatocytes in primary culture. *J Cell Physiol* 1995; **165**: 398-405
62 **Fabregat I**, Sánchez A, Alvarez AM, Nakamura T, Benito M. Epidermal growth factor, but not hepatocyte growth factor, suppresses the apoptosis induced by transforming growth factor-beta in fetal hepatocytes in primary culture. *FEBS Lett* 1996; **384**: 14-18
63 **Pagan R**, Martín I, Llobera M, Vilaró S. Growth and differentiation factors inhibit the migratory phenotype of cultured neonatal rat hepatocytes induced by HGF/SF. *Exp Cell Res* 1997; **235**: 170-179
64 **Pagan R**, Sánchez A, Martin I, Llobera M, Fabregat I, Vilaró S. Effects of growth and differentiation factors on the epithelial-mesenchymal transition in cultured neonatal rat hepatocytes. *J Hepatol* 1999; **31**: 895-904
65 **Block GD**, Locker J, Bowen WC, Petersen BE, Katyal S, Strom SC, Riley T, Howard TA, Michalopoulos GK. Population expansion, clonal growth, and specific differentiation patterns in primary cultures of hepatocytes induced by HGF/SF, EGF and TGF alpha in a chemically defined (HGM) medium. *J Cell Biol* 1996; **132**: 1133-1149
66 **Sonnenberg E**, Meyer D, Weidner KM, Birchmeier C. Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J Cell Biol* 1993; **123**: 223-235
67 **Schirmacher P**, Geerts A, Pietrangelo A, Dienes HP, Rogler CE. Hepatocyte growth factor/hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology* 1992; **15**: 5-11
68 **Ramadori G**, Neubauer K, Odenthal M, Nakamura T, Knittel T, Schwögler S, Meyer zum Büschenfelde KH. The gene of hepatocyte growth factor is expressed in fat-storing cells of rat liver and is downregulated during cell growth and by transforming growth factor-beta. *Biochem Biophys Res Commun* 1992; **183**: 739-742
69 **LeCouter J**, Moritz DR, Li B, Phillips GL, Liang XH, Gerber HP, Hillan KJ, Ferrara N. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. *Science* 2003; **299**: 890-893
70 **Prat M**, Narsimhan RP, Crepaldi T, Nicotra MR, Natali PG, Comoglio PM. The receptor encoded by the human c-MET oncogene is expressed in hepatocytes, epithelial cells and solid tumors. *Int J Cancer* 1991; **49**: 323-328
71 **Matsumoto K**, Fujii H, Michalopoulos G, Fung JJ, Demetris AJ. Human biliary epithelial cells secrete and respond to cytokines and hepatocyte growth factors in vitro: interleukin-6, hepatocyte growth factor and epidermal growth factor promote DNA synthesis in vitro. *Hepatology* 1994; **20**: 376-382
72 **Burgess AW**, Cho HS, Eigenbrot C, Ferguson KM, Garrett TP, Leahy DJ, Lemmon MA, Sliwkowski MX, Ward CW, Yokoyama S. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. *Mol Cell* 2003; **12**: 541-552
73 **Harris RC**, Chung E, Coffey RJ. EGF receptor ligands. *Exp Cell Res* 2003; **284**: 2-13
74 **Citri A**, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* 2006; **7**: 505-516
75 **Bublil EM**, Yarden Y. The EGF receptor family: spearheading a merger of signaling and therapeutics. *Curr Opin Cell Biol* 2007; **19**: 124-134
76 **Mitsudomi T**, Yatabe Y. Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. *FEBS J* 2010; **277**: 301-308
77 **Bucher NL**, Patel U, Cohen S. Hormonal factors concerned with liver regeneration. *Ciba Found Symp* 1977; 95-107
78 **Liu ML**, Mars WM, Zarnegar R, Michalopoulos GK. Collagenase pretreatment and the mitogenic effects of hepatocyte growth factor and transforming growth factor-alpha in adult rat liver. *Hepatology* 1994; **19**: 1521-1527
79 **Draghi E**, Armato U, Andreis PG, Mengato L. The stimulation by epidermal growth factor (urogastrone) of the growth of neonatal rat hepatocytes in primary tissue culture and its modulation by serum and associated pancreatic hormones. *J Cell Physiol* 1980; **103**: 129-147
80 **McGowan JA**, Strain AJ, Bucher NL. DNA synthesis in primary cultures of adult rat hepatocytes in a defined medium: effects of epidermal growth factor, insulin, glucagon, and cyclic-AMP. *J Cell Physiol* 1981; **108**: 353-363
81 **Sawada N**, Lee GH, Mochizuki Y, Ishikawa T. Active proliferation of mouse hepatocytes in primary culture under defined conditions as compared to rat hepatocytes. *Jpn J Cancer Res* 1988; **79**: 983-988
82 **de Juan C**, Benito M, Alvarez A, Fabregat I. Differential proliferative response of cultured fetal and regenerating hepatocytes to growth factors and hormones. *Exp Cell Res* 1992; **202**: 495-500
83 **Natarajan A**, Wagner B, Sibilia M. The EGF receptor is required for efficient liver regeneration. *Proc Natl Acad Sci USA* 2007; **104**: 17081-17086
84 **Fabregat I**. Dysregulation of apoptosis in hepatocellular carcinoma cells. *World J Gastroenterol* 2009; **15**: 513-520
85 **Musallam L**, Ethier C, Haddad PS, Bilodeau M. Role of EGF receptor tyrosine kinase activity in antiapoptotic effect of EGF on mouse hepatocytes. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1360-G1369
86 **de Juan C**, Benito M, Fabregat I. Regulation of albumin expression in fetal rat hepatocytes cultured under proliferative conditions: role of epidermal growth factor and hormones. *J Cell Physiol* 1992; **152**: 95-101
87 **Sánchez A**, Pagan R, Alvarez AM, Roncero C, Vilaró S, Benito M, Fabregat I. Transforming growth factor-beta (TGF-beta) and EGF promote cord-like structures that indicate terminal differentiation of fetal hepatocytes in primary culture. *Exp Cell Res* 1998; **242**: 27-37
88 **Evarts RP**, Nakatsukasa H, Marsden ER, Hu Z, Thorgeirsson SS. Expression of transforming growth factor-alpha in regenerating liver and during hepatic differentiation. *Mol Carcinog* 1992; **5**: 25-31
89 **Evarts RP**, Hu Z, Fujio K, Marsden ER, Thorgeirsson SS. Activation of hepatic stem cell compartment in the rat: role of transforming growth factor alpha, hepatocyte growth factor, and acidic fibroblast growth factor in early proliferation. *Cell Growth Differ* 1993; **4**: 555-561
90 **Cheung RS**, Brooling JT, Johnson MM, Riehle KJ, Campbell JS, Fausto N. Interactions between MYC and transforming growth factor alpha alter the growth and tumorigenicity of liver progenitor cells. *Carcinogenesis* 2007; **28**: 2624-2631
91 **del Castillo G**, Alvarez-Barrientos A, Carmona-Cuenca I, Fernández M, Sánchez A, Fabregat I. Isolation and characterization of a putative liver progenitor population after

treatment of fetal rat hepatocytes with TGF-beta. *J Cell Physiol* 2008; **215**: 846-855
92 **Akhurst B**, Matthews V, Husk K, Smyth MJ, Abraham LJ, Yeoh GC. Differential lymphotoxin-beta and interferon gamma signaling during mouse liver regeneration induced by chronic and acute injury. *Hepatology* 2005; **41**: 327-335
93 **Matthews VB**, Klinken E, Yeoh GC. Direct effects of interleukin-6 on liver progenitor oval cells in culture. *Wound Repair Regen* 2004; **12**: 650-656
94 **Presnell SC**, Hooth MJ, Borchert KM, Coleman WB, Grisham JW, Smith GJ. Establishment of a functional HGF/C-MET autocrine loop in spontaneous transformants of WB-F344 rat liver stem-like cells. *Hepatology* 1998; **28**: 1253-1259
95 **Laird AD**, Brown PI, Fausto N. Inhibition of tumor growth in liver epithelial cells transfected with a transforming growth factor alpha antisense gene. *Cancer Res* 1994; **54**: 4224-4232
96 **Presnell SC**, Stolz DB, Mars WM, Jo M, Michalopoulos GK, Strom SC. Modifications of the hepatocyte growth factor/c-met pathway by constitutive expression of transforming growth factor-alpha in rat liver epithelial cells. *Mol Carcinog* 1997; **18**: 244-255
97 **Zaret KS**. Regulatory phases of early liver development: paradigms of organogenesis. *Nat Rev Genet* 2002; **3**: 499-512
98 **Zhao R**, Duncan SA. Embryonic development of the liver. *Hepatology* 2005; **41**: 956-967
99 **Lemaigre FP**. Mechanisms of liver development: concepts for understanding liver disorders and design of novel therapies. *Gastroenterology* 2009; **137**: 62-79
100 **Marsden ER**, Hu Z, Fujio K, Nakatsukasa H, Thorgeirsson SS, Evarts RP. Expression of acidic fibroblast growth factor in regenerating liver and during hepatic differentiation. *Lab Invest* 1992; **67**: 427-433
101 **Hu Z**, Evarts RP, Fujio K, Marsden ER, Thorgeirsson SS. Expression of fibroblast growth factor receptors flg and bek during hepatic ontogenesis and regeneration in the rat. *Cell Growth Differ* 1995; **6**: 1019-1025
102 **Spagnoli FM**, Cicchini C, Tripodi M, Weiss MC. Inhibition of MMH (Met murine hepatocyte) cell differentiation by TGF(beta) is abrogated by pre-treatment with the heritable differentiation effector FGF1. *J Cell Sci* 2000; **113** (Pt 20): 3639-3647
103 **Michalopoulos GK**. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. *Am J Pathol* 2010; **176**: 2-13
104 **Inagaki Y**, Okazaki I. Emerging insights into Transforming growth factor beta Smad signal in hepatic fibrogenesis. *Gut* 2007; **56**: 284-292
105 **Matsuzaki K**. Modulation of TGF-beta signaling during progression of chronic liver diseases. *Front Biosci* 2009; **14**: 2923-2934
106 **Thenappan A**, Li Y, Kitisin K, Rashid A, Shetty K, Johnson L, Mishra L. Role of transforming growth factor beta signaling and expansion of progenitor cells in regenerating liver. *Hepatology* 2010; **51**: 1373-1382
107 **Massagué J**. TGFbeta in Cancer. *Cell* 2008; **134**: 215-230
108 **Nakamura T**, Tomita Y, Hirai R, Yamaoka K, Kaji K, Ichihara A. Inhibitory effect of transforming growth factor-beta on DNA synthesis of adult rat hepatocytes in primary culture. *Biochem Biophys Res Commun* 1985; **133**: 1042-1050
109 **Braun L**, Mead JE, Panzica M, Mikumo R, Bell GI, Fausto N. Transforming growth factor beta mRNA increases during liver regeneration: a possible paracrine mechanism of growth regulation. *Proc Natl Acad Sci USA* 1988; **85**: 1539-1543
110 **Oberhammer FA**, Pavelka M, Sharma S, Tiefenbacher R, Purchio AF, Bursch W, Schulte-Hermann R. Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. *Proc Natl Acad Sci USA* 1992; **89**: 5408-5412
111 **Sánchez A**, Alvarez AM, Benito M, Fabregat I. Apoptosis induced by transforming growth factor-beta in fetal hepatocyte primary cultures: involvement of reactive oxygen intermediates. *J Biol Chem* 1996; **271**: 7416-7422
112 **Preisegger KH**, Factor VM, Fuchsbichler A, Stumptner C, Denk H, Thorgeirsson SS. Atypical ductular proliferation and its inhibition by transforming growth factor beta1 in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine mouse model for chronic alcoholic liver disease. *Lab Invest* 1999; **79**: 103-109
113 **Clark JB**, Rice L, Sadiq T, Brittain E, Song L, Wang J, Gerber DA. Hepatic progenitor cell resistance to TGF-beta1's proliferative and apoptotic effects. *Biochem Biophys Res Commun* 2005; **329**: 337-344
114 **Nguyen LN**, Furuya MH, Wolfraim LA, Nguyen AP, Holdren MS, Campbell JS, Knight B, Yeoh GC, Fausto N, Parks WT. Transforming growth factor-beta differentially regulates oval cell and hepatocyte proliferation. *Hepatology* 2007; **45**: 31-41
115 **Ding W**, Mouzaki M, You H, Laird JC, Mato J, Lu SC, Rountree CB. CD133+ liver cancer stem cells from methionine adenosyl transferase 1A-deficient mice demonstrate resistance to transforming growth factor (TGF)-beta-induced apoptosis. *Hepatology* 2009; **49**: 1277-1286
116 **Isfort RJ**, Cody DB, Stuard SB, Randall CJ, Miller C, Ridder GM, Doersen CJ, Richards WG, Yoder BK, Wilkinson JE, Woychik RP. The combination of epidermal growth factor and transforming growth factor-beta induces novel phenotypic changes in mouse liver stem cell lines. *J Cell Sci* 1997; **110** (Pt 24): 3117-3129
117 **Hirata M**, Amano K, Miyashita A, Yasunaga M, Nakanishi T, Sato K. Establishment and characterization of hepatic stem-like cell lines from normal adult rat liver. *J Biochem* 2009; **145**: 51-58
118 **Mishra L**, Cai T, Yu P, Monga SP, Mishra B. Elf3 encodes a novel 200-kD beta-spectrin: role in liver development. *Oncogene* 1999; **18**: 353-364
119 **Weinstein M**, Monga SP, Liu Y, Brodie SG, Tang Y, Li C, Mishra L, Deng CX. Smad proteins and hepatocyte growth factor control parallel regulatory pathways that converge on beta1-integrin to promote normal liver development. *Mol Cell Biol* 2001; **21**: 5122-5131
120 **Tang Y**, Katuri V, Dillner A, Mishra B, Deng CX, Mishra L. Disruption of transforming growth factor-beta signaling in ELF beta-spectrin-deficient mice. *Science* 2003; **299**: 574-577
121 **Mishra L**, Banker T, Murray J, Byers S, Thenappan A, He AR, Shetty K, Johnson L, Reddy EP. Liver stem cells and hepatocellular carcinoma. *Hepatology* 2009; **49**: 318-329
122 **Tang Y**, Kitisin K, Jogunoori W, Li C, Deng CX, Mueller SC, Ressom HW, Rashid A, He AR, Mendelson JS, Jessup JM, Shetty K, Zasloff M, Mishra B, Reddy EP, Johnson L, Mishra L. Progenitor/stem cells give rise to liver cancer due to aberrant TGF-beta and IL-6 signaling. *Proc Natl Acad Sci USA* 2008; **105**: 2445-2450
123 **Thiery JP**, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; **139**: 871-890
124 **Turley EA**, Veiseh M, Radisky DC, Bissell MJ. Mechanisms of disease: epithelial-mesenchymal transition--does cellular plasticity fuel neoplastic progression? *Nat Clin Pract Oncol* 2008; **5**: 280-290
125 **Valcourt U**, Kowanetz M, Niimi H, Heldin CH, Moustakas A. TGF-beta and the Smad signaling pathway support transcriptomic reprogramming during epithelial-mesenchymal cell transition. *Mol Biol Cell* 2005; **16**: 1987-2002
126 **Zavadil J**, Böttinger EP. TGF-beta and epithelial-to-mesenchymal transitions. *Oncogene* 2005; **24**: 5764-5774
127 **Gotzmann J**, Huber H, Thallinger C, Wolschek M, Jansen B, Schulte-Hermann R, Beug H, Mikulits W. Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGF-beta1 and Ha-Ras: steps towards invasiveness. *J Cell Sci* 2002; **115**: 1189-1202
128 **Valdés F**, Alvarez AM, Locascio A, Vega S, Herrera B, Fernández M, Benito M, Nieto MA, Fabregat I. The epithelial mesen-

chymal transition confers resistance to the apoptotic effects of transforming growth factor Beta in fetal rat hepatocytes. *Mol Cancer Res* 2002; **1**: 68-78
129 **Caja L**, Ortiz C, Bertran E, Murillo MM, Miró-Obradors MJ, Palacios E, Fabregat I. Differential intracellular signalling induced by TGF-beta in rat adult hepatocytes and hepatoma cells: implications in liver carcinogenesis. *Cell Signal* 2007; **19**: 683-694
130 **Kaimori A**, Potter J, Kaimori JY, Wang C, Mezey E, Koteish A. Transforming growth factor-beta1 induces an epithelial-to-mesenchymal transition state in mouse hepatocytes in vitro. *J Biol Chem* 2007; **282**: 22089-22101
131 **Shin I**, Bakin AV, Rodeck U, Brunet A, Arteaga CL. Transforming growth factor beta enhances epithelial cell survival via Akt-dependent regulation of FKHRL1. *Mol Biol Cell* 2001; **12**: 3328-3339
132 **Valdés F**, Murillo MM, Valverde AM, Herrera B, Sánchez A, Benito M, Fernández M, Fabregat I. Transforming growth factor-beta activates both pro-apoptotic and survival signals in fetal rat hepatocytes. *Exp Cell Res* 2004; **292**: 209-218
133 **Murillo MM**, del Castillo G, Sánchez A, Fernández M, Fabregat I. Involvement of EGF receptor and c-Src in the survival signals induced by TGF-beta1 in hepatocytes. *Oncogene* 2005; **24**: 4580-4587
134 **Nicolás FJ**, Lehmann K, Warne PH, Hill CS, Downward J. Epithelial to mesenchymal transition in Madin-Darby canine kidney cells is accompanied by down-regulation of Smad3 expression, leading to resistance to transforming growth factor-beta-induced growth arrest. *J Biol Chem* 2003; **278**: 3251-3256
135 **Gal A**, Sjöblom T, Fedorova L, Imreh S, Beug H, Moustakas A. Sustained TGF beta exposure suppresses Smad and non-Smad signalling in mammary epithelial cells, leading to EMT and inhibition of growth arrest and apoptosis. *Oncogene* 2008; **27**: 1218-1230
136 **Vega S**, Morales AV, Ocaña OH, Valdés F, Fabregat I, Nieto MA. Snail blocks the cell cycle and confers resistance to cell death. *Genes Dev* 2004; **18**: 1131-1143
137 **Leroy P**, Mostov KE. Slug is required for cell survival during partial epithelial-mesenchymal transition of HGF-induced tubulogenesis. *Mol Biol Cell* 2007; **18**: 1943-1952
138 **Sánchez A**, Alvarez AM, López Pedrosa JM, Roncero C, Benito M, Fabregat I. Apoptotic response to TGF-beta in fetal hepatocytes depends upon their state of differentiation. *Exp Cell Res* 1999; **252**: 281-291
139 **Dudas J**, Mansuroglu T, Batusic D, Saile B, Ramadori G. Thy-1 is an in vivo and in vitro marker of liver myofibroblasts. *Cell Tissue Res* 2007; **329**: 503-514
140 **Peñuelas S**, Anido J, Prieto-Sánchez RM, Folch G, Barba I, Cuartas I, García-Dorado D, Poca MA, Sahuquillo J, Baselga J, Seoane J. TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell* 2009; **15**: 315-327
141 **You H**, Ding W, Rountree CB. Epigenetic regulation of cancer stem cell marker CD133 by transforming growth factor-beta. *Hepatology* 2010; **51**: 1635-1644
142 **McMahon AP**, Ingham PW, Tabin CJ. Developmental roles and clinical significance of hedgehog signaling. *Curr Top Dev Biol* 2003; **53**: 1-114
143 **Mehlen P**, Mille F, Thibert C. Morphogens and cell survival during development. *J Neurobiol* 2005; **64**: 357-366
144 **Goodrich LV**, Johnson RL, Milenkovic L, McMahon JA, Scott MP. Conservation of the hedgehog/patched signaling pathway from flies to mice: induction of a mouse patched gene by Hedgehog. *Genes Dev* 1996; **10**: 301-312
145 **Becker S**, Wang ZJ, Massey H, Arauz A, Labosky P, Hammerschmidt M, St-Jacques B, Bumcrot D, McMahon A, Grabel L. A role for Indian hedgehog in extraembryonic endoderm differentiation in F9 cells and the early mouse embryo. *Dev Biol* 1997; **187**: 298-310
146 **Grabel L**, Becker S, Lock L, Maye P, Zanders T. Using EC and ES cell culture to study early development: recent observations on Indian hedgehog and Bmps. *Int J Dev Biol* 1998; **42**: 917-925
147 **Deutsch G**, Jung J, Zheng M, Lóra J, Zaret KS. A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development* 2001; **128**: 871-881
148 **Hirose Y**, Itoh T, Miyajima A. Hedgehog signal activation coordinates proliferation and differentiation of fetal liver progenitor cells. *Exp Cell Res* 2009; **315**: 2648-2657
149 **Sicklick JK**, Li YX, Melhem A, Schmelzer E, Zdanowicz M, Huang J, Caballero M, Fair JH, Ludlow JW, McClelland RE, Reid LM, Diehl AM. Hedgehog signaling maintains resident hepatic progenitors throughout life. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G859-G870
150 **Jung Y**, Brown KD, Witek RP, Omenetti A, Yang L, Vandongen M, Milton RJ, Hines IN, Rippe RA, Spahr L, Rubbia-Brandt L, Diehl AM. Accumulation of hedgehog-responsive progenitors parallels alcoholic liver disease severity in mice and humans. *Gastroenterology* 2008; **134**: 1532-1543
151 **Jung Y**, Diehl AM. Non-alcoholic steatohepatitis pathogenesis: role of repair in regulating the disease progression. *Dig Dis* 2010; **28**: 225-228
152 **Jung Y**, Witek RP, Syn WK, Choi SS, Omenetti A, Premont R, Guy CD, Diehl AM. Signals from dying hepatocytes trigger growth of liver progenitors. *Gut* 2010; **59**: 655-665
153 **Fleig SV**, Choi SS, Yang L, Jung Y, Omenetti A, VanDongen HM, Huang J, Sicklick JK, Diehl AM. Hepatic accumulation of Hedgehog-reactive progenitors increases with severity of fatty liver damage in mice. *Lab Invest* 2007; **87**: 1227-1239
154 **Sicklick JK**, Li YX, Choi SS, Qi Y, Chen W, Bustamante M, Huang J, Zdanowicz M, Camp T, Torbenson MS, Rojkind M, Diehl AM. Role for hedgehog signaling in hepatic stellate cell activation and viability. *Lab Invest* 2005; **85**: 1368-1380
155 **Omenetti A**, Yang L, Li YX, McCall SJ, Jung Y, Sicklick JK, Huang J, Choi S, Suzuki A, Diehl AM. Hedgehog-mediated mesenchymal-epithelial interactions modulate hepatic response to bile duct ligation. *Lab Invest* 2007; **87**: 499-514
156 **Richardson MM**, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, Purdie DM, Demetris AJ, Clouston AD. Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. *Gastroenterology* 2007; **133**: 80-90
157 **Yang L**, Wang Y, Mao H, Fleig S, Omenetti A, Brown KD, Sicklick JK, Li YX, Diehl AM. Sonic hedgehog is an autocrine viability factor for myofibroblastic hepatic stellate cells. *J Hepatol* 2008; **48**: 98-106
158 **Omenetti A**, Porrello A, Jung Y, Yang L, Popov Y, Choi SS, Witek RP, Alpini G, Venter J, Vandongen HM, Syn WK, Baroni GS, Benedetti A, Schuppan D, Diehl AM. Hedgehog signaling regulates epithelial-mesenchymal transition during biliary fibrosis in rodents and humans. *J Clin Invest* 2008; **118**: 3331-3342
159 **Syn WK**, Jung Y, Omenetti A, Abdelmalek M, Guy CD, Yang L, Wang J, Witek RP, Fearing CM, Pereira TA, Teaberry V, Choi SS, Conde-Vancells J, Karaca GF, Diehl AM. Hedgehog-mediated epithelial-to-mesenchymal transition and fibrogenic repair in nonalcoholic fatty liver disease. *Gastroenterology* 2009; **137**: 1478-1488.e8
160 **Capdevila J**, lzpisúa Belmonte JC. Extracellular modulation of the Hedgehog, Wnt and TGF-beta signalling pathways during embryonic development. *Curr Opin Genet Dev* 1999; **9**: 427-433
161 **Jung Y**, McCall SJ, Li YX, Diehl AM. Bile ductules and stromal cells express hedgehog ligands and/or hedgehog target genes in primary biliary cirrhosis. *Hepatology* 2007; **45**: 1091-1096
162 **Cadigan KM**, Peifer M. Wnt signaling from development to disease: insights from model systems. *Cold Spring Harb Perspect Biol* 2009; **1**: a002881

163 **Monga SP**. Role of Wnt/beta-catenin signaling in liver metabolism and cancer. *Int J Biochem Cell Biol* 2009; Epub ahead of print

164 **van Amerongen R**, Nusse R. Towards an integrated view of Wnt signaling in development. *Development* 2009; **136**: 3205-3214

165 **MacDonald BT**, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell* 2009; **17**: 9-26

166 **Fischer AN**, Fuchs E, Mikula M, Huber H, Beug H, Mikulits W. PDGF essentially links TGF-beta signaling to nuclear beta-catenin accumulation in hepatocellular carcinoma progression. *Oncogene* 2007; **26**: 3395-3405

167 **Breuhahn K**, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. *Oncogene* 2006; **25**: 3787-3800

168 **Monga SP**, Mars WM, Pediaditakis P, Bell A, Mulé K, Bowen WC, Wang X, Zarnegar R, Michalopoulos GK. Hepatocyte growth factor induces Wnt-independent nuclear translocation of beta-catenin after Met-beta-catenin dissociation in hepatocytes. *Cancer Res* 2002; **62**: 2064-2071

169 **Apte U**, Zeng G, Muller P, Tan X, Micsenyi A, Cieply B, Dai C, Liu Y, Kaestner KH, Monga SP. Activation of Wnt/beta-catenin pathway during hepatocyte growth factor-induced hepatomegaly in mice. *Hepatology* 2006; **44**: 992-1002

170 **Hu M**, Kurobe M, Jeong YJ, Fuerer C, Ghole S, Nusse R, Sylvester KG. Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. *Gastroenterology* 2007; **133**: 1579-1591

171 **Apte U**, Thompson MD, Cui S, Liu B, Cieply B, Monga SP. Wnt/beta-catenin signaling mediates oval cell response in rodents. *Hepatology* 2008; **47**: 288-295

172 **Williams JM**, Oh SH, Jorgensen M, Steiger N, Darwiche H, Shupe T, Petersen BE. The role of the Wnt family of secreted proteins in rat oval "stem" cell-based liver regeneration: Wnt1 drives differentiation. *Am J Pathol* 2010; **176**: 2732-2742

173 **McLin VA**, Rankin SA, Zorn AM. Repression of Wnt/beta-catenin signaling in the anterior endoderm is essential for liver and pancreas development. *Development* 2007; **134**: 2207-2217

174 **Yang W**, Yan HX, Chen L, Liu Q, He YQ, Yu LX, Zhang SH, Huang DD, Tang L, Kong XN, Chen C, Liu SQ, Wu MC, Wang HY. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res* 2008; **68**: 4287-4295

175 **Yamashita T**, Budhu A, Forgues M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. *Cancer Res* 2007; **67**: 10831-10839

176 **Zulehner G**, Mikula M, Schneller D, van Zijl F, Huber H, Sieghart W, Grasl-Kraupp B, Waldhör T, Peck-Radosavljevic M, Beug H, Mikulits W. Nuclear beta-catenin induces an early liver progenitor phenotype in hepatocellular carcinoma and promotes tumor recurrence. *Am J Pathol* 2010; **176**: 472-481

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EDITORIAL

Paediatric and adult colonic manometry: A tool to help unravel the pathophysiology of constipation

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Abstract

Colonic motility subserves large bowel functions, including absorption, storage, propulsion and defaecation. Colonic motor dysfunction remains the leading hypothesis to explain symptom generation in chronic constipation, a heterogeneous condition which is extremely prevalent in the general population, and has huge socioeconomic impact and individual suffering. Physiological testing plays a crucial role in patient management, as it is now accepted that symptom-based assessment, although important, is unsatisfactory as the sole means of directing therapy. Colonic manometry provides a direct method for studying motor activities of the large bowel, and this review provides a contemporary understanding of how this technique has enhanced our knowledge of normal colonic motor physiology, as well as helping to elucidate pathophysiological mechanisms underlying constipation. Methodological approaches, including available catheter types, placement technique and recording protocols, are covered, along with a detailed description of recorded colonic motor activities. This review also critically examines the role of colonic manometry in current clinical practice, and how manometric assessment may aid diagnosis, classification and guide therapeutic intervention in the constipated individual. Most importantly, this review considers both adult and paediatric patients. Limitations of the procedure and a look to the future are also addressed.

Key words: Colon; Constipation; Manometry; Paediatric; Adult

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INTRODUCTION

Constipation is a common condition, with approximately 15% of adults and 9% of children reporting symptoms[1-4]. The direct and indirect costs are substantial; in the USA alone, an estimated $US1.7 billion/year is spent on adults[5] and a further $US3.9 billion/year on childhood constipation[6]. While anatomical malformation (e.g. anal stenosis, imperforate anus), metabolic and gastrointestinal causes (e.g. hypothyroidism, celiac disease, and cystic fibrosis),

intestinal nerve or muscle disorders (e.g. Hirschsprung disease, chronic idiopathic intestinal pseudo-obstruction), childbirth or pelvic surgery are known to cause constipation, for many patients, their condition is regarded as "idiopathic". Indeed, in children, it has been reported that less than 10% of those suffering from constipation have a recognizable underlying organic cause[7,8]. Defects in the nerves and pacemaker cells (e.g. decreased ICC density[9]) could be responsible in a proportion of cases, but as yet, techniques are not sufficiently developed to clearly identify such altered morphology, rendering any attribution to patho-aetiological significance (cause, effect, or epiphenomenon) speculative[8,10,11].

Given that both aetiology and pathophysiology are unclear in many cases, it is perhaps therefore not surprising that up to one-third of adults and children who seek medical help will fail non-surgical therapy[12,13]. Patients refractory to such interventions are debilitated, with physical functioning, mental health, general health and bodily pain all scoring poorly on quality of life questionnaires, compared to the non-constipated population[14]; indeed, the impact is comparable to other chronic conditions, such as inflammatory bowel disease in adults[15] and cancer in children[16]. In refractory cases, surgery, including subtotal or total colectomy, repair of anatomical anomalies believed to impede defaecation[17,18], appendicostomies[19], and more recently sacral nerve stimulation[20] may be considered. However surgical procedures are not without risk, with reported post-operative morbidity often high, and functional outcomes suboptimal[17,21,22]; this, in turn, equates to poor quality of life[23].

Improving therapies for functional bowel disorders is a fundamental goal for all researchers and clinicians working in this field, and to achieve this objective, a better understanding of pathophysiologies that may underpin such disorders is essential. In chronic constipation, altered colonic motor function remains the leading hypothesis to explain symptom generation, and this may be true not only for constipation associated with delayed colonic transit[24], but also for constipation allied to an evacuatory disorder[25]. In clinical practice, routine investigation of colonic motor function is achieved by use of transit studies (typically radio-opaque markers[24,26]), while defecatory function typically is assessed using evacuation proctography or balloon expulsion. Diagnostic manometry, which provides a direct assessment of those contractile activities subserving intestinal functions, is well developed for other parts of the gastrointestinal (GI) tract, notably the oesophagus and anorectum, but its diagnostic potential in the colon remains less certain. This is due to a number of factors including the relative inaccessibility of the proximal large bowel, the technical constrains of current manometric recording systems, and inadequate understanding of normal colonic motor physiology, which is fundamental to confidently diagnosing motor abnormalities in disease states. This review examines the equipment and protocols used to record colonic motor patterns *in vivo* in humans, with particular focus on the diagnostic relevance of data obtained, and the ability of colonic manometry to guide treatment. Current limitations and future direction for the procedure are also covered.

COLONIC MANOMETRY

Manometric studies of true colonic (as opposed to recto-sigmoid) motor function have been reported upon since the late 1980s[27]. However, the colon remains the least well understood section of the GI tract, particularly its proximal portions. For constipation, around 20 studies in adults, and fewer in paediatric populations, have been published. As such, colonic manometry is still largely used as a research tool in adults, with relatively few authors advocating diagnostic use[28,29]. Nevertheless, a recent American Neurogastroenterology and Motility Society consensus statement has recommended this test for the assessment of severely constipated patients (both adult and paediatric) who are unresponsive to medical therapy, and who have evidence of slow colonic transit in the absence of an evacuatory disorder[30]. Furthermore, in children, colonic manometry has been proposed to discriminate normal colonic motility from colonic neuromuscular disorders[31]. It may also help to clarify the pathophysiology of persistent lower GI symptoms after surgery for Hirschsprung disease, evaluate colonic involvement in a child diagnosed with idiopathic intestinal pseudo-obstruction syndrome, assess function of a diverted colon prior to possible re-anastomosis, and to assess colonic motor activity prior to intestinal transplantation to determine if the colon should be kept at the time of transplant[32]. What is certainly true is that colonic manometric procedures lack standardisation, with a variety of catheter types, placement techniques and protocols currently used.

It must also be remembered that colonic manometry is an invasive procedure, and as such should only be performed in those patients with symptoms severe enough to have a marked impact on their quality of life and social well-being. Extensive conventional treatment should be offered before testing colonic motility.

Catheter types

In general, there are two broad types of catheters used: (1) water-perfused; and (2) solid-state. As yet, there have been no studies which directly compare the two types. Preference for one over the other is guided by existing equipment, desired application, study design, and particularly cost.

Water-perfused manometry catheters: Water-perfused manometry is used in children and has been the preferred choice in the vast majority of studies in adults. Catheters are made of flexible PVC[27,33] or extruded lengths of silicone rubber[34]. The latter is highly flexible, and is desirable for patient tolerability if an oral route is used for intubation (see below). The catheters incorporate between 4 and 16 recording ports or side-holes, with an inter-side-hole distance of between 1 and 15 cm[24,35]. Each recording port

consists of an open lumen, which is connected to a pneumohydraulic infusion pump that ensures constant flow of water. The basic premise for recording is that contractions of the colonic wall occlude the manometric ports, thus impeding flow of perfusate (degassed water). Resistance to flow is transmitted as pressure change to external transducers, and the degree of resistance depends upon the amplitude and duration of the contraction. Such catheters are relatively inexpensive and highly versatile with regard to configuration (number, position and orientation of side-holes); in addition, silicone catheters are autoclaveable, and therefore can be re-used many times without fear of contamination. However, due to the requirement of being attached to the manometry system, and need for constant water infusion, studies are always performed in the laboratory setting. This confines the subject to a bed for the length of the recording period with minimal mobility allowed, thus creating a somewhat unphysiological environment. In addition, the volume of water introduced into the lumen is considerable (> 3 litres) if recording from multiple side-holes for prolonged periods of time. Whilst the colon does have the ability to absorb up to 6 litres of fluid a day[36], the effects of introduction of this amount of fluid upon colonic motility remains unknown.

Solid state manometry catheters: So-called 'solid-state' assemblies traditionally consist of strain gauges embedded into a flexible PVC catheter. Each strain gauge is attached to an amplification/recording system *via* fine connective wiring[35]. Until recently, solid-state catheters have typically had between 6 and 10 sensors spaced at 7-15 cm intervals[24]; however, recent technological advances now allow in excess of 20 sensors. Recorded pressure signals can be stored on portable digital recorders enabling the subject to be ambulant[29,37]. However, the cost of these catheters is considerable in comparison to water-perfused catheters, and there has been a tendency in the past for recording channels to break[38,39]. Nevertheless, with further technological improvements, and recent developments in fibre-optic technology, which has seen the emergence of an optical manometry system (see Future directions below), such limitations should be overcome[40,41].

Placement techniques

In human subjects, gaining access to the colon can generally be achieved through two routes: *antegrade* placement *via* the nose or mouth, or *retrograde* placement *via* the anus. Colonic catheters can alternatively be placed through a variety of stomas, a method used particularly in children[42-44] but also in adults[45].

Retrograde placement: This provides the easiest access to the colon, and is by far the most commonly used procedure in both adults and children[33,37,46,47]. In adults, catheters are placed with the aid of a colonoscope and can be positioned in the desired location. To date, most studies utilising this method have located the tip of the catheter at the hepatic flexure or mid-transverse colon, meaning that recordings are only achieved from sites distal to this. However, the Sydney group have achieved true pancolonic recordings over the past few years by securing the tip of the catheter to ascending colonic/caecal mucosal folds using endoclips[20,29,48,49]; this has the advantage of helping to prevent catheter displacement or excretion during defecation[50,51].

In children, the retrograde placement of colonic manometry catheters has also utilised colonoscopic procedures[46]; however, in recent years colonic catheters have also been successfully placed using fluoroscopic guidance alone, both through stomas and *via* the rectum[44]. However, this technique may be associated with prolonged exposure to radiation, in some cases up to 27 min[44].

One of the major advantages of retrograde catheter intubation is subject tolerability. Once the catheter has been sited, there is little or no discomfort. However, in addition to concerns over catheter displacement, there are other potential disadvantages, most notably the requirement for bowel preparation and usually some form of anaesthesia or sedation. The removal of faeces from the colon has recently been shown to result in an increase in the frequency of high amplitude propagating contractions[52,53], and also to disrupt the spatiotemporal organisation of propagating sequences (PSs)[53]. However, the colonic responses to physiological stimuli, such as a meal and morning waking, remain unchanged[53]. Nevertheless, it could be argued that bowel preparation is advantageous, in that the 'starting point' for each study, provided that bowel preparation is performed in an equivalent manner, is standardised.

Antegrade placement: This is a less common method for recording colonic motility and is restricted to adult studies[25,34,39,54]. The catheter is fed through the nasal cavity or mouth, and once through the pylorus (often achieved under fluoroscopic guidance), a balloon on the tip of the catheter is inflated with air or water to facilitate transit through the small and large bowel[25,34]. The main advantage of this procedure is that it obviates the need for bowel preparation, and thus the study is performed under essentially normal basal physiological conditions. The procedure is, however, time-consuming in comparison to retrograde catheter placement. Even in healthy controls, it may take up to 36 h to intubate the distal descending colon[34,35]. Furthermore, with the catheter *in situ* for a prolonged period of time, subject tolerability becomes an issue with nasal/oral/pharyngeal discomfort and nausea commonly reported. Finally, while antegrade placement can be used to study colonic motility in health and in patients with relatively normal colonic transit[25], its use in patients with slow transit constipation (STC) is problematic. As antegrade catheter placement relies on peristalsis to promote the catheter tip through the gut, the process can be greatly prolonged in patients with a disorder characterised by delayed gut transit (the primary clinical indication for this investigation[30]), especially when small bowel motility may also be also abnormal[55].

Placement through a stoma: Intubation *via* appendicostomy, caecostomy, ileostomy or colostomy has been used to successfully investigate children with STC[19,44,56]. In such subjects, after a preceding colonic washout, the in-dwelling device (e.g. Chait caecostomy button) is removed. Two techniques can then be adapted: (1) following sedation, the catheter can be advanced through the stoma with the aid of a guidewire, and positioned under fluoroscopic guidance[44]; and (2) a 10F feeding tube can be positioned through the stoma through which a Bisacodyl solution (2-4 mg) can be instilled directly into the caecum. A manometry catheter can then be introduced through the feeding tube and advanced in an antegrade direction along the colon. The Bisacodyl induces propulsive activity, and a balloon on the tip of the catheter can be inflated with water to aid propulsion through the large bowel[42,43].

RECORDED COLONIC MOTOR ACTIVITIES

Non-propagating motor activity

Non-propagating activity makes up the majority of a colonic manometric recording, consisting of apparently random pressure waves recorded at single or multiple recording sites, often alternating with phases of motor quiescence. Pressure waves may be sub-classified on the basis of duration, and are considered to be of either long duration (the majority) or short duration (often superimposed on long duration pressure waves). The presence of two types of phasic contraction in the colon is unique, compared with the remainder of the GI tract[57]. The functionality of non-propagating activity is outlined below.

Propagating motor activity

Propagating pressure waves are categorised into PSs (also termed propagating contractions) and high amplitude PSs (HAPSs; also termed high amplitude propagating contractions; Figure 1); the latter are recognised as the motor correlate of mass intraluminal movement and are involved in defaecation (Figure 2)[59-62]. Both PSs and HAPSs can be further qualified by the terms antegrade (aboral) or retrograde (oral), depending upon the direction of propagation. However, criteria used to define these events are inconsistent[35]. A general classification includes an array of 3 or more pressure waves recorded from adjacent recording sites, with a trough to peak amplitude $\geqslant$ 5 mmHg per pressure wave, in which the conduction velocity between wave onsets is between 0.2 and 12 cm/s[34,50]. The definition of what constitutes a HAPS is based on a threshold value for amplitude of one or more of the component pressure waves[35]; this varies markedly in the literature from > 50 to 136 mmHg[34,50,63-67]. Consequently, the range of published frequencies is wide[35]; in many cases, the chosen threshold value appears to be arbitrary. Some, however, are based on proven functional characteristics[34].

PSs are identified throughout the large bowel, although they originate with greater frequency in the proximal compared to the distal colon[37,38,58,59,68,69]. The majority of ascending colonic PSs do not migrate beyond the splenic flexure, and conduction velocity of PSs increases as the PS moves distally from the proximal colon, as does the amplitude of the propagating pressure waves[50,58,59,70].

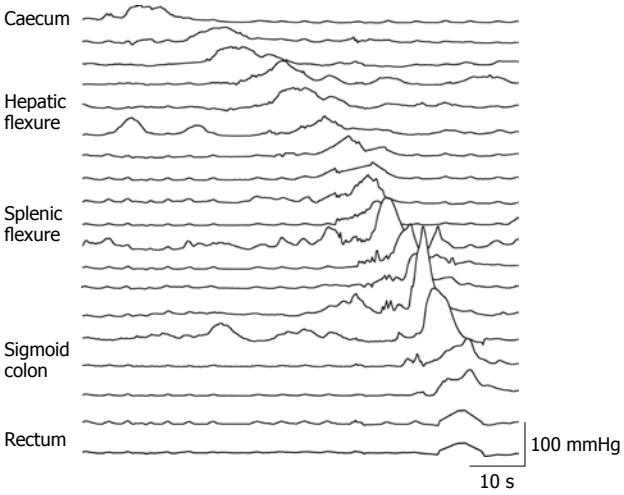

Figure 1 The high amplitude propagating sequence was first described in 1988[27]. The majority of these propagating events originate in the proximal colon and extend to the sigmoid colon. The amplitude of the component pressure waves increase as the motor patterns reaches the descending colon[58].

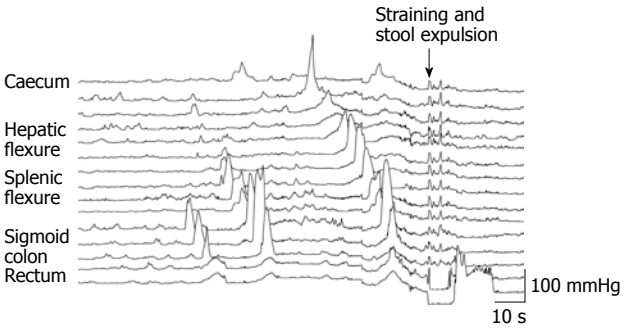

Figure 2 High amplitude propagating sequences are commonly associated with defaecation in healthy controls. Note the series of three high amplitude propagating sequences (HAPSs) prior to stool expulsion. The first originates at the distal descending colon, and with each subsequent HAPS, the site of origin moves to a more proximal location with the final HAPS extending the entire length of the colon[34].

Given their functional significance, HAPSs have garnered most interest in terms of pathological implication, and indeed decreased HAPS frequency is widely accepted as one of the principal hallmarks of STC (see below[27,29]). However, focus on propagated pressure waves alone, as is the case in many published reports, has perhaps led to their clinical impact being over-stressed, particularly given that they occur only a minority of the time during a colonic manometric recording; the organisation of other, more common motor activities may be of similar (or even greater) importance.

Organised colonic motor patterns

Akin to the interdigestive migrating motor complex of the upper GI tract[71], the colon exhibits periodic bursts of regular phasic pressure wave activity, termed either colonic

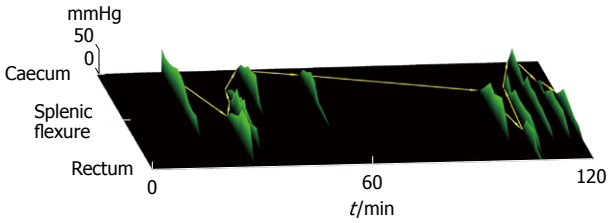

Figure 3 Two hour spatiotemporal maps of colonic propagating sequences in a female healthy control. In this map each individual ridge represents an antegrade propagating sequence. The start of each ridge indicates the site of origin and the time of day the propagating sequence occurred. The length of the ridge indicates the extent of propagation. The heights of the ridge indicate the amplitude of the component pressure waves. The yellow arrows link the site of origin of sequential propagating sequences. While no single propagating sequence spans the entire colon collectively, a linked series of propagating sequences can do so[76].

motor complexes[38,39,58] or rectal motor complexes (MC)[35,72], depending upon their site of origin. MCs predominate in the sigmoid or rectum, where they have also been termed "periodic rectal motor activity"[37,73,74]. The functional significance of these events is unclear, although it has been suggested that they provide a "brake" around the rectosigmoid junction[73], and reflect enteric neuromotor integrity, given that they occur in extrinsically denervated colon preparations[75].

In addition to MCs, sequential PSs have been shown to be linked in organized spatiotemporal patterns throughout the colon[76]. Many of these PSs form series in which three or more consecutive PSs demonstrate a regional shift in the colonic region from which they originate, i.e. each PS in a linked series will originate in a progressively more proximal or distal colonic location. Whilst most single PSs do not span the length the colon, collectively, a series of linked PSs can do so. It is likely that such linkage is important for the transport of content over longer lengths of the colon (Figure 3)[76].

COLONIC MANOMETRY PROTOCOLS

Paediatric studies

Despite the variety of placement techniques and catheter types, once the catheter is positioned, there is commonality to the actual recording protocol. In children, clinical studies generally do not last more than 4-5 h[77], but 24 h studies have been used in the research setting to provide more detailed information on motility characteristics[42,43]. Paediatric colonic manometry catheters typically contain 6-8 recording side holes, evenly spaced at 7.5, 10 or 15 cm intervals[42,43,77]. The paediatric colonic motility test includes at least 1 h of fasting and 1 h postprandial after finishing a high calorie meal. During the test, the child is required to remain in bed and it is important to have a trained observer at the bedside. If HAPSs are not recorded during the fasted or postprandial period, then drug stimulation with Bisacodyl (0.2 mg/kg, max 10 mg, diluted with 0.9% NaCl) is administered. HAPSs induced by Bisacodyl usually occur within a few minutes and are identical to spontaneous HAPSs. A cramping sensation and the urge to defaecate often accompany the presence of HAPSs[47]. If the paediatric test extends to 24 h, then the nocturnal frequency of motor patterns and the colonic response to waking are also measured[42,43,77].

Adult studies

In published adult colonic manometry studies, similar protocols are followed, although the catheter length is generally longer, containing up 16 sensors (water-perfused) spaced at 7.5 cm intervals[25,34]. Recordings are typically of 24 h duration. All studies examine two or more of the following: (1) fasting; (2) meal responses; (3) nocturnal suppression; (4) morning waking; and (5) response to chemical stimuli[24,35].

Normative data

For a clinical diagnosis, data collected from patients is compared to that obtained from healthy controls. In the adult population, recruitment of volunteers is generally not problematic, allowing normal ranges to be developed for various parameters of the motility recording (though it must again be stated that the size of published normative data sets remains inadequate).

By contrast, colonic motility recordings in truly healthy paediatric control subjects are lacking for obvious ethical reasons. Di Lorenzo *et al*[78] attempted colonic manometry in 32 "healthy" paediatric patients, though they were not strictly healthy controls, as they had diagnoses ranging from functional faecal retention (n = 15) and non-ulcer dyspepsia (n = 10), to Munchausen by proxy (n = 7). Other paediatric studies in adolescents with STC have used healthy young adults as the control group; whether this is appropriate is unknown[42,43].

OBSERVATIONS FROM COLONIC MANOMETRY STUDIES

Both propagating and non-propagating motor patterns may be temporally associated with propulsion and mixing of colonic content[59,60,79], signifying that these are important determinants of normal physiology in health and of pathophysiology in patient groups. In adult and paediatric patients with chronic constipation, a number of findings have been made which suggest pathological significance, including: (1) a lack of the normal[37,58,80] increase in colonic motor activity after a high calorie meal[29,43,46,49,80-86] (Figure 4); (2) a lack of the normal[29,50,51,58] suppression of colonic motor activity at night, and a suppressed or absent increase in colonic activity in response to morning waking[25,29,43,80] (Figure 4); (3) a decrease in frequency of HAPSs[27-29,39,47,49,80,83,85-88], although normal daily frequencies have also been reported in some constipated subjects[25,43] (Figure 4); (4) increased non-propagating activity in the left colon[89]; (5) absence[25] of the characteristic spatiotemporal patterning of PSs preceding normal spontaneous defecation[34]; (6) diminished co-ordination between

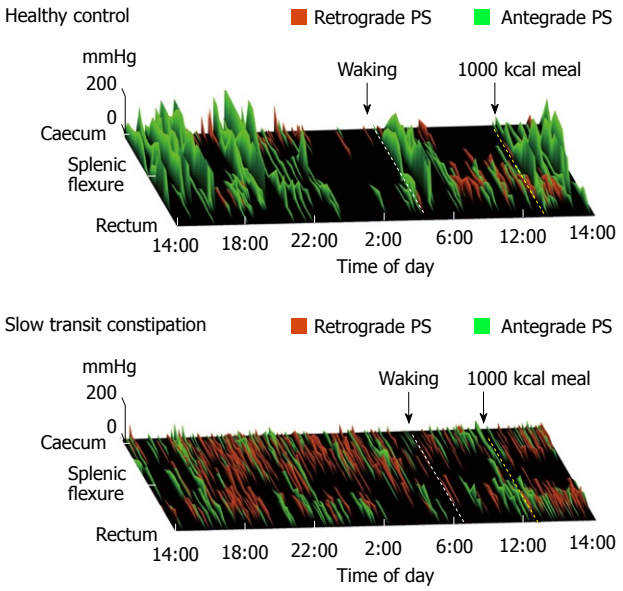

Figure 4 Twenty-four hour spatiotemporal maps[80] of colonic propagating sequences in a female healthy control and a female patient with slow transit constipation. Within each map the ridges represent antegrade (green) and retrograde (red) propagating sequences (PS). The antegrade PSs originate at the orad end of the green ridge and retrograde PSs originate at the anal end of the red ridges. The start of each antegrade and retrograde ridge indicates the site of origin and the time of day the PS occurred. The length of the ridge indicates the extent of propagation. The height of the ridge indicates the amplitude of the component pressure waves. The yellow-hatched line indicates when the 1000 kcal lunch was given, the white-hatched line indicates the time of morning waking. In health the frequency of propagating sequences are reduced during sleep and increase upon morning waking and in response to a high calorie meal. The map in the STC patients is characterized by an increased frequency of short extent PSs and a lack of high amplitude propagating sequences. There is an absent meal response (no increase in PSs) and an absent nocturnal suppression of PSs.

sequential pan-colonic PSs[76,80]; (7) a blunting of the normal[49] increase in PS or HAPS frequency in response to rectal or colonic infusion of Bisacodyl has been shown in a proportion of individuals[47,49,85], although in the majority of patients a normal response is recorded[49,83,85,90-92]; (8) a blunting of the normal increase in colonic HAPS frequency to intravenous injection of the cholinergic agonist edrophonium chloride[92]; (9) a blunting of the normal proximal colonic increase in motor activity in response to a rectal infusion of chenodeoxycholic acid[93]; (10) an increase in the frequency of HAPSs in response to sacral nerve stimulation in patients with STC[20]; and (11) a significant increase in the frequency of rectosigmoid motor complex activity[73,74].

CAN COLONIC MANOMETRY FINDINGS HELP TO DISTINGUISH SUBTYPES OF CONSTIPATION?

Constipation can be conceptualized into two broad overlapping categories: STC, and disorders of evacuation[1,94-96]. In some cases, this distinction among subgroups, based on aberrant physiological measurement, has proven beneficial in planning treatment and in predicting therapeutic outcome[96-99]. For example, identification of dyssynergic defaecation by anorectal manometry and balloon expulsion testing, in the absence of delayed colonic transit, is predictive of a high success rate from biofeedback[97,98]. Likewise, in carefully selected patients with severe STC, where the transit delay is restricted to the colon[17,22,100,101], and there is no evidence of evacuatory dysfunction, surgery such as colectomy with ileorectal anastomosis may be effective. In children this group is successfully managed with an appendix stoma and antegrade enemas[19].

In terms of colonic manometric investigation, specific biomarkers or biosignatures that may help to define constipation subtypes (and thus provide therapeutic targets) have, as yet, not been fully established. In adults, only three studies have addressed the validity of manometric findings between "recognised" subgroups[102-104]. The first concluded that intraluminal measurements could not be used to discriminate between subgroups of patients with either STC or an evacuation disorder[102]. The second study examined three subgroups of constipated patients with either STC, an evacuation disorder, or 'normal transit' constipation, and found that fasting and postprandial motor parameters were not useful for discriminating amongst these subtypes, although colonic compliance was[103]. Bassotti *et al*[104] noted that no colonic motor patterns were able to differentiate between constipated patients with or without delayed transit.

A further study in patients with constipation secondary to antidepressants revealed minimal motility differences compared to patients with idiopathic constipation[85]. Finally, Hervé *et al*[83] took the approach that manometric findings themselves should be used as the basis for subclassification. In a study of 40 adults with STC, they identified 4 subgroups: group 1 displayed no HAPS or a colonic response to a high calorie meal; group 2 showed increased sigmoid motor activity; group 3 had a reduced frequency of HAPSs; and group 4 displayed normal colonic motility. However the clinical impact of such findings, in terms of guiding successful management, remained undetermined. Nevertheless, through sufficiently large scale studies, a classification system incorporating findings from pancolonic manometric investigations, rather than being based on traditional studies of colonic transit and evacuatory function alone, may be merited. Current stratification, where the colon is seen to be responsible for transit, and the anorectum solely responsible for evacuation, is likely a gross oversimplification.

CAN COLONIC MANOMETRY HELP TO GUIDE TREATMENT AND IMPROVE OUTCOMES?

The primary indicators of abnormal colonic motor function in constipation (see above) are reduced HAPS frequency and a diminished or absent response to eating, morning waking or chemical stimulation. The colonic re-

sponse to ingestion of a meal is likely to involve the CNS and neurohormonal pathways; a suppressed response has been proposed as indicative of colonic myopathy and an absent response as a possible neuropathy[29]. Similarly, diurnal variation in colonic motor activity is likely to be mediated by the CNS, and therefore a diminished or absent response to sleep or morning waking supports a neuropathic cause in such patients[29]. With regard to chemical stimuli, a failed response may indicate an abnormality within the myenteric plexus[105], cholinergic pathways[92] or recto-colonic neural pathways[93].

Adult studies

In adults, very few interventional studies have been based upon evidence gained from colonic manometric investigation. In 3 patients with severe constipation who had failed conservative therapy, Bassotti *et al*[28] demonstrated globally reduced colonic motility, a minimal meal response and no response to Edrophonium. Based upon these data, 2 patients underwent a total colectomy and 1 a hemicolectomy, with "fairly good results at follow-up" reported. No long-term data were provided.

In a larger study, Rao *et al*[29] performed 24 h colonic manometry in 21 patients with STC. Utilising the response to ingestion of a meal, response to morning waking, and HAPS frequency as diagnostic criteria, patients were classified as having a neuropathy (n = 10) if they had an absence of two or more, a myopathy (n = 5) if they had a reduced response to two or more, or being normal (n = 6). Those with a suspected neuropathy were offered a colectomy (performed in 7/10, with "improved" bowel symptoms reported; no follow-up duration specified). Those with a myopathy were offered a regime of biofeedback and laxatives, and reported "modest improvement" at 1 year.

Paediatric studies

In children, colonic manometry studies are far more likely to guide treatment options. In a study by Pensabene *et al*[106], results of colonic manometry testing resulted in a recommendation to change therapy (mostly surgical) in 93% of the patients with intractable organic or functional constipation. Importantly, 88% of the 98 parents believed that the therapeutic suggestions made had been helpful in improving their child's health[106].

Di Lorenzo *et al*[107] studied 46 children with constipation and/or faecal incontinence after surgery for Hirschsprung disease. Four groups were established based upon results from colonic manometry: (1) HAPSs propagating from the proximal colon through the neorectum to the anal sphincter, which was associated with faecal incontinence (n = 18); (2) an absence of HAPSs and lack of colonic meal response, which was predominantly associated with constipation (n = 15); (3) a normal manometric study, with a hypertensive anal sphincter, which was invariably associated with constipation (n = 4); and (4) a normal manometric study, but the child presented with fear of defaecation and retentive posturing, which was predominantly associated with constipation and soiling (n = 9). Those in group one were treated with anticholinergics and Loperamide. In group two, resection of the abnormal section of colon was recommended. In the third group, four-quadrant intrasphincteric botulinum toxin injections were recommended, and in the fourth group, a programme of behaviour modification and stool softeners were used[107]. Based upon these treatments, the authors reported an improvement in global health and emotional health scores as well as an improvement in the number of bowel movements and resolution of abdominal pain[107].

Also in paediatric patients, colonic manometry is reported to have value in determining poor outcomes for the surgical treatment of intractable constipation using an appendicostomy or a caecostomy with antegrade irrigation of the colon[47]. For example, those children with a poor outcome also displayed an absence of colonic response to Bisacodyl. However, the fact that 50% of children with no production of HAPS still had good outcomes to antegrade irrigation suggests that the Bisacodyl response is by no means a solid predictor[47].

CURRENT LIMITATIONS

While colonic manometry studies in both children and adults have helped to define some of the physiological and pathophysiological aspects of colonic motor function, there are still no published quantitative data on propagating or non-propagating activity that clearly differentiates healthy subjects from constipated patients[30]. Furthermore, at least in children, morphological changes reflecting possible myogenic or neurogenic disorders in muscle tissue do not correlate with particular features of colonic manometric recordings[108] or anorectal manometry[109]. It also remains unclear whether data derived from adult studies can be extrapolated to children and *vice versa*. No manometric studies using the same equipment and protocols have compared childhood and adult constipation. Indeed, because a normal frequency of HAPSs has been identified in children with STC (contradicting most reported findings in adults), childhood constipation may represent a different entity[43]. The impact of sensory, as opposed to motor dysfunction on the development of constipation is gaining increasing recognition[110,111]. Nevertheless, the two are inextricably linked, and future studies should look to explore both domains concurrently, by the best methods available, rather than consider certain functional bowel conditions as principally motor disorders (e.g. constipation), and others as sensory disorders (e.g. the irritable bowel syndrome).

With variations in protocols, catheter types, numbers of recording sites, spacing between recording sites, placement techniques and definitions of recorded activities, it is perhaps not surprising that consistent findings have not been reported in the literature[24,35]. Furthermore (and as stated above[24]), almost all studies that detail "colonic" manometry have, in reality, recorded colonic motor patterns distal to the mid-transverse colon. Support for the use of true pancolonic manometry resides in the fact that

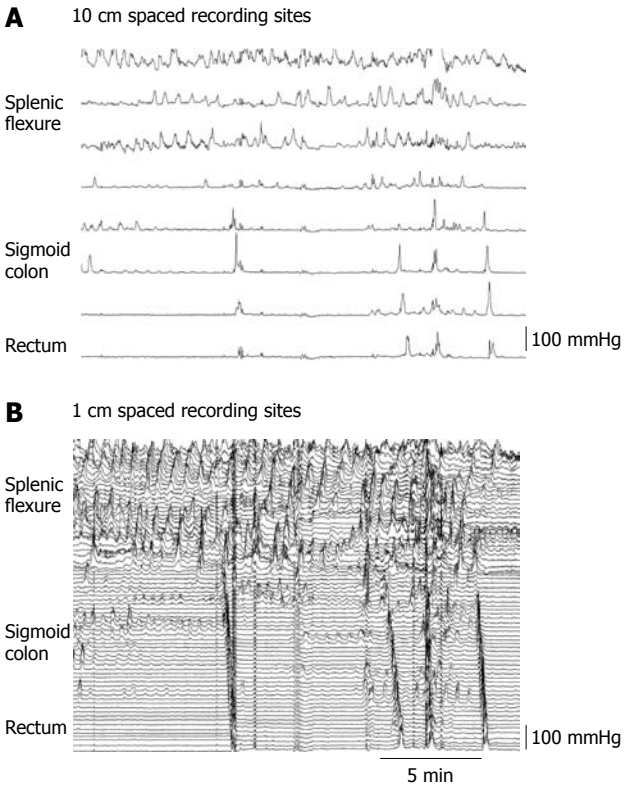

Figure 5 Manometric traces of colonic motor activity recorded by a fibre-optic manometry catheter with 90 sensors spaced at 1 cm intervals. A: To represent common sensor spacing for current published colonic recordings, the data set, obtained through "high resolution" manometry, is sub-sampled to present every 10th channel (10 cm spacing, top); B: The complete data set, captured at 1 cm intervals, is displayed at the bottom. Note that at 10 cm spacing, the most proximal three channels reveal what appears to be non-propagating pressure waves. However, when the full data set is viewed (B), a series of short-extent retrograde propagating events are detected. These events are missed when spatial resolution is poor. Modified from[41].

PS and HAPS are not distributed evenly throughout the colon, and predominantly originate in the ascending and proximal transverse colon, and that the entire colon appears to function in a coordinated fashion[76,112]. 'Partial' colonic recordings thus provide an incomplete picture of colonic (patho)physiology.

FUTURE DIRECTIONS

In the oesophagus and the anorectum, manometry is an established diagnostic tool. In comparison to the colon, these regions afford easier access and therefore have been subjected to far more research and clinical investigation. More recently, development of high resolution manometry (HRM) has further enhanced diagnostic potential, particularly in the oesophagus[113], most notably with regard to standardisation of recording procedures. With the advent of fibre-optic manometry[40,114], HRM recordings are now feasible throughout the entire colon. Catheters incorporating up to 120 sensors spaced at 1 cm intervals have already demonstrated that the vast majority of PSs propagating over short distances are missed by recording sites spaced > 7 cm apart[40,41] (Figure 5). If such technology is able to identify those elusive biomarkers that can help to reliably distinguish constipation subtypes and guide management, then this provides the opportunity to take colonic manometry out of the research arena and into clinical practice. Such progress is only possible through appropriately designed and powered studies, utilising equivalent hardware and software, both in adults and children. The revolution that has been seen with oesophageal HRM, such that traditional manometry is now regarded as obsolete, can serve as a template for the clinical potential of pancolonic manometric investigation.

CONCLUSION

Pancolonic manometric investigation provides a unique window onto motor functions of the large bowel, and through its use we now have a greater understanding of normal colonic physiology and also the pathophysiology of constipation in both adults and children. Although a recent consensus statement[30] advocates use of colonic manometry in patients with "significant motility disorders", the technique is still not refined enough for widespread clinical use, and no prospective and controlled studies have been performed evaluating the clinical value of this tool. Nevertheless, technological advances offer the potential of adding colonic manometry to the available diagnostic armamentarium in broader clinical practice. To achieve such a goal, there is a fundamental requirement for standardisation of methodologies and recording protocols, further development of automated analysis software, and a vital need for generating larger normative data sets, which will enable diagnostic yield to improve. This will be achieved through the coordinated efforts of those dedicated to this field. Further clinical exploration of this technique affords the possibility of a more directed therapeutic approach in patients with chronic constipation. Colonic manometric studies, performed in large numbers of constipated patients, will hopefully establish biomarkers that can distinguish existing (largely predetermined) constipation subtypes (i.e. STC, evacuation disorders), or aid a more contemporary phenotypic classification.

REFERENCES

1 **Cook IJ**, Talley NJ, Benninga MA, Rao SS, Scott SM. Chronic constipation: overview and challenges. *Neurogastroenterol Motil* 2009; **21** Suppl 2: 1-8
2 **McCrea GL**, Miaskowski C, Stotts NA, Macera L, Varma MG. A review of the literature on gender and age differences in the prevalence and characteristics of constipation in North America. *J Pain Symptom Manage* 2009; **37**: 737-745
3 **van den Berg MM**, Benninga MA, Di Lorenzo C. Epidemiology of childhood constipation: a systematic review. *Am J Gastroenterol* 2006; **101**: 2401-2409
4 **Peppas G**, Alexiou VG, Mourtzoukou E, Falagas ME. Epidemiology of constipation in Europe and Oceania: a systematic review. *BMC Gastroenterol* 2008; **8**: 5
5 **Everhart JE**, Ruhl CE. Burden of digestive diseases in the United States part I: overall and upper gastrointestinal diseases. *Gastroenterology* 2009; **136**: 376-386
6 **Liem O**, Harman J, Benninga M, Kelleher K, Mousa H, Di

Lorenzo C. Health utilization and cost impact of childhood constipation in the United States. *J Pediatr* 2009; **154**: 258-262
7 **Constipation Guideline Committee of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition**. Evaluation and treatment of constipation in infants and children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2006; **43**: e1-e13
8 **Southwell BR**, King SK, Hutson JM. Chronic constipation in children: organic disorders are a major cause. *J Paediatr Child Health* 2005; **41**: 1-15
9 **Wedel T**, Spiegler J, Soellner S, Roblick UJ, Schiedeck TH, Bruch HP, Krammer HJ. Enteric nerves and interstitial cells of Cajal are altered in patients with slow-transit constipation and megacolon. *Gastroenterology* 2002; **123**: 1459-1467
10 **Knowles CH**, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K, Gershon MD, Hutson J, Lindberg G, Martin JE, Meier-Ruge WA, Milla PJ, Smith VV, Vandervinden JM, Veress B, Wedel T. Gastrointestinal neuromuscular pathology: guidelines for histological techniques and reporting on behalf of the Gastro 2009 International Working Group. *Acta Neuropathol* 2009; **118**: 271-301
11 **King SK**, Sutcliffe JR, Hutson JM, Southwell BR. Paediatric constipation for adult surgeons - article 2: new microscopic abnormalities and therapies. *ANZ J Surg* 2004; **74**: 890-894
12 **Rantis PC Jr**, Vernava AM 3rd, Daniel GL, Longo WE. Chronic constipation--is the work-up worth the cost? *Dis Colon Rectum* 1997; **40**: 280-286
13 **van Ginkel R**, Reitsma JB, Büller HA, van Wijk MP, Taminiau JA, Benninga MA. Childhood constipation: longitudinal follow-up beyond puberty. *Gastroenterology* 2003; **125**: 357-363
14 **Dennison C**, Prasad M, Lloyd A, Bhattacharyya SK, Dhawan R, Coyne K. The health-related quality of life and economic burden of constipation. *Pharmacoeconomics* 2005; **23**: 461-476
15 **Belsey J**, Greenfield S, Candy D, Geraint M. Systematic review: impact of constipation on quality of life in adults and children. *Aliment Pharmacol Ther* 2010; **31**: 938-949
16 **Clarke MC**, Chow CS, Chase JW, Gibb S, Hutson JM, Southwell BR. Quality of life in children with slow transit constipation. *J Pediatr Surg* 2008; **43**: 320-324
17 **Knowles CH**, Dinning PG, Pescatori M, Rintala R, Rosen H. Surgical management of constipation. *Neurogastroenterol Motil* 2009; **21** Suppl 2: 62-71
18 **Wong SW**, Lubowski DZ. Slow-transit constipation: evaluation and treatment. *ANZ J Surg* 2007; **77**: 320-328
19 **King SK**, Sutcliffe JR, Southwell BR, Chait PG, Hutson JM. The antegrade continence enema successfully treats idiopathic slow-transit constipation. *J Pediatr Surg* 2005; **40**: 1935-1940
20 **Dinning PG**, Fuentealba SE, Kennedy ML, Lubowski DZ, Cook IJ. Sacral nerve stimulation induces pan-colonic propagating pressure waves and increases defecation frequency in patients with slow-transit constipation. *Colorectal Dis* 2007; **9**: 123-132
21 **Lubowski DZ**, Chen FC, Kennedy ML, King DW. Results of colectomy for severe slow transit constipation. *Dis Colon Rectum* 1996; **39**: 23-29
22 **Knowles CH**, Scott M, Lunniss PJ. Outcome of colectomy for slow transit constipation. *Ann Surg* 1999; **230**: 627-638
23 **Thaler K**, Dinnewitzer A, Oberwalder M, Weiss EG, Nogueras JJ, Efron J, Vernava AM 3rd, Wexner SD. Quality of life after colectomy for colonic inertia. *Tech Coloproctol* 2005; **9**: 133-137
24 **Dinning PG**, Smith TK, Scott SM. Pathophysiology of colonic causes of chronic constipation. *Neurogastroenterol Motil* 2009; **21** Suppl 2: 20-30
25 **Dinning PG**, Bampton PA, Andre J, Kennedy ML, Lubowski DZ, King DW, Cook IJ. Abnormal predefecatory colonic motor patterns define constipation in obstructed defecation. *Gastroenterology* 2004; **127**: 49-56
26 **Southwell BR**, Clarke MC, Sutcliffe J, Hutson JM. Colonic transit studies: normal values for adults and children with comparison of radiological and scintigraphic methods. *Pediatr Surg Int* 2009; 25: 559-572
27 **Bassotti G**, Gaburri M, Imbimbo BP, Rossi L, Farroni F, Pelli MA, Morelli A. Colonic mass movements in idiopathic chronic constipation. *Gut* 1988; **29**: 1173-1179
28 **Bassotti G**, Betti C, Pelli MA, Morelli A. Extensive investigation on colonic motility with pharmacological testing is useful for selecting surgical options in patients with inertia colica. *Am J Gastroenterol* 1992; **87**: 143-147
29 **Rao SS**, Sadeghi P, Beaty J, Kavlock R. Ambulatory 24-hour colonic manometry in slow-transit constipation. *Am J Gastroenterol* 2004; **99**: 2405-2416
30 **Camilleri M**, Bharucha AE, di Lorenzo C, Hasler WL, Prather CM, Rao SS, Wald A. American Neurogastroenterology and Motility Society consensus statement on intraluminal measurement of gastrointestinal and colonic motility in clinical practice. *Neurogastroenterol Motil* 2008; **20**: 1269-1282
31 **Di Lorenzo C**, Hillemeier C, Hyman P, Loening-Baucke V, Nurko S, Rosenberg A, Taminiau J. Manometry studies in children: minimum standards for procedures. *Neurogastroenterol Motil* 2002; **14**: 411-420
32 **Hussain SZ**, Di Lorenzo C. Motility disorders. Diagnosis and treatment for the pediatric patient. *Pediatr Clin North Am* 2002; **49**: 27-51
33 **Bassotti G**, Gaburri M. Manometric investigation of high-amplitude propagated contractile activity of the human colon. *Am J Physiol* 1988; **255**: G660-G664
34 **Bampton PA**, Dinning PG, Kennedy ML, Lubowski DZ, deCarle D, Cook IJ. Spatial and temporal organization of pressure patterns throughout the unprepared colon during spontaneous defecation. *Am J Gastroenterol* 2000; **95**: 1027-1035
35 **Scott SM**. Manometric techniques for the evaluation of colonic motor activity: current status. *Neurogastroenterol Motil* 2003; **15**: 483-513
36 **Debongnie JC**, Phillips SF. Capacity of the human colon to absorb fluid. *Gastroenterology* 1978; **74**: 698-703
37 **Rao SS**, Sadeghi P, Beaty J, Kavlock R, Ackerson K. Ambulatory 24-h colonic manometry in healthy humans. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G629-G639
38 **Hagger R**, Kumar D, Benson M, Grundy A. Periodic colonic motor activity identified by 24-h pancolonic ambulatory manometry in humans. *Neurogastroenterol Motil* 2002; **14**: 271-278
39 **Hagger R**, Kumar D, Benson M, Grundy A. Colonic motor activity in slow-transit idiopathic constipation as identified by 24-h pancolonic ambulatory manometry. *Neurogastroenterol Motil* 2003; **15**: 515-522
40 **Arkwright JW**, Underhill ID, Maunder SA, Blenman N, Szczesniak MM, Wiklendt L, Cook IJ, Lubowski DZ, Dinning PG. Design of a high-sensor count fibre optic manometry catheter for in-vivo colonic diagnostics. *Opt Express* 2009; 17: 22423-22431
41 **Dinning PG**, Arkwright JW, Gregersen H, o'grady G, Scott SM. Technical advances in monitoring human motility patterns. *Neurogastroenterol Motil* 2010; **22**: 366-380
42 **Stanton MP**, Hutson JM, Simpson D, Oliver MR, Southwell BR, Dinning P, Cook I, Catto-Smith AG. Colonic manometry via appendicostomy shows reduced frequency, amplitude, and length of propagating sequences in children with slow-transit constipation. *J Pediatr Surg* 2005; **40**: 1138-1145
43 **King SK**, Catto-Smith AG, Stanton MP, Sutcliffe JR, Simpson D, Cook I, Dinning P, Hutson JM, Southwell BR. 24-Hour colonic manometry in pediatric slow transit constipation shows significant reductions in antegrade propagation. *Am J Gastroenterol* 2008; **103**: 2083-2091
44 **van den Berg MM**, Hogan M, Mousa HM, Di Lorenzo C. Colonic manometry catheter placement with primary fluoroscopic guidance. *Dig Dis Sci* 2007; **52**: 2282-2286
45 **Garcia D**, Hita G, Mompean B, Hernandez A, Pellicer E, Morales G, Parrilla P. Colonic motility: electric and manometric

description of mass movement. *Dis Colon Rectum* 1991; **34**: 577-584
46 **Di Lorenzo C**, Flores AF, Reddy SN, Hyman PE. Use of colonic manometry to differentiate causes of intractable constipation in children. *J Pediatr* 1992; **120**: 690-695
47 **van den Berg MM**, Hogan M, Caniano DA, Di Lorenzo C, Benninga MA, Mousa HM. Colonic manometry as predictor of cecostomy success in children with defecation disorders. *J Pediatr Surg* 2006; **41**: 730-736; discussion 730-736
48 **Fajardo N**, Hussain K, Korsten MA. Prolonged ambulatory colonic manometric studies using endoclips. *Gastrointest Endosc* 2000; **51**: 199-201
49 **De Schryver AM**, Samsom M, Smout AI. Effects of a meal and bisacodyl on colonic motility in healthy volunteers and patients with slow-transit constipation. *Dig Dis Sci* 2003; **48**: 1206-1212
50 **Furukawa Y**, Cook IJ, Panagopoulos V, McEvoy RD, Sharp DJ, Simula M. Relationship between sleep patterns and human colonic motor patterns. *Gastroenterology* 1994; **107**: 1372-1381
51 **Narducci F**, Bassotti G, Gaburri M, Morelli A. Twenty four hour manometric recording of colonic motor activity in healthy man. *Gut* 1987; **28**: 17-25
52 **Lémann M**, Flourié B, Picon L, Coffin B, Jian R, Rambaud JC. Motor activity recorded in the unprepared colon of healthy humans. *Gut* 1995; **37**: 649-653
53 **Dinning PG**, Zarate N, Szczesniak MM, Mohammed SD, Preston SL, Fairclough PD, Lunniss PJ, Cook IJ, Scott SM. Bowel preparation affects the amplitude and spatiotemporal organization of colonic propagating sequences. *Neurogastroenterol Motil* 2010; **22**: 633-e176
54 **Jouet P**, Coffin B, Lémann M, Gorbatchef C, Franchisseur C, Jian R, Rambaud JC, Flourié B. Tonic and phasic motor activity in the proximal and distal colon of healthy humans. *Am J Physiol* 1998; **274**: G459-G464
55 **Panagamuwa B**, Kumar D, Ortiz J, Keighley MR. Motor abnormalities in the terminal ileum of patients with chronic idiopathic constipation. *Br J Surg* 1994; **81**: 1685-1688
56 **Marshall J**, Hutson JM, Anticich N, Stanton MP. Antegrade continence enemas in the treatment of slow-transit constipation. *J Pediatr Surg* 2001; **36**: 1227-1230
57 **Sarna SK**. Physiology and pathophysiology of colonic motor activity (1). *Dig Dis Sci* 1991; **36**: 827-862
58 **Bampton PA**, Dinning PG, Kennedy ML, Lubowski DZ, Cook IJ. Prolonged multi-point recording of colonic manometry in the unprepared human colon: providing insight into potentially relevant pressure wave parameters. *Am J Gastroenterol* 2001; **96**: 1838-1848
59 **Cook IJ**, Furukawa Y, Panagopoulos V, Collins PJ, Dent J. Relationships between spatial patterns of colonic pressure and individual movements of content. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G329-G341
60 **Dinning PG**, Szczesniak MM, Cook IJ. Proximal colonic propagating pressure waves sequences and their relationship with movements of content in the proximal human colon. *Neurogastroenterol Motil* 2008; **20**: 512-520
61 **Hardcastle JD**, Mann CV. Study of large bowel peristalsis. *Gut* 1968; **9**: 512-520
62 **Torsoli A**, Ramorino ML, Ammaturo MV, Capurso L, Paoluzi P, Anzini F. Mass movements and intracolonic pressures. *Am J Dig Dis* 1971; **16**: 693-696
63 **Bassotti G**, Crowell MD, Whitehead WE. Contractile activity of the human colon: lessons from 24 hour studies. *Gut* 1993; **34**: 129-133
64 **Spiller RC**, Brown ML, Phillips SF. Decreased fluid tolerance, accelerated transit, and abnormal motility of the human colon induced by oleic acid. *Gastroenterology* 1986; **91**: 100-107
65 **Crowell MD**, Bassotti G, Cheskin LJ, Schuster MM, Whitehead WE. Method for prolonged ambulatory monitoring of high-amplitude propagated contractions from colon. *Am J Physiol* 1991; **261**: G263-G268
66 **von der Ohe MR**, Hanson RB, Camilleri M. Comparison of simultaneous recordings of human colonic contractions by manometry and a barostat. *Neurogastroenterol Motil* 1994; **6**: 213-222
67 **Herbst F**, Kamm MA, Morris GP, Britton K, Woloszko J, Nicholls RJ. Gastrointestinal transit and prolonged ambulatory colonic motility in health and faecal incontinence. *Gut* 1997; **41**: 381-389
68 **Dinoso VP Jr**, Murthy SN, Goldstein J, Rosner B. Basal motor activity of the distal colon: a reappraisal. *Gastroenterology* 1983; **85**: 637-642
69 **Bassotti G**, Clementi M, Antonelli E, Pelli MA, Tonini M. Low-amplitude propagated contractile waves: a relevant propulsive mechanism of human colon. *Dig Liver Dis* 2001; **33**: 36-40
70 **Rao SS**, Beaty J, Chamberlain M, Lambert PG, Gisolfi C. Effects of acute graded exercise on human colonic motility. *Am J Physiol* 1999; **276**: G1221-G1226
71 **Wingate DL**. Backwards and forwards with the migrating complex. *Dig Dis Sci* 1981; **26**: 641-666
72 **Kumar D**, Williams NS, Waldron D, Wingate DL. Prolonged manometric recording of anorectal motor activity in ambulant human subjects: evidence of periodic activity. *Gut* 1989; **30**: 1007-1011
73 **Rao SS**, Sadeghi P, Batterson K, Beaty J. Altered periodic rectal motor activity: a mechanism for slow transit constipation. *Neurogastroenterol Motil* 2001; **13**: 591-598
74 **Rao SS**, Welcher K. Periodic rectal motor activity: the intrinsic colonic gatekeeper? *Am J Gastroenterol* 1996; **91**: 890-897
75 **Spencer NJ**. Control of migrating motor activity in the colon. *Curr Opin Pharmacol* 2001; **1**: 604-610
76 **Dinning PG**, Szczesniak MM, Cook IJ. Spatio-temporal analysis reveals aberrant linkage among sequential propagating pressure wave sequences in patients with symptomatically defined obstructed defecation. *Neurogastroenterol Motil* 2009; **21**: 945-e975
77 **Baker SS**, Liptak GS, Colletti RB, Croffie JM, Di Lorenzo C, Ector W, Nurko S. Constipation in infants and children: evaluation and treatment. A medical position statement of the North American Society for Pediatric Gastroenterology and Nutrition. *J Pediatr Gastroenterol Nutr* 1999; **29**: 612-626
78 **Di Lorenzo C**, Flores AF, Hyman PE. Age-related changes in colon motility. *J Pediatr* 1995; **127**: 593-596
79 **Reddy SN**, Bazzocchi G, Chan S, Akashi K, Villanueva-Meyer J, Yanni G, Mena I, Snape WJ Jr. Colonic motility and transit in health and ulcerative colitis. *Gastroenterology* 1991; **101**: 1289-1297
80 **Dinning PG**, Zarate N, Hunt LM, Fuentealba SE, Mohammed SD, Szczesniak MM, Lubowski DZ, Preston SL, Fairclough PD, Lunniss PJ, Scott SM, Cook IJ. Pancolonic spatiotemporal mapping reveals regional deficiencies in, and disorganization of colonic propagating pressure waves in severe constipation. *Neurogastroenterol Motil* 2010; Epub ahead of print
81 **Brown AJ**, Horgan AF, Anderson JH, McKee RF, Finlay IG. Colonic motility is abnormal before surgery for rectal prolapse. *Br J Surg* 1999; **86**: 263-266
82 **Bazzocchi G**, Ellis J, Villanueva-Meyer J, Jing J, Reddy SN, Mena I, Snape WJ Jr. Postprandial colonic transit and motor activity in chronic constipation. *Gastroenterology* 1990; **98**: 686-693
83 **Hervé S**, Savoye G, Behbahani A, Leroi AM, Denis P, Ducrotté P. Results of 24-h manometric recording of colonic motor activity with endoluminal instillation of bisacodyl in patients with severe chronic slow transit constipation. *Neurogastroenterol Motil* 2004; **16**: 397-402
84 **Bassotti G**, Imbimbo BP, Betti C, Dozzini G, Morelli A. Impaired colonic motor response to eating in patients with slow-transit constipation. *Am J Gastroenterol* 1992; **87**: 504-508
85 **Leroi AM**, Lalaude O, Antonietti M, Touchais JY, Ducrotte P,

Menard JF, Denis P. Prolonged stationary colonic motility recording in seven patients with severe constipation secondary to antidepressants. *Neurogastroenterol Motil* 2000; **12**: 149-154
86 **Di Lorenzo C**, Flores AF, Reddy SN, Snape WJ Jr, Bazzocchi G, Hyman PE. Colonic manometry in children with chronic intestinal pseudo-obstruction. *Gut* 1993; **34**: 803-807
87 **Bassotti G**, Chistolini F, Nzepa FS, Morelli A. Colonic propulsive impairment in intractable slow-transit constipation. *Arch Surg* 2003; **138**: 1302-1304
88 **Bassotti G**, Chistolini F, Marinozzi G, Morelli A. Abnormal colonic propagated activity in patients with slow transit constipation and constipation-predominant irritable bowel syndrome. *Digestion* 2003; **68**: 178-183
89 **Connell AM**. The motility of the pelvic colon. II. Paradoxical motility in diarrhoea and constipation. *Gut* 1962; **3**: 342-348
90 **Kamm MA**, van der Sijp JR, Lennard-Jones JE. Observations on the characteristics of stimulated defaecation in severe idiopathic constipation. *Int J Colorectal Dis* 1992; **7**: 197-201
91 **Bassotti G**, Chiarioni G, Germani U, Battaglia E, Vantini I, Morelli A. Endoluminal instillation of bisacodyl in patients with severe (slow transit type) constipation is useful to test residual colonic propulsive activity. *Digestion* 1999; **60**: 69-73
92 **Bassotti G**, Chiarioni G, Imbimbo BP, Betti C, Bonfante F, Vantini I, Morelli A, Whitehead WE. Impaired colonic motor response to cholinergic stimulation in patients with severe chronic idiopathic (slow transit type) constipation. *Dig Dis Sci* 1993; **38**: 1040-1045
93 **Dinning PG**, Bampton PA, Kennedy ML, Lubowski DZ, King D, Cook IJ. Impaired proximal colonic motor response to rectal mechanical and chemical stimulation in obstructed defecation. *Dis Colon Rectum* 2005; **48**: 1777-1784
94 **American College of Gastroenterology Chronic Constipation Task Force**. An evidence-based approach to the management of chronic constipation in North America. Am J Gastroenterol 2005; 100 Suppl 1: S1-S4
95 **Bharucha AE**. Constipation. *Best Pract Res Clin Gastroenterol* 2007; **21**: 709-731
96 **Nyam DC**, Pemberton JH, Ilstrup DM, Rath DM. Long-term results of surgery for chronic constipation. *Dis Colon Rectum* 1997; **40**: 273-279
97 **Rao SS**, Seaton K, Miller M, Brown K, Nygaard I, Stumbo P, Zimmerman B, Schulze K. Randomized controlled trial of biofeedback, sham feedback, and standard therapy for dyssynergic defecation. *Clin Gastroenterol Hepatol* 2007; **5**: 331-338
98 **Chiarioni G**, Salandini L, Whitehead WE. Biofeedback benefits only patients with outlet dysfunction, not patients with isolated slow transit constipation. *Gastroenterology* 2005; **129**: 86-97
99 **Battaglia E**, Serra AM, Buonafede G, Dughera L, Chistolini F, Morelli A, Emanuelli G, Bassotti G. Long-term study on the effects of visual biofeedback and muscle training as a therapeutic modality in pelvic floor dyssynergia and slow-transit constipation. *Dis Colon Rectum* 2004; **47**: 90-95
100 **Kamm MA**, Hawley PR, Lennard-Jones JE. Outcome of colectomy for severe idiopathic constipation. *Gut* 1988; **29**: 969-973
101 **Kuijpers HC**. Application of the colorectal laboratory in diagnosis and treatment of functional constipation. *Dis Colon Rectum* 1990; **33**: 35-39
102 **O'Brien MD**, Camilleri M, von der Ohe MR, Phillips SF, Pemberton JH, Prather CM, Wiste JA, Hanson RB. Motility and tone of the left colon in constipation: a role in clinical practice? *Am J Gastroenterol* 1996; **91**: 2532-2538
103 **Ravi K**, Bharucha AE, Camilleri M, Rhoten D, Bakken T, Zinsmeister AR. Phenotypic variation of colonic motor functions in chronic constipation. *Gastroenterology* 2010; **138**: 89-97
104 **Bassotti G**, Chiarioni G, Vantini I, Betti C, Fusaro C, Pelli MA, Morelli A. Anorectal manometric abnormalities and colonic propulsive impairment in patients with severe chronic idiopathic constipation. *Dig Dis Sci* 1994; **39**: 1558-1564
105 **Preston DM**, Lennard-Jones JE. Pelvic motility and response to intraluminal bisacodyl in slow-transit constipation. *Dig Dis Sci* 1985; **30**: 289-294
106 **Pensabene L**, Youssef NN, Griffiths JM, Di Lorenzo C. Colonic manometry in children with defecatory disorders. role in diagnosis and management. *Am J Gastroenterol* 2003; **98**: 1052-1057
107 **Di Lorenzo C**, Solzi GF, Flores AF, Schwankovsky L, Hyman PE. Colonic motility after surgery for Hirschsprung's disease. *Am J Gastroenterol* 2000; **95**: 1759-1764
108 **van den Berg MM**, Di Lorenzo C, Mousa HM, Benninga MA, Boeckxstaens GE, Luquette M. Morphological changes of the enteric nervous system, interstitial cells of cajal, and smooth muscle in children with colonic motility disorders. *J Pediatr Gastroenterol Nutr* 2009; **48**: 22-29
109 **Treepongkaruna S**, Hutson JM, Hughes J, Cook D, Catto-Smith AG, Chow CW, Oliver MR. Gastrointestinal transit and anorectal manometry in children with colonic substance P deficiency. *J Gastroenterol Hepatol* 2001; **16**: 624-630
110 **Gladman MA**, Lunniss PJ, Scott SM, Swash M. Rectal hyposensitivity. *Am J Gastroenterol* 2006; **101**: 1140-1151
111 **van den Berg MM**, Bongers ME, Voskuijl WP, Benninga MA. No role for increased rectal compliance in pediatric functional constipation. *Gastroenterology* 2009; **137**: 1963-1969
112 **Dinning PG**, Szczesniak MM, Cook IJ. Twenty-four hour spatiotemporal mapping of colonic propagating sequences provides pathophysiological insight into constipation. *Neurogastroenterol Motil* 2008; **20**: 1017-1021
113 **Pandolfino JE**, Kwiatek MA, Nealis T, Bulsiewicz W, Post J, Kahrilas PJ. Achalasia: a new clinically relevant classification by high-resolution manometry. *Gastroenterology* 2008; **135**: 1526-1533
114 **Arkwright JW**, Blenman NG, Underhill ID, Maunder SA, Szczesniak MM, Dinning PG, Cook IJ. In-vivo demonstration of a high resolution optical fiber manometry catheter for diagnosis of gastrointestinal motility disorders. *Opt Express* 2009; **17**: 4500-4508

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TOPIC HIGHLIGHT

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Transient elastography in chronic hepatitis B: An Asian perspective

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Abstract

Transient elastography (TE) is a new non-invasive tool for assessing liver stiffness, which is correlated with the histologic stage of liver fibrosis. Many studies have reported a good accuracy of TE in predicting significant fibrosis and an optimal accuracy in predicting cirrhosis. Furthermore, the potential role of TE in screening the general population has also been proven. TE thus helps physicians to decide treatment strategies, predict prognosis, and monitor disease progression in patients with chronic liver disease and to screen the general population to identify high risk patients with potential liver disease. However, most data on the clinical roles of TE have been gathered in European patients with chronic hepatitis C (CHC), because TE was first developed in France. Accordingly, much data on the usefulness of TE in patients with CHC has accumulated. Recently, however, vigorous efforts have been made to apply TE to patients with chronic hepatitis B (CHB), and TE has also proved to have acceptable accuracy in diagnosing liver fibrosis and cirrhosis in these patients. Thus, we focused on TE in the Asian population with CHB in comparison with the European population with CHC and found that the diagnostic performance and cutoff values were different between the 2 populations possibly as a result of several different confounders between Asian and European populations (the etiology of chronic liver disease, histologic features, major fluctuation in alanine aminotransferase levels, and the prevalence of high body mass index and metabolic syndrome). Therefore, further studies tailored to the Asian population with CHB should be performed before the widespread application of TE in Asian populations with CHB.

Key words: Asia; Chronic hepatitis B; Fibroscan; Hepatitis B virus; Liver stiffness measurement; Transient elastography

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INTRODUCTION

Transient elastography (TE) using FibroScan® (EchoSens, Paris, France) is a newly introduced non-invasive tool, that generates an elastic wave using a vibrator applied to the thoracic wall at the level of the right lobe of the liver and measures the propagation velocity of the wave,

which is directly associated with liver stiffness (LS)[1]. To date, the clinical utility of TE has been widely reviewed, and it is regarded as having considerable accuracy for predicting liver cirrhosis in patients with chronic liver disease (CLD) of diverse etiologies[2-5]. TE is quick, highly reproducible, and completely harmless to patients. In addition, it can be learned and performed easily after a short training period (by nurses or technicians) without consuming the time of clinicians. For these reasons, TE has become popular in clinical practice as a tool for aiding in the diagnosis and follow-up of liver disease.

Because TE was first developed in France, most studies on its benefits have been explored in European countries where chronic hepatitis C (CHC) is prevalent. Accordingly, extensive data on the clinical roles of TE in assessing liver fibrosis in patients with CHC have been gathered. Recently, several meta-analysis studies reported that TE is a reliable non-invasive tool to detect advanced liver fibrosis and liver cirrhosis[6-8]. However, most studies included in the meta-analysis investigated European populations with CHC. Data from Europe cannot be extrapolated to the Asian population, as subsequent trials of TE in Asian countries where hepatitis B virus (HBV) infection is more prevalent than hepatitis C virus (HCV), displayed divergent results. In this Topic Highlight, we will focus on TE in the Asian population with chronic hepatitis B (CHB).

METHODOLOGICAL CONSIDERATION OF TE IN THE ASIAN POPULATION

LS values are defined as the median of 10 valid measurements and at least 60% of TE shots should be successful for each examination according to the manufacturer's recommendations. Accordingly, to date, only the results of TE examinations satisfying the above criteria have been considered as reliable and have been included for the analysis in reported studies.

Another important parameter for confirming the validity of LS values is the interquartile range (IQR), which is defined as the values between which 50% of observations fall. The lower boundary is the 25th percentile (lower quartile), the upper boundary is the 75th percentile (upper quartile). Generally, the IQR/median LS value ratio (IQR/M) should not exceed 30% if the reliability of LS values is to be preserved. However, recently, Lucidarme *et al*[9] proposed new criteria for reliable LS values, using the IQR/M based on the data from more than 250 French patients with CHC. They found, in a multivariate analysis, the fibrosis stage (F0-2 *vs* F3-4) and IQR/M were significantly associated with significant discordances between TE and liver biopsy (LB), with an optimal discriminant cutoff value of 0.21. They thus concluded that the IQR/M can overestimate liver fibrosis using TE, irrespective of success rate, when the significance discordance was defined as a discordance of at least 2 stages of fibrosis between LS values by TE examination and the METAVIR scoring system. From these results, they suggested a novel algorithm for clinical practice as first-line assessment of liver fibrosis in patients with CHC (Figure 1).

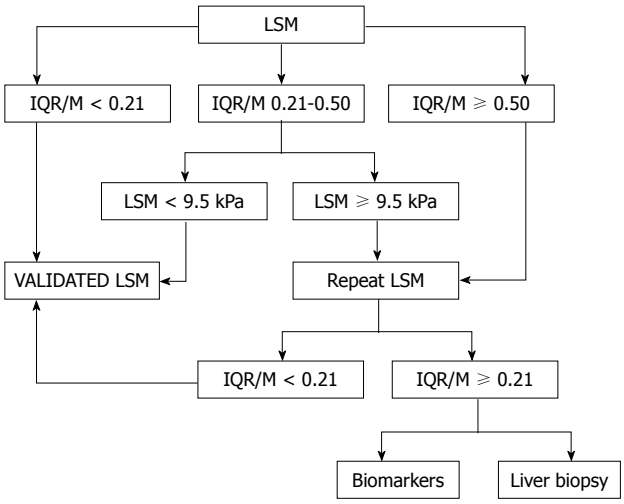

Figure 1 Suggested algorithm for clinical practice as first-line assessment of hepatic fibrosis in patients with chronic hepatitis C. IQR/M: Interquartile range/median liver stiffness value ratio; LSM: Liver stiffness measurement.

Because the IQR/M was confirmed in only one European study with CHC showing relatively high body mass index (BMI) (25 ± 4.1 kg/m^2), its application to the Asian population with CHB (who generally show a lower BMI) is difficult and requires further validation. Although the data are preliminary, we have attempted to validate the IQR/M for our cohort of 156 patients with CHB (mean age 38.8 years, male 75.0%, mean BMI 23.2 kg/m^2) who underwent LB and TE prior to starting antiviral treatment. In this study, we selected the cutoff LS values of Marcellin *et al*[10] (7.2 kPa for ⩾ F2, 8.1 kPa ⩾ F3, and 11.0 kPa for F4) as the reference values between 2 external studies that investigated the performance of TE and then set up the optimal cutoff LS values for each fibrosis stage in patients with CHB[10,11]. We identified 28 (18.0%) patients showing significant discordance between TE and LB. However, the IQR/M was not significantly different between patients with discordance and those with non-discordance (0.125 ± 0.083 *vs* 0.129 ± 0.079, $P = 0.831$), and the success rate also failed to show a significant difference.

Interestingly, the mean IQR/M of our study population was greatly reduced compared to that of subjects in Lucidarme *et al*[9] (0.30 in patients with discordance and 0.22 in those with non-discordance) and the proportion of patients with an IQR/M > 0.03 ($n = 7$, 4.5%) was very small. The reasons are not clear why the IQR/M in our study was reduced in comparison with that in the previous study, and was not selected as a discriminant factor to predict discordance. However, we think that the optimal cutoff value of the IQR/M will be lower than that from patients with CHC even if the IQR/M can be validated in the future for the Asian population with CHB, or that the IQR/M is not a predictor of discordance in the Asian population with CHB due to other potential confounders that overwhelm the influence of the IQR/M, such as the inhomogeneous histological features of CHB which often

Table 1 Normal liver stiffness values of transient elastography

	Asian study		European study				
	Kim *et al*[14]	Fung *et al*[19]	Corpechot *et al*[15]	Roulot *et al*[13]	Sirli *et al*[16]	Colombo *et al*[17]	Colombo *et al*[18]
Type	Full article	Full article	Letter	Full article	Full article	Abstract	Abstract
No. of subjects	69	28	71	429	144	327	746
Population	Liver and kidney donors	Liver donors	Healthy volunteers	Medical check-up	Healthy volunteers	Blood donors	Blood donors
Liver stiffness (kPa)	4.6 (mean)	4.6 (median)	4.8 (median)	5.5 (mean)	4.8 (mean)	4.9 (mean)	4.4 (median)
95th percentile (kPa)	4.7	-	-	8.6	-	7.8	6.7
BMI (kg/m^2)	22.6 (mean)	-	22.5 (median)	25.8 (mean)	-	-	-
Effects on TE							
Age	No	-	No	No	No	No	No
Gender	M = F	-	M > F	M > F	M > F	M = F	M > F
High BMI	No	-	No	Increased TE values	No	Increased TE values	No
Metabolic syndrome	-	-	-	Increased TE values	-	-	-
Fatty liver	-	-	-	-	-	Increased TE values	Increased TE values

BMI: Body mass index; TE: Transient elastography.

makes liver texture inherently macronodular. This would result in LS values that vary depending on the area of the liver and necroinflammatory activity, which has been demonstrated to raise LS values. Therefore, the validation of the IQR/M in the Asian population with CHB requires further studies using increased sample sizes to allow sufficient cases with a spectrum of IQR/M values.

Several issues still remain to be resolved regarding the Asian population with CHB: How many valid measurements do we need to maintain the performance of TE[12]? What is the optimal success rate of the TE measurement to accurately exclude unreliable LS values? Can the IQR/M alone be applied to increase the performance of TE without a sufficient number of valid measurements and an adequate success rate?

WHAT ARE THE NORMAL LS VALUES FOR THE ASIAN POPULATION

Before discussing data on TE in the Asian population with CHB, it is important to first confirm that TE can reliably identify patients with CLD from the normal population. If TE cannot, the clinical meaning of further analysis on the performance of TE in grading the severity of CLD would be significantly lessened. Despite the importance of this, to date, most studies on TE have focused on patients with CLD.

The concept of "what are normal LS values assessed by TE?" is a critical issue that should be addressed to determine whether TE can be used for screening the general population and for subsequent selection of high-risk patients who require periodic follow-up and adequate treatment measures. Indeed, concerns regarding normal LS values are increasing, and several studies (including 2 Asian studies) have proposed normal LS values despite variations in study design[13-19].

In a study by Corpechot *et al*[15], the authors found that the normal LS values in the healthy population were significantly higher in men than in women and that the median LS value was 4.8 kPa (range, 2.5-6.9 kPa). Roulot *et al*[13] examined a large cohort of 429 apparently healthy subjects to establish normal LS values [5.81 ± 1.54 kPa (range, 3.8-8.0 kPa) in men *vs* 5.23 ± 1.59 kPa (range, 3.3-7.8 kPa) in women, $P < 0.01$]. Another study conducted in Romania demonstrated that the mean values of TE in 144 normal subjects were 4.8 ± 1.3 kPa (range, 2.3-8.8 kPa) and that the normal LS values were significantly different between genders[16]. Colombo *et al*[17] also assessed the LS values of 327 voluntary blood donors and found that the mean LS value was 4.9 kPa (95% CI: 4.6-5.1 kPa) and the normal LS values showed no significant differences between genders. However, Colombo *et al*[18] in their follow-up study that enrolled more than 1000 healthy blood donors reported that the normal LS value was 4.4 kPa (95th percentile, 6.7 kPa) and that the male gender presents with increased LS values.

All the aforementioned studies were conducted in Europe. However, normal LS values in the Asian population are now available. Fung *et al*[19] reported a mean LS value in 28 healthy living-related liver donors of 4.6 kPa (range, 2.0-7.1 kPa), and all subjects had LS values of < 7.2 kPa, which indicated that they had no significant fibrosis. We have previously reported that the normal range of LS values was 3.9-5.3 kPa, which was calculated from 69 strictly selected living liver and kidney donors[14].

Table 1 indicates the results of all studies on normal LS values. The upper normal range of LS values was consistently lower than the range generally used for identifying significant liver disease (7-8 kPa) in the majority of previous studies[20-22]. These data demonstrated that TE can perform reliably in identifying high-risk subpopulations without an overlap between the normal and abnormal ranges of LS values. Interestingly, the mean LS values in Asian studies appear lower in comparison with European studies, and the effects of gender on the performance of TE are variable among studies. The discrepancy cannot be fully explained, because the complete histological data of the normal subjects were largely unavailable except a small

Table 2 Failure rate of transient elastography measurement

	Asian study				European study		
	Kim *et al*[14]	Chan *et al*[24]	Masuzaki *et al*[25]	Wong *et al*[26]	Ziol *et al*[2]	Marcellin *et al*[10]	Maimone *et al*[27]
Etiology	HBV	HBV	HCV	HBV	HCV	HBV	HBV
Total patients (*n*)	74	1136	876	487	327	202	230
Excluded patients (*n*)	5	30	10	34	76	29	10
Excluded patients due to TE failure (*n*)	1	30	10	17	23	14	10
TE failure rate (%)	1.4	2.6	1.1	3.5	7	6.9	4.3
Enrolled patients (*n*)	69	1106	866	453	251	173	220
Age (yr)	38.9 (mean)	47 (mean)	66.2 (mean)	37 (median)	47.5 (mean)	40.1 (mean)	45.2 (mean)
Male, *n* (%)	35 (50.7)	-	398 (46.0)	270 (60.0)	155 (61.8)	115 (66.5)	99 (45.0)
BMI (kg/m^2)	22.6 (mean)	-	22.5 (mean)	22.4 (median)	23.9 (mean)	24.5 (mean)	-
Failure criteria							
Success rate	< 60%	< 60%	< 60%	< 60%	< 60%	< 50%	< 60%
The number of VMs	< 10 VMs	< 10 VMs	< 8 VMs	< 10 VMs	< 10 VMs	< 7 VMs	< 10 VMs

HBV: Hepatitis B virus; HCV: Hepatitis C virus; TE: Transient elastography; BMI: Body mass index; VM: Valid measurement.

number of liver donors in the studies by our group[14] and Fung *et al*[19]. Lower mean BMI, younger age, and lower prevalence of metabolic syndrome in the Asian studies can explain, at least in part, the lower mean LS values observed. Another reasons are that other confounders of TE performance, such as high alanine aminotransferase (ALT) levels and fatty liver, were not sufficiently excluded[17,18] and that the hepatic imaging studies and/or cardiologic evaluation were not fully completed in European studies[13,16]. These reasons may have increased the normal range of LS values in the European studies.

More carefully designed studies with a large number of subjects are thus still required to fully assess the normal LS values for both Western and Asian populations. However, it should be remembered that the actual normal range of LS values cannot be precisely defined without a definition of a "normal liver", sufficient histological evaluation of normal subjects, and further stratified analysis with lifestyle factors between genders (such as alcohol consumption). If these issues are resolved, we can identify the true normal LS value and use this as a reference for future studies.

INFLUENCE OF BMI ON THE PERFORMANCE OF TE IN THE ASIAN POPULATION

Previous work has demonstrated that BMI can affect the performance of TE. This can be explained in 2 ways. First, BMI influences the failure rate of TE, and second, BMI acts to increase LS values. Although previous studies have reported that the success rate of TE decreases in subjects displaying higher BMI[13,23], TE failure may not be significant, because these patients were largely excluded from the analysis. However, in another point of view, this effect of high BMI on TE failure can be a significant pitfall of TE, as the majority of patients with high BMI have an increased chance of suffering from fatty liver and, if TE examination fails, they miss the opportunity to receive rapid and non-invasive methods to exclude significant fibrosis. From the knowledge of the varying failure rates between Asian and European study populations, we can discern the possible influence of BMI on TE failure rate according to the study population.

Table 2 summarizes the failure rates of previous studies conducted in Asian and European populations. Overall, TE failure rates and BMI both appear to be lower in Asian compared to European studies, indicating that the lower BMI found in Asian populations is possibly associated with a lower TE failure rate.

In spite of debate, several European studies have reported that a high BMI (which might cause hepatic steatosis) could potentially increase LS values[13,17], although research to date has not determined whether steatosis itself increases LS value in patients with chronic viral hepatitis[28-30]. For the Asian population, the effects of high BMI on TE performance have not been fully validated, because most Asian studies on TE lack sufficient numbers of patients with high BMI (> 30 kg/m^2). In our previous study, BMI did not influence LS values, although our study population was younger and showed lower BMI values than European studies[14]. This potential effect of high BMI on TE performance should therefore be further investigated for the Asian population.

INFLUENCE OF METABOLIC SYNDROME ON THE PERFORMANCE OF TE IN THE ASIAN POPULATION

Recently, the effects of metabolic syndrome (MS) on TE have been examined in a French study[13]. Here, BMI > 30 kg/m^2 was more frequent among subjects with MS than among normal subjects (49.1% *vs* 8.9%, respectively, $P < 0.001$). The mean LS value was higher in subjects with MS compared to controls (6.51 ± 1.64 kPa *vs* 5.33 ± 1.51 kPa, $P < 0.001$). In a multivariate analysis, LS values were still significantly different between subjects with and without MS, irrespective of BMI and other variables. Because MS

has been demonstrated to increase LS values significantly, and hepatic steatosis which can result in histological progression to cirrhosis and hepatocellular carcinoma is strongly associated with MS[31-33], a potential role of TE in detecting MS was proposed. However, 88% of the subjects with MS had LS values within the defined normal range. This result indicated that not only could TE not precisely perform in the diagnosis of MS, but also that hepatic steatosis differentially affected by MS may have influenced LS values differently for different individuals. One drawback of the study[13] was a lack of ultrasonographic evaluation of hepatic steatosis. It is thus unclear whether the increased LS values in the subjects with MS were dependent on MS itself or on hepatic steatosis associated with MS. Other studies in Italy[17,18] attempted to overcome this drawback by including ultrasonographic evaluation of the healthy blood donors and concluded that the severity of hepatic steatosis was independently related to LS values.

The association between MS or hepatic steatosis and LS values was investigated primarily in healthy subjects, because the effects of MS or hepatic steatosis on TE can be attenuated if the study population presented with background fibrotic liver (the most significant factor for high LS values). Therefore, it is difficult to identify the main factors that increase LS values among MS, hepatic steatosis, associated steatofibrosis, and background fibrosis, if the study enrolls patients already presenting with CLD.

As mentioned above, only one published European study reporting the effects of MS on TE in healthy individuals is currently available. Furthermore, no available European and Asian data address the effects of MS on TE performance in patients with chronic viral hepatitis. Considering the different clinicopathological course of hepatic steatosis in patients with CHC and CHB and the varied distribution of body fat according to race, further studies on the effects of MS and hepatic steatosis should be performed with the Asian populations.

INFLUENCE OF ALT ON THE PERFORMANCE OF TE IN THE ASIAN POPULATION WITH CHB

The most important confounding factor of TE is serum ALT levels. Hepatic inflammation, as reflected by higher ALT levels, tends to increase LS values[11,20,34]. Even minor changes in ALT levels have been shown to influence LS values[35]. Because HBV, unlike HCV, frequently exhibits acute ALT flares[36,37], the interpretation of LS values becomes increasingly difficult in the setting of CHB when major fluctuations of necrosis and inflammatory activity occur[24,38]. It thus becomes more relevant to consider ALT levels when examining TE in the Asian population with CHB.

Considering the significant effects of high ALT levels, several Asian studies with CHB patients attempted to establish varying cutoff LS values according to ALT levels. Chan *et al*[11] proposed ALT-based algorithms when interpreting LS results, and established different optimal cutoff values according to ALT levels (one group with normal ALT *vs* the other group with ALT > upper limit of normal (ULN) and ≤ 5 × ULN). In our previous study, we also stratified the study population according to ALT levels and calculated the optimal cutoff values for each group[34]. For patients with normal ALT levels, 6.0, 7.5 and 10.1 kPa were selected as the optimal cutoff values for ≥ F2, ≥ F3, and F4, respectively, whereas 8.9, 11.0, and 15.5 kPa were selected in those with ALT > ULN and ≤ 2 × ULN. Considering this unreliability of TE performance in patients with high ALT levels, more recent studies began to exclude patients displaying ALT > 5 × ULN for analysis[24]. In addition, the optimal time interval for TE to recover its reliability in patients who experience acute exacerbation of CHB was recently reported[38].

Although it seems reasonable to use different cutoff values according to ALT levels, large-scale validation of these LS values has not been performed for Asian patients with CHB. Furthermore, some issues still remain unresolved, such as how we stratify patients with CHB according to ALT levels to establish the cutoff values, and who should be excluded for TE examination because of unreliability of TE among patients with high ALT levels.

DIAGNOSTIC PERFORMANCE OF TE IN THE ASIAN POPULATION WITH CHB

Few studies have investigated the performance of TE in the Asian population with CHB[4,11,39-43]. The characteristics of studies and the identified performance of TE to predict significant fibrosis and cirrhosis are summarized in Tables 3 and 4 (Marcellin *et al*[10] is listed for comparison with other Asian studies). The listed Asian studies were selected if they evaluated TE in Asian populations with CHB, they used LB as a reference standard, and they assessed the diagnostic accuracy of TE [using area under the receiver operating characteristic curve (AUROC)] for F ≥ 2 or F = 4 fibrosis stage and/or diagnostic indexes (sensitivity, specificity, positive predictive value, or negative predictive value based on some cutoff LS values). Most Asian studies were conducted in Korea and China (Hong Kong) and some studies were available in abstract form from Korea, Singapore, and Thailand.

The AUROCs for predicting F ≥ 2 fibrosis and F = 4 fibrosis stage in patients with CHC were reported as 0.79-0.83 and 0.97-0.95, respectively in the studies by Ziol *et al*[2] and Castera *et al*[44]. However, the AUROCs in Asian studies seems to be lower than those reported in European studies (0.76-0.88 for F ≥ 2 and 0.80-0.93 for F4, Table 4), although TE diagnosed cirrhosis more accurately than significant fibrosis in Asian studies.

Overall, TE is accepted as a promising and accurate tool for the early detection of cirrhosis irrespective of the etiology of CLD, although the optimal cutoff values remain debatable. The range of the optimal cutoff values for diagnosing HBV-related cirrhosis in the Asian population were between 9.0 and 10.1 kPa based on the full-length articles[4,11,39] (Table 4) and the range for cirrhosis was less than 11.0 kPa, which is consistently lower than in

Table 3 Characteristics of studies evaluating the performance of transient elastography for the diagnosis of liver fibrosis in patients with chronic hepatitis B

	Type	Country	Total sample (*n*)	Sample size (*n*)	Excluded due to failure		Age (yr)	Male (%)	BMI (kg/m^2)	LB length (mm)	Staging
					TE (reason)	LB (reason)					
Kim *et al*[4]	Original	Korea	194	103	0 (SR < 60%, < 10 VMs)	4 (< 10 mm, < 10 PTs)	40.0	80.2	23.8	16.7	METAVIR
Chan *et al*[11]	Original	China	186	161	1 (SR < 60%, < 10 VMs)	22 (< 15 mm, < 6 PTs)	45.0	76.0	24.0	19.0	METAVIR
Kim *et al*[36]	Original	Korea	130	130	0 (SR < 60%, < 10 VMs)	0 (< 10 mm, < 6 PTs)	42.5	79.2	25.3	14.5	METAVIR
Marcellin *et al*[10]	Original	France	202	173	14 (SR < 50%, < 7 VMs)	15 (< 10 PTs)	40.1	66.5	24.5	16.6	METAVIR
Chang *et al*[40]	Abstract	Singapore	35	33	2 (obesity, narrow ICS)	0 (-)	43.0	-	25.6	-	Ishak
Tanwandee *et al*[41]	Abstract	Thailand	104	104	0 (-)	0 (-)	44.0	63.0	23.6	-	METAVIR
Choi *et al*[42]	Abstract	Korea	48	48	0 (-)	0 (-)	41.7	58.3	23.3	-	-
Chang *et al*[43]	Abstract	Singapore	88	84	3 (-)	1 (-)	49.0	71.6	-	-	-

TE: Transient elastography; VM: Valid measurement; LB: Liver biopsy; BMI: Body mass index; SR: Success rate; PT: Portal tract; ICS: Intercostal space.

Table 4 Histologic distribution and the performance of transient elastography for the diagnosis of liver fibrosis in patients with chronic hepatitis B

	n (%)					METAVIR and other scoring system (≥ F2/F4)							
	F0	F1	F2	F3	F4	AUROC	Cutoff (kPa)	Se (%)	Sp (%)	PPV (%)	NPV (%)	LR (+)	LR (-)
Kim *et al*[4]	0	9 (9.9)	33 (36.3)	10 (11.0)	39 (42.9)	-/0.803	-/9.7	-/82	-/62	-/63	-/76	-/4.97	-/0.13
Chan *et al*[11]	10 (6.2)	27 (16.8)	47 (29.2)	37 (23.0)	40 (24.8)	-/0.93	-/9	-/98	-/75	-/57	-/98	-/1	-/0.01
Kim *et al*[36]	0	10 (7.7)	37 (28.5)	16 (12.3)	67 (51.5)	-/0.84	-/10.1	-/76	-/81	-/76.1	-/80.9	-/-	-/-
Marcellin *et al*[10]	16 (9.2)	70 (40.5)	44 (25.4)	29 (16.8)	14 (8.1)	0.81/0.93	7.2/11	70/98	83/75	80/57	73/98	4/1	-/0.01
Chang *et al*[40]	7 (20.0)	16 (45.7)	F2-3 (5, 14.3)		7 (20.0)	-/-	11.8/-	90/-	78/-	-/-	-/-	-/-	-/-
Tanwandee *et al*[41]	-	-	-	-	-	0.757/-	6.9/7.3	70/93	79/61	82/31	66/98	-/-	-/-
Choi *et al*[42]	-	-	-	-	-	0.88/0.86	7.7/10.4	88/79	88/83	-/-	-/-	-/-	-/-
Chang *et al*[43]	-14.8	-30.7	-14.8	-21.6	-17.1	0.801/-	8.8/-	-/-	-/-	-/-	-/-	-/-	-/-

AUROC: Area under the receive operating characteristic curve; Se: Sensitivity, Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; LR: Likelihood ratio.

patients with CHC[45,46]. These findings can be explained in several ways. The histopathological characteristics of CHC (including portal lymphoid follicles, bile duct damage, lobular activity, and steatosis) may contribute to the variations in cutoff values compared with those in patients with CHB[47]. Another explanation is that the total fibrotic material in CHB may be lower than that in CHC, because CHB tends to make the liver macronodular and heterogeneous[48]. Other researchers have proposed that the different types and extent of liver inflammatory infiltrate within the liver may account for the different cutoff LS values between CHB and CHC[20].

The performance of TE in prediction of cirrhosis is acceptable for the Asian population with CHB, but increased performance in prediction of significant fibrosis and subsequent precise staging, particularly for patients with CHB who are candidates for antiviral treatment, is needed and should be pursued through future studies. This is particularly important, because the decision to start antiviral treatment and the type of antiviral agents to be used may be affected by fibrosis stage.

CONCLUSION

Because of limitations of LB, prohibiting its routine use for the evaluation of liver fibrosis in patients with CHB, interest in the use of noninvasive TE has increased. Ideally, TE can be used to screen the general population to detect high-risk patients with potential liver disease, to identify patients with significant fibrosis who could benefit from the initiation of antiviral treatment, to select patients with cirrhosis who are at a high risk of developing HCC[25], and to identify patients with cirrhosis and portal hypertension. To date, although numerous European studies have demonstrated that this is possible, data on TE for the Asian population are scarce.

We are now fully armed with nuggets of knowledge that the etiologies of CLD, BMI, MS, cardiac function, cholestasis, hepatic steatosis, and ALT levels can influence the performance of TE, and also that several confounders vary between Asian and European populations, for example, the etiology of CLD, major fluctuation in ALT levels, and the prevalence of high BMI and MS. However, because almost all information on TE has thus far originated from European data, for the Asian population with CHB, TE is not popular clinically. Therefore, further studies tailored to the Asian population with CHB will restore the confidence of Asian clinicians to utilize TE.

REFERENCES

1 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C,

Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713
2 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Ledinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54
3 **Corpechot C**, El Naggar A, Poujol-Robert A, Ziol M, Wendum D, Chazouilleres O, de Ledinghen V, Dhumeaux D, Marcellin P, Beaugrand M, Poupon R. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; **43**: 1118-1124
4 **Kim do Y**, Kim SU, Ahn SH, Park JY, Lee JM, Park YN, Yoon KT, Paik YH, Lee KS, Chon CY, Han KH. Usefulness of FibroScan for detection of early compensated liver cirrhosis in chronic hepatitis B. *Dig Dis Sci* 2009; **54**: 1758-1763
5 **de Ledinghen V**, Beaugrand M, Kelleher TB, Foucher J, Castera L, Ziol M, Ganne N, Lai M, Afdhal NH. Prediction of liver fibrosis in non-alcoholic steatohepatitis (NASH): Risk factors and diagnostic potential of liver elasticity using fibroscan. *J Hepatol* 2006; **44**: S39
6 **Talwalkar JA**, Kurtz DM, Schoenleber SJ, West CP, Montori VM. Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2007; **5**: 1214-1220
7 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974
8 **Stebbing J**, Farouk L, Panos G, Anderson M, Jiao LR, Mandalia S, Bower M, Gazzard B, Nelson M. A meta-analysis of transient elastography for the detection of hepatic fibrosis. *J Clin Gastroenterol* 2010; **44**: 214-219
9 **Lucidarme D**, Foucher J, Le Bail B, Vergniol J, Castera L, Duburque C, Forzy G, Filoche B, Couzigou P, de Ledinghen V. Factors of accuracy of transient elastography (fibroscan) for the diagnosis of liver fibrosis in chronic hepatitis C. *Hepatology* 2009; **49**: 1083-1089
10 **Marcellin P**, Ziol M, Bedossa P, Douvin C, Poupon R, de Ledinghen V, Beaugrand M. Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. *Liver Int* 2009; **29**: 242-247
11 **Chan HL**, Wong GL, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ, Wong VW. Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B. *J Viral Hepat* 2009; **16**: 36-44
12 **Kettaneh A**, Marcellin P, Douvin C, Poupon R, Ziol M, Beaugrand M, de Ledinghen V. Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *J Hepatol* 2007; **46**: 628-634
13 **Roulot D**, Czernichow S, Le Clesiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. *J Hepatol* 2008; **48**: 606-613
14 **Kim SU**, Choi GH, Han WK, Kim BK, Park JY, Kim do Y, Choi JS, Yang SC, Choi EH, Ahn SH, Han KH, Chon CY. What are 'true normal' liver stiffness values using FibroScan?: a prospective study in healthy living liver and kidney donors in South Korea. *Liver Int* 2010; **30**: 268-274
15 **Corpechot C**, El Naggar A, Poupon R. Gender and liver: is the liver stiffness weaker in weaker sex? *Hepatology* 2006; **44**: 513-514
16 **Sirli R**, Sporea I, Tudora A, Deleanu A, Popescu A. Transient elastographic evaluation of subjects without known hepatic pathology: does age change the liver stiffness? *J Gastrointestin Liver Dis* 2009; **18**: 57-60
17 **Colombo S**, Belloli L, Buonocore M, Jamoletti C, Zaccanelli M, Badia E, Del Poggio P. Liver Stiffness values in the normal population: a studying voluntary blood donors. *Hepatology* 2008; **48** Suppl: A995
18 **Colombo S**, Belloli L, Buonocore M, Jamoletti C, Zaccanelli E, Del Poggio P. True normal liver stiffness measurement (LSM) and its determinants. *Hepatology* 2009; **50** Suppl: A741
19 **Fung J**, Lai CL, Chan SC, But D, Seto WK, Cheng C, Wong DK, Lo CM, Fan ST, Yuen MF. Correlation of liver stiffness and histological features in healthy persons and in patients with occult hepatitis B, chronic active hepatitis B, or hepatitis B cirrhosis. *Am J Gastroenterol* 2010; **105**: 1116-1122
20 **Oliveri F**, Coco B, Ciccorossi P, Colombatto P, Romagnoli V, Cherubini B, Bonino F, Brunetto MR. Liver stiffness in the hepatitis B virus carrier: a non-invasive marker of liver disease influenced by the pattern of transaminases. *World J Gastroenterol* 2008; **14**: 6154-6162
21 **Kelleher T**, MacFarlane C, de Ledinghen V, Beaugrand M, Foucher J, Castera L. Risk factors and hepatic elastography (FibroScan) in the prediction of hepatic fibrosis in nonalcoholic steatohepatitis. *Gastroenterology* 2006; **130**: A736
22 **Yoneda M**, Yoneda M, Fujita K, Inamori M, Tamano M, Hiriishi H, Nakajima A. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). *Gut* 2007; **56**: 1330-1331
23 **Foucher J**, Castera L, Bernard PH, Adhoute X, Laharie D, Bertet J, Couzigou P, de Ledinghen V. Prevalence and factors associated with failure of liver stiffness measurement using FibroScan in a prospective study of 2114 examinations. *Eur J Gastroenterol Hepatol* 2006; **18**: 411-412
24 **Chan HL**, Wong GL, Tse CH, Chim AM, Yiu KK, Chan HY, Sung JJ, Wong VW. Hepatitis B virus genotype C is associated with more severe liver fibrosis than genotype B. *Clin Gastroenterol Hepatol* 2009; **7**: 1361-1366
25 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954-1961
26 **Wong GL**, Wong VW, Choi PC, Chan AW, Chim AM, Yiu KK, Chan HY, Chan FK, Sung JJ, Chan HL. Clinical factors associated with liver stiffness in hepatitis B e antigen-positive chronic hepatitis B patients. *Clin Gastroenterol Hepatol* 2009; **7**: 227-233
27 **Maimone S**, Calvaruso V, Pleguezuelo M, Squadrito G, Amaddeo G, Jacobs M, Khanna P, Raimondo G, Dusheiko G. An evaluation of transient elastography in the discrimination of HBeAg-negative disease from inactive hepatitis B carriers. *J Viral Hepat* 2009; **16**: 769-774
28 **Lupsor M**, Stefanescu H, Sparchez Z, Serban A, Grigorescu M, Iancu S, Suteu T, Badea R. The influence of fatty load on liver stiffness in chronic hepatitis c patients. *J Hepatol* 2008; **48** Suppl 2: S278
29 **Gaia S**, Carenzi S, Brunello F, Barilli AL, Lagger M, Bugianesi E, Smedile A, Rizzetto M. Is liver stiffness measurement different in patients with NASH or with viral hepatitis? *Dig Liver Dis* 2008; **40**: A128
30 **Kim SU**, Kim DY, Ahn SH, Kim HM, Lee JM, Chon CY, Park YN, Han KH, Park JY. The impact of steatosis on liver stiffness measurement in patients with chronic hepatitis B. *Hepatogastroenterology* 2010; In press
31 **Wong VW**, Chan HL, Hui AY, Chan KF, Liew CT, Chan FK, Sung JJ. Clinical and histological features of non-alcoholic fatty liver disease in Hong Kong Chinese. *Aliment Pharmacol Ther* 2004; **20**: 45-49
32 **Nugent C**, Younossi ZM. Evaluation and management of obesity-related nonalcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 432-441
33 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastro-*

enterology 2005; **129**: 113-121
34 **Kim SU**, Kim do Y, Park JY, Lee JH, Ahn SH, Kim JK, Paik YH, Lee KS, Chon CY, Choi EH, Song KJ, Park YN, Han KH. How can we enhance the performance of liver stiffness measurement using FibroScan in diagnosing liver cirrhosis in patients with chronic hepatitis B? *J Clin Gastroenterol* 2010; **44**: 66-71
35 **Calvaruso V**, Cammà C, Di Marco V, Maimone S, Bronte F, Enea M, Pleguezuelo M, Xirouchakis E, Misseri M, Manousou P, Dusheiko M, Burroughs A, Craxi A. Error factors for transient elastography in chronic hepatitis C. *Hepatology* 2008; **48** Suppl: A313
36 **Yuen MF**, Yuan HJ, Hui CK, Wong DK, Wong WM, Chan AO, Wong BC, Lai CL. A large population study of spontaneous HBeAg seroconversion and acute exacerbation of chronic hepatitis B infection: implications for antiviral therapy. *Gut* 2003; **52**: 416-419
37 **Liaw YF**. Hepatitis flares and hepatitis B e antigen seroconversion: implication in anti-hepatitis B virus therapy. *J Gastroenterol Hepatol* 2003; **18**: 246-252
38 **Wong GL**, Wong VW, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ, Chan HL. Increased liver stiffness measurement by transient elastography in severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol* 2009; **24**: 1002-1007
39 **Kim SU**, Ahn SH, Park JY, Kang W, Kim do Y, Park YN, Chon CY, Han KH. Liver stiffness measurement in combination with noninvasive markers for the improved diagnosis of B-viral liver cirrhosis. *J Clin Gastroenterol* 2009; **43**: 267-271
40 **Chang J**, Tan HH, Yew BS, Thia K, Ang D, Yap YM, Chau C, Tan CK, Chow WC, Lui HF. Transient elastography (FibroScan®) to assess hepatic fibrosis in Chinese with chronic hepatitis B. *Hepatol Int* 2007; **1** Suppl: A206
41 **Tanwandee T**, Charatcharoenwitthaya P, Viboolsirikul V, Chotiyaputta W, Chainuvati S, Maneerattanaporn M, Prachayakul V, Pongprasobchai S, Manatsathit S, Leelakucolvong S, Pausawasdi N, Srikureja W, Kachintom U. Utility of liver stiffness measured by transient elastography for determining significant liver fibrosis in patients with chronic hepatitis B. *Hepatology* 2008; **48** Suppl: A709
42 **Choi JW**, Kim DY, Park JY, Ahn SH, Yoon KT, Lee JM, Kim JK, Paik YH, Lee KS, Moon BS, Chon CY, Han KH. Clinical usefulness of liver stiffness measurement in HBeAg-positive chronic hepatitis B patients with ALT level <2 times upper limit of normal. *Hepatology* 2008; **48** Suppl: A750
43 **Chang J**, Lui HF, Tan CK, Chow WC. Transient elastography (FibroScan®) is reliable for non-invasive diagnosis of significant fibrosis in chronic hepatitis B with mild transaminitis but becomes less reliable at higher ALT levels. *Hepatology* 2008; **50** Suppl: A501
44 **Castera L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Ledinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350
45 **Colletta C**, Smirne C, Fabris C, Toniutto P, Rapetti R, Minisini R, Pirisi M. Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. *Hepatology* 2005; **42**: 838-845
46 **Foucher J**, Chanteloup E, Vergniol J, Castera L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Ledinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403-408
47 **Scheuer PJ**, Davies SE, Dhillon AP. Histopathological aspects of viral hepatitis. *J Viral Hepat* 1996; **3**: 277-283
48 **Castera L**, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; **48**: 835-847

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REVIEW

Consequences of *Helicobacter pylori* infection in children

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Abstract

Although evidence is emerging that the prevalence of *Helicobacter pylori* (*H. pylori*) is declining in all age groups, the understanding of its disease spectrum continues to evolve. If untreated, *H. pylori* infection is lifelong. Although *H. pylori* typically colonizes the human stomach for many decades without adverse consequences, children infected with *H. pylori* can manifest gastrointestinal diseases. Controversy persists regarding testing (and treating) for *H. pylori* infection in children with recurrent abdominal pain, chronic idiopathic thrombocytopenia, and poor growth. There is evidence of the role of *H. pylori* in childhood iron deficiency anemia, but the results are not conclusive. The possibility of an inverse relationship between *H. pylori* and gastroesophageal reflux disease, as well as childhood asthma, remains a controversial question. A better understanding of the *H. pylori* disease spectrum in childhood should lead to clearer recommendations about testing for and treating *H. pylori* infection in children who are more likely to develop clinical sequelae.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is one of the most common chronic bacterial infections world-wide, and it is currently estimated that approximately half of the world's population is infected with the bacterium[1,2]. However, the prevalence of *H. pylori* is not homogeneous world-wide[1,3]. In western countries, the prevalence of infection has been decreasing during the past few decades[4-6]. *H. pylori* infection is acquired early in life (almost always before the age of 10 years), and in the absence of antibiotic therapy, it generally persists for life[1].

It is widely accepted that *H. pylori* infection is the main etiological factor for gastritis and peptic ulcer[3]. Its eradication is associated with healing of these diseases and significant reduction of ulcer recurrence and rebleeding[7,8]. Several studies have demonstrated that inflammation caused by *H. pylori* infection might contribute to the development of adenocarcinoma of the stomach; moreover, it has been involved in the development of low-grade B-cell lymphoma of gastric mucosa-associated lymphoid tissue

type (MALT)[3,9]. Recently, a potential role of *H. pylori* infection in other digestive diseases (gastroesophageal reflux disease; GERD) as well as several extra-intestinal pathologies [iron deficiency anemia (IDA), growth retardation, idiopathic thrombocytopenic purpura (ITP), asthma and allergic disorders] has been suggested[10]. The postulated role of *H. pylori* in the pathogenesis of extra-intestinal manifestations is based on the facts that: (1) local inflammation has systemic effects; (2) *H. pylori* gastric infection is a chronic process that lasts for several decades; and (3) persistent infection induces a chronic inflammatory and immune response that is able to induce lesions both locally and remote to the primary site of infection[11].

The aim of this report is to provide a critical review of the available literature about digestive and extradigestive manifestations of *H. pylori* infection in children. Pertinent articles have been identified through a MEDLINE search. Studies published in English during the past two decades have been identified and reviewed.

GASTRITIS AND PEPTIC ULCERS

During childhood, *H. pylori* is associated with predominant antral gastritis, and duodenal ulcers[12-14]. Successful eradication of *H. pylori* markedly reduces the rate of recurrence of duodenal ulcers in affected children[2,15,16]. Gastric ulcers are much less common in children than they are in adults[17].

A pooled analysis of early reports (1983-1994) has demonstrated that the rate ratio of antral gastritis for children with *H. pylori* infection (compared with uninfected subjects) ranged from 1.9 to 71.0 (median, 4.6)[17]. The prevalence of *H. pylori* in children with duodenal ulcer was high (range, 33%-100%; median, 92%) compared with children with gastric ulcer (range, 11%-75%; median, 25%)[17]. Thus, there was strong evidence for an association between *H. pylori* infection and antral gastritis and duodenal ulcer in children; there was weak evidence for an association with gastric ulcer. Nevertheless, a subsequent retrospective study (1995-2001) from Japan has confirmed that the prevalence of *H. pylori* was very high in antral (nodular) gastritis and duodenal ulcer (98.5% and 83%, respectively), but it has also demonstrated that *H. pylori* was a definite risk factor for the development of gastric ulcer, although the prevalence of infection did not reach 50%[18]. *H. pylori* was significantly linked to duodenal and gastric ulcers in the age group 10-16 years, but not ⩽ 9 years.

More recently, a decreasing proportion of *H. pylori*-positive peptic ulcers in adults has been observed, along with a decrease in the prevalence rate of the infection[19]. In children, there have been few data published in the literature to investigate the trend of *H. pylori* prevalence in peptic ulcer[20-23]. In a prospective European multicenter pilot study on the incidence of gastric and duodenal ulcer disease in children, Kalach *et al*[20] have found that ulcers occurred in 10.6% of cases, with *H. pylori* infection in only 26.7% of these. From January 2001 to December 2002, information on 518 children was collected from the pediatric European register for treatment of *H. pylori*[21]. At endoscopy, 454 children had *H. pylori*-associated gastritis and 64 had an ulcer (12.3%). However, this series included children from Russia and they had a significantly higher prevalence of peptic ulcer (35% *vs* 6.7% in the remainder of European children, $P < 0.0001$; OR: 7.5; 95% CI: 4-13). Thus the prevalence of *H. pylori*-positive ulcers in children differed between countries, and this was not completely explained by the prevalence of the infection in the population studied[22]. In a retrospective review (1998-2006) of 619 Chinese children who had undergone upper endoscopy for investigation of upper gastrointestinal symptoms, Tam *et al*[23] have found that 43 (6.9%) had peptic ulcer. Of these 43 patients, 37 and six had duodenal and gastric ulcer, respectively. The prevalence of *H. pylori* infection was 56.8% (21/37) in duodenal ulcer and 33.3% (2/6) in gastric ulcer. When they arbitrarily divided the study period into two, 1998-2001 and > 2002, no significant difference in the prevalence of *H. pylori* infection between the two periods was found.

GASTRIC MALIGNANCIES

In relation to *H. pylori*-associated gastric malignancies in children, there have been a few cases of gastric MALT lymphoma[14,24,25], but there have been no reports of adenocarcinoma.

There is evidence to support an association between long-standing *H. pylori* infection, gastric atrophy, and intestinal metaplasia with the development of intestinal-type and undifferentiated adenocarcinomas in adults[3]. It has been suggested that chronic gastritis, gastric atrophy, intestinal metaplasia and gastric cancer develop progressively, stepwise over decades, in predisposed individuals infected by *H. pylori*[26]. However, gastric atrophy and intestinal metaplasia have indeed been described in children living in countries with high gastric cancer incidence[27], and they are sometimes found in very young subjects[28-30]. These findings provide support to the hypothesis that host genetic factors that affect the inflammatory and immune response to *H. pylori* infection might determine why some individuals infected with this bacterium develop precancerous lesions and gastric carcinoma while others do not[31,32]. Thus, it is probable that the prevalence of gastric atrophy and intestinal metaplasia varies according to the geographic/genetic origins as well as environmental factors[27-30,33-40], as shown in Table 1. Yet, sampling problems exist. In fact, the non-systematic search during pediatric gastroscopy for these histological states might mask their true prevalence[32]. Most reported studies of the histological features of *H. pylori* infection in children have used either random biopsies[41] or a small number of targeted biopsies[29,34,42,43], taken primarily from the antrum[29,44]. In most studies, the identification of atrophy has been focused on the presence of intestinal metaplasia or has been ill defined[29].

In clinical practice, the updated Sydney system is widely used for grading gastric histopathological findings (density

Table 1 Prevalence of gastric atrophy in children

Author (yr)/country	Updated Sydney System	Mean age (range, yr)	No. of patients		Gastric atrophy and/or intestinal metaplasia (%)	
			H. pylori+	*H. pylori*-	*H. pylori*+	*H. pylori*-
Whitney *et al*[28] (2000)/USA	Yes	10.7 (1-21)	42	0	Antral: 16.6/0 Fundic: 2.3/0	-/-
Kolho *et al*[33] (2000)/Finland	Not reported	9.5 (2-16)	71	0	0/0	-/-
Campbell *et al*[34] (2001)/Gambia	Yes	1.4 (-/-)	21	16	0/0	0/0
Guiraldes *et al*[35] (2002)/Chile	No	12.2 (5-17)	59	14	0/0	0/0
Oztürk *et al*[36] (2003)/Turkey	Yes	12.2 (6-16)	18	9	Antral: 72.2/77.7	11.1/0
Guarner *et al*[29] (2003)/USA	Yes	- (1-17)	19	45	Antral: 52.6/15.7 Fundic: 0/5.2	22.2/0 0/0
Usta *et al*[37] (2004)/Turkey	Yes	11.8 (4-17)	175	0	2.2/1.1	-/-
Levine *et al*[38] (2004)/Israel	Not reported	14.2 (-/-)	55	40	0/0	2.5/0
Ricuarte *et al*[27] (2005)/Colombia, Korea	No	12.0 (4-18) 14.0 (8-18)	97 10	18 48	16.4[1] 0	[1] 0
Kato *et al*[39] (2006)/Japan	Yes	11.3 (1-16)	131	65	Antral: 51.9/4.6 Fundic: 34.8/0	10.8/4.6 8.3/4.2
Tutar *et al*[30] (2009)/Turkey	Yes	1.3 (0.1-2.0)	40	112	2.5/0	0/0
Kalach *et al*[40] (2009)/France	Yes	5.3 (0.1-17.7)	66	553	0/0	0.2/0.2

[1]Atrophic mucosa: 31% as intestinal metaplasia; 63% as pseudopyloric metaplasia; 6% as both. *H. pylori*: *Helicobacter pylori*.

of *H. pylori* organisms, acute and chronic inflammation, atrophy and intestinal metaplasia)[45]. Although this system has been validated in adult patients, interobserver variability is still a problem, primarily in the evaluation of mucosal atrophy[46,47]. Thus, further validation of atrophy parameters needs to be obtained for pediatric biopsy samples[29]. In contrast, metaplastic epithelium is easily detected by the pathologist, owing to the characteristic goblet cells[32].

In adults, five gastric biopsy samples are recommended (two antral, two corporeal and one from the angulus)[45], but no consensus is available about the optimal number and site location of gastric biopsies in children. Clinical practices in this domain are very heterogeneous, which could in part account for the different prevalence figures of atrophic gastritis in children[32]. Of note, in a study of 173 children from countries with high gastric cancer incidence, Ricuarte *et al*[27] emphasized the importance of biopsy site location for identifying the presence of corpus atrophy. In children, atrophy is only identified in biopsies taken near the normal antrum-corpus junction, which is consistent with the notion that atrophy progresses as an advancing antrum-corpus border[27]. Therefore, identification and characterization of the natural history of *H. pylori* gastritis requires, in addition to biopsies that target the antrum and the cardia, targeted biopsies to include the lesser and greater curvature of the corpus, starting just proximal to the anatomical junction of the antrum and corpus. Unfortunately, the sites recommended by the updated Sydney system can identify corpus atrophy only when it is extensive.

Studies from the 1990s have established that the development of low-grade gastric MALT lymphoma is strongly associated with chronic *H. pylori* gastritis. In two large series of adult patients, *H. pylori* was detected by histological examination in 92% and 100% of those with gastric MALT lymphoma[48,49]. Recognition of the responsiveness of MALT lymphoma to antibiotic therapy aimed at eradication of *H. pylori* has changed the approach to its management. Remission rates in the literature range from 60% to 80%, although recurrence can be expected in 5% of cases[50-53]. MALT lymphoma occurs commonly in middle and old age; only a small number of cases have been reported in both immunocompetent and immunocompromised children[14,24,25]. Individual case reports have described the regression of MALT lymphoma after *H. pylori* eradication therapy alone[24,25].

FUNCTIONAL DYSPEPSIA

The role of *H. pylori* infection as a cause of non-ulcer or functional dyspepsia has been one of the most debated controversies in the medical community since the discovery of this bacterium.

Dyspepsia is very common in children with chronic or recurrent abdominal pain (RAP), with as many as 80% reporting this symptom[54]. The relation between RAP in childhood and *H. pylori* infection is not clear[17]. Pediatric studies are limited by the lack of a clear definition for RAP or by the use of nonspecific criteria for the diagnosis of chronic abdominal pain[54]. A pooled analysis of early reports (1983-1994) has demonstrated that prevalence rates of infection in children with RAP were inconsistent (range, 0%-81%; median, 22%),with lower rates (range, 0%-9%; median, 6%), in children who met Apley's criteria (i.e. at least three discrete episodes of abdominal pain of sufficient severity to interrupt normal daily activities or performance occurring over a period of $\geq$ 3 mo)[17].

In adults, several controlled trials have shown a vague connection between *H. pylori* colonization and dyspeptic symptoms[55]. In children, controlled randomized treatment studies have been scant. The results of two uncontrolled trials have suggested improvement of clinical symptoms after treatment of *H. pylori* infection[56,57]. However, the double-blind, randomized placebo-controlled trial by Ashorn *et al*[58] has suggested that RAP is not an indication

for a test and treatment strategy for *H. pylori* infection in children. In fact, in that study, at 52 wk, dyspeptic symptoms improved to the same extent in the treatment group and in children who received placebo, irrespective of the healing of gastritis, which was more commonly achieved along with eradication[58]. Nonetheless, due to the limited number of patients who could finally be included, the results by Ashorn *et al*[58] have to be interpreted with care. Large-scale multicenter trials performed in children are still needed to answer definitively the question whether a connection exists between *H. pylori* infection and RAP. Neither did the results of a very recent study give support for the use of *H. pylori* eradication in children with RAP[59]. Based on a meta-analysis of 38 studies between 1966 and 2009, the study by Spee *et al*[59] has found no association between RAP (fulfilling Apley's criteria) and *H. pylori* infection in children. However, the authors have demonstrated that children who are referred to a gastroenterologist with unspecified abdominal pain (i.e. including children who do not fulfill Apley's criteria) or pain in the epigastric region are at 2-3-fold higher risk for *H. pylori* infection than children without these symptoms. Thus, these authors have postulated that unspecified abdominal pain in a hospital-based setting and epigastric pain in general might be associated with (acute) *H. pylori* infection. The potential for *H. pylori* to cause clinical symptoms that arise from gastric infection, in the absence of mucosal ulceration, requires additional studies using a strict study group definition. Functional dyspepsia and non-ulcer dyspepsia (i.e. epigastric pain in the absence of mucosal ulceration at esophago-gastro-duodenoscopy) must be evaluated as separate entities.

GASTROESOPHAGEAL REFLUX DISEASE

The interaction between *H. pylori* infection and GERD has been widely debated in the literature over the past decade, and the hypothesis that eradication of *H. pylori* leads to increased GERD has been the subject of many publications with contradictory conclusions in children as well as in adults[38,60-76]. There are limited data in children because the prevalence of *H. pylori* infection is low and no randomized controlled trials have been conducted; therefore, inferences about the effects of *H. pylori* eradication and GERD need to be drawn from studies on adult patients[77].

H. pylori has been found to be inversely correlated with the prevalence of GERD, and certain studies have shown aggravation of esophagitis after eradication[60-66]. Suggested mechanisms include presence of atrophic or significant body gastritis that leads to a post-eradication increase in acid output; decreased buffering as a result of elimination of *H. pylori*, which produces ammonia *via* bacterial urease; masking of reflux by acid neutralizing medications given for *H. pylori*-related disease; and increased appetite with weight-gain- mediated reflux. These observations are controversial, because several studies have not found an association between eradication of *H. pylori* and reflux disease[38, 66,70,74,78-80].

Most studies aimed at evaluating the effect of *H. pylori* eradication on reflux in adults have used selected populations such as those with duodenal ulcer or patients with GERD before eradication[63,65,78,80]. The spectrum of risk factors found in adults, such as atrophic gastritis, duodenal ulcer, or significant esophagitis, might influence the outcome of the study. These factors are less common in children, therefore, these results might not be relevant to the decision to eradicate *H. pylori* when found in children[38].

Dent has proposed that the effect of *H. pylori* eradication on GERD is most likely determined by the population studied[67]. Acid secretion in predominant antral gastritis with preserved body mucosa is hyper-responsive, thus enabling increased duodenal or esophageal injury. In these patients, eradication should improve or not affect GERD. This hypothesis is consistent with the results of other studies that have shown improvement in GERD symptoms in patients with duodenal ulcer[80,81]. However, in patients with atrophic gastritis or severe body gastritis, *H. pylori* eradication might result in increased acid secretion. Children and adolescents are more likely to behave like the first group, with predominant antral gastritis[38].

The risks and benefits of *H. pylori* eradication are less well-defined for patients with gastritis alone, and vary according to the severity and pattern of gastritis[67]. Although this is the patient group most likely to develop reflux esophagitis[63], the risks from this are outweighed by those of continued *H. pylori* infection. Reflux esophagitis following *H. pylori* eradication is believed to carry little risk and, in particular, not to lead to intestinal metaplasia. By contrast, the risks of continued *H. pylori* infection are relatively high in patients who have had an episode of chronic duodenal, gastric or gastroduodenal ulceration[67]. What is certain, is that *H. pylori* is a major risk factor for non-cardia gastric adenocarcinoma[82], and children with this infection have at least a fivefold increased risk of developing stomach neoplasia in later life. This risk is likely to be reversed with *H. pylori* eradication[77]. In a study of high-risk adults, no reduction in gastric cancer risk at the end of 7.5 years of follow-up was observed in *H. pylori* carriers who had previously undergone eradication therapy[83]. However, in a subgroup analysis of patients who had no precancerous lesions at baseline, cancer risk was significantly reduced[83]. Additional studies have suggested that prevention of gastric cancer might be possible in infected individuals without precancerous lesions[84,85]. Therefore, children could well be the group to target in an effort to prevent the future development of gastric cancer.

IRON DEFICIENCY ANEMIA

In addition to the already known causes of IDA, over the past two decades, an association between *H. pylori* and pediatric IDA has been established[86-92]. However, the issues of whether *H. pylori* infection is linked causally to IDA in children and whether treatment or resolution of *H. pylori* infection would improve iron stores or resolve IDA in children are still matters of great debate. In 1991,

Table 2 Randomized trials of *Helicobacter pylori* eradication for iron deficiency anemia and iron deficiency in children

Author (yr)/country	No. of children with IDA/*H. pylori*	Follow-up (No. of children)	Outcome	
			IDA	ID
Choe *et al*[89] (1999)/Korea	43/25	8 wk (18)	Hb increased with: eradication + iron, eradication + placebo *vs* iron + placebo ($P = 0.0086$)	No significant differences in serum iron or ferritin
Sarker *et al*[106] (2008)/Bangladesh	260/200	3 mo (260)	IDA persisted with: eradication + iron, 11%; eradication alone, 33%; iron alone, 0%; placebo, 45%	ID persisted with: eradication + iron, 19%; eradication alone, 65%; iron alone, 7%; placebo, 78%
Gessner *et al*[105] (2006)/Alaska	219/219	14 mo (201)	IDA persisted with: eradication + iron, 22%; iron alone, 14%	ID persisted with: eradication + iron, 65%; iron alone, 72%
Fagan *et al*[104] (2009)/Alaska	219/219	40 mo (176)	IDA persisted with: eradication + iron, 5%; iron alone, 19%	ID persisted with: eradication + iron, 52%; iron alone, 58%

Hb: Hemoglobin; ID: Iron deficiency; IDA: Iron deficiency anemia; *H. pylori*: *Helicobacter pylori*.

an association between *H. pylori* infection and IDA due to microscopic blood loss was described in a 15-year-old girl with *H. pylori*-positive chronic active hemorrhagic gastritis, who showed no signs of gastrointestinal symptoms[88]. Two years later, Dufour *et al*[93] reported a 7-year-old child who presented with *H. pylori*-associated chronic antral gastritis without evidence of hemorrhage or clinical symptoms other than sideropenic anemia, which was refractory to oral iron administration and subsided after *H. pylori* eradication. These case reports were followed by other studies that have identified an association between *H. pylori* infection and pediatric unexplained or refractory IDA, and have indicated improvement of iron stores and anemia after successful *H. pylori* eradication[94-100]. Yet, some pediatric studies have implicated *H. pylori* as a cause of IDA that is refractory to oral iron treatment[94,95,99,100]. Thus, the above studies have supported a clinically significant influence of *H. pylori* infection on body iron stores and have led to a recommendation for *H. pylori* eradication in infected individuals with unexplained IDA[101-103]. However, small sample sizes, lack of control groups, and other methodological issues, including the use of validated measures of active *H. pylori* infection such as biopsy-related tests to confirm *H. pylori* infection, are among factors that have limited the interpretation and ability to generalize the importance of the results of these studies in children.

To the best of our knowledge, only four population-based randomized trials of the effect of *H. pylori* infection treatment on IDA have been performed in children[89,104-106], as shown in Table 2. Three of them lacked true placebo groups[89,104,105]. Choe *et al*[89] have demonstrated a beneficial effect of *H. pylori* eradication therapy plus iron or placebo in increasing hemoglobin levels. However, no significant differences among study groups were found at 8 wk follow-up for serum iron level, total iron-binding capacity, and ferritin level. Sarker *et al*[106] have shown, in a relatively large cohort of Bangladeshi children with IDA, a similar improvement of IDA as well as iron deficiency, with anti-*H. pylori* therapy plus iron compared with iron therapy alone. Therefore, the improvement of iron status in children who receive combined therapy could be attributed to the effect of iron rather than anti-*H. pylori* therapy. The findings from the Bangladeshi children corroborated well those of the study from rural Alaska, which showed that treatment and resolution of *H. pylori* infection did not substantially decrease levels of iron deficiency or mild anemia at 14 mo after treatment initiation, despite the relatively low rate of reinfection once the initial infection resolved[105]. When an additional follow-up evaluation at 40 mo after treatment initiation was performed, it was found that sustained resolution of *H. pylori* infection substantially reduced the prevalence of mild IDA but modestly improved iron status[104].

How can *H. pylori* gastritis cause IDA? Several theoretical mechanisms have been proposed to explain the possible relationship between *H. pylori* infection and decreased iron stores. It appears that chronic gastrointestinal blood loss is not the likely culprit, because most published cases and case series have found no bleeding lesions at the time of endoscopy, and have reported negative testing for fecal occult blood[107]. Another explanation for a relationship between *H. pylori* infection and IDA involves the possible effect of *H. pylori* gastritis on gastric acid secretion and iron absorption. Non-heme iron accounts for 80% of dietary iron in industrialized countries[107]. Crucial to the effective absorption of non-heme iron is hydrochloric acid in acid secretions. Reduction of the ferric to ferrous form is dependent upon the pH of the gastric juice, and reduction to the ferrous form facilitates membrane transport[108]. An important promoter of iron absorption is ascorbic acid, which appears to act in two ways: by promoting reduction to the ferrous form, and by forming an absorbable molecular complex with ferric iron, which is insoluble at pH > 5[107,109]. Gastric acid hyposecretion results from atrophy of the gastric glands and fundic mucosa, which has been associated with chronic *H. pylori* infection[107]. It has been shown that adult patients with IDA and *H. pylori* infection are more likely to have a pattern of gastritis that involves the gastric corpus, with related decreases in gastric acid secretion and increases in intragastric pH that might impair iron absorption[110]. There are no comparable data in children.

Another hypothesized mechanism is that *H. pylori* might lead to IDA by sequestering and utilizing iron, thus

Table 3 Cross-sectional studies on the association between *Helicobacter pylori* infection and growth retardation

Author (yr)/country	Total No. of patients (*H. pylori* +)	Age (range, yr)	Diagnostic test	Conclusion
Perri *et al*[114] (1997)/Italy	216 (49)	3-14	Urea breath test	*H. pylori* infection was associated with growth delay, and poor socioeconomic status
Oderda *et al*[115] (1998)/Italy	134 with short stature (27)	5-13	Serology	No association with short stature
	134 controls (18)	5-13		
Quiñonez *et al*[116] (1999)/Guatemala	211 (107)	5-10	Serology	No association with height for age and nutritional status
Choe *et al*[90] (2000)/Korea	375 (63)	10-15	Serology	*H. pylori* infection accompanied by IDA, rather than *H. pylori* infection *per se*, was associated with delayed pubertal growth
Richter *et al*[117] (2001)/Germany	3315 (213)	5-7	Urea breath test	*H. pylori* infection was associated with growth delay
Ertem *et al*[118] (2002)/Turkey	327 (162)	3-12	Urea breath test	*H. pylori* infection was associated with short stature independently of poor living standards
Sood *et al*[119] (2005)/UK	257 (97)	-	Urea breath test	No association with height and weight z scores, after adjustment for socioeconomic status and ethnicity
Süoglu *et al*[120] (2007)/Turkey	70 (35)	4-16	Endoscopy	*H. pylori* infection and IDA had a significant effect on height z scores, after adjustment for economic status
Mohammad *et al*[121] (2008)/Egypt	286 (208)	6-15	Urea breath test	*H. pylori* infection affected both body weight and height
Soylu *et al*[122] (2008)/Turkey	108 with dyspepsia (57)	7-17	Endoscopy	No association with anthropometry. But, dyspeptic children had worse nutritional status compared to controls, regardless of *H. pylori* status
	50 healthy controls	8-17		
Cherian *et al*[123] (2009)/Australia	182 (149)	< 16	Stool antigen	No association with BMI or other anthropometric measures
Gulcan *et al*[124] (2010)/Turkey	181 with RAP (121)	6-15	Endoscopy, 181	RAP associated with gastric mucosal injury had a negative effect on BMI independent of *H. pylori* infection
	309 asymptomatic (110)	6-15	Serology, 309	RAP originating from *H. pylori* infection affected both BMI and linear growth

BMI: Body mass index; RAP: Recurrent abdominal pain; IDA: Iron deficiency anemia; *H. pylori*: *Helicobacter pylori*.

competing with the human host[107]. Ferrokinetic studies have suggested the diversion of iron to some extramedullary focus, hypothesized but not proven to be *H. pylori*-associated gastric infection[95]. Like many bacteria, *H. pylori* requires iron as a growth factor, and it possesses a 19-kDa iron-binding protein that resembles ferritin, which has been considered to play a role in storage of excess iron sequestered by the bacterium[111]. Another possible mechanism for IDA in *H. pylori*-infected subjects involves sequestration of iron in lactoferrin in the gastric mucosa, and uptake of iron by *H. pylori*. Lactoferrin is an iron-binding glycoprotein that is found in body fluids, and its secretion in the gastric mucosa seems to be influenced by some signal from *H. pylori*[112]. It appears that *H. pylori* then absorbs the iron from lactoferrin *via* a specific lactoferrin-binding protein that is expressed by *H. pylori*[107]. Lactoferrin levels in the gastric mucosa have been shown to be significantly higher in *H. pylori*-positive patients with IDA compared to those who are non-anemic *H. pylori*-negative, non-anemic *H. pylori*-positive, and *H. pylori*-negative with IDA[112].

We conclude that future work in this area is needed. Randomized, double-blind, and placebo-controlled trials of sufficient size and power should evaluate the long-term effect of *H. pylori* eradication in children with IDA, who are living in developing as well as in developed countries. Additionally, these studies should evaluate the effect of *H. pylori* treatment among different pediatric populations, such as those with and without concurrent gastrointestinal symptoms, and those with a wide spectrum of IDA severity.

Based on our present knowledge, children with a first episode of IDA and no complications should be initially treated with iron supplementation alone, irrespective of *H. pylori* status[2]. Eradication of *H. pylori* could be considered in cases that are refractory to iron supplementation and in the case of frequent relapses, assuming that other causes, such as celiac disease and inflammatory bowel disease, have been excluded[2,102]. Of particular interest is the work by Memeo *et al*[113] who have shown the frequent occurrence of duodenal intraepithelial lymphocyte expansion in individuals with *H. pylori* gastritis, and the considerable overlap of the intraepithelial lymphocyte counts as well as the distribution patterns with those described for celiac disease and other small bowel diseases.

GROWTH RETARDATION

The available evidence regarding *H. pylori* infection and its effect on growth in children is controversial. There have been many cross-sectional studies that point to either the presence or absence of such an association[90,114-124], as shown in Table 3.

The Italian cross-sectional study by Perri *et al*[114] suggests that *H. pylori* infection (as diagnosed by urea breath test) is associated with growth delay in older children, poor socioeconomic conditions, and household overcrowding. The findings by Perri *et al*[114] are consistent with the hypothesis that *H. pylori* infection is one of the environmental factors capable of affecting growth. The cross-section-

al study by Richter *et al*[117] of a large number of 5-7-year-old preschool and school children suggests that *H. pylori* infection (as diagnosed by urea breath test) is associated in German children with growth delay, growth retardation, or both, despite similar socioeconomic status between *H. pylori*-positive and -negative children. Likewise, Ertem *et al*[118] also have suggested that *H. pylori* is associated with short stature through mechanisms that are independent of poor living conditions.

Other investigators have suggested that growth suppression reported in children with *H. pylori* infection could be due to socioeconomic, genetic and environmental factors. In their retrospective chart review of the growth parameters of children with dyspepsia referred to the Regional Paediatric Gastroenterology unit in Manchester, United Kingdom, Sood *et al*[119] found that children with dyspepsia and *H. pylori* infection (as diagnosed by urea breath test) were shorter and lighter than patients with similar symptoms but no infection. Sood *et al*[119] concluded that the differences in anthropometry might have been due to socioeconomic and ethnic factors rather than *H. pylori* infection. Yet, in a cross-sectional study of Turkish dyspeptic children who were evaluated by endoscopic gastric biopsy for *H. pylori* infection, as well as a control group of age and sex cross-matched children, Soylu and Ozturk found that dyspeptic children with and without *H. pylori* infection had worse nutritional status compared to healthy controls[122]. The authors concluded that *H. pylori* infection as a major cause of dyspepsia might be considered to cause malnutrition secondary to decreased caloric intake associated with dyspepsia[122].

In a case-control study of children aged 5-13 years whose height was below the third centile, matched with children of the same age and sex whose height was above the 25th centile, Oderda *et al*[115] found that *H. pylori* (diagnosed by serologic methods) was not a risk factor for short stature, and that reduced growth was related to genetic determinants such as parental height and to mixed genetic and environmental factors such as birth weight. Low socioeconomic status was also relevant[115]. In a cross-sectional study of children aged 5-10 years who were attending an all-girl public school in inner Guatemala City, Quiñonez *et al*[116] investigated the effect of *H. pylori* infection (diagnosed by serologic methods) on the anthropometric nutritional parameters (weight-for-height and height-for-age). After controlling for sociodemographic variables, the authors did not find significant differences in the nutritional parameters between infected and uninfected children. In another cross-sectional study of Turkish children aged 4-16 years who underwent upper gastrointestinal endoscopy for RAP and dyspeptic complaints, Süoglu *et al*[120] found that the effect of *H. pylori* infection on mean SD scores of height for age was statistically insignificant after correction for breast feeding, IDA and socioeconomic level. In contrast, even after controlling for socioeconomic level, *H. pylori* infection remained the single and most important variable that had an effect on mean weight SD score. Among *H. pylori*-positive as well as -negative children[120], IDA had no significant effect on anthropometric measurements *per se*. However, when *H. pylori* and IDA were present, mean weight was found to be significantly lower than that of the *H. pylori*-negative patients without IDA[120].

There are also longitudinal studies that support the hypothesis that *H. pylori* infection might influence growth rate in children. Thomas *et al*[125] conducted two consecutive prospective, longitudinal cohort studies in Gambia, and found that, in both cohorts, children with early *H. pylori* colonization had lower values for both length- and weight-for-age Z scores than their peers in late infancy. No socioeconomic or demographic confounding variables were identified to explain this, and the weight deficit was no longer detectable when the children were aged 5-8 years. The authors concluded that *H. pylori* colonization at critical vulnerable ages might lead to malnutrition and growth retardation among infants in countries such as Gambia[125]. In the prospective, longitudinal study by Bravo *et al*[126], lower-middle class children from Colombia, in general good health, aged 1-5 years, who tested negative by urea breath test at baseline, were monitored over the following 2.5 years for anthropometric measurements every 2 mo, and for *H. pylori* by urea breath test every 4 mo. The authors found significant slowing of growth velocity in children infected with *H. pylori*, independent of socioeconomic variables or overcrowding[126]. Likewise, Mera *et al*[127] prospectively investigated in Colombian children, in general good health, aged 1-5 years, whether a newly acquired *H. pylori* infection affected height and weight of children within 16 mo, by performing breath tests and anthropometry every 2-4 mo. The authors observed that the impact of a new infection on height growth velocity was more pronounced right after the infection was diagnosed and slowly ebbed, and continued to be significant for up to 6 mo after infection, and became borderline significant at 8 mo after infection. No catch-up growth was evident in infected children, with crowding retarding linear growth. Compared with uninfected children, newly infected children also experienced a significant, but small, decrease in weight at the first follow-up visit, which was not statistically significant at 4 mo after infection. There was no catch-up in weight. The authors concluded that *H. pylori* caused a non-transient negative effect on height and weight in affected children, regardless of age at the time of infection[127].

Taken together, the results of these studies pointing to the presence or absence of an association between *H. pylori* and growth are subject to some potential limitations. First, most studies were cross-sectional or retrospective and therefore were unable to evaluate the possible effect of new infections on growth velocity. Second, the definition of socioeconomic status is complex and no set of parameters was fully descriptive in most studies. Third, some studies used serological methods to determine *H. pylori* infection. However, serology does not indicate whether there is active or past infection. Even in those patients who are treated and cured of their *H. pylori* infection, evidence of IgG antibodies may exist for several months

and possibly for years. Serological assays also have varying levels of sensitivity and specificity when used in different populations, particularly in children[128]. Fourth, in community-based studies, for example, in those that involve a blood specimen, participation rate is rarely more than 50%[116,129]. This might also be the case for studies that involve gastrointestinal endoscopy. As such, we have no information on the non-participating children in some of the above studies; thus, we cannot estimate if any bias was introduced in the results or the generalizability of their findings to other populations.

We conclude that future work in this area is needed to elucidate the importance of these factors. One would expect growth velocity to improve following *H. pylori* eradication, if the infection were the primary cause of growth suppression.

IDIOPATHIC THROMBOCYTOPENIC PURPURA

ITP is an autoimmune disease that is characterized by a low circulating platelet count caused by the destruction of antibody-sensitized platelets in the reticuloendothelial system[130]. The mechanisms that trigger the production of platelet autoantibodies remain poorly understood. Persistent thrombocytopenia for > 6 mo defines the chronic form of this disorder (cITP)[130]. Lately, eradication of *H. pylori* from the gastric mucosa has been associated with an improvement of cITP.

Several studies in adults have reported improved platelet counts in *H. pylori*-positive patients following standard triple *H. pylori* eradication therapy[131-140]. A meta-analysis of 13 cohort reports in adult cITP, with combined data from 193 patients, has indicated an overall response rate of 52% of patients after *H. pylori* eradication therapy[141]. A recent systematic review of 25 published studies, with combined data from 696 adult patients with cITP, has demonstrated an overall response in platelet count after *H. pylori* eradication in 50.3% of patients (95% CI: 41.6%-59.0%)[142]. Cohorts from Japan and Italy[132,134,138,140] have reported higher response rates than from other countries[143,144]. Several theories, including direct antigen mimicry between *H. pylori cagA* and platelet glycoprotein antigens, *H. pylori* binding to von Willebrand factor, and the immunomodulatory effect of antibiotics (e.g. macrolides) used in *H. pylori* eradication, have been proposed to explain the platelet response to anti-*H. pylori* therapy[139,141]. It has also been postulated that platelet autoantibodies might be produced by autoreactive clonal B cells that are induced by chronic immunological stimulus by *H. pylori*[141,145]. The relative toxicity profiles of triple therapy compared to standard ITP therapy certainly make eradication an attractive and generally safe option in adults. However, large controlled clinical trials of adult patients from various ethnic backgrounds are necessary to determine the response rate and mechanism of platelet response to *H. pylori* eradication therapy[141].

In children, the natural history of cITP is clearly different from that observed in adults. Spontaneous recovery occurs in one third of childhood cITP cases from several months to many years after their diagnosis, whereas only 5% of adults recover[130,146]. Thus, the effects of *H. pylori* eradication in childhood cITP could be different from those in adults. The issue of whether *H. pylori* eradication has a beneficial effect on the course of cITP in children has been the subject of few, apparently contradictory studies with small sample sizes[147-153]. Yet, the results of pediatric studies are difficult to compare because the prevalence of *H. pylori* infection and diagnostic methods vary among them[154]. Of note, in children with cITP, no studies have assessed, at the initial as well as follow-up visits, *H. pylori* status by upper gastrointestinal endoscopy.

In a study from Taiwan, Jaing *et al*[147] evaluated 22 cITP children, nine of whom were *H. pylori*-infected and were treated with a 1-wk course of triple therapy [including clarithromycin, amoxicillin, and proton pump inhibitors (PPIs)]. Of these nine patients, five (55.6%) were in complete or partial remission over a median of 16 mo follow-up, while four showed no improvement in platelet counts during 8-19 mo follow-up. In a study from the Netherlands, Neefjes *et al*[149] evaluated 47 children with cITP, three of whom were *H. pylori*-infected and were treated with a 2-wk course of the triple therapy mentioned above. Over a 6-mo follow-up, all three children achieved complete or partial remission. In a study from Iran, Hamidieh *et al*[150] evaluated 31 cITP children, four of whom were *H. pylori*-infected and were treated with a 2-wk course of the same triple therapy. None of the four patients achieved complete or partial remission after *H. pylori* eradication. In a study from Japan, Hayashi *et al*[148] evaluated 10 children with cITP, one of whom was *H. pylori*-infected and was treated with a 1-wk course of the same triple therapy. This child achieved complete remission throughout > 1 year of follow-up. In a study from Italy, Bisogno *et al*[151] evaluated 25 children with cITP, nine of whom were *H. pylori*-infected and had *H. pylori* eradication following 1-2 courses of 2 wk of the same triple therapy. Over 6-mo follow-up, of these nine patients, three had an increase in platelet count after eradication therapy, and one had complete remission and two had partial, transient remission followed by relapse a few months later. Over the same follow-up period, no significant increase in platelet count was seen in the other six eradicated patients. In the same study, Bisogno *et al*[151] also reported the platelet response in the 16 *H. pylori*-negative children with cITP. At 6-mo follow-up, two of these 16 patients achieved partial remission without any specific treatment. Yet, four of the *H. pylori*-negative children with cITP achieved spontaneous partial remission 1 year after diagnosis of *H. pylori* infection was excluded[151]. At latest follow-up, the remaining 10 *H. pylori*-negative children presented with a count above 50×10^9/L without any treatment. In another study from Italy, Loffredo *et al*[152] evaluated 39 children (median age, 136 mo) with cITP, eight of whom were *H. pylori*-infected and had *H. pylori* eradication following 1-3 courses of 2 wk of the same triple therapy. Over 1-year follow-up, none of the eight patients achieved complete or partial remission after *H. pylori* eradication[152].

Recently, Treepongkaruna *et al*[153] reported a multicenter randomized controlled trial of *H. pylori* eradication in 55 children with cITP. Of the 16 (29.1%) patients with cITP and *H. pylori* infection, seven were randomly treated with PPI-based triple therapy, while the remaining nine did not receive any specific treatment. If the first-line therapy failed to eradicate *H. pylori,* then second-line eradication therapy was used. Although eradication of *H. pylori* infection was successful in all patients in the treated group, the platelet recovery rate was not significantly different between the *H. pylori* treatment group and control group during the 6-mo period.

From the foregoing, whether antibiotic treatment of *H. pylori* infection should be considered in children with cITP is an unresolved question[154]. In conclusion, in view of the published evidence in children with cITP and the sporadic benefit of *H. pylori* eradication on the platelet response, the relationship between *H. pylori* and cITP in children warrants further investigation with large randomized controlled trials of sufficient size and power and across different ethnic populations[154].

ASTHMA AND ALLERGIC DISORDERS

In industrialized countries, the incidence of asthma, especially childhood asthma, has risen in recent years[155]. Conversely, in developed countries, the rate of acquisition of *H. pylori* has decreased substantially over recent decades[3,9]. The lack of early exposure to *H. pylori* has been suggested to be an important determinant of asthma risk in childhood[156]. In a recent retrospective, cross-sectional study, using data from 3327 participants, aged 3-19 years, *H. pylori* seropositivity was found to be inversely associated with onset of asthma before 5 years of age and current asthma in children aged 3-13 years[157]. *H. pylori* seropositivity also was inversely related to recent wheezing, allergic rhinitis, and dermatitis, eczema, or rash.

Allergic diseases and asthma are caused by exaggerated T-helper 2 (Th2)-biased immune response in genetically susceptible individuals[158]. A number of recent studies have indicated that regulatory T cells (Tregs) play an important role in controlling such Th2-biased responses. Impaired expansion of natural and/or adaptive Tregs is hypothesized to lead to the development of allergy and asthma[158]. Increased numbers of Tregs have been reported in the *H. pylori*-infected human gastric mucosa[159,160]. Thus, the absence of early exposure to *H. pylori* might cause the loss of a metabolically active lymphoid compartment in the stomach, including Tregs, which ultimately could affect the activity of T cells present in other mucosal and cutaneous sites[156,160]. Although this is an undoubtedly interesting theory, future prospective, longitudinal studies are needed to test the strength of the association between *H. pylori* status and asthma risk in children from developed and developing countries[161].

CONCLUSION

Many areas of active scientific inquiry have been reported in the recent literature about the disease spectrum of *H. pylori*. Several studies have demonstrated that *H. pylori* infection is not associated with specific symptomatology in children. Therefore, identification of children with *H. pylori*-associated gastritis on the basis of clinical presentation alone is not possible. Based on the best available evidence, testing for (and treating) *H. pylori* infection should be performed in children with endoscopically proven duodenal ulcer. Evidence from studies in adults supports the recommendation that testing for *H. pylori* should also be performed in children with a documented gastric ulcer. Endoscopy and biopsy are also recommended for children with persistent symptoms.

Pursuing *H. pylori* in asymptomatic children should be indicated for patients at increased risk of gastric cancer, for example, first-degree relatives of patients with gastric cancer, and individualized in populations at increased risk for gastric cancer, taking into consideration comorbid illness. Studies suggest that prevention of gastric cancer is possible in infected individuals with no precancerous lesions. Therefore, children might well be the group to target in an effort to prevent future development of gastric cancer.

Although dyspepsia and *H. pylori* infection are common in the general population, current data in the literature regarding a causal association between *H. pylori* gastritis and dyspepsia are conflicting. It is uncertain whether eradication of the infection leads to an improvement of symptoms. Randomized, placebo-controlled, double-blind trials with minimal loss to follow-up, strict group definition, and standardized and validated outcome measures are needed.

There is no compelling evidence to support routine testing in children with cITP, poor growth, and GERD. In children with refractory IDA, where other causes have been ruled out, testing for (and treating) *H. pylori* infection can be considered. Prospective, longitudinal studies are needed to test the strength of the newly reported association between *H. pylori* status and asthma risk in children. In the absence of these studies, there is little call to leave *H. pylori* infection untreated in patients with asthma and allergy. We believe that *H. pylori* eradication is strongly beneficial for curing peptic ulcer disease and gastric lymphoma and for prevention of gastric cancer, as well as other diseases that are putatively linked to infection, and it must be done in *H. pylori*-infected patients, whether or not they have asthma.

REFERENCES

1 **Sherman PM**. Appropriate strategies for testing and treating Helicobacter pylori in children: when and how? *Am J Med* 2004; **117** Suppl 5A: 30S-35S

2 **Kato S**, Sherman PM. What is new related to Helicobacter pylori infection in children and teenagers? *Arch Pediatr Adolesc Med* 2005; **159**: 415-421

3 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186

4 **Perez-Perez GI**, Salomaa A, Kosunen TU, Daverman B, Rautelin H, Aromaa A, Knekt P, Blaser MJ. Evidence that

cagA(+) Helicobacter pylori strains are disappearing more rapidly than cagA(-) strains. *Gut* 2002; **50**: 295-298
5 **Banatvala N**, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and Helicobacter pylori. *J Infect Dis* 1993; **168**: 219-221
6 **Kindermann A**, Lopes AI. Helicobacter pylori infection in pediatrics. *Helicobacter* 2009; **14** Suppl 1: 52-57
7 **Graham DY**, Hepps KS, Ramirez FC, Lew GM, Saeed ZA. Treatment of Helicobacter pylori reduces the rate of rebleeding in peptic ulcer disease. *Scand J Gastroenterol* 1993; **28**: 939-942
8 **Treiber G**, Lambert JR. The impact of Helicobacter pylori eradication on peptic ulcer healing. *Am J Gastroenterol* 1998; **93**: 1080-1084
9 **Atherton JC**. The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. *Annu Rev Pathol* 2006; **1**: 63-96
10 **Pellicano R**, Franceschi F, Saracco G, Fagoonee S, Roccarina D, Gasbarrini A. Helicobacters and extragastric diseases. *Helicobacter* 2009; **14** Suppl 1: 58-68
11 **Realdi G**, Dore MP, Fastame L. Extradigestive manifestations of Helicobacter pylori infection: fact and fiction. *Dig Dis Sci* 1999; **44**: 229-236
12 **Gold BD**. Helicobacter pylori infection in children. *Curr Probl Pediatr Adolesc Health Care* 2001; **31**: 247-266
13 **Sherman P**, Czinn S, Drumm B, Gottrand F, Kawakami E, Madrazo A, Oderda G, Seo JK, Sullivan P, Toyoda S, Weaver L, Wu TC. Helicobacter pylori infection in children and adolescents: Working Group Report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2002; **35** Suppl 2: S128-S133
14 **Blecker U**. Helicobacter pylori-associated gastroduodenal disease in childhood. *South Med J* 1997; **90**: 570-576; quiz 577
15 **Goggin N**, Rowland M, Imrie C, Walsh D, Clyne M, Drumm B. Effect of Helicobacter pylori eradication on the natural history of duodenal ulcer disease. *Arch Dis Child* 1998; **79**: 502-505
16 **Chiesa C**, Pacifico L, Anania C, Poggiogalle E, Chiarelli F, Osborn JF. Helicobacter pylori therapy in children: overview and challenges. *Int J Immunopathol Pharmacol* 2010; **23**: 405-416
17 **Macarthur C**, Saunders N, Feldman W. Helicobacter pylori, gastroduodenal disease, and recurrent abdominal pain in children. *JAMA* 1995; **273**: 729-734
18 **Kato S**, Nishino Y, Ozawa K, Konno M, Maisawa S, Toyoda S, Tajiri H, Ida S, Fujisawa T, Iinuma K. The prevalence of Helicobacter pylori in Japanese children with gastritis or peptic ulcer disease. *J Gastroenterol* 2004; **39**: 734-738
19 **Arents NL**, Thijs JC, van Zwet AA, Kleibeuker JH. Does the declining prevalence of Helicobacter pylori unmask patients with idiopathic peptic ulcer disease? Trends over an 8 year period. *Eur J Gastroenterol Hepatol* 2004; **16**: 779-783
20 **Kalach N**, Bontems P, Mourad-Baars P, Koletzko S, Scherbakov P, Celinska-Cedro D, Iwanczak B, Gottrand F, Martinez Gomez M, Phelivanouglu E, Oderda G, Urruzuno P, Casswall T, Lamireau T, Sykora J, Roma E, Veres G, Wewer V, Charkaluk M, Megraud F, Cadranel S. Prospective European Multicenter Pilot Study on gastric and duodenal ulcer disease or erosions in children. *Helicobacter* 2007; **12**: 394
21 **Oderda G**, Shcherbakov P, Bontems P, Urruzuno P, Romano C, Gottrand F, Gómez MJ, Ravelli A, Gandullia P, Roma E, Cadranel S, De Giacomo C, Canani RB, Rutigliano V, Pehlivanoglu E, Kalach N, Roggero P, Celinska-Cedro D, Drumm B, Casswall T, Ashorn M, Arvanitakis SN. Results from the pediatric European register for treatment of Helicobacter pylori (PERTH). *Helicobacter* 2007; **12**: 150-156
22 **Oderda G**, Mura S, Valori A, Brustia R. Idiopathic peptic ulcers in children. *J Pediatr Gastroenterol Nutr* 2009; **48**: 268-270
23 **Tam YH**, Lee KH, To KF, Chan KW, Cheung ST. Helicobacter pylori-positive versus Helicobacter pylori-negative idiopathic peptic ulcers in children with their long-term outcomes. *J Pediatr Gastroenterol Nutr* 2009; **48**: 299-305
24 **Blecker U**, McKeithan TW, Hart J, Kirschner BS. Resolution of Helicobacter pylori-associated gastric lymphoproliferative disease in a child. *Gastroenterology* 1995; **109**: 973-977
25 **Ohno Y**, Kosaka T, Muraoka I, Kanematsu T, Tsuru A, Kinoshita E, Moriuchi H. Remission of primary low-grade gastric lymphomas of the mucosa-associated lymphoid tissue type in immunocompromised pediatric patients. *World J Gastroenterol* 2006; **12**: 2625-2628
26 **Correa P**, Haenszel W, Cuello C, Zavala D, Fontham E, Zarama G, Tannenbaum S, Collazos T, Ruiz B. Gastric precancerous process in a high risk population: cross-sectional studies. *Cancer Res* 1990; **50**: 4731-4736
27 **Ricuarte O**, Gutierrez O, Cardona H, Kim JG, Graham DY, El-Zimaity HM. Atrophic gastritis in young children and adolescents. *J Clin Pathol* 2005; **58**: 1189-1193
28 **Whitney AE**, Guarner J, Hutwagner L, Gold BD. Helicobacter pylori gastritis in children and adults: comparative histopathologic study. *Ann Diagn Pathol* 2000; **4**: 279-285
29 **Guarner J**, Bartlett J, Whistler T, Pierce-Smith D, Owens M, Kreh R, Czinn S, Gold BD. Can pre-neoplastic lesions be detected in gastric biopsies of children with Helicobacter pylori infection? *J Pediatr Gastroenterol Nutr* 2003; **37**: 309-314
30 **Tutar E**, Ertem D, Kotiloglu Karaa E, Pehlivanoglu E. Endoscopic and histopathologic findings associated with H. pylori infection in very young children. *Dig Dis Sci* 2009; **54**: 111-117
31 **Machado JC**, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simões M. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology* 2003; **125**: 364-371
32 **Dimitrov G**, Gottrand F. Does gastric atrophy exist in children? *World J Gastroenterol* 2006; **12**: 6274-6279
33 **Kolho KL**, Jusufovic J, Miettinen A, Savilahti E, Rautelin H. Parietal cell antibodies and Helicobacter pylori in children. *J Pediatr Gastroenterol Nutr* 2000; **30**: 265-268
34 **Campbell DI**, Warren BF, Thomas JE, Figura N, Telford JL, Sullivan PB. The African enigma: low prevalence of gastric atrophy, high prevalence of chronic inflammation in West African adults and children. *Helicobacter* 2001; **6**: 263-267
35 **Guiraldes E**, Peña A, Duarte I, Triviño X, Schultz M, Larraín F, Espinosa MN, Harris P. Nature and extent of gastric lesions in symptomatic Chilean children with Helicobacter pylori-associated gastritis. *Acta Paediatr* 2002; **91**: 39-44
36 **Oztürk Y**, Büyükgebiz B, Arslan N, Ozer E. Antral glandular atrophy and intestinal metaplasia in children with Helicobacter pylori infection. *J Pediatr Gastroenterol Nutr* 2003; **37**: 96-97; author reply 97-98
37 **Usta Y**, Saltk-Temizel IN, Ozen H. Gastric atrophy and intestinal metaplasia in Helicobacter pylori infection. *J Pediatr Gastroenterol Nutr* 2004; **38**: 548
38 **Levine A**, Milo T, Broide E, Wine E, Dalal I, Boaz M, Avni Y, Shirin H. Influence of Helicobacter pylori eradication on gastroesophageal reflux symptoms and epigastric pain in children and adolescents. *Pediatrics* 2004; **113**: 54-58
39 **Kato S**, Nakajima S, Nishino Y, Ozawa K, Minoura T, Konno M, Maisawa S, Toyoda S, Yoshimura N, Vaid A, Genta RM. Association between gastric atrophy and Helicobacter pylori infection in Japanese children: a retrospective multicenter study. *Dig Dis Sci* 2006; **51**: 99-104
40 **Kalach N**, Papadopoulos S, Asmar E, Spyckerelle C, Gosset P, Raymond J, Dehecq E, Decoster A, Creusy C, Dupont C. In French children, primary gastritis is more frequent than Helicobacter pylori gastritis. *Dig Dis Sci* 2009; **54**: 1958-1965
41 **Kim KM**, Oh YL, Ko JS, Choe YH, Seo JK. Histopathology and expression of Ki-67 and cyclooxygenase-2 in childhood Helicobacter pylori gastritis. *J Gastroenterol* 2004; **39**: 231-237
42 **Hassall E**, Dimmick JE. Unique features of Helicobacter pylori disease in children. *Dig Dis Sci* 1991; **36**: 417-423

43 **Camorlinga-Ponce M**, Aviles-Jimenez F, Cabrera L, Hernández-Pando R, Muñoz O, Soza J, Torres J. Intensity of inflammation, density of colonization and interleukin-8 response in the gastric mucosa of children infected with Helicobacter pylori. *Helicobacter* 2003; **8**: 554-560
44 **Ozen H**, Dinler G, Akyön Y, Koçak N, Yüce A, Gürakan F. Helicobacter pylori infection and recurrent abdominal pain in Turkish children. *Helicobacter* 2001; **6**: 234-238
45 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181
46 **Guarner J**, Herrera-Goepfert R, Mohar A, Sanchez L, Halperin D, Ley C, Parsonnet J. Interobserver variability in application of the revised Sydney classification for gastritis. *Hum Pathol* 1999; **30**: 1431-1434
47 **el-Zimaity HM**, Graham DY, al-Assi MT, Malaty H, Karttunen TJ, Graham DP, Huberman RM, Genta RM. Interobserver variation in the histopathological assessment of Helicobacter pylori gastritis. *Hum Pathol* 1996; **27**: 35-41
48 **Wotherspoon AC**, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991; **338**: 1175-1176
49 **Eidt S**, Stolte M, Fischer R. Helicobacter pylori gastritis and primary gastric non-Hodgkin's lymphomas. *J Clin Pathol* 1994; **47**: 436-439
50 **Fischbach W**, Goebeler-Kolve ME, Dragosics B, Greiner A, Stolte M. Long term outcome of patients with gastric marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) following exclusive Helicobacter pylori eradication therapy: experience from a large prospective series. *Gut* 2004; **53**: 34-37
51 **Stolte M**, Bayerdörffer E, Morgner A, Alpen B, Wündisch T, Thiede C, Neubauer A. Helicobacter and gastric MALT lymphoma. *Gut* 2002; **50** Suppl 3: III19-III24
52 **Steinbach G**, Ford R, Glober G, Sample D, Hagemeister FB, Lynch PM, McLaughlin PW, Rodriguez MA, Romaguera JE, Sarris AH, Younes A, Luthra R, Manning JT, Johnson CM, Lahoti S, Shen Y, Lee JE, Winn RJ, Genta RM, Graham DY, Cabanillas FF. Antibiotic treatment of gastric lymphoma of mucosa-associated lymphoid tissue. An uncontrolled trial. *Ann Intern Med* 1999; **131**: 88-95
53 **Thiede C**, Morgner A, Alpen B, Wündisch T, Herrmann J, Ritter M, Ehninger G, Stolte M, Bayerdörffer E, Neubauer A. What role does Helicobacter pylori eradication play in gastric MALT and gastric MALT lymphoma? *Gastroenterology* 1997; **113**: S61-S64
54 **Perez ME**, Youssef NN. Dyspepsia in childhood and adolescence: insights and treatment considerations. *Curr Gastroenterol Rep* 2007; **9**: 447-455
55 **Talley NJ**, Vakil N, Ballard ED 2nd, Fennerty MB. Absence of benefit of eradicating Helicobacter pylori in patients with nonulcer dyspepsia. *N Engl J Med* 1999; **341**: 1106-1111
56 **Frank F**, Stricker T, Stallmach T, Braegger CP. Helicobacter pylori infection in recurrent abdominal pain. *J Pediatr Gastroenterol Nutr* 2000; **31**: 424-427
57 **Uc A**, Chong SK. Treatment of Helicobacter pylori gastritis improves dyspeptic symptoms in children. *J Pediatr Gastroenterol Nutr* 2002; **34**: 281-285
58 **Ashorn M**, Rägö T, Kokkonen J, Ruuska T, Rautelin H, Karikoski R. Symptomatic response to Helicobacter pylori eradication in children with recurrent abdominal pain: double blind randomized placebo-controlled trial. *J Clin Gastroenterol* 2004; **38**: 646-650
59 **Spee LA**, Madderom MB, Pijpers M, van Leeuwen Y, Berger MY. Association between helicobacter pylori and gastrointestinal symptoms in children. *Pediatrics* 2010; **125**: e651-e669
60 **Labenz J**, Blum AL, Bayerdörffer E, Meining A, Stolte M, Börsch G. Curing Helicobacter pylori infection in patients with duodenal ulcer may provoke reflux esophagitis. *Gastroenterology* 1997; **112**: 1442-1447
61 **Vakil N**, Hahn B, McSorley D. Recurrent symptoms and gastro-oesophageal reflux disease in patients with duodenal ulcer treated for Helicobacter pylori infection. *Aliment Pharmacol Ther* 2000; **14**: 45-51
62 **Fallone CA**, Barkun AN, Friedman G, Mayrand S, Loo V, Beech R, Best L, Joseph L. Is Helicobacter pylori eradication associated with gastroesophageal reflux disease? *Am J Gastroenterol* 2000; **95**: 914-920
63 **Wu JC**, Sung JJ, Chan FK, Ching JY, Ng AC, Go MY, Wong SK, Ng EK, Chung SC. Helicobacter pylori infection is associated with milder gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2000; **14**: 427-432
64 **Richter JE**. Effect of Helicobacter pylori eradication on the treatment of gastro-oesophageal reflux disease. *Gut* 2004; **53**: 310-311
65 **Laine L**, Sugg J. Effect of Helicobacter pylori eradication on development of erosive esophagitis and gastroesophageal reflux disease symptoms: a post hoc analysis of eight double blind prospective studies. *Am J Gastroenterol* 2002; **97**: 2992-2997
66 **Schwizer W**, Thumshirn M, Dent J, Guldenschuh I, Menne D, Cathomas G, Fried M. Helicobacter pylori and symptomatic relapse of gastro-oesophageal reflux disease: a randomised controlled trial. *Lancet* 2001; **357**: 1738-1742
67 **Dent J**. Review Article: is Helicobacter pylori relevant in the management of reflux disease? *Aliment Pharmacol Ther* 2001; **15** Suppl 1: 16-21
68 **Pollet S**, Gottrand F, Vincent P, Kalach N, Michaud L, Guimber D, Turck D. Gastroesophageal reflux disease and Helicobacter pylori infection in neurologically impaired children: inter-relations and therapeutic implications. *J Pediatr Gastroenterol Nutr* 2004; **38**: 70-74
69 **Ozçay F**, Gürakan F, Demir H, Saltik IN, Ozen H, Yüce A, Koçak N. Helicobacter pylori infection and reflux esophagitis in children. *Helicobacter* 2002; **7**: 328-329
70 **Elitsur Y**, Durst P, Lawrence Z, Rewalt M. Does Helicobacter pylori protect children from reflux disease? *J Clin Gastroenterol* 2008; **42**: 215-216
71 **Emiroglu HH**, Sokucu S, Suoglu OD, Gulluoglu M, Gokce S. Is there a relationship between Helicobacter pylori infection and erosive reflux disease in children? *Acta Paediatr* 2010; **99**: 121-125
72 **Gold BD**. Outcomes of pediatric gastroesophageal reflux disease: in the first year of life, in childhood, and in adults...oh, and should we really leave Helicobacter pylori alone? *J Pediatr Gastroenterol Nutr* 2003; **37** Suppl 1: S33-S39
73 **Daugule I**, Rumba I, Alksnis J, Ejderhamn J. Helicobacter pylori infection among children with gastrointestinal symptoms: a high prevalence of infection among patients with reflux oesophagitis. *Acta Paediatr* 2007; **96**: 1047-1049
74 **Moon A**, Solomon A, Beneck D, Cunningham-Rundles S. Positive association between Helicobacter pylori and gastroesophageal reflux disease in children. *J Pediatr Gastroenterol Nutr* 2009; **49**: 283-288
75 **Malfertheiner P**. Helicobacter pylori eradication does not exacerbate gastro-oesophageal reflux disease. *Gut* 2004; **53**: 312-313
76 **Yaghoobi M**, Farrokhyar F, Yuan Y, Hunt RH. Is there an increased risk of GERD after Helicobacter pylori eradication?: a meta-analysis. *Am J Gastroenterol* 2010; **105**: 1007-1013; quiz 1006, 1014
77 **Moayyedi P**. Should we test for Helicobacter pylori before treating gastroesophageal reflux disease? *Can J Gastroenterol* 2005; **19**: 425-427
78 **Moayyedi P**, Bardhan C, Young L, Dixon MF, Brown L, Axon AT. Helicobacter pylori eradication does not exacerbate reflux symptoms in gastroesophageal reflux disease. *Gastroenterology* 2001; **121**: 1120-1126
79 **Rosioru C**, Glassman MS, Halata MS, Schwarz SM. Esopha-

gitis and Helicobacter pylori in children: incidence and therapeutic implications. *Am J Gastroenterol* 1993; **88**: 510-513
80 **McColl KE**, Dickson A, El-Nujumi A, El-Omar E, Kelman A. Symptomatic benefit 1-3 years after H. pylori eradication in ulcer patients: impact of gastroesophageal reflux disease. *Am J Gastroenterol* 2000; **95**: 101-105
81 **Moayyedi P**, Feltbower R, Brown J, Mason S, Mason J, Nathan J, Richards ID, Dowell AC, Axon AT. Effect of population screening and treatment for Helicobacter pylori on dyspepsia and quality of life in the community: a randomised controlled trial. Leeds HELP Study Group. *Lancet* 2000; **355**: 1665-1669
82 **Helicobacter and Cancer Collaborative Group**. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001; **49**: 347-353
83 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194
84 **Leung WK**, Lin SR, Ching JY, To KF, Ng EK, Chan FK, Lau JY, Sung JJ. Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on Helicobacter pylori eradication. *Gut* 2004; **53**: 1244-1249
85 **Ley C**, Mohar A, Guarner J, Herrera-Goepfert R, Figueroa LS, Halperin D, Johnstone I, Parsonnet J. Helicobacter pylori eradication and gastric preneoplastic conditions: a randomized, double-blind, placebo-controlled trial. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 4-10
86 **Baggett HC**, Parkinson AJ, Muth PT, Gold BD, Gessner BD. Endemic iron deficiency associated with Helicobacter pylori infection among school-aged children in Alaska. *Pediatrics* 2006; **117**: e396-e404
87 **Baysoy G**, Ertem D, Ademoğlu E, Kotiloğlu E, Keskin S, Pehlivanoğlu E. Gastric histopathology, iron status and iron deficiency anemia in children with Helicobacter pylori infection. *J Pediatr Gastroenterol Nutr* 2004; **38**: 146-151
88 **Blecker U**, Renders F, Lanciers S, Vandenplas Y. Syncopes leading to the diagnosis of a Helicobacter pylori positive chronic active haemorrhagic gastritis. *Eur J Pediatr* 1991; **150**: 560-561
89 **Choe YH**, Kim SK, Son BK, Lee DH, Hong YC, Pai SH. Randomized placebo-controlled trial of Helicobacter pylori eradication for iron-deficiency anemia in preadolescent children and adolescents. *Helicobacter* 1999; **4**: 135-139
90 **Choe YH**, Kim SK, Hong YC. Helicobacter pylori infection with iron deficiency anaemia and subnormal growth at puberty. *Arch Dis Child* 2000; **82**: 136-140
91 **Choe YH**, Kim SK, Hong YC. The relationship between Helicobacter pylori infection and iron deficiency: seroprevalence study in 937 pubescent children. *Arch Dis Child* 2003; **88**: 178
92 **Choe YH**, Kwon YS, Jung MK, Kang SK, Hwang TS, Hong YC. Helicobacter pylori-associated iron-deficiency anemia in adolescent female athletes. *J Pediatr* 2001; **139**: 100-104
93 **Dufour C**, Brisigotti M, Fabretti G, Luxardo P, Mori PG, Barabino A. Helicobacter pylori gastric infection and sideropenic refractory anemia. *J Pediatr Gastroenterol Nutr* 1993; **17**: 225-227
94 **Ashorn M**, Ruuska T, Mäkipernaa A. Helicobacter pylori and iron deficiency anaemia in children. *Scand J Gastroenterol* 2001; **36**: 701-705
95 **Barabino A**, Dufour C, Marino CE, Claudiani F, De Alessandri A. Unexplained refractory iron-deficiency anemia associated with Helicobacter pylori gastric infection in children: further clinical evidence. *J Pediatr Gastroenterol Nutr* 1999; **28**: 116-119
96 **Carnicer J**, Badía R, Argemí J. Helicobacter pylori gastritis and sideropenic refractory anemia. *J Pediatr Gastroenterol Nutr* 1997; **25**: 441
97 **Hershko C**, Hoffbrand AV, Keret D, Souroujon M, Maschler I, Monselise Y, Lahad A. Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. *Haematologica* 2005; **90**: 585-595
98 **Konno M**, Muraoka S, Takahashi M, Imai T. Iron-deficiency anemia associated with Helicobacter pylori gastritis. *J Pediatr Gastroenterol Nutr* 2000; **31**: 52-56
99 **Kostaki M**, Fessatou S, Karpathios T. Refractory iron-deficiency anaemia due to silent Helicobacter pylori gastritis in children. *Eur J Pediatr* 2003; **162**: 177-179
100 **Russo-Mancuso G**, Branciforte F, Licciardello M, La Spina M. Iron deficiency anemia as the only sign of infection with Helicobacter pylori: a report of 9 pediatric cases. *Int J Hematol* 2003; **78**: 429-431
101 **Barabino A**. Helicobacter pylori-related iron deficiency anemia: a review. *Helicobacter* 2002; **7**: 71-75
102 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781
103 **Rowland M**, Imrie C, Bourke B, Drumm B. How should Helicobacter pylori infected children be managed? *Gut* 1999; **45** Suppl 1: I36-I39
104 **Fagan RP**, Dunaway CE, Bruden DL, Parkinson AJ, Gessner BD. Controlled, household-randomized, open-label trial of the effect of treatment of Helicobacter pylori infection on iron deficiency among children in rural Alaska: results at 40 months. *J Infect Dis* 2009; **199**: 652-660
105 **Gessner BD**, Baggett HC, Muth PT, Dunaway E, Gold BD, Feng Z, Parkinson AJ. A controlled, household-randomized, open-label trial of the effect that treatment of Helicobacter pylori infection has on iron deficiency in children in rural Alaska. *J Infect Dis* 2006; **193**: 537-546
106 **Sarker SA**, Mahmud H, Davidsson L, Alam NH, Ahmed T, Alam N, Salam MA, Beglinger C, Gyr N, Fuchs GJ. Causal relationship of Helicobacter pylori with iron-deficiency anemia or failure of iron supplementation in children. *Gastroenterology* 2008; **135**: 1534-1542
107 **DuBois S**, Kearney DJ. Iron-deficiency anemia and Helicobacter pylori infection: a review of the evidence. *Am J Gastroenterol* 2005; **100**: 453-459
108 **Lombard M**, Chua E, O'Toole P. Regulation of intestinal non-haem iron absorption. *Gut* 1997; **40**: 435-439
109 **Charlton RW**, Bothwell TH. Iron absorption. *Annu Rev Med* 1983; **34**: 55-68
110 **Capurso G**, Lahner E, Marcheggiano A, Caruana P, Carnuccio A, Bordi C, Delle Fave G, Annibale B. Involvement of the corporal mucosa and related changes in gastric acid secretion characterize patients with iron deficiency anaemia associated with Helicobacter pylori infection. *Aliment Pharmacol Ther* 2001; **15**: 1753-1761
111 **Doig P**, Austin JW, Trust TJ. The Helicobacter pylori 19.6-kilodalton protein is an iron-containing protein resembling ferritin. *J Bacteriol* 1993; **175**: 557-560
112 **Choe YH**, Oh YJ, Lee NG, Imoto I, Adachi Y, Toyoda N, Gabazza EC. Lactoferrin sequestration and its contribution to iron-deficiency anemia in Helicobacter pylori-infected gastric mucosa. *J Gastroenterol Hepatol* 2003; **18**: 980-985
113 **Memeo L**, Jhang J, Hibshoosh H, Green PH, Rotterdam H, Bhagat G. Duodenal intraepithelial lymphocytosis with normal villous architecture: common occurrence in H. pylori gastritis. *Mod Pathol* 2005; **18**: 1134-1144
114 **Perri F**, Pastore M, Leandro G, Clemente R, Ghoos Y, Peeters M, Annese V, Quitadamo M, Latiano A, Rutgeerts P, Andriulli A. Helicobacter pylori infection and growth delay in older children. *Arch Dis Child* 1997; **77**: 46-49
115 **Oderda G**, Palli D, Saieva C, Chiorboli E, Bona G. Short stature and Helicobacter pylori infection in italian children: prospective multicentre hospital based case-control study. The Italian Study Group on Short Stature and H pylori. *BMJ* 1998;

317: 514-515
116 **Quiñonez JM**, Chew F, Torres O, Bégué RE. Nutritional status of Helicobacter pylori-infected children in Guatemala as compared with uninfected peers. *Am J Trop Med Hyg* 1999; **61**: 395-398
117 **Richter T**, Richter T, List S, Müller DM, Deutscher J, Uhlig HH, Krumbiegel P, Herbarth O, Gutsmuths FJ, Kiess W. Five- to 7-year-old children with Helicobacter pylori infection are smaller than Helicobacter-negative children: a cross-sectional population-based study of 3,315 children. *J Pediatr Gastroenterol Nutr* 2001; **33**: 472-475
118 **Ertem D**, Pehlivanoglu E. Helicobacter pylori may influence height in children independent of socioeconomic factors. *J Pediatr Gastroenterol Nutr* 2002; **35**: 232-233
119 **Sood MR**, Joshi S, Akobeng AK, Mitchell J, Thomas AG. Growth in children with Helicobacter pylori infection and dyspepsia. *Arch Dis Child* 2005; **90**: 1025-1028
120 **Süoglu OD**, Gökçe S, Saglam AT, Sökücü S, Saner G. Association of Helicobacter pylori infection with gastroduodenal disease, epidemiologic factors and iron-deficiency anemia in Turkish children undergoing endoscopy, and impact on growth. *Pediatr Int* 2007; **49**: 858-863
121 **Mohammad MA**, Hussein L, Coward A, Jackson SJ. Prevalence of Helicobacter pylori infection among Egyptian children: impact of social background and effect on growth. *Public Health Nutr* 2008; **11**: 230-236
122 **Soylu OB**, Ozturk Y. Helicobacter pylori infection: effect on malnutrition and growth failure in dyspeptic children. *Eur J Pediatr* 2008; **167**: 557-562
123 **Cherian S**, Forbes D, Sanfilippo F, Cook A, Burgner D. Helicobacter pylori, helminth infections and growth: a cross-sectional study in a high prevalence population. *Acta Paediatr* 2009; **98**: 860-864
124 **Gulcan M**, Ozen A, Karatepe HO, Gulcu D, Vitrinel A. Impact of H. pylori on growth: is the infection or mucosal disease related to growth impairment? *Dig Dis Sci* 2010; **55**: 2878-2886
125 **Thomas JE**, Dale A, Bunn JE, Harding M, Coward WA, Cole TJ, Weaver LT. Early Helicobacter pylori colonisation: the association with growth faltering in The Gambia. *Arch Dis Child* 2004; **89**: 1149-1154
126 **Bravo LE**, Mera R, Reina JC, Pradilla A, Alzate A, Fontham E, Correa P. Impact of Helicobacter pylori infection on growth of children: a prospective cohort study. *J Pediatr Gastroenterol Nutr* 2003; **37**: 614-619
127 **Mera RM**, Correa P, Fontham EE, Reina JC, Pradilla A, Alzate A, Bravo LE. Effects of a new Helicobacter pylori infection on height and weight in Colombian children. *Ann Epidemiol* 2006; **16**: 347-351
128 **Khanna B**, Cutler A, Israel NR, Perry M, Lastovica A, Fields PI, Gold BD. Use caution with serologic testing for Helicobacter pylori infection in children. *J Infect Dis* 1998; **178**: 460-465
129 **Begue RE**, Castellares G, Ruiz R, Hayashi KE, Sanchez JL, Gotuzzo E, Oberst RB, Taylor DN, Svennerholm AM. Community-based assessment of safety and immunogenicity of the whole cell plus recombinant B subunit (WC/rBS) oral cholera vaccine in Peru. *Vaccine* 1995; **13**: 691-694
130 **Blanchette VS**, Price V. Childhood chronic immune thrombocytopenic purpura: unresolved issues. *J Pediatr Hematol Oncol* 2003; **25** Suppl 1: S28-S33
131 **Ando K**, Shimamoto T, Tauchi T, Ito Y, Kuriyama Y, Gotoh A, Miyazawa K, Kimura Y, Kawai T, Ohyashiki K. Can eradication therapy for Helicobacter pylori really improve the thrombocytopenia in idiopathic thrombocytopenic purpura? Our experience and a literature review. *Int J Hematol* 2003; **77**: 239-244
132 **Emilia G**, Longo G, Luppi M, Gandini G, Morselli M, Ferrara L, Amarri S, Cagossi K, Torelli G. Helicobacter pylori eradication can induce platelet recovery in idiopathic thrombocytopenic purpura. *Blood* 2001; **97**: 812-814
133 **Emilia G**, Luppi M, Zucchini P, Morselli M, Potenza L, Forghieri F, Volzone F, Jovic G, Leonardi G, Donelli A, Torelli G. Helicobacter pylori infection and chronic immune thrombocytopenic purpura: long-term results of bacterium eradication and association with bacterium virulence profiles. *Blood* 2007; **110**: 3833-3841
134 **Gasbarrini A**, Franceschi F, Tartaglione R, Landolfi R, Pola P, Gasbarrini G. Regression of autoimmune thrombocytopenia after eradication of Helicobacter pylori. *Lancet* 1998; **352**: 878
135 **Hino M**, Yamane T, Park K, Takubo T, Ohta K, Kitagawa S, Higuchi K, Arakawa T. Platelet recovery after eradication of Helicobacter pylori in patients with idiopathic thrombocytopenic purpura. *Ann Hematol* 2003; **82**: 30-32
136 **Kohda K**, Kuga T, Kogawa K, Kanisawa Y, Koike K, Kuroiwa G, Hirayama Y, Sato Y, Niitsu Y. Effect of Helicobacter pylori eradication on platelet recovery in Japanese patients with chronic idiopathic thrombocytopenic purpura and secondary autoimmune thrombocytopenic purpura. *Br J Haematol* 2002; **118**: 584-588
137 **Nomura S**, Inami N, Kanazawa S. The effects of Helicobacter pylori eradication on chemokine production in patients with immune thrombocytopenic purpura. *Eur J Haematol* 2004; **72**: 304-305
138 **Sato R**, Murakami K, Watanabe K, Okimoto T, Miyajima H, Ogata M, Ohtsuka E, Kodama M, Saburi Y, Fujioka T, Nasu M. Effect of Helicobacter pylori eradication on platelet recovery in patients with chronic idiopathic thrombocytopenic purpura. *Arch Intern Med* 2004; **164**: 1904-1907
139 **Takahashi T**, Yujiri T, Shinohara K, Inoue Y, Sato Y, Fujii Y, Okubo M, Zaitsu Y, Ariyoshi K, Nakamura Y, Nawata R, Oka Y, Shirai M, Tanizawa Y. Molecular mimicry by Helicobacter pylori CagA protein may be involved in the pathogenesis of H. pylori-associated chronic idiopathic thrombocytopenic purpura. *Br J Haematol* 2004; **124**: 91-96
140 **Veneri D**, Franchini M, Gottardi M, D'Adda M, Ambrosetti A, Krampera M, Zanetti F, Pizzolo G. Efficacy of Helicobacter pylori eradication in raising platelet count in adult patients with idiopathic thrombocytopenic purpura. *Haematologica* 2002; **87**: 1177-1179
141 **Jackson S**, Beck PL, Pineo GF, Poon MC. Helicobacter pylori eradication: novel therapy for immune thrombocytopenic purpura? A review of the literature. *Am J Hematol* 2005; **78**: 142-150
142 **Stasi R**, Sarpatwari A, Segal JB, Osborn J, Evangelista ML, Cooper N, Provan D, Newland A, Amadori S, Bussel JB. Effects of eradication of Helicobacter pylori infection in patients with immune thrombocytopenic purpura: a systematic review. *Blood* 2009; **113**: 1231-1240
143 **Jarque I**, Andreu R, Llopis I, De la Rubia J, Gomis F, Senent L, Jiménez C, Martín G, Martínez JA, Sanz GF, Ponce J, Sanz MA. Absence of platelet response after eradication of Helicobacter pylori infection in patients with chronic idiopathic thrombocytopenic purpura. *Br J Haematol* 2001; **115**: 1002-1003
144 **Michel M**, Cooper N, Jean C, Frissora C, Bussel JB. Does Helicobater pylori initiate or perpetuate immune thrombocytopenic purpura? *Blood* 2004; **103**: 890-896
145 **Franchini M**, Veneri D. Helicobacter pylori infection and immune thrombocytopenic purpura: an update. *Helicobacter* 2004; **9**: 342-346
146 **George JN**, Woolf SH, Raskob GE, Wasser JS, Aledort LM, Ballem PJ, Blanchette VS, Bussel JB, Cines DB, Kelton JG, Lichtin AE, McMillan R, Okerbloom JA, Regan DH, Warrier I. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. *Blood* 1996; **88**: 3-40
147 **Jaing TH**, Yang CP, Hung IJ, Chiu CH, Chang KW. Efficacy of Helicobacter pylori eradication on platelet recovery in children with chronic idiopathic thrombocytopenic purpura. *Acta Paediatr* 2003; **92**: 1153-1157
148 **Hayashi H**, Okuda M, Aoyagi N, Yoshiyama M, Miyashiro

E, Kounami S, Yoshikawa N. Helicobacter pylori infection in children with chronic idiopathic thrombocytopenic purpura. *Pediatr Int* 2005; **47**: 292-295

149 **Neefjes VM**, Heijboer H, Tamminga RY. H. pylori infection in childhood chronic immune thrombocytopenic purpura. *Haematologica* 2007; **92**: 576

150 **Hamidieh AA**, Arzanian MT, Gachkar L, Pasha F. Helicobacter pylori infection in children with chronic idiopathic thrombocytopenic purpura. *J Pediatr Hematol Oncol* 2008; **30**: 96-97

151 **Bisogno G**, Errigo G, Rossetti F, Sainati L, Pusiol A, Da Dalt L, Colleselli P, Grotto P, Carli M. The role of Helicobacter pylori in children with chronic idiopathic thrombocytopenic purpura. *J Pediatr Hematol Oncol* 2008; **30**: 53-57

152 **Loffredo G**, Marzano MG, Migliorati R, Miele E, Menna F, Poggi V, Staiano A. The relationship between immune thrombocytopenic purpura and Helicobacter pylori infection in children: where is the truth? *Eur J Pediatr* 2007; **166**: 1067-1068

153 **Treepongkaruna S**, Sirachainan N, Kanjanapongkul S, Winaichatsak A, Sirithorn S, Sumritsopak R, Chuansumrit A. Absence of platelet recovery following Helicobacter pylori eradication in childhood chronic idiopathic thrombocytopenic purpura: a multi-center randomized controlled trial. *Pediatr Blood Cancer* 2009; **53**: 72-77

154 **Kühne T**, Michaels LA. Helicobacter pylori in children with chronic idiopathic thrombocytopenic purpura: are the obstacles in the way typical in pediatric hematology? *J Pediatr Hematol Oncol* 2008; **30**: 2-3

155 **Eder W**, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006; **355**: 2226-2235

156 **Cover TL**, Blaser MJ. Helicobacter pylori in health and disease. *Gastroenterology* 2009; **136**: 1863-1873

157 **Chen Y**, Blaser MJ. Helicobacter pylori colonization is inversely associated with childhood asthma. *J Infect Dis* 2008; **198**: 553-560

158 **Umetsu DT**, DeKruyff RH. The regulation of allergy and asthma. *Immunol Rev* 2006; **212**: 238-255

159 **Lundgren A**, Trollmo C, Edebo A, Svennerholm AM, Lundin BS. Helicobacter pylori-specific CD4+ T cells home to and accumulate in the human Helicobacter pylori-infected gastric mucosa. *Infect Immun* 2005; **73**: 5612-5619

160 **Robinson K**, Kenefeck R, Pidgeon EL, Shakib S, Patel S, Polson RJ, Zaitoun AM, Atherton JC. Helicobacter pylori-induced peptic ulcer disease is associated with inadequate regulatory T cell responses. *Gut* 2008; **57**: 1375-1385

161 **Raj SM**, Choo KE, Noorizan AM, Lee YY, Graham DY. Evidence against Helicobacter pylori being related to childhood asthma. *J Infect Dis* 2009; **199**: 914-915; author reply 915-916

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ORIGINAL ARTICLE

Prognostic relevance of *β-catenin* expression in T2-3N0M0 esophageal squamous cell carcinoma

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Abstract

AIM: To study the expression of *β-catenin* in esophageal squamous cell carcinoma (ESCC) at stage T2-3N0M0 and its relation with the prognosis of ESCC patients.

METHODS: Expression of *β-catenin* in 227 ESCC specimens was detected by immunohistochemistry (IHC). A reproducible semi-quantitative method which takes both staining percentage and intensity into account was applied in IHC scoring, and receiver operating characteristic curve analysis was used to select the cut-off score for high or low IHC reactivity. Then, correlation of *β-catenin* expression with clinicopathological features and prognosis of ESCC patients was determined.

RESULTS: No significant correlation was observed between *β-catenin* expression and clinicopathological parameters in terms of gender, age, tumor size, tumor grade, tumor location, depth of invasion and pathological stage. The Kaplan-Meier survival curve showed that the up-regulated expression of *β-catenin* indicated a poorer post-operative survival rate of ESCC patients at stage T2-3N0M0 (P = 0.004), especially of those with T3 lesions (P = 0.014) or with stage ⅡB diseases (P = 0.007). Multivariate analysis also confirmed that *β-catenin* was an independent prognostic factor for the overall survival rate of ESCC patients at stage T2-3N0M0 (relative risk = 1.642, 95% CI: 1.159-2.327, P = 0.005).

CONCLUSION: Elevated *β-catenin* expression level may be an adverse indicator for the prognosis of ESCC patients at stage T2-3N0M0, especially for those with T3 lesions or stage ⅡB diseases.

Key words: Esophageal squamous cell carcinoma; *β-catenin*; Prognosis; Receiver operating characteristic curve; Immunohistochemistry

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INTRODUCTION

Esophageal carcinoma, an aggressive tumor with a poor prognosis, is one of the most common malignant tumors in Asia, especially in certain areas of China, and South

America[1]. Esophageal squamous cell carcinoma (ESCC) accounts for over 90% of all esophageal cancers worldwide[2]. Despite advances in imaging technologies enabling earlier diagnosis of ESCC, surgery and response rates of radiotherapy and chemotherapy, the clinical outcome of ESCC patients remains unsatisfactory. Even in the developed world, more than 85% of ESCC patients die within 2 years after its diagnosis[3]. In China, the esophageal cancer mortality rate ranks fourth of cancer-related deaths[4]. Thus, improvement in the efficacy of ESCC treatment is a major public health goal. New modalities based on a better understanding of the ESCC biology are indispensable. Since the classical staging criteria fail to differentiate the prognostic characteristics of ESCC patients adequately, molecular tumor analysis may provide a necessary means for defining the prognosis of ESCC patients. In order to further improve the survival rate of ESCC patients, it is essential to identify the relevant biomarkers with adverse prognostic significance, and to modify the therapeutic strategies for individual patients according to their molecular tumor status.

β-catenin is an 88kDa versatile protein that has at least two different cellular functions[5-7]. First, *β-catenin* is an important structural component of both normal epithelium and malignant cells. Together with a structurally homologous *γ-catenin*, *β-catenin* participates in cell-cell and cell-matrix adhesion by binding to the intracellular domain of *E-cadherin*, a homotypic cell-to-cell interaction molecule ubiquitously expressed in epithelial cells[6,8,9]. In addition, these catenins play an important role in cell polarity by binding to the actin filament network of cytoskeleton through *a-catenin* as a linker[6,8]. Unlike other known catenins, *β-catenin* is also a key mediator in the Wingless/Wnt signal transduction pathway[7,8,10]. In cytoplasm of normal cells, the amount and location of *β-catenin* are controlled exquisitely through its association with the adenomatous polyposis coli (*APC*) tumor-suppressor gene product, a scaffolding protein *Axin*, and a glycogen synthetase kinase (*GSK-3β*) enabling phosphorylation and degradation of free *β-catenin*[6-10]. Once located in nuclei, *β-catenin* can act as a transcription factor by serving as a coactivator of the lymphoid enhancer factor/TCF family of DNA-binding proteins[10]. Activation of Wnt signaling involves the inhibition of catenin degradation by proteasomes, resulting in its cytoplasmic and nuclear accumulation and transcriptional activation of the target gene[5-7,10]. It is believed that *β-catenin* integrity impairment -related intracellular network may be closely associated with the dedifferentiation, hyperproliferation, invasion and metastatic potential of malignancy[10,11]. This biomarker has thereby been extensively studied in a variety of neoplasms, such as hepatocellular carcinoma[12], colorectal carcinoma[13], gastric cancer[14,15], pancreatic cancer[16], ovarian cancer[17], lung cancer[18-20], breast cancer[21], nasopharyngeal carcinoma[22], prostate cancer[23] and even lymphoma[24], with regard to its potential role as a prognostic factor in cell polarity. The present findings in ESCC are controversial in the literature[25-28]. The role of *β-catenin* in development of ESCC and its prognostic significance remain to be defined. In the present study, the expression level of *β-catenin* was measured in specimens from a relatively homogeneous cohort of ESCC patients with no lymph node involved, which was correlated with the clinical outcome of ESCC patients.

MATERIALS AND METHODS

Patients and tissue samples

The study was approved by the Ethics Committee of Sun Yat-Sen University Cancer Center. A total of 227 consecutive patients with node-negative ESCC at stage ⅠB-ⅡB who underwent curative surgery from January 1993 to August 2004 were enrolled in this study. *β-catenin* expression level was measured in resected specimens with immunohistochemistry (IHC). Patients were followed up prospectively and their survival data were recorded through October 2009. The inclusion criteria were patients with histopathologically-proven ESCC, those with esophageal cancer at T2-3N0M0 based on the seventh edition of the American Joint Committee on Cancer staging system[29], those with at least 15 lymph nodes to be removed for pathological evaluation, those at the age of at least 18 years, those with no evidence of metastatic disease as determined by history, physical examination, and blood chemistry analysis or routine computed tomography, those with no history of adjuvant therapy. Patients with a history of previously treated cancer other than basal or squamous cell carcinoma of the skin, preoperative chemotherapy and/or radiotherapy, or with unknown causes of death in follow-up were excluded from the study.

Immunohistochemistry

β-catenin (CAT-5H10, Fuzhou Maxim Inc., Fuzhou, Fujian province, China) was diluted at 1:100. ESCC tissue was cut into 4 μm-thick paraffin sections, which were stained with immunoperoxidase. The sections were deparaffinized in xylene, hydrated prior to antigen retrieval by microwaving in sodium citrate buffer (pH 6.0), and incubated with a peroxidase block followed by primary antibody. After washed with PBS, the sections were incubated first with secondary antibody followed by 3,3'-diaminobenzidine, and then counterstained with hematoxylin (Hematoxylin 7211; Richard-Allen Scientific, Kalamazoo, Michigan, USA). The peroxidase block, secondary antibody and 3,3'-diaminobenzidine were all obtained from the Dako-Cytomation EnVision System (Glostrup, Denmark).

Immunohistochemical scoring

β-catenin was scored with IHC using a semi-quantitative system as previously described[30,31]. Each section was assigned a score and the score of tumor cell staining was multiplied by the score of staining intensity. Tumor cell staining was scored using a semi-quantitative six-category grading system: 0 = no tumor cell staining, 1 = 1%-10% of tumor cells staining, 2 = 11%-25% of tumor cells staining, 3 =

26%-50% of tumor cells staining, 4 = 51%-75% of tumor cells staining, 5 = over 75% of tumor cells staining. Stain intensity was scored using a semi-quantitative four-category grading system: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining. Two experienced pathologists blinded to the clinical follow-up data independently scored the 400 ESCC samples including the cases used in this study. The complete score agreement of the two pathologists was 87% of all cases, indicating that the scoring method is reasonably reproducible. A third blinded pathologist intervened and evaluated the patients with different IHC scores. If the third pathologist agreed with one of the previous scores, it was used for analysis. The three pathologists were asked to reach an agreement on the cases from which three different scores were obtained.

Selection of cut-off scores

Cut-off scores for *β-catenin* expression were selected based on receiver operating characteristic (ROC) curve analysis. ROC curve was plotted for the outcome of ESCC patients under study by calculating the sensitivity and specificity on its points. The score closest to the points (0.0, 1.0) on the curve with a maximum sensitivity and specificity was selected as the cut-off score leading to the greatest number of tumors classified with or without clinical outcome. The area under the ROC curve was calculated to estimate the discriminatory power of *β-catenin* over the entire range of scores for overall survival (OS) rate of ESCC patients. The ROC curve was generated and analyzed using the MedCalc statistical software package 11.0.1 (MedCalc Software bvba, Belgium).

Statistical analysis

Association between categorical variables was analyzed by χ^2 test. Survival curves were calculated with the Kaplan-Meier method and compared by the log-rank test. Time of death was calculated from the date of surgery to the date of death. The time variable was censored on the date of last follow-up of event-free subjects. Multivariate analysis of prognostic factors was performed using the Cox's regression model. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS 13.0 for Windows software system (SPSS Inc., Chicago, IL).

RESULTS

Characteristics of patients and expression of β-catenin

The demographic and clinicopathological parameters of ESCC patients included in this study are listed in Table 1. Various intensities of positive *β-catenin* reaction were detected in cytoplasm and membrane of cancer cells (Figure 1). According to the ROC curves for OS rate, a threshold value of 1.3333 was the optimal point for maximum sensitivity and specificity, and selected as the cut-off score (Figure 2). The 227 ESCC specimens were then catego-

Table 1 Demographic and clinicopathological parameters of esophageal squamous cell carcinoma patients included in this study *n* (%)

Demographic and clinicopathological parameters	Patients with ESCC
Sex	
Male	165 (72.7)
Female	62 (27.3)
Age (yr)	
Median	58
Range	33-77
Tumor size (cm)	4.92 ± 2.046
Tumor grade	
Grade 1	57 (25.1)
Grade 2	114 (50.2)
Grade 3	56 (24.7)
Tumor location	
Upper	25 (11.0)
Middle	152 (67.0)
Lower	50 (22.0)
Depth of invasion	
T2	88 (38.8)
T3	139 (61.2)
AJCC staging system (7th ed)	
Ⅰ B	12 (5.3)
Ⅱ A	83 (36.6)
Ⅱ B	132 (58.1)

ESCC: Esophageal squamous cell carcinoma; AJCC: American Joint Committee on Cancer.

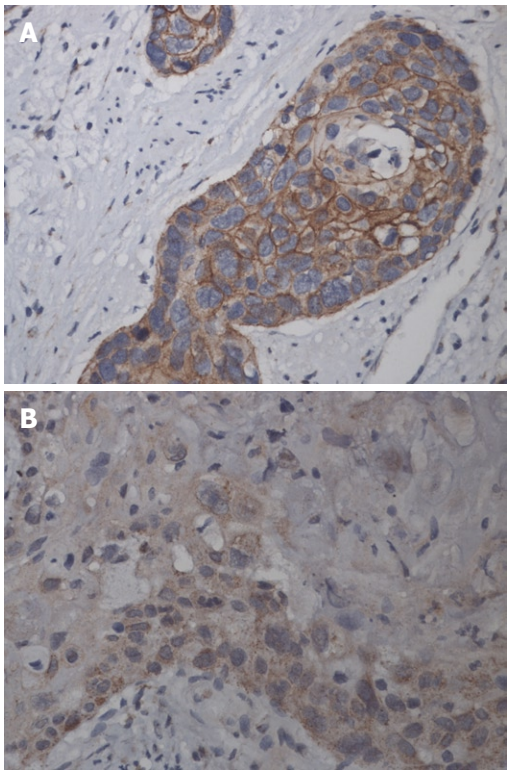

Figure 1 Expression of *β-catenin* in esophageal squamous cell carcinoma tissue samples (magnification × 400). A: Immunohistochemical staining of *β-catenin* in cytoplasm and membrane of cancer cells (immunohistochemistry score: 15); B: Immunohistochemical staining of *β-catenin* in cytoplasm of cancer cells (immunohistochemistry score: 3).

Table 2 *β-catenin* expression in esophageal squamous cell carcinoma patients *n* (%)

Parameters of ESCC patients	*n*	Expression of *β-catenin*		*P*-value
		Low	High	
Sex				
Male	165	57 (34.5)	108 (65.5)	0.354
Female	62	26 (41.9)	36 (58.1)	
Age (yr)				
≤ 60	138	49 (35.5)	89 (64.5)	0.778
> 60	89	34 (38.2)	55 (61.8)	
Tumor size (cm)				
≤ 5.0	155	54 (34.8)	101 (65.2)	0.461
> 5.0	72	29 (40.3)	43 (59.7)	
Tumor grade				
Grade 1	57	17 (29.8)	40 (70.2)	
Grade 2	114	44 (38.6)	70 (61.4)	0.473
Grade 3	56	22 (39.3)	34 (60.7)	
Tumor location				
Upper	25	11 (44.0)	14 (56.0)	
Middle	152	52 (34.2)	100 (65.8)	0.545
Lower	50	20 (40.0)	30 (60.0)	
Depth of invasion				
T2	88	34 (38.6)	54 (61.4)	0.672
T3	139	49 (35.3)	90 (64.7)	
AJCC staging system (7th ed)				
Ⅰ B	12	3 (25.0)	9 (75.0)	
Ⅱ A	83	31 (37.3)	52 (62.7)	0.694
Ⅱ B	132	49 (37.1)	83 (62.9)	

ESCC: Esophageal squamous cell carcinoma; AJCC: American Joint Committee on Cancer.

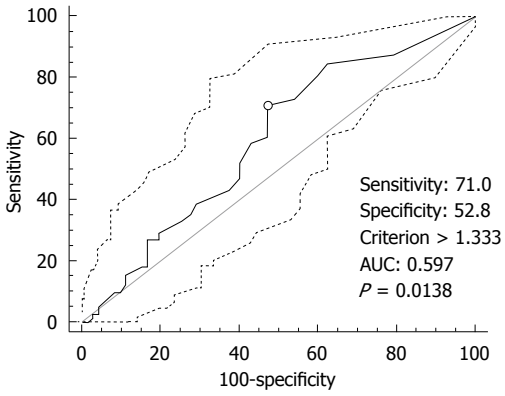

Figure 2 Receiver operating characteristic curve analysis of *β-catenin* and selection of cut-off score.

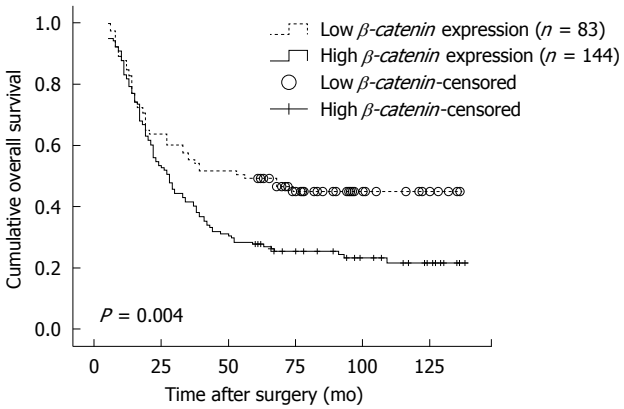

Figure 3 Kaplan-Meier survival curves for patients with esophageal squamous cell carcinoma at stage T2-3N0M0 according to *β-catenin* expression.

rized into high and low *β-catenin* expression groups. The expression level of *β-catenin* was up-regulated in 144 cases (63.4%) and down-regulated in 83 cases (36.6%).

Correlation between β-catenin expression and clinicopathological features

The correlation between *β-catenin* expression in and clinicopathological features of ESCC patients are shown in Table 2. No significant correlation was identified between *β-catenin* expression and any clinicopathological parameters, including gender, age, tumor size, tumor grade, tumor location, depth of invasion and pathological stage based on the seventh edition of AJCC staging system[29].

β-catenin expression and survival rate

At the time of data analysis (October 2009), 72 patients (31.7%), with a median follow-up time of 32 mo (range 5-138 mo), remained alive and 155 patients (68.3%) died. The overall 1-, 3- and 5-year survival rates for the patients were 58%, 39%, and 33%, respectively.

The Kaplan-Meier survival curves (Figure 3) showed that the post-operative survival rate of patients with a low *β-catenin* expression level was significantly higher than that of those with a high *β-catenin* expression level ($P = 0.004$). Further stratified analysis split by depth of invasion (Figure 4) showed that the expression of *β-catenin* had a statistically significant influence on the survival rate of patients with T3 diseases ($P = 0.014$) rather than on the survival rate of those with T2 lesions ($P = 0.145$). Furthermore, the stratified analysis split by pathological stage based on the new staging system (Figure 5) revealed that *β-catenin* expression had a significant influence on the prognosis of patients with ESCC at stage ⅡB ($P = 0.007$) but not on the prognosis of those with ⅡA diseases ($P = 0.253$). Patients with ESCC at stage ⅠB were not included in this analysis due to a small sample size.

Factors involved in OS rate of ESCC patients were identified using the Cox proportional hazards model (Table 3). Univariate analysis showed that tumor grade, depth of invasion and *β-catenin* expression were found to be the significant prognostic indicators for the OS rate of ESCC patients, and thereby selected as the parameters to be included in the same Cox regression model. Further multivariate analysis also confirmed that *β-catenin* expression (relative risk = 1.642, 95% CI: 1.159-2.327, $P = 0.005$), tumor grade (relative risk = 1.549, 95% CI: 1.095-2.190, $P = 0.013$) and depth of invasion (relative risk = 1.493, 95% CI: 1.066-2.089, $P = 0.020$) were the independent prognostic factors for the OS rate of ESCC patients.

DISCUSSION

To date, several IHC studies have been performed in order

Table 3 Univariate and multivariate analysis of overall survival rate of esophageal squamous cell carcinoma patients with Cox proportional hazards model

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	*P*-value	Hazard ratio	95% CI	*P*-value
Age (yr)						
≤ 60 *vs* > 60	1.023	0.740-1.414	0.889			
Gender						
Male *vs* female	0.692	0.474-1.009	0.056			
Tumor size (cm)						
≤ 5 *vs* > 5	0.987	0.704-1.386	0.942			
Grade						
G1 *vs* G2 and 3	1.498	1.060-2.116	0.022[a]	1.549	1.095-2.190	0.013[a]
Tumor location						
Upper and middle *vs* lower	0.954	0.657-1.385	0.804			
Depth of invasion						
T2 *vs* T3	1.461	1.045-2.043	0.026[a]	1.493	1.066-2.089	0.020[a]
AJCC staging system (7th ed)						
ⅠB and ⅡA *vs* ⅡB	1.192	0.865-1.643	0.284			
β-catenin						
Low *vs* high	1.644	1.161-2.329	0.005[b]	1.642	1.159-2.327	0.005[b]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* univariate analysis. AJCC: American Joint Committee on Cancer; CI: Confidence interval.

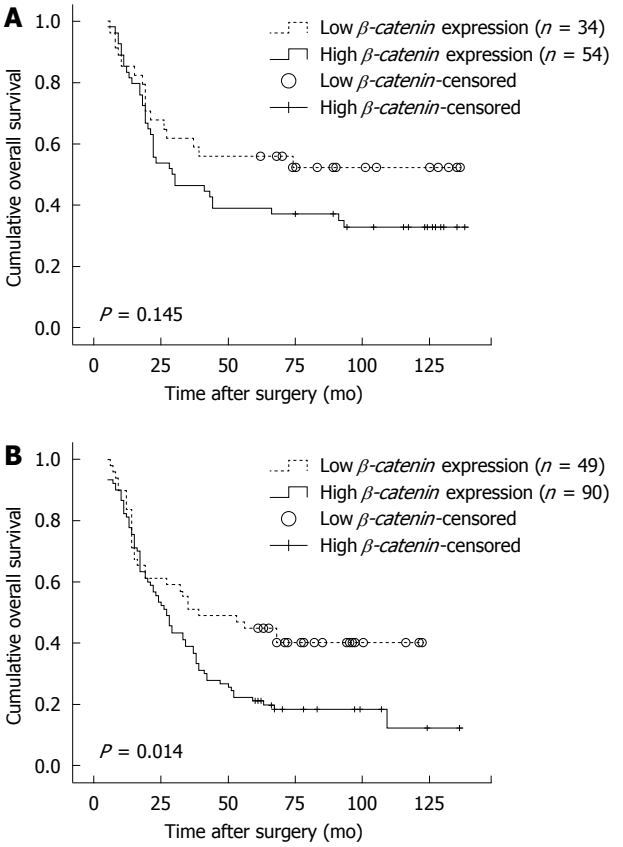

Figure 4 Kaplan-Meier survival curves for patients with esophageal squamous cell carcinoma according to *β-catenin* expression. A: Correlation between *β-catenin* expression and post-operative survival rate of patients with T2 lesions; B: Correlation between *β-catenin* expression and post-operative survival rate of patients with T3 lesions.

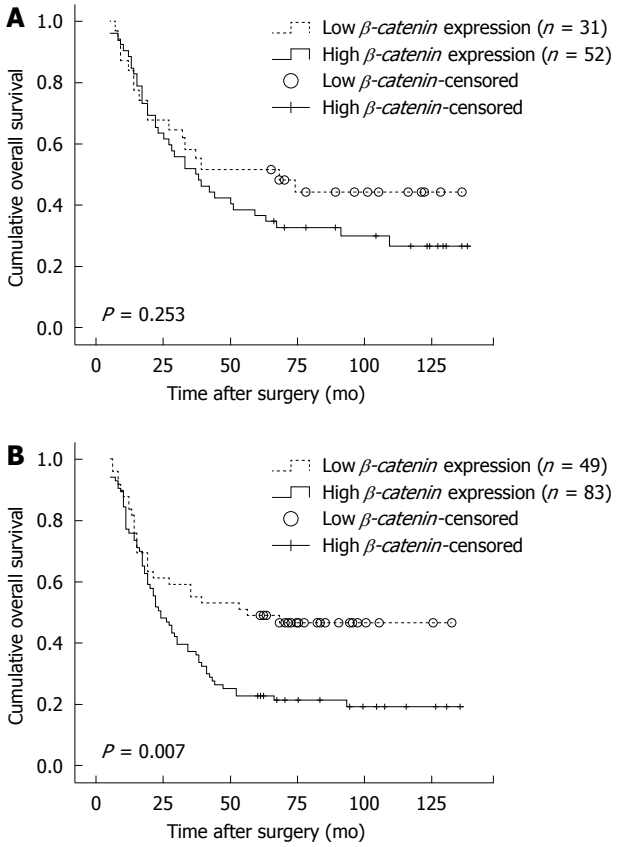

Figure 5 Kaplan-Meier survival curves for patients with esophageal squamous cell carcinoma stratified for pathological stage according to *β-catenin* expression. A: Correlation between *β-catenin* expression and post-operative survival rate of patients with ⅡA diseases; B: Correlation between *β-catenin* expression and post-operative survival rate of patients with ⅡB diseases.

to elucidate the role of *β-catenin* in ESCC, but the current findings in terms of its expression pattern and potential involvement in formation and progression of ESCC are contradictory in the literature[25-28]. One problem faced by researchers is the determination of tumor immunohistochemical positivity for *β-catenin* which is clinically and biologically relevant. Previous studies have applied different scoring systems in predetermination of cut-off scores

which might be set arbitrarily[30-33]. A lack of consistent, widely applicable methodology may have been primarily responsible for the contradictory results of these studies evaluating *β-catenin* and its prognostic value in ESCC patients. Therefore, our study used a reproducible scoring method which takes both staining percentage and intensity into account, and the cut-off score was selected based on ROC curve analysis, so that the trade-off between sensitivity and specificity was the smallest, leading to the greatest overall number of correctly classified tumors with and without clinical outcome.

In the present study, univariate and multivariate analyses showed that high expression level of *β-catenin* in completely resected samples from patients with ESCC at stage T2-3N0M0 was significantly correlated with the worse post-operative survival rate of ESCC patients, which is consistent with the reported findings[27]. Krishnadath *et al*[34] reported that low expression level of *β-catenin* is significantly correlated with the poor prognosis of esophageal adenocarcinoma patients, especially at early-stage. It has been shown that the higher the expression level of *β-catenin* is, the better the outcome of esophageal adenocarcinoma patients is, and the *β-catenin* expression level is higher in more invasive tumors than in superficial tumors[35]. However, Zhao *et al*[26] and Lin *et al*[28] demonstrated that *β-catenin* expression does not imply more aggressive malignant behaviors of ESCC or predict the poor prognosis of ESCC patients. Krishnadath *et al*[34] and Osterheld *et al*[35] showed a different histological type of tumor (adenocarcinoma) when they analyzed these contradictory results. In addition, esophageal carcinoma at stages I -Ⅳ was involved in the studies[26,28,34,35], suggesting that different therapeutic strategies including adjuvant or neoadjuvant chemotherapy and radiotherapy for more advanced disease may introduce confounding factors affecting the application of molecular analysis in assessing the prognosis of ESCC patients.

β-catenin protein not only serves as a pivotal component of *E-cadherin/catenin* complex which participates in cell-cell and cell-matrix adhesion[6,8,9], but also as a key mediator in the Wingless/Wnt signal transduction pathway[5,7], indicating that disruption of *E-cadherin/catenin* complex or physical and functional loss of *β-catenin* protein can lead to loosening of cell-cell contact and promote tumor invasion and metastasis. On the other hand, *β-catenin* can be oncogenically activated either by direct gene mutation[36] and inactivation of the *APC* tumor suppressor[37], or by activation of the Wingless/Wnt signal transduction pathway[38], thus resulting in post-translational stabilization of *β-catenin* protein. Excess cytoplasmic accumulation of *β-catenin* protein can then increase the influx of this molecule into nuclei, leading to over-expression of tumor-promoting genes, such as *cyclin-D1* and *c-myc*[5,6], and promote cell mitosis and growth[39,40]. Therefore, either up-regulated or down-regulated expression of *β-catenin* contributes to invasive and metastatic potentials of esophageal cancer. Obviously, the question is which physiopathological process takes the advantage in different circumstances, such as different histological types or different pathological stages. Further analysis of the role of *β-catenin* gene and its products in formation and progression of esophageal carcinomas may provide a better understanding of this pathogenic process.

In this study, further stratified analysis split by pathological stage and depth of invasion showed that *β-catenin* exhibited its effect on the prognosis of patients with ESCC at stage ⅡB or with T3 lesions, indicating that this biomarker is more valuable in predicting the outcome of ESCC patients at advanced stages, which is consistent with the findings in other studies[2,22]. Further study is needed to verify this trend. In this study, no significant correlation was found between *β-catenin* expression and prognostic parameters, including tumor grade, tumor location, depth of invasion and pathological stage. Multivariate survival analysis of all potential prognostic variables also confirmed that *β-catenin* was an absolutely independent prognostic factor, which is in accordance with the reported findings[25,28]. However, other studies showed that *β-catenin* is significantly correlated with the accepted prognostic parameters of ESCC[26,27,41]. Further study with a large sample size is needed to obtain a clearer picture.

In conclusion, elevated *β-catenin* expression level is an adverse prognostic factor for ESCC patients at stage T2-3N0M0, especially for those with T3 lesions or with stage ⅡB diseases. However, further study with a larger cohort of patients is required to verify this observation, especially in view of the contradictory results.

COMMENTS

Background

Esophageal squamous cell carcinoma (ESCC), an aggressive tumor with a poor prognosis, is one of the most common malignant tumors in Asia, especially in certain areas of China. Despite advances in early diagnosis and therapies, the clinical outcome of ESCC patients remains unsatisfactory. Since the classical staging criteria fail to differentiate prognostic characteristics of ESCC patients adequately, many efforts have been made to identify relevant biomarkers with adverse prognostic significance, and to modify therapeutic strategies for individual patients. *β-catenin*, as a prognostic factor for ESCC, has been extensively studied in a variety of neoplasms. However, the exact role of *β-catenin* and its prognostic significance in ESCC remain to be defined.

Research frontiers

β-catenin protein not only serves as a pivotal component of *E-cadherin/catenin* complex which participates in cell-cell and cell-matrix adhesion, but also as a key mediator in the Wingless/Wnt signal transduction pathway. The hotspot in molecular tumor analysis of *β-catenin* is whether *β-catenin* involves and how it involves in the formation and progression of esophageal carcinoma.

Innovations and breakthroughs

Previous immunohistochemistry studies showed contradictory results in *β-catenin* expression pattern and its prognostic value for ESCC, due to lack of consistent, widely applicable methods. Therefore, the present study used a reproducible scoring method which takes both staining percentage and intensity into account, and the cut-off score was selected based on receiver operating characteristic (ROC) curve analysis so that the trade-off between sensitivity and specificity was the smallest, leading to the greatest overall number of correctly classified tumors with and without clinical outcome. This is the first study to evaluate *β-catenin* expression in ESCC patients with this novel method, showing that elevated *β-catenin* expression is an adverse prognostic factor for ESCC patients at stage T2-3N0M0, especially for those with T3 lesions or stage ⅡB diseases.

Applications

The novel methods used in this study can be applied in treatment of ESCC patients at stage T2-3N0M0.

Terminology

ROC curve analysis: In signal detection theory, a ROC curve is a graphical plot of the sensitivity, or true positivity *vs* (1-specificity), or false positivity for a binary classifier system as its discrimination threshold is varied. The ROC curve can also be represented equivalently by plotting the fraction of true positivity (TPR = true positive rate) *vs* the fraction of false positivity (FPR = false positive rate). ROC curve analysis provides tools to select possibly optimal models and to discard suboptimal ones independently from (and prior to specifying) the cost context or the class distribution. ROC curve analysis is related in a direct and natural way to cost/benefit analysis of diagnostic decision making. *β-catenin*: *β-catenin* protein was originally identified as a component of adherence junction, a multi-protein complex supporting tight cell-cell contacts in the presence of extracellular calcium. However, *β-catenin* also plays a key role in the Wnt signaling transduction pathway.

Peer review

The authors studied the expression of *β-catenin* in ESCC at stage T2-3N0M0 and its prognostic significance by analyzing the expression of *β-catenin* in 227 ESCC specimens with IHC and ROC curve analysis to select the cut-off score for high or low IHC reactivity. Then, they correlated the *β-catenin* expression with clinicopathological features of ESCC patients and its relation with the prognosis of ESCC patients. No significant correlation was observed between *β-catenin* expression in and clinicopathological parameters of ESCC patients, but multivariate analysis confirmed that *β-catenin* was an independent prognostic factor for the overall survival rate of ESCC patients at stage T2-3N0M0. The manuscript reads nicely and is easy to follow. Tables are legible and easy to understand. I believe that the method used in this study is plausible and the conclusion is well supported by the data. This manuscript adds to the current knowledge on this topic and it is a pleasure to read it.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249
2 **Stoner GD**, Gupta A. Etiology and chemoprevention of esophageal squamous cell carcinoma. *Carcinogenesis* 2001; **22**: 1737-1746
3 **Munro AJ**. Oesophageal cancer: a view over overviews. *Lancet* 2004; **364**: 566-568
4 **Su M**, Lu SM, Tian DP, Zhao H, Li XY, Li DR, Zheng ZC. Relationship between ABO blood groups and carcinoma of esophagus and cardia in Chaoshan inhabitants of China. *World J Gastroenterol* 2001; **7**: 657-661
5 **Conacci-Sorrell M**, Zhurinsky J, Ben-Ze'ev A. The cadherin-catenin adhesion system in signaling and cancer. *J Clin Invest* 2002; **109**: 987-991
6 **Hajra KM**, Fearon ER. Cadherin and catenin alterations in human cancer. *Genes Chromosomes Cancer* 2002; **34**: 255-268
7 **Nelson WJ**, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* 2004; **303**: 1483-1487
8 **Bremnes RM**, Veve R, Hirsch FR, Franklin WA. The E-cadherin cell-cell adhesion complex and lung cancer invasion, metastasis, and prognosis. *Lung Cancer* 2002; **36**: 115-124
9 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70
10 **Polakis P**. The oncogenic activation of beta-catenin. *Curr Opin Genet Dev* 1999; **9**: 15-21
11 **Kudo Y**, Kitajima S, Ogawa I, Hiraoka M, Sargolzaei S, Keikhaee MR, Sato S, Miyauchi M, Takata T. Invasion and metastasis of oral cancer cells require methylation of E-cadherin and/or degradation of membranous beta-catenin. *Clin Cancer Res* 2004; **10**: 5455-5463
12 **Zhai B**, Yan HX, Liu SQ, Chen L, Wu MC, Wang HY. Reduced expression of E-cadherin/catenin complex in hepatocellular carcinomas. *World J Gastroenterol* 2008; **14**: 5665-5673
13 **Elzagheid A**, Buhmeida A, Korkeila E, Collan Y, Syrjanen K, Pyrhonen S. Nuclear beta-catenin expression as a prognostic factor in advanced colorectal carcinoma. *World J Gastroenterol* 2008; **14**: 3866-3871
14 **Pan KF**, Liu WG, Zhang L, You WC, Lu YY. Mutations in components of the Wnt signaling pathway in gastric cancer. *World J Gastroenterol* 2008; **14**: 1570-1574
15 **Cheng XX**, Wang ZC, Chen XY, Sun Y, Kong QY, Liu J, Li H. Correlation of Wnt-2 expression and beta-catenin intracellular accumulation in Chinese gastric cancers: relevance with tumour dissemination. *Cancer Lett* 2005; **223**: 339-347
16 **Li YJ**, Ji XR. Relationship between expression of E-cadherin-catenin complex and clinicopathologic characteristics of pancreatic cancer. *World J Gastroenterol* 2003; **9**: 368-372
17 **Faleiro-Rodrigues C**, Macedo-Pinto I, Pereira D, Ferreira VM, Lopes CS. Association of E-cadherin and beta-catenin immunoexpression with clinicopathologic features in primary ovarian carcinomas. *Hum Pathol* 2004; **35**: 663-669
18 **Smythe WR**, Williams JP, Wheelock MJ, Johnson KR, Kaiser LR, Albelda SM. Cadherin and catenin expression in normal human bronchial epithelium and non-small cell lung cancer. *Lung Cancer* 1999; **24**: 157-168
19 **Leinonen T**, Pirinen R, Böhm J, Johansson R, Ropponen K, Kosma VM. Expression of matrix metalloproteinases 7 and 9 in non-small cell lung cancer. Relation to clinicopathological factors, beta-catenin and prognosis. *Lung Cancer* 2006; **51**: 313-321
20 **Choi YS**, Shim YM, Kim SH, Son DS, Lee HS, Kim GY, Han J, Kim J. Prognostic significance of E-cadherin and beta-catenin in resected stage I non-small cell lung cancer. *Eur J Cardiothorac Surg* 2003; **24**: 441-449
21 **Jiang Y**, Zou L, Zhang C, He S, Cheng C, Xu J, Lu W, Zhang Y, Zhang H, Wang D, Shen A. PPARgamma and Wnt/beta-Catenin pathway in human breast cancer: expression pattern, molecular interaction and clinical/prognostic correlations. *J Cancer Res Clin Oncol* 2009; **135**: 1551-1559
22 **Zheng Z**, Pan J, Chu B, Wong YC, Cheung AL, Tsao SW. Downregulation and abnormal expression of E-cadherin and beta-catenin in nasopharyngeal carcinoma: close association with advanced disease stage and lymph node metastasis. *Hum Pathol* 1999; **30**: 458-466
23 **Richmond PJ**, Karayiannakis AJ, Nagafuchi A, Kaisary AV, Pignatelli M. Aberrant E-cadherin and alpha-catenin expression in prostate cancer: correlation with patient survival. *Cancer Res* 1997; **57**: 3189-3193
24 **Groen RW**, Oud ME, Schilder-Tol EJ, Overdijk MB, ten Berge D, Nusse R, Spaargaren M, Pals ST. Illegitimate WNT pathway activation by beta-catenin mutation or autocrine stimulation in T-cell malignancies. *Cancer Res* 2008; **68**: 6969-6977
25 **Ninomiya I**, Endo Y, Fushida S, Sasagawa T, Miyashita T, Fujimura T, Nishimura G, Tani T, Hashimoto T, Yagi M, Shimizu K, Ohta T, Yonemura Y, Inoue M, Sasaki T, Miwa K. Alteration of beta-catenin expression in esophageal squamous-cell carcinoma. *Int J Cancer* 2000; **85**: 757-761
26 **Zhao XJ**, Li H, Chen H, Liu YX, Zhang LH, Liu SX, Feng QL. Expression of e-cadherin and beta-catenin in human esophageal squamous cell carcinoma: relationships with prognosis. *World J Gastroenterol* 2003; **9**: 225-232
27 **Ji L**, Cao XF, Wang HM, Li YS, Zhu B, Xiao J, Wang D. Expression level of beta-catenin is associated with prognosis of esophageal carcinoma. *World J Gastroenterol* 2007; **13**: 2622-2625
28 **Lin YC**, Wu MY, Li DR, Wu XY, Zheng RM. Prognostic and clinicopathological features of E-cadherin, alpha-catenin, beta-catenin, gamma-catenin and cyclin D1 expression in human esophageal squamous cell carcinoma. *World J Gastroenterol* 2004; **10**: 3235-3239
29 **Hsu PK**, Wu YC, Chou TY, Huang CS, Hsu WH. Comparison of the 6th and 7th editions of the American Joint Committee on Cancer tumor-node-metastasis staging system in patients with resected esophageal carcinoma. *Ann Thorac Surg* 2010; **89**: 1024-1031
30 **Situ D**, Long H, Lin P, Zhu Z, Wang J, Zhang X, Xie Z, Rong T.

Expression and prognostic relevance of CD44v6 in stage I non-small cell lung carcinoma. *J Cancer Res Clin Oncol* 2010; **136**: 1213-1219
31 **Zhu ZH**, Sun BY, Ma Y, Shao JY, Long H, Zhang X, Fu JH, Zhang LJ, Su XD, Wu QL, Ling P, Chen M, Xie ZM, Hu Y, Rong TH. Three immunomarker support vector machines-based prognostic classifiers for stage IB non-small-cell lung cancer. *J Clin Oncol* 2009; **27**: 1091-1099
32 **Hanley JA**. Receiver operating characteristic (ROC) methodology: the state of the art. *Crit Rev Diagn Imaging* 1989; **29**: 307-335
33 **Zlobec I**, Steele R, Terracciano L, Jass JR, Lugli A. Selecting immunohistochemical cut-off scores for novel biomarkers of progression and survival in colorectal cancer. *J Clin Pathol* 2007; **60**: 1112-1116
34 **Krishnadath KK**, Tilanus HW, van Blankenstein M, Hop WC, Kremers ED, Dinjens WN, Bosman FT. Reduced expression of the cadherin-catenin complex in oesophageal adenocarcinoma correlates with poor prognosis. *J Pathol* 1997; **182**: 331-338
35 **Osterheld MC**, Bian YS, Bosman FT, Benhattar J, Fontolliet C. Beta-catenin expression and its association with prognostic factors in adenocarcinoma developed in Barrett esophagus. *Am J Clin Pathol* 2002; **117**: 451-456
36 **Sparks AB**, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. *Cancer Res* 1998; **58**: 1130-1134
37 **Tanaka S**, Akiyoshi T, Mori M, Wands JR, Sugimachi K. A novel frizzled gene identified in human esophageal carcinoma mediates APC/beta-catenin signals. *Proc Natl Acad Sci USA* 1998; **95**: 10164-10169
38 **Mizushima T**, Nakagawa H, Kamberov YG, Wilder EL, Klein PS, Rustgi AK. Wnt-1 but not epidermal growth factor induces beta-catenin/T-cell factor-dependent transcription in esophageal cancer cells. *Cancer Res* 2002; **62**: 277-282
39 **Ilyas M**, Tomlinson IP. The interactions of APC, E-cadherin and beta-catenin in tumour development and progression. *J Pathol* 1997; **182**: 128-137
40 **Nakamura Y**. Cleaning up on beta-catenin. *Nat Med* 1997; **3**: 499-500
41 **Norwood MG**, Bailey N, Nanji M, Gillies RS, Nicholson A, Ubhi S, Darnton JJ, Steyn RS, Womack C, Hughes A, Hemingway D, Harrison R, Waters R, Jankowski JA. Cytoplasmic beta-catenin accumulation is a good prognostic marker in upper and lower gastrointestinal adenocarcinomas. *Histopathology* 2010; **57**: 101-111

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ORIGINAL ARTICLE

Classification of histological severity of *Helicobacter pylori*-associated gastritis by confocal laser endomicroscopy

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Abstract

AIM: To classify the histological severity of *Helicobacter pylori* (*H. pylori*) infection-associated gastritis by confocal laser endomicroscopy (CLE).

METHODS: Patients with upper gastrointestinal symptoms or individuals who were screened for gastric cancer were enrolled in this study. Histological severity of *H. pylori* infection-associated gastritis was graded according to the established CLE criteria. Diagnostic value of CLE for histological gastritis was investigated and compared with that of white light endoscopy (WLE). Targeted biopsies from the sites observed by CLE were performed.

RESULTS: A total of 118 consecutive patients with *H. pylori* infection-associated gastritis were enrolled in this study. Receiver operating characteristic curve analysis showed that the sensitivity and specificity of CLE were 82.9% and 90.9% for the diagnosis of *H. pylori* infection, 94.6% and 97.4% for predicting gastric normal mucosa, 98.5% and 94.6% for predicting histological active inflammation, 92.9% and 95.2% for predicting glandular atrophy, 98.6% and 100% for diagnosing intestinal metaplasia, respectively. Post-CLE image analysis showed that goblet cells and absorptive cells were the two most common parameters on the CLE-diagnosed intestinal metaplasia (IM) images ($P < 0.001$). More histological lesions of the stomach could be found by CLE than by WLE ($P < 0.001$).

CONCLUSION: CLE can accurately show the histological severity of *H. pylori* infection-associated gastritis. Mapping IM by CLE has a rather good diagnostic accuracy.

Key words: Confocal laser endomicroscopy; *Helicobacter pylori*; Gastritis; Classification; Histology

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a well accepted major etiological factor for gastric diseases, such as chronic gastritis, peptic ulcer and gastric carcinoma[1-3]. Therefore, endos-

copists must determine the presence of *H. pylori* infection in the stomach during or after endoscopy. The updated Sydney system for classification of gastritis has established the association between *H. pylori* infection and histological evidence of gastritis[4]. Gastric atrophy especially accompanying intestinal metaplasia (IM), which is the severest stage of gastritis and has a high risk for gastric cancer, is closely associated with *H. pylori* infection[5]. Although the Sydney system has been widely used, multiple biopsies are rarely performed for gastritis evaluation but only for suspected lesions of cancer in clinical practice, thus leading to omission of some precancerous lesions which are difficult to find by white light endoscopy (WLE)[6]. Therefore, *in vivo* detection of *H. pylori* infection and its related complications by endoscopy is a simple, noninvasive and inexpensive procedure. *H. pylori*-induced gastritis must be evaluated and graded especially in Eastern countries because of a high prevalence of *H. pylori* infection and a high incidence of gastric cancer[7]. Furthermore, it can facilitate the clinical assessment and timely treatment of gastritis. The association between *H. pylori* infection and endoscopic findings has been extensively studied using modern endoscopic techniques such as magnification endoscopy and narrow-band imaging[8-10].

Confocal laser endomicroscopy (CLE) can detect gastrointestinal diseases, such as Barrett esophagus, gastric carcinoma and colonic neoplasia[11,12]. CLE can observe real-time histological-like cellular and subcellular conditions of gastric mucosal layer at the magnification × 500-1000. It was reported that acriflavine-aided CLE can observe *H. pylori in vivo*[13]. In addition, gastric pit patterns of various gastropathies have been accurately classified and IM has been studied by CLE[14,15]. However, no systematic data are available on the CLE characteristics of chronic gastritis and the classification of chronic gastritis has not been investigated by CLE.

In this study, the CLE features of gastritis mainly caused by *H. pylori* infection were compared to evaluate the accuracy of CLE in diagnosing *H. pylori* infection and the severity of gastritis.

MATERIALS AND METHODS

Patients

Consecutive patients with upper gastrointestinal symptoms or individuals who were screened for gastric carcinoma, admitted to our hospital from June to November 2009, were enrolled in this study. The following patients were excluded from the study, including those who received proton pump inhibitors, antibiotics, or bismuth subsalicylate in the previous 6 wk, those with a history of using nonsteroidal anti-inflammatory drugs and medication for *H. pylori* infection, those undergone stomach surgery, those with systematic diseases or known gastric carcinoma, pregnant or breast-feeding females, those who did not give their informed consent or had an allergy to fluorescein. All participants gave their written informed consent before endoscopy. The study was approved by the Ethics Committee of Qilu Hospital.

CLE system

A confocal laser endomicroscope (Pentax ISC-1000, Pentax, Tokyo, Japan) was used in this study. It is novel digestive endoscope with a confocal laser microscope integrated into the distal tip, can realize a histological-like examination during routine endoscopy and accurately diagnose gastrointestinal diseases at the magnification × 1000. CLE was performed to scan the gastric mucosa from the top layer to 250 μm beneath the surface. The CLE and white-light endoscopy (WLE) images were captured and stored.

CLE procedure

CLE was performed by an endoscopist experienced with the system. All patients received oral chymotrypsin (20000 U) to eliminate the slime layer of the stomach for better visualization. One mL of 2% fluorescein (a contrast agent) was intravenously injected before endoscopy. After a first WLE of the stomach, 10 mL of 10% fluorescein was intravenously injected, and CLE images could be seen after a few seconds. Five standardized sites (2 from the lesser and greater curvatures of the antrum about 2-3 cm near the pylorus, 2 from the middle portion of the lesser and greater curvatures of the corpus about 8 cm from the cardia, and 1 from the angulus), as recommended by the updated Sydney system, were examined separately on CLE images. Shallow-deep CLE images were captured at each site and stored as digital files for further analysis. At the standardized locations, real-time image assessment and targeted biopsies were performed by CLE for histopathology. For *H. pylori* testing, 2 specimens were taken from the greater curvature of the antrum and corpus, respectively. If necessary, other scanning and biopsies were performed for lesions such as those with color changes or erosions, polyps, ulcers, or abnormal folds.

CLE classification of H. pylori infection-associated gastritis severity

In our study, the severity of gastritis was divided into 6 levels by CLE: normal mucosa, *H. pylori*-associated active inflammation (3 levels), glandular atrophy and IM.

CLE criteria for normal gastric mucosa and *H. pylori*-associated active inflammation: The published CLE classification of gastric pit patterns was used for one part of our classification of *H. pylori*-associated active inflammation[14]. In addition, a new marker, fluorescein leakage, was introduced to define the active inflammation in stomach on CLE images (Figure 1). Each of the 5 standardized sites was assigned to a confocal gastritis score (CGS) and a corresponding histologic gastritis score (HGS).The CGS for normal mucosa, and mild, moderate and marked active inflammation was 0, 1, 2 and 3, respectively. The severity of histological activity and chronic inflammation was also defined as normal, mild, moderate and marked with a score of 0-3, respectively. Finally, the mean CGS and HGS (mCGS and mHGS) were calculated for specimens from each stomach.

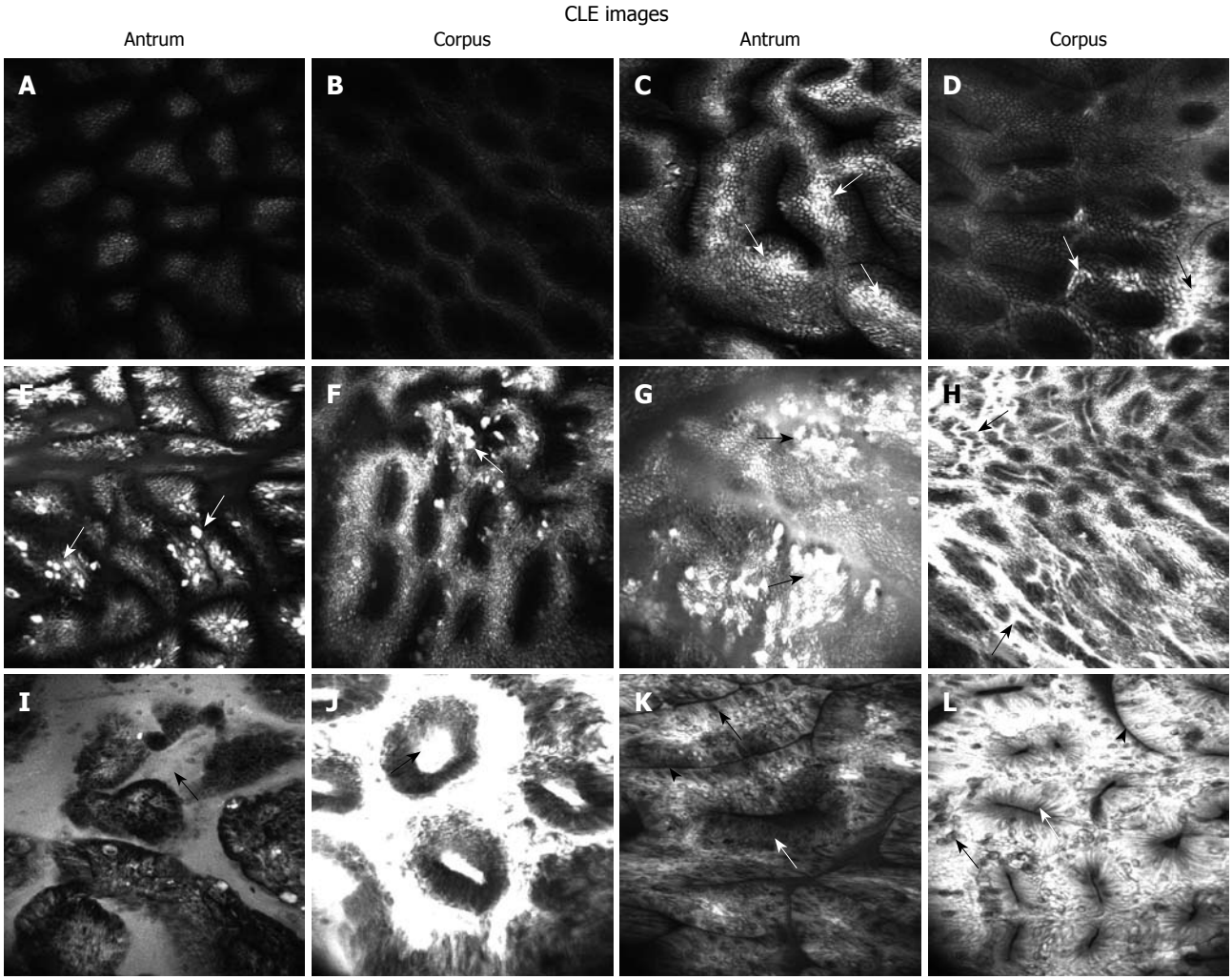

Figure 1 Confocal laser endomicroscopy classification of *Helicobacter pylori*-associated gastritis severity in gastric antrum and corpus. A, B: Normal mucosa with normal antral and corporal pits, and free of fluorescein leakage; C, D: Active inflammation (mild) with slightly distorted pits and intact epithelium, scattered focal fluorescein leakage (arrows); E, F: Active inflammation (moderate) with more distorted pits and partly destroyed epithelium (arrows), and more fluorescein leakage; G, H: Active inflammation (marked) with markedly distorted pits and dilated opening, destroyed epithelium (arrows), and widespread fluorescein leakage; I, J: Glandular atrophy with decreased gastric pits and markedly dilated opening (arrows); K, L: Intestinal metaplasia with villous-like gastric pits and goblet cells (black arrows), absorptive cells (white arrows) and brush border (arrowheads) appearing. CLE: Confocal laser endomicroscopy.

CLE criteria for glandular atrophy and IM: Glandular atrophy and IM are the severest stage of chronic gastritis. The CLE classification of glandular atrophy and IM was defined as decreased gastric pits with a dilated opening and as villous-like gastric pits with goblet cells, absorptive cells and brush border, respectively (Figure 1). To assess the CLE images, the mean number of gastric pits on each grade image was calculated and compared, except for that on IM images. The clinical values for IM features on CLE images were assessed by calculating their mean ratio present in each IM site on CLE images.

Final diagnosis of H. pylori infection

Rapid urease testing and Giemsa staining confirmed *H. pylori* infection. If only one test was positive for *H. pylori* infection, ^{13}C-urea breath test was performed for further confirmation. The *H. pylori* infection was classified into Hp (-), Hp (+), Hp (++) and Hp (+++).

Histopathology

All specimens were fixed in 10% formalin. An experienced histopathologist analyzed the histological features of each sample with hematoxylin and eosin staining and made the diagnosis according to the updated Sydney classification. The histological parameters assessed in this study included histological activity, chronic inflammation, glandular atrophy and intestinal metaplasia, which were identified blinding to the results of CLE or WLE.

Statistical analysis

Data were collected from CLE images and histological examination. analysis of variance and box-plot analysis of mCGS were used in pairwise comparison of mCGS among the 4 *H. pylori* test groups [Hp (-), Hp (+), Hp (++) and Hp (+++)]. $P < 0.05$ was considered statistically significant. The efficacy of CGS for predicting *H. pylori* infection was evaluated by area under the receiver oper-

Table 1 Characteristics of patients enrolled in this study

Patients	118
Gender	
Female	44
Male	74
Age (yr), median (range)	49.8 (19-67)
H. pylori infection (mCGS, mean ± SD)	
Positive	41
+	15 (1.11 ± 0.53)
++	15 (1.68 ± 0.51)
+++	11 (2.42 ± 0.45)
Negative	77 (0.61 ± 0.39)
Indication for CLE	
Upper GI symptoms	87
Screened for gastric cancer	31
Endoscopic diagnosis	
Normal stomach	6
Gastritis	92
Peptic ulcer	5
Polyps	13
Early gastric cancer	2

H. pylori: *Helicobacter pylori*; mCGS: Mean confocal gastritis score; CLE: Confocal laser endomicroscopy; GI: Gastrointestinal.

ating characteristic (ROC) curve analysis. On the other hand, the sensitivity, specificity, positive and negative predictive values (PPV, NPV) of the CLE criteria for diagnosing normal gastric mucosa, *H. pylori*-associated active inflammation, glandular atrophy and IM were calculated, respectively. Correlation between mCGS and mHGS was analyzed with the coefficient of determination. SPSS v16.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses.

RESULTS

Collection of data

One hundred and eighteen patients including 74 males, at a mean age of 49.8 years (range 19-67 years) were enrolled in this study. A total of 12243 CLE images (mean 104 images per patient) and 653 optical biopsy images (mean 5.5 biopsy images per patient) were obtained. The median endoscopy time was 23.2 min (range 15-33 min) (Table 1). The quality of over 90% of CLE images was good, while that of the remaining 10% was not satisfactory because it was difficult to fix the distal tip on the gastric angle and the artifacts were motile in gastrointestinal tract. No endoscopic complications or adverse reactions to fluorescein were observed.

CLE diagnosis of H. pylori infection

Association between mCGS and *H. pylori* infection: Of the 118 patients enrolled in this study, 41 were positive for *H. pylori*. Detailed data on *H. pylori* level are shown in Table 1. The mCGS was significantly differed among Hp (-), Hp (+),Hp (++), and Hp (+++) groups ($P < 0.001$). The median mCGS was significantly higher in Hp (+) and Hp (+++) groups than in Hp (-) and Hp (++) groups ($P < 0.001$), and higher in Hp (++) group than in Hp (+) and Hp (-) groups ($P < 0.001$) (Figure 2).

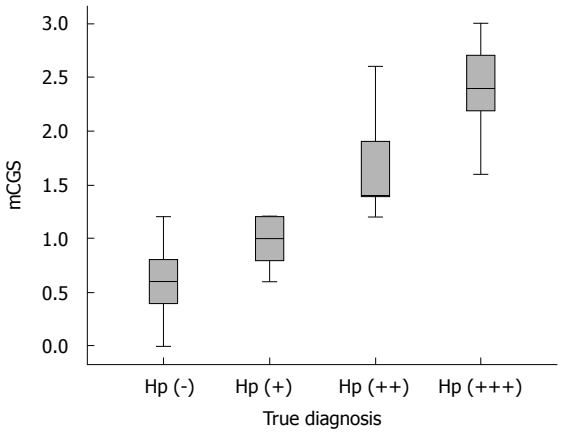

Figure 2 Box plot analysis of mean confocal gastritis score in 4 *Helicobacter pylori* test groups. mCGS: Mean confocal gastritis score.

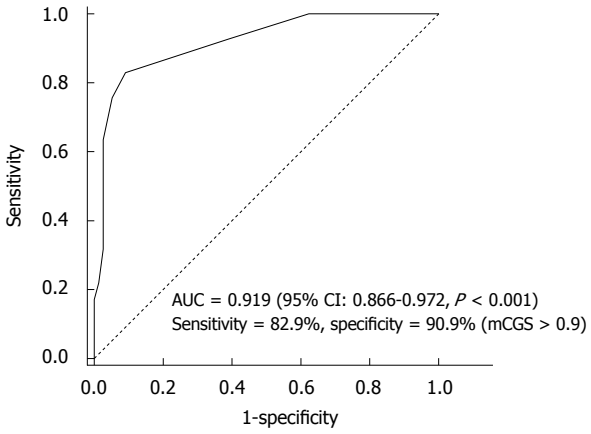

Figure 3 Receiver operating characteristic curve analysis showing the sensitivity and specificity of confocal laser endomicroscopy and mean confocal gastritis score for diagnosing *Helicobacter pylori* infection. AUC: Area under curve; mCGS: Mean confocal gastritis score.

Sensitivity and specificity of CLE for *H. pylori* detection: The ROC curve for mCGS was plotted to determine a cut-off value of sensitivity relative to specificity for diagnosing *H. pylori* infection. The area under the ROC curve was 0.919 (95% CI: 0.866-0.972), showing an excellent accuracy (Figure 3). The sensitivity and specificity of CEL were 82.9% and 90.9%, respectively, for mCGS > 0.9. With this cut-off value, CLE could correctly diagnose *H. pylori* infection.

CLE classification of histological gastritis severity

Normal gastric mucosa: A good correlation was found between normal mucosa detected by CLE and histology. The sensitivity, specificity, PPV and NPV of CLE were 94.6% (95% CI: 91.9%-97.4%), 97.4% (95% CI: 95.8%-99.1%), 96.5% (95% CI: 94.2%-98.7%), and 96.1% (95% CI: 94.1%-98.1%), respectively, for predicting gastric normal mucosa lesions (Table 2).

Chronic active inflammation: The CLE criteria correlated well with the active inflammation detected by histology. The sensitivity, specificity, PPV and NPV of CLE

Table 2 Sensitivity, specificity, positive predictive values and negative predictive values of confocal laser endomicroscopy for the diagnosis of normal mucosa, *Helicobacter pylori*-associated active inflammation, glandular atrophy and intestinal metaplasia (per specimen) [% (95% CI)]

CLE classification	Sensitivity	Specificity	PPV	NPV
Normal mucosa	94.6 (91.9-97.4)	97.4 (95.8-99.1)	96.5 (94.2-98.7)	96.1 (94.1-98.1)
Active inflammation	98.5 (97.2-99.8)	94.6 (91.9-97.4)	95.9 (93.7-97.9)	98.0 (96.3-99.7)
Glandular atrophy	92.9 (87.0-98.9)	95.2 (93.3-97.0)	72.5 (63.4-81.7)	98.9 (98.1-99.9)
Intestinal metaplasia	98.6 (95.7-100.0)	100 (100.0-100.0)	100 (100.0-100.0)	99.8 (99.4-100.0)

CLE: Confocal laser endomicroscopy; PPV: Positive predictive value; NPV: Negative predictive value.

Table 3 Confocal laser endomicroscopy and white-light endoscopy in predicting histological changes

Biopsy sites	CLE normal sites[1]	CLE abnormal sites[1]			
		Inflammation	Atrophy	IM	Early cancer
WLE normal sites	216	173	41	45	0
WLE abnormal sites[2]	31	83	25	23	2

[1]The normal and abnormal sites on confocal laser endomicroscopy (CLE) images were confirmed by histology; [2]Abnormal sites include erythema, erosion, nodules and petechiae, *etc.*, with no noticeable lesions such as ulcers or polyps on white light endoscopy (WLE) images. IM: Intestinal metaplasia.

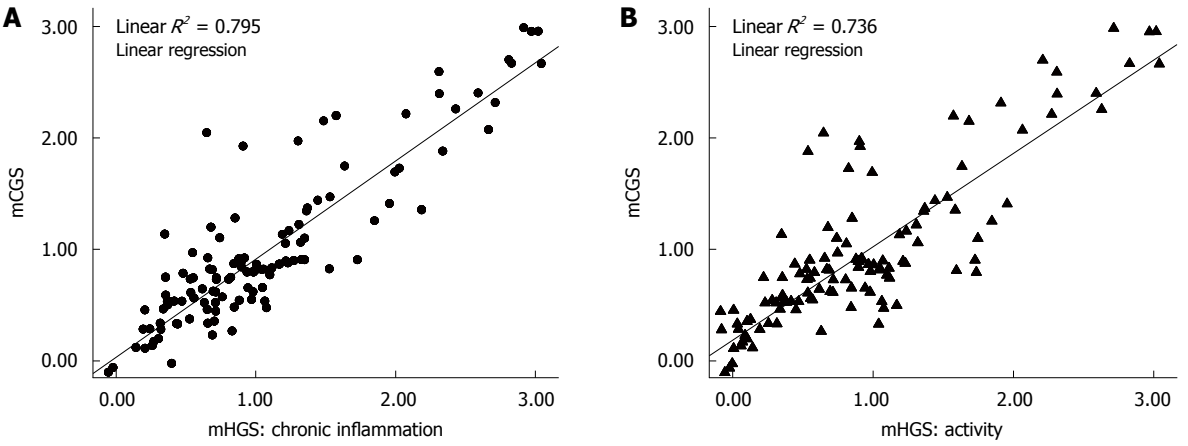

Figure 4 Correlation between mean confocal gastritis score and mean histologic gastritis score for histological chronic inflammation (R^2 = 0.795, A) and activity (R^2 = 0.736, B). mCGS: Mean confocal gastritis score; mHGS: Mean histologic gastritis score.

were 98.5% (95% CI: 97.2%-99.8%), 94.6% (95% CI: 91.9%-97.4%), 95.9% (95% CI: 93.7%-97.9%), and 98.0% (95% CI: 96.3%-99.7%), respectively, for predicting active inflammation detected by histology (Table 2). In particular, the histological activity and chronic inflammation were significantly differed among the 3 levels of active inflammation on CLE images, based on the evidence that histological activity and chronic inflammation were positively and linearly correlated with the mCGS (R^2 = 0.795, R^2 = 0.736, $P < 0.001$) (Figure 4).

Glandular atrophy and IM: The sensitivity, specificity, PPV and NPV of CLE were 92.9% (95% CI: 87.0%-98.9%), 95.2% (95% CI: 3.3%-97.0%), 72.5% (95% CI: 63.4%-81.7%) and 98.9% (95% CI: 98.1%-99.9%), respectively, for glandular atrophy (Table 2). Furthermore, a post-CLE analysis of CLE images showed that the mean number of gastric pits in glandular atrophy was significantly less than that in IM on CLE images ($P < 0.001$) (Figure 5).

The sensitivity, specificity, PPV and NPV of CLE for IM were 98.6% (95% CI: 95.7%-100.0%), 100% (95% CI: 100.0%-100.0%), 100% (95% CI: 100.0%-100.0%), and 99.8% (95% CI: 99.4%-100.0%), respectively, for predicting IM (Table 2). Of the IM features on CLE images, goblet cells and absorptive cells were more common and easier to note than the other features ($P < 0.001$) (Figure 6).

CLE and WLE for predicting histological conditions: In our study, 54.5% (259/475) normal sites were found to be abnormal on WLE by CLE (173 inflamed sites, 41 atrophic sites and 45 IM sites). In contrast, of the 164 abnormal sites on WLE images, 18.9% (31/164) were found to be normal on CLE images. All the findings on CLE images were confirmed by histology, indicating that CLE can show more histological lesions than WLE ($P < 0.001$) (Table 3).

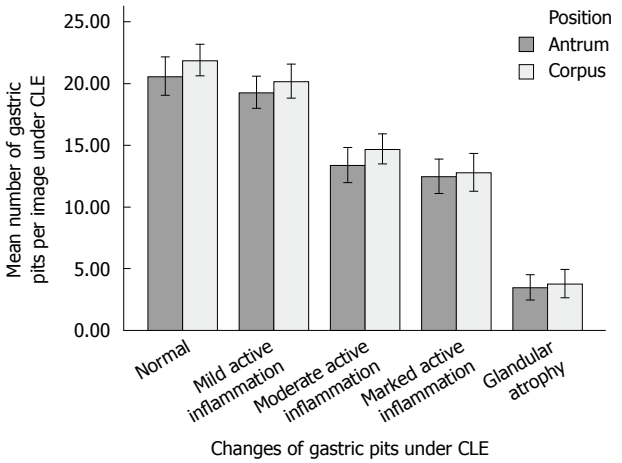

Figure 5 Gastric pits on confocal laser endomicroscopy images. CLE: Confocal laser endomicroscopy.

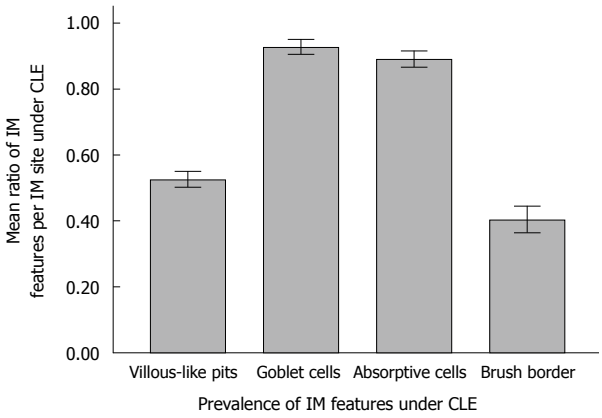

Figure 6 Prevalence of intestinal metaplasia features on confocal laser endomicroscopy images. IM: Intestinal metaplasia; CLE: Confocal laser endomicroscopy.

DISCUSSION

Conventional endoscopy is not frequently correlated with histological alterations, WLE is not accurate for predicting histological gastritis with nonspecific erythema, prominent area gastrica, nodularity, or erosions, *etc.*[16], and gastroscopy without biopsy is incomplete in routine clinical practice. In recent years, novel endoscopies, such as magnification endoscopy and narrow band imaging, have been evaluated for gastric pathologic conditions with a good accuracy[17]. High resolution, magnification and advanced electronic dye techniques, can identify the mucosal and vascular details of *H. pylori* infection and its related complications, with a high diagnostic sensitivity and specificity[18].

More details of gastric pits can be clearly observed on CLE images at the magnification × 1000. Zhang *et al*[14] reported that CLE can reveal inflamed pits with marked morphological alterations. The sensitivity and specificity of type B'+D' pits are 81.9% and 99.3%, respectively, for predicting moderate to severe active inflammation. In our study, *H. pylori* infection was positively correlated with progressively increasing changes in gastric pits due to direct injury caused by *H. pylori* and inflammatory infiltration. CLE could accurately distinguish normal mucosa from *H. pylori*-infected mucosa in the stomach. ROC curve analysis showed a high sensitivity (82.9%) and specificity (90.9%) of mCGS for diagnosing *H. pylori* infection. The mCGS cut-off value was 0.9, indicating an accurate and balanced result. Fluorescein leakage can classify active inflammation in ulcerative colitis[19] and fluorescein leakage into crypt lumen is positively correlated with histological active inflammation, because of increased colonic permeability. Fluorescein leakage is often present in the stomach, which contributes to the diagnosis of *H. pylori*-associated gastritis. When normal gastric mucosa is observed on CLE images, the fluorescence is confined to beneath the subepithelium and evenly distributed, epithelial cells are clearly visible and well-demarcated, showing a uniform gray cytoplasm and black border (Figure 1A and B). However, in *H. pylori*-infected mucosa, fluorescein easily leaks onto the surface through the damaged epithelium or normal epithelium with a high permeability. That is why this region is brighter than the surrounding normal epithelium. Sometimes, with a mass of the seepage, the entire field of vision can be white (Figure 1C-H). However, because fluorescein leakage is only an indirect response to mucosal damage and histological inflammation, the specificity of fluorescein is lower. In our study, fluorescein in combination with other CLE features was used to diagnose *H. pylori* infection following the active inflammation criteria defined by CLE[4]. The CLE scoring system used in our study could offer an objective evaluation of the entire stomach. High mCGS of the stomach indicated severe *H. pylori* infection, whereas low mCGS indicated mild or no *H. pylori* infection. Because the CGS agrees with the grading of confocal images, a high score represents severely injured gastric mucosa. It was reported that *H. pylori* organisms are usually observed singly or in groups along the surface epithelium but rarely in deeper mucosa[20]. Adherence of *H. pylori* to epithelial cells is the first step in *H. pylori* infection. When urease is secreted by *H. pylori,* and inflammatory and immune processes are activated by Vac A and Cag A toxin, the epithelium is depleted of mucin with irregular and missing cells ("drop-out cells"). In our study, similar alterations in moderate and severe active inflammation were found on CLE images, and clusters of destroyed epithelial cells (drop-out cells) on the two grade images were closely correlated with *H. pylori* infection, suggesting that CLE can display the drop-out cells because such microscopic lesions may progress to microerosions and precursors of gastric ulcer. However, when mCGS is less than 0.9, it can result in 7 false-positive and 7 false-negative diagnoses. False-positive cases are mainly diagnosed as mild active inflammation based on the CLE images of antrum, because normal mucosa on CLE images is sometimes misdiagnosed as mild inflammation when some fibrin or debris appears on the mucosal surface. The 7 false-negative cases are diagnosed as mild inflammation with a low *H. pylori* level and the corresponding mucosal changes are thus not obvious to be identified.

The second endpoint was to demonstrate the histologi-

cal severity of gastritis by CLE, which showed that the 6 CLE grades were well correlated with the corresponding parameters of histological gastritis. Active inflammation was classified into 3 levels by CLE. On the other hand, obvious drop-out cells were observed at the moderate and marked levels with a high risk for gastric ulcer formation and management of these groups of patients. The severity of chronic inflammation was much higher at moderate and marked levels on CLE images (R^2 = 0.736 and 0.795), probably because interstitial infiltration of inflammatory cells can easily squeeze the glands and damage the epithelium with an increasing severity of inflammation and *H. pylori* infection, leading to aggravated pit distortion and fluorescein leakage.

Gastric glands would be destroyed, and glandular atrophy and/or intestinal metaplasia would occur when they are infected with *H. pylori* and infiltrated with inflammatory cells. Glandular atrophy is manifested as thinner mucosa without gastric rugae but with visibly vascular pattern on WLE images[21]. However, gastric atrophy is poorly correlated with histological atrophy on WLE images[22]. It was reported that CLE shows a good sensitivity and specificity for gastric atrophy[14]. In our study, the diagnostic sensitivity and specificity of CLE were 92.9% and 95.2% for glandular atrophy. A further analysis of CLE-diagnosed glandular atrophy was performed with a more objective method. The mean number of gastric pits was calculated on different CLE images, and the mean number of pits in glandular atrophy was significantly less than that in gastritis on CLE images ($P < 0.001$). However, IM on CLE images was not analyzed because the gastric pits were replaced by intestinal mucosa, which became another form of atrophy or metaplasia. Although the sensitivity and specificity of CLE were high for glandular atrophy, the PPV was relatively low (72.5%) probably due to the low prevalence of atrophy in histology (71 sites/590 sites, 12.0%), indicating that there is a certain false positive rate when CLE is used to diagnose glandular atrophy, and identification of mild glandular atrophy is a big challenge for CLE.

Although the Sydney system has suggested a definition of endoscopic intestinal metaplasia for "grey-white patches with a slight opalescent tinge and/or a villous appearance on close inspection", studies demonstrated that these features are quite difficult to be identified by conventional endoscopy[21,23]. The detection of IM needs multiple biopsy specimens. Tahara *et al*[18] reported that magnifying narrow-band imaging endoscopy can show IM with a sensitivity of 73.3% and a specificity of 95.6%. In this study, IM was further analyzed following the CLE criteria[15]. The sensitivity and specificity of CLE were 98.6% and 100%, respectively, for IM in our study, and were higher than those reported by Tahara *et al*[18]. In addition, goblet cells and absorptive cells were more frequently and easily observed than other features or parameters in IM on CLE images in our study ($P < 0.001$). Because diagnosis of IM is often disregarded by naked eyes or by chromoendoscopy in clinical practice, more powerful endoscopic techniques, such as CLE and magnifying endoscopy, should be used to map IM[24].

Microscopic examination is essential for endoscopists to make a final diagnosis of gastritis, because the term of gastritis has been used indiscriminately and whether histological inflammation is present or not. However, CLE plays a pathological role in improving the diagnostic rate of WLE. In this study, CLE could discover 54.5% of lesions missed by WLE and correct 18.9% of normal mucosal sites misdiagnosed as abnormal sites by WLE, because the micro-gastric inflammation may appear completely normal on WLE images, suggesting that the presence or absence of gastritis should not be determined only based on gross WLE images. However, CLE can detect these micro-changes.

The main limitations of our study are as follows. First, we did not establish a control group, or a follow-up group to validate our hypothesis from multiple aspects, leading some bias in determination of *H. pylori* infection and histological gastritis. Second, we did not evaluate inter- and intra-observer variability. The repeatability of our CLE criteria should be assessed in a future single study. Third, due to the scanning depth and resolution limitations of CLE, there is still some gap when compared with *in vitro* histology. Therefore, the CLE equipment needs to be constantly upgraded.

In conclusion, *H. pylori* infection is related to its related CLE image features. Histologically active inflammation can be classified by CLE. Two most accurate markers, goblet cells and absorptive cells for IM, are established. Although CLE itself still has some limitations as a novel technique, it is a promising new procedure for accurate histological assessment *in vivo*.

COMMENTS

Background

The accurate diagnosis of chronic gastritis still relies on histopathology. Confocal laser endomicroscopy (CLE) can show gastric pit patterns and epithelial cells *in vivo*, thus, contributing to an accurate diagnosis of chronic gastritis *in vivo*.

Research frontiers

CLE has been extensively investigated in cancerous or precancerous mucosa of Barrett's esophagus and its related neoplasia, early esophageal cancer, gastric intestinal metaplasia, neoplasia and early cancer, colonic neoplasia and cancer, and ulcerative colitis, *etc*.

Innovations and breakthroughs

Endoscopic diagnosis of chronic gastritis and *Helicobacter pylori* (*H. pylori*) infection is still challenge to clinical endoscopists. This study first used CLE to classify *H. pylori* infection-associated chronic gastritis, which could accurately diagnose chronic gastritis *in vivo* without mutiple biopsy specimens.

Applications

By using the classification criteria for chronic gastritis established in this study, clinical endoscopits could use CLE to evaluate the conditions of the entire stomach, and calculate the abnormal mucosal sites in the entire stomach.

Terminology

CLE is novel digestive endoscope. It is a conventional white-light endoscope with a confocal laser microscope integrated into the distal tip. CLE can realize a histological-like examination during routine endoscopy and accurately diagnose gastrointestinal diseases at the magnification × 1000.

Peer review

The authors established the classification criteria for chronic gastritis, and compared CLE and white light endoscopy in diagnosis of gastritis and intestinal metaplasia, thus proving a novel method for the diagnosis of gastritis and intestinal metaplasia by CLE, which has a higher sensitivity and specificity than conventional endoscopy.

REFERENCES

1 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789

2 **Goldblum JR**, Vicari JJ, Falk GW, Rice TW, Peek RM, Easley K, Richter JE. Inflammation and intestinal metaplasia of the gastric cardia: the role of gastroesophageal reflux and H. pylori infection. *Gastroenterology* 1998; **114**: 633-639

3 **Fox JG**, Correa P, Taylor NS, Zavala D, Fontham E, Janney F, Rodriguez E, Hunter F, Diavolitsis S. Campylobacter pylori-associated gastritis and immune response in a population at increased risk of gastric carcinoma. *Am J Gastroenterol* 1989; **84**: 775-781

4 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181

5 **Leung WK**, Lin SR, Ching JY, To KF, Ng EK, Chan FK, Lau JY, Sung JJ. Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on Helicobacter pylori eradication. *Gut* 2004; **53**: 1244-1249

6 **Carpenter HA**, Talley NJ. Gastroscopy is incomplete without biopsy: clinical relevance of distinguishing gastropathy from gastritis. *Gastroenterology* 1995; **108**: 917-924

7 **Wong BC**, Lam SK, Ching CK, Hu WH, Kwok E, Ho J, Yuen ST, Gao Z, Chen JS, Lai KC, Ong LY, Chen BW, Wang WH, Jiang XW, Hou XH, Lu JY. Differential Helicobacter pylori infection rates in two contrasting gastric cancer risk regions of South China. China Gastric Cancer Study Group. *J Gastroenterol Hepatol* 1999; **14**: 120-125

8 **Yagi K**, Nakamura A, Sekine A. Characteristic endoscopic and magnified endoscopic findings in the normal stomach without Helicobacter pylori infection. *J Gastroenterol Hepatol* 2002; **17**: 39-45

9 **Bansal A**, Ulusarac O, Mathur S, Sharma P. Correlation between narrow band imaging and nonneoplastic gastric pathology: a pilot feasibility trial. *Gastrointest Endosc* 2008; **67**: 210-216

10 **Anagnostopoulos GK**, Yao K, Kaye P, Fogden E, Fortun P, Shonde A, Foley S, Sunil S, Atherton JJ, Hawkey C, Ragunath K. High-resolution magnification endoscopy can reliably identify normal gastric mucosa, Helicobacter pylori-associated gastritis, and gastric atrophy. *Endoscopy* 2007; **39**: 202-207

11 **Kitabatake S**, Niwa Y, Miyahara R, Ohashi A, Matsuura T, Iguchi Y, Shimoyama Y, Nagasaka T, Maeda O, Ando T, Ohmiya N, Itoh A, Hirooka Y, Goto H. Confocal endomicroscopy for the diagnosis of gastric cancer in vivo. *Endoscopy* 2006; **38**: 1110-1114

12 **Dunbar KB**, Okolo P 3rd, Montgomery E, Canto MI. Confocal laser endomicroscopy in Barrett's esophagus and endoscopically inapparent Barrett's neoplasia: a prospective, randomized, double-blind, controlled, crossover trial. *Gastrointest Endosc* 2009; **70**: 645-654

13 **Kiesslich R**, Goetz M, Burg J, Stolte M, Siegel E, Maeurer MJ, Thomas S, Strand D, Galle PR, Neurath MF. Diagnosing Helicobacter pylori in vivo by confocal laser endoscopy. *Gastroenterology* 2005; **128**: 2119-2123

14 **Zhang JN**, Li YQ, Zhao YA, Yu T, Zhang JP, Guo YT, Liu H. Classification of gastric pit patterns by confocal endomicroscopy. *Gastrointest Endosc* 2008; **67**: 843-853

15 **Guo YT**, Li YQ, Yu T, Zhang TG, Zhang JN, Liu H, Liu FG, Xie XJ, Zhu Q, Zhao YA. Diagnosis of gastric intestinal metaplasia with confocal laser endomicroscopy in vivo: a prospective study. *Endoscopy* 2008; **40**: 547-553

16 **Elta GH**, Appelman HD, Behler EM, Wilson JA, Nostrant TJ. A study of the correlation between endoscopic and histological diagnoses in gastroduodenitis. *Am J Gastroenterol* 1987; **82**: 749-753

17 **Yagi K**, Honda H, Yang JM, Nakagawa S. Magnifying endoscopy in gastritis of the corpus. *Endoscopy* 2005; **37**: 660-666

18 **Tahara T**, Shibata T, Nakamura M, Yoshioka D, Okubo M, Arisawa T, Hirata I. Gastric mucosal pattern by using magnifying narrow-band imaging endoscopy clearly distinguishes histological and serological severity of chronic gastritis. *Gastrointest Endosc* 2009; **70**: 246-253

19 **Li CQ**, Xie XJ, Yu T, Gu XM, Zuo XL, Zhou CJ, Huang WQ, Chen H, Li YQ. Classification of inflammation activity in ulcerative colitis by confocal laser endomicroscopy. *Am J Gastroenterol* 2010; **105**: 1391-1396

20 **Smith VC**, Genta RM. Role of Helicobacter pylori gastritis in gastric atrophy, intestinal metaplasia, and gastric neoplasia. *Microsc Res Tech* 2000; **48**: 313-320

21 **Tytgat GN**. The Sydney System: endoscopic division. Endoscopic appearances in gastritis/duodenitis. *J Gastroenterol Hepatol* 1991; **6**: 223-234

22 **Capelle LG**, de Vries AC, Haringsma J, Ter Borg F, de Vries RA, Bruno MJ, van Dekken H, Meijer J, van Grieken NC, Kuipers EJ. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. *Gastrointest Endosc* 2010; **71**: 1150-1158

23 **Guarner J**, Herrera-Goepfert R, Mohar A, Sanchez L, Halperin D, Ley C, Parsonnet J. Interobserver variability in application of the revised Sydney classification for gastritis. *Hum Pathol* 1999; **30**: 1431-1434

24 **Wo JM**, Ray MB, Mayfield-Stokes S, Al-Sabbagh G, Gebrail F, Slone SP, Wilson MA. Comparison of methylene blue-directed biopsies and conventional biopsies in the detection of intestinal metaplasia and dysplasia in Barrett's esophagus: a preliminary study. *Gastrointest Endosc* 2001; **54**: 294-301

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BRIEF ARTICLE

Smad7 dependent expression signature highlights BMP2 and HK2 signaling in HSC transdifferentiation

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Abstract

AIM: To analyse the influence of Smad7, antagonist of transforming growth factor (TGF)-β canonical signaling pathways on hepatic stellate cell (HSC) transdifferentiation in detail.

METHODS: We systematically analysed genes regulated by TGF-β/Smad7 in activated HSCs by microarray analysis and validated the results using real time polymerase chain reaction and Western blotting analysis.

RESULTS: We identified 100 known and unknown targets underlying the regulation of Smad7 expression and delineated 8 gene ontology groups. Hk2, involved in glycolysis, was one of the most downregulated proteins, while BMP2, activator of the Smad1/5/8 pathway, was extremely upregulated by Smad7. However, BMP2 dependent Smad1 activation could be inhibited *in vitro* by Smad7 overexpression in HSCs.

CONCLUSION: We conclude (1) the existence of a tight crosstalk of TGF-β and BMP2 pathways in HSCs and (2) a Smad7 dependently decreased sugar metabolism ameliorates HSC activation probably by energy withdrawal.

Key words: Transforming growth factor-β; Smad7; Hepatic stellate cell; Gene regulation; Glucose metabolism; BMP2

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INTRODUCTION

Histopathological changes of chronic liver diseases usually

start with inflammatory hepatitis, followed by fibrosis and the final stage of cirrhosis, possibly leading to liver cancer. Hepatic fibrosis is characterized by increased and altered deposition of newly generated or deficiently degraded extracellular matrix (ECM) in response to injury[1]. Hepatic stellate cells (HSCs) are the major fibrotic precursor cells that transdifferentiate in inflammatory liver tissue to fibrogenic myofibroblasts (MFBs), by undergoing morphological changes, increased expression of α-SMA and synthesis of large amounts of ECM components[2].

Transdifferentiation of HSCs is driven by a variety of cytokines with transforming growth factor (TGF)-β playing a master role. It stimulates quiescent HSCs by paracrine and transdifferentiated MFBs by autocrine mechanisms activating intracellular Smad cascades. A great variety of cytokines, chemokines and mitogens (TNF-α, IFN-γ, EGF, PDGF, CTGF, ID1, YB1) display complex crosstalk with TGF-β[3-6].

Smad7 is a powerful antagonist of TGF-β in HSCs blunting downstream signaling by inhibiting receptor (R)-Smad phosphorylation[7]. In quiescent HSCs, expression of Smad7 itself is induced by the R-Smad cascade, thereby providing a negative feedback loop to terminate TGF-β signals[8]. We demonstrated before phenotypically and functionally that overexpressed Smad7 inhibits HSC transdifferentiation and attenuates the extent of fibrosis[7] suggesting that Smad7 is a promising antifibrotic tool for treatment approaches.

Therefore, in this study we analyzed the influence of Smad7 on the HSC gene expression pattern in great detail using microarray analysis. Its overexpression affects a great variety of cellular pathways involved in development, angiogenesis, differentiation, transcription, immune response, apoptosis, proliferation, signal transduction, ion and electron transport, sugar and lipid metabolism, morphogenesis, protein synthesis and modification, DNA synthesis and repair, cell adhesion, stress response, blood circulation, cell cycle and growth, cell motility, muscle contraction and organization of the cytoskeleton. The strongest regulated proteins are Pla2g2a, Cyp4b1, both upregulated and, Hk2 and VEGFa, which were downregulated significantly. Interestingly, BMP2, a member of the TGF-β family and alternative activator of the Smad1/5/8 pathway, was strongly induced by Smad7 overexpression in HSCs.

MATERIALS AND METHODS

Affymetrix gene chip array

Primary HSCs of male Sprague-Dawley were isolated as previously described[9,10]. To identify Smad7 dependent gene responses, HSCs were infected with adenoviruses encoding for Smad7 (AdSmad7; kindly provided by C. Heldin (Ludwig Institute for Cancer Research, Uppsala, Sweden)) or LacZ (AdLacZ) as control 2 d after seeding[7].

RNA sample collection and generation of biotinylated complementary RNA probes was carried out according to the Affymetrix GeneChip® Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA,USA). In brief, total RNA was prepared at day 4 from 5×10^6 cultured primary HSCs that were infected with AdLacZ or AdSmad7 at day 2. Twenty five micrograms total RNA was reversely transcribed into double-stranded cDNA using HPLC-purified T7-(dt) 24 primers (MWG, Ebersberg, Germany) and the Superscript choice cDNA synthesis system (Invitrogen Corp., Carlsbad, CA). Purified cDNA was used to synthesize biotinylated complementary RNA using the BioArray High Yield RNA Transcription Labeling Kit (Enzo Diagnostics, Enzo Life Science Inc., Farmingdale, NY, USA). Each sample was hybridized to an Affymetrix rat Genome RG-U34A microarray (8799 probe sets) for 16h at 45℃. Expression values of each probe set were determined and AdSmad7 infected samples were compared to AdLacZ infected controls using the Affymetrix Microarray Suite 5.0 software.

Intensities across multiple arrays were normalized to a target intensity of 2500 using global normalization scaling. Two separate experiments with HSCs from different animals were performed under identical conditions. Genes whose expression levels were changed more than 2-fold with $P < 0.001$ in both experiments were considered to be significantly regulated by Smad7. These genes were investigated according to their molecular function and biological process by searching the gene ontology (GO) term database. Genes differentially expressed in AdSmad7 treated compared to controls were classified by "pathway" analysis [KEGG (http://www.genome.jp/kegg/pathway.html), PathwayArchitect, Stratagene].

Reverse-transcription and quantitative real-time polymerase chain reaction

Total RNA was collected from 3 (3 d-) or 7 d old (7 d-)HSCs, which were either infected with AdSmad7 or AdLacZ 2 d earlier or were uninfected[11]. cDNA from cell culture samples was synthesized as described[11]. Quantitative real-time polymerase chain reaction (RT-qPCR) was performed as in[11] with modified conditions: 95℃ for 60 s, then 40 cycles (50 cycles for low copy genes) of 95℃ for 10 s, 60℃ for 10 s and 72℃ for 15 s. Annealing temperature was set at 58℃ for U92564 and 62℃ for rat VEGF.1. Primers are listed in Table 1. The quantity of target mRNA was determined using a TGF-β RI standard curve[11]: A cDNA fragment was amplified and column-purified using the QIAquick PCR purification kit (Qiagen) and the following primers: TGFβ RI (GI: 416397) 180 bp; F (5'-CGTCTGCATTGCACTTATGC-3'), R (5'-AGCAGTGGTAAACCTGATCC-3'). A standard curve was generated from serial 10 time logarithmic dilutions of the cRNA by reverse transcription.

Western blotting analysis

Isolated primary HSCs of female Wistar rats were cultured as in[7]. Following overnight starvation (0.5% FCS) HSCs were stimulated with 5 ng/mL human recombinant TGF-β (Peprotech, Hamburg, Germany) or 20 ng/mL BMP2 (R&D, Minneapolis, MN), respectively.

Table 1 Primer used for quantitative real-time polymerase chain reaction validation of array results

Gene	Probe set ID	Forward	Reverse
CYP4B1	M29853	5'CCGAAGGCTGCAGATGTGT3'	5'TTTGGCCCATCCAGAACTAGTAG3'
mSmad7		5'GGTGCTCAAGAAACTCAAGG3'	5'CAGCCTGCAGTCCAGGCG3'
BMP2	L02678_at	5'TGCCCCCTAGTGCTTCTTAGAC3'	5'GGGAAGCAGCAACACTAGAAGAC3'
SGⅢ	U02983	5'CAAGCAGGACCGAGAATCAG3'	5'CGTTGGACAAGGTCAAGGTG3'
Zfp423	U92564	5'GCAGTGCTACACCTGACTCG3'	5'GTCATCCCGCATCTTCTTCTG3'
Pla2g2a	x51529	5'GCTCAATTCAGGTCCAGGG3'	5'CCACCCACACCACAATGG3'
EST189231	AA799734	5'CGGCTCACTGAGCTTGAAGTAG3'	5'ACACGACGGAGGAGCTTCTG3'
Olr1	AB005900	5'CAGAGAGAACTGAAGGAACAG3'	5'GGACCTGAAGAGTTTGCAGC3'
ID1	L23148_g_at	5'TGGACGAACAGCAGGTGAAC3'	5'TCTCCACCTTGCTCACTTTGC3'
HK2	D26393exon_s_at	5'CTCAGAGCGCCTCAAGACAAG3'	5'GATGGCACGAACCTGTAGCA3'
Slc16a3	U87627	5'CTCATCGGACCCCCATCAG3'	5'CGCCAGGATGAACACATACTTG3'
ratVEGF.1		5'TGCCAAGTGGTCCCAGGC3'	5'ATTGGACGGCAATAGCTGCG3'

For Smad7 overexpression studies, HSCs were infected on day 3 or day 6 with 50 IFU/cell (infectious units) Smad7 encoding adenovirus for 24 hr in medium containing 5% FBS. Four days old HSCs are considered to be in the transactivation process, while 7 d old HSCs are considered to be fully activated. After infection cells were serum-starved overnight and stimulated with 5 ng/mL TGF-β1 or 20 ng/mL BMP2. Generally, more than 90% of HSCs were infected.

For Western blotting analysis 20 μg protein was separated (4%-12% Bis-Tris Gel, NuPAGE, Invitrogen) and transferred to nitrocellulose membranes (Pierce, Rockford, IL). Nonspecific binding was blocked with 5% milk/TBST for Smad7 and GAPDH (Santa Cruz, CA, USA) or 5% BSA/TBST for pSmad1/3 antibodies (Epitomics/Biomol). Horseradish peroxidase-linked goat anti-rabbit antibody (Santa Cruz, CA, USA) served as secondary antibody. Membranes were developed with Supersignal Ultra (Pierce, Hamburg, Germany).

RESULTS

Smad7 dependent gene expression pattern

At day 2 of culture, primary rat HSCs were infected with AdSmad7 or AdLacZ (control). Two days later, when HSCs are in the process of transdifferentiation, the expression of genes displayed on 8799 probe sets was compared between cells overexpressing Smad7 and controls. Confirming Western blotting analysis of HSC lysates[12], microarray data revealed tremendous overexpression of Smad7 in AdSmad7 infected HSCs (40.79 times).

One hundred and twenty-nine probe sets were found differentially expressed due to Smad7, including 10 unknown proteins, 1 predicted protein and 89 known proteins (Table 2 provides a full list). According to their biological role, these genes were classified into eight main GO groups (Figure 1A). 37% of the regulated genes are involved in development. 22% can be assigned to signal transduction processes, which was expected since Smad7 represses TGF-β signaling and thus has impact on manifold different cross-talking signaling pathways. 15% refer to multicellular organismal processes (i.e. processes involved in intercellular interaction of any kind), 35% to response to stimulus, 21% to localization, 38% to metabolic processes, 25% to cell differentiation and 5% to cell adhesion. Note that the total percentage is greater than 100% as some regulated genes can be assigned to different ontology groups. A similar classification of all differentially expressed genes was carried out according to their molecular function (Figure 1B). Figures 2 and 3 graphically summarize regulation of all genes according to their ontology groups.

In general, many known mediators of TGF-β signaling were differentially expressed in AdSmad7 infected HSCs, confirming a direct link of Smad7 effects to TGF-β signalling (Table 2). ECM proteins like Col1a1 and Fn1 which are induced during HSC activation and fibrogenesis were negatively regulated upon Smad7 overexpression. Further profibrogenic cytokines like CXCL10 and HGF were upregulated. In addition, Cyp proteins like Cyp1b1, Cyp2E1 and Cyp4B1, Id proteins 1, 2, and 3, as well as PDGFR A were identified as Smad7 dependent in activated HSCs. Unexpectedly, several genes involved in glucose metabolism, so far annotated as predominantly associated with hepatocytes were influenced by Smad7 overexpression in HSCs.

As expected, Smad7 led to an opposite regulation of a number of recently systematically identified genes induced during HSC activation[14]. Table 3 contains a complete list of proteins identified to be regulated in both studies. In total, 37 genes of our study overlapped with the array results reported by[14]. Twenty-two of those (60%), e.g. HK2, were induced during activation[14] and decreased by Smad7 (this study) and therefore probably represent profibrogenic TGF-β target genes. There were also a few genes strongly upregulated by Smad7, which were downregulated during *in vivo* HSC activation, e.g. BMP2. Some of the proteins found to be differently regulated by activation *vs* Smad7 overexpression are already known to be TGF-β target genes and related to fibrogenesis, i.e. BMP2, Cnn1, Col1a1, Ddah1, Fn1, Lox, Pdgfra, Slc2a1, Slc16a3, and VEGF. Others might represent yet unidentified target genes of profibrogenic TGF-β signaling and/or new markers of HSC activation. Their specific influence on HSC transdifferentiation *in vivo* needs to be carefully investigated in future as they display potential antifibrotic target genes. In some cases De Minicis *et al*[14] reported opposite effects in regards to regulation of gene expression

Table 2 One hundred genes selected as being differentially expressed after Smad7 overexpression in hepatic stellate cells (note that some specific transcripts are detected by more than one probe set)

Official symbol	Average log2 fold	SD log2 fold	Affymetrix probe set ID	Official full name
Downregulated (n = 72)				
Acta2	-0.85	0.21	X06801cds_i_at	Smooth muscle α-actin
Ak3l1	-1.20	0.42	rc_AA891949_at	Adenylate kinase 3-like 1
Akap12	-1.00	0.57	U75404UTR#1_s_at	A kinase (PRKA) anchor protein (gravin) 12
Akr1b1	-0.70	0.42	M60322_g_at	Aldo-keto reductase family 1, member B1 (aldose reductase)
Atp6v1b2	-1.10	0.14	Y12635_at	ATPase, H transporting, lysosomal V1 subunit B2
Btg1	-0.65	0.49	L26268_g_at	B-cell translocation gene 1, anti-proliferative
Clec4f	-2.40	3.39	M55532_at	C-type lectin domain family 4, member f
Cml5	-1.30	0.42	rc_AA894273_at	Camello-like 5
Cnn1	-1.30	0.57	D14437_s_at	Calponin 1
Col1a1	-1.51	0.53	M27207mRNA_s_at/rc_AI231472_s_at/U75405UTR#1_f_at/Z78279_at/Z78279_g_at	Procollagen, type 1, α 1
Cryab	-1.13	0.32	M55534mRNA_s_at/X60351cds_s_at	Crystallin, α B
Cyp1b1	-1.10	0.28	rc_AI176856_at/U09540_at/U09540_g_at	Cytochrome P450, family 1, subfamily b, polypeptide 1
Ddah1	-0.95	0.21	D86041_at	Dimethylarginine dimethylaminohydrolase 1
Dpysl2	-0.95	0.64	rc_AA875444_at	Dihydropyrimidinase-like 2
Egr2	-1.25	0.64	U78102_at	Early growth response 2
Eif4ebp1	-1.05	0.07	U05014_at	Eukaryotic translation initiation factor 4E binding protein 1
Emp1	-0.65	0.78	Z54212_at	Epithelial membrane protein 1
Eno2	-0.80	0.71	X07729exon#5_s_at	Enolase 2, γ
Ercc1	-0.75	0.49	rc_AA892791_at	Excision repair cross-complementing rodent repair deficiency, complementation group 1
EST (unknown)	-2.65	0.64	rc_AI102814_at	EST
EST (unknown)	-2.60	0.28	rc_AI230256_at	EST
EST (unknown)	-2.00	0.14	rc_AA874889_g_at	EST
EST (unknown)	-1.40	0.85	rc_AA866419_at	EST
EST (unknown)	-1.35	0.64	X62950mRNA_f_at	EST
EST (unknown)	-1.10	0.99	rc_AA859740_at	EST
EST (unknown)	-0.85	0.35	rc_AA800708_at	EST
EST (unknown)	-0.40	1.41	X62951mRNA_s_at	EST
F3	-1.85	0.92	U07619_at	Coagulation factor Ⅲ
Fabp5	-0.80	0.57	S69874_s_at	Fatty acid binding protein 5, epidermal
Fkbp1a	-0.65	0.49	rc_AI228738_s_at	FK506 binding protein 1a
Fn1	-1.15	0.36	L00190cds#1_s_at/U82612cds_g_at/X05834_at	Fibronectin 1
Fntb	-1.28	0.49	rc_AI136396_at/rc_AI230914_at	Farnesyltransferase, CAAX box, β
Gabbr1	-1.25	0.92	rc_AI639395_at	γ-aminobutyric acid (GABA) B receptor 1
Gpx3	-1.10	0.14	D00680_at	Glutathione peroxidase 3
Hig1	-0.95	0.49	rc_AA891422_at	Hypoxia induced gene 1
Hk2	-3.20	0.00	D26393exon_s_at	Hexokinase 2
Id1	-2.55	0.35	L23148_g_at	Inhibitor of DNA binding 1
Id2	-2.45	0.21	rc_AI137583_at	Inhibitor of DNA binding 2
Id3	-1.85	0.13	AF000942_at/rc_AI009405_s_at	Inhibitor of DNA binding 3
Idi1	-0.70	0.57	AF003835_at	Isopentenyl-diphosphate delta isomerase
LOC686781	-1.25	0.21	rc_AA799657_at	Similar to NFκB interacting protein 1
Lox	-1.10	0.17	rc_AA875582_at/rc_AI234060_s_at/S77494_s_at	Lysyl oxidase
Lpl	-1.20	0.71	L03294_at/L03294_g_at/rc_AI237731_s_at	Lipoprotein lipase
Lrrc59	-0.65	0.64	D13623_at	Leucine rich repeat containing 59
Lum	-0.80	0.42	X84039_at	Lumican
Ncam1	-1.35	0.78	X06564_at	Neural cell adhesion molecule 1
Olr1	-2.43	0.99	AB005900_at/AB018104cds_s_at/rc_AI071531_s_at	Oxidized low density lipoprotein (lectin-like) receptor 1
P4ha1	-0.85	0.21	X78949_at	Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), α 1 polypeptide
Pcsk6	-1.10	0.71	rc_AI230712_at	Proprotein convertase subtilisin/kexin type 6
Pfkp	-1.25	0.54	L25387_at/L25387_g_at	Phosphofructokinase, platelet
Plaur	-1.20	0.71	X71898_at	Plasminogen activator, urokinase receptor
Plod2	-1.00	0.28	rc_AA892897_at	Procollagen lysine, 2-oxoglutarate 5-dioxygenase 2
Pmepa1	-1.55	0.07	rc_AI639058_s_at	Prostate transmembrane protein, androgen induced 1
Ptk2	-0.85	0.35	S83358_s_at	PTK2 protein tyrosine kinase 2
Rasl11a	-2.25	0.49	rc_AI169372_at	RAS-like family 11 member A
Rasl11b	-1.03	0.24	rc_AA800853_at/rc_AA800853_g_at	RAS-like family 11 member B
Rcn2	-0.90	0.57	U15734_at	Reticulocalbin 2

RGD1306841	-1.10	0.14	rc_AI639203_at	Similar to RIKEN cDNA 2410006F12
RGD1310444_predicted	-1.25	0.21	rc_AA866432_at	LOC363015 (predicted)
Rgs4	-1.45	0.64	U27767_at	Regulator of G-protein signaling 4
Sc4mol	-1.10	0.45	E12625cds_at/rc_AI172293_at	Sterol-C4-methyl oxidase-like
Schip1	-1.00	0.42	rc_AA800036_at	Schwannomin interacting protein 1
Serpine1	-1.90	0.00	M24067_at	Serine (or cysteine) peptidase inhibitor, clade E, member 1
Slc12a2	-0.80	0.99	AF051561_s_at	Solute carrier family 12, member 2
Slc16a3	-2.05	0.35	U87627_at	Solute carrier family 16 (monocarboxylic acid transporters), member 3
Slc2a1	-1.15	0.35	S68135_s_at	Solute carrier family 2 (facilitated glucose transporter), member 1
Spink8	-2.55	1.48	rc_AA799734_at	Serine peptidase inhibitor, kazal type 8
Tfrc	-0.90	0.42	M58040_at	Transferrin receptor
Tnc	-0.90	0.28	U09401_s_at	Tenascin C
Tnnt2	-1.70	0.42	M80829_at	Troponin T2, cardiac
Vegfa	-2.25	1.28	L20913_s_at/M32167_g_at/rc_AA850734_at	Vascular endothelial growth factor A
Wfdc1	-1.70	0.14	AF037272_at	WAP four-disulfide core domain 1
Up-regulated (n = 28)				
Adora2a	0.85	0.35	S47609_s_at	Adenosine A2a receptor
Agtr1a	0.95	0.21	M74054_s_at/X62295cds_s_at	Angiotensin Ⅱ receptor, type 1 (AT1A)
Bmp2	2.83	1.31	L02678_at/rc_AA997410_s_at	Bone morphogenetic protein 2
Col3a1	0.90	0.42	M21354_s_at/X70369_s_at/	Procollagen, type Ⅲ, α 1
Cxcl10	1.05	0.21	U17035_s_at	Chemokine (C-X-C motif) ligand 10
Cyp2e1	1.00	0.14	M20131cds_s_at	Cytochrome P450, family 2, subfamily e, polypeptide 1
Cyp4b1	3.25	0.49	M29853_at	Cytochrome P450, family 4, subfamily b, polypeptide 1
Ednrb	0.70	0.42	rc_AA818970_s_at	Endothelin receptor type B
Ephx1	1.15	0.21	M26125_at	Epoxide hydrolase 1, microsomal
EST (unknown)	0.80	0.28	rc_AA874873_g_at	EST
EST (unknown)	0.90	0.28	rc_AI177256_at	EST
Glul	0.90	0.23	M91652complete_seq_at/rc_AA852004_s_at	Glutamate-ammonia ligase (glutamine synthase)
Hgf	1.03	0.05	E03190cds_s_at/X54400_r_at	Hepatocyte growth factor
Hsd11b1	0.95	0.49	rc_AI105448_at	Hydroxysteroid 11-β dehydrogenase 1
Igfbp3	1.15	0.30	M31837_at	Insulin-like growth factor binding protein 3
Kif4	1.05	0.07	rc_AA859926_at	Kinesin family member 4
Lhx2	0.95	0.21	L06804_at	LIM homeobox protein 2
Notch1	0.80	0.42	X57405_g_at	Notch gene homolog 1 (Drosophila)
Nr2f1	0.95	0.21	U10995_g_at	Nuclear receptor subfamily 2, group F, member 1
Pdcd4	1.00	0.14	rc_AI172247_at	Programmed cell death 4
Pdgfra	1.10	0.28	rc_AI232379_at	Platelet derived growth factor receptor, α polypeptide
Pla2g2a	3.60	0.00	X51529_at	Phospholipase A2, group ⅡA (platelets, synovial fluid)
Ptn	2.10	0.57	rc_AI102795_at	Pleiotrophin
Scg3	2.70	0.85	U02983	Secretogranin Ⅲ
Serping1	0.85	0.21	rc_AA800318_at	Serine (or cysteine) peptidase inhibitor, clade G, member 1
Smad7	5.35	1.20	AF042499_at	MAD homolog 7 (Drosophila)
Sod3	1.05	0.07	Z24721_at	superoxide dismutase 3, extracellular
Zfp423	1.85	1.47	U92564_at/U92564_g_at	Zinc finger protein 423

The average change in expression after Smad7 overexpression is given as log2 fold. SD: Square root of the variance; NFκB: Nuclear factor κB.

in "culture activated" cells compared to "*in vivo* activated" cells. This leaves a final evaluation of Smad7 influence on the regulation of these genes in HSC activation processes open.

Confirmation of array data using quantitative real-time PCR

To validate our microarray results, we selected 12 genes from array data identified as highly regulated in dependency to Smad7 for RT-qPCR analysis. Transdifferentiating (3 d in culture) and fully activated (7 d in culture) HSCs were investigated. TGF-β RI mRNA expression is not modulated during transdifferentiation[15,16] and was used as the expression reference. A synopsis of Smad7 associated modulation of gene expression, given in Figure 4 as log2 fold of LacZ, generally supports the array results. We confirmed upregulation of Cyp4B1, BMP2, SGIII, Zfp423, Pla2g2a and downregulation of EST189231, Olr1 and Id1 (Table 4) independent of time during the transdifferentiation process.

Interestingly, when comparing 3 d- with 7 d-HSCs, opposite effects of Smad7 were found for HK2 (0.38-fold in 3 d-, 3.85-fold in 7 d-HSCs), Slc16a3 (0.59-fold in 3 d, 2.25-fold in 7 d-HSCs) and VEGF.1 (0.51-fold in 3 d-, 1.07-fold in 7 d-HSCs), underlining temporal differences and modulation of the TGF-β signal during HSC activation[15].

Smad7 inhibits BMP2 dependent Smad1 expression

BMP2 was strongly upregulated in Smad7 expressing HSCs (Table 2, Figure 4A and B). Here, we further demonstrate that nevertheless Smad7 blunted BMP2 and

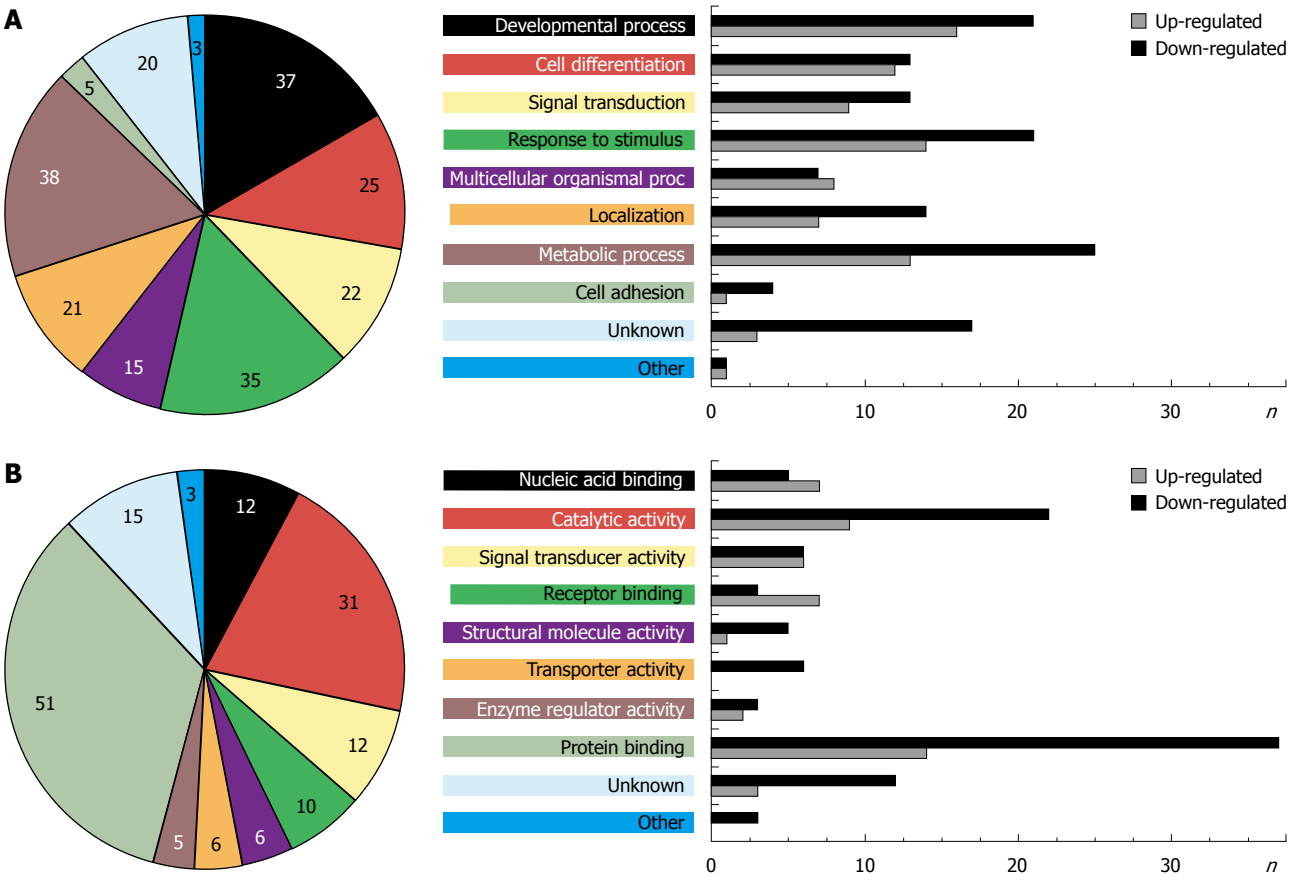


Figure 1 Genes whose expression levels were changed (*n* = 100) after overexpression of Smad7 in hepatic stellate cells are matched to 8 gene ontology annotations using GoMiner[13]. Left part: Percentage of genes matching to the given gene ontology. Note the total percentage is greater than 100% as the same regulated genes can be assigned to different annotations; Right part: Bar diagram shows number of upregulated (grey bars) and downregulated (black bars) genes matching to the given gene ontology (unknown = percentage/number of genes without annotation, other = percentage/number of genes which are not assignable to the given annotations). A: GO group: biological process; B: GO group: molecular function.

TGF-β dependent signalling *via* Smad1 upon AdSmad7 infection in transdifferentiating (4 d in culture) rat HSCs (Figure 5) and infected CFSC (data not shown). Fully activated (7 d in culture) HSCs, which are insensitive to TGF-β, remain responsive to BMP2 mediated Smad1 phosphorylation, show the same tendency when stimulated with BMP2.

DISCUSSION

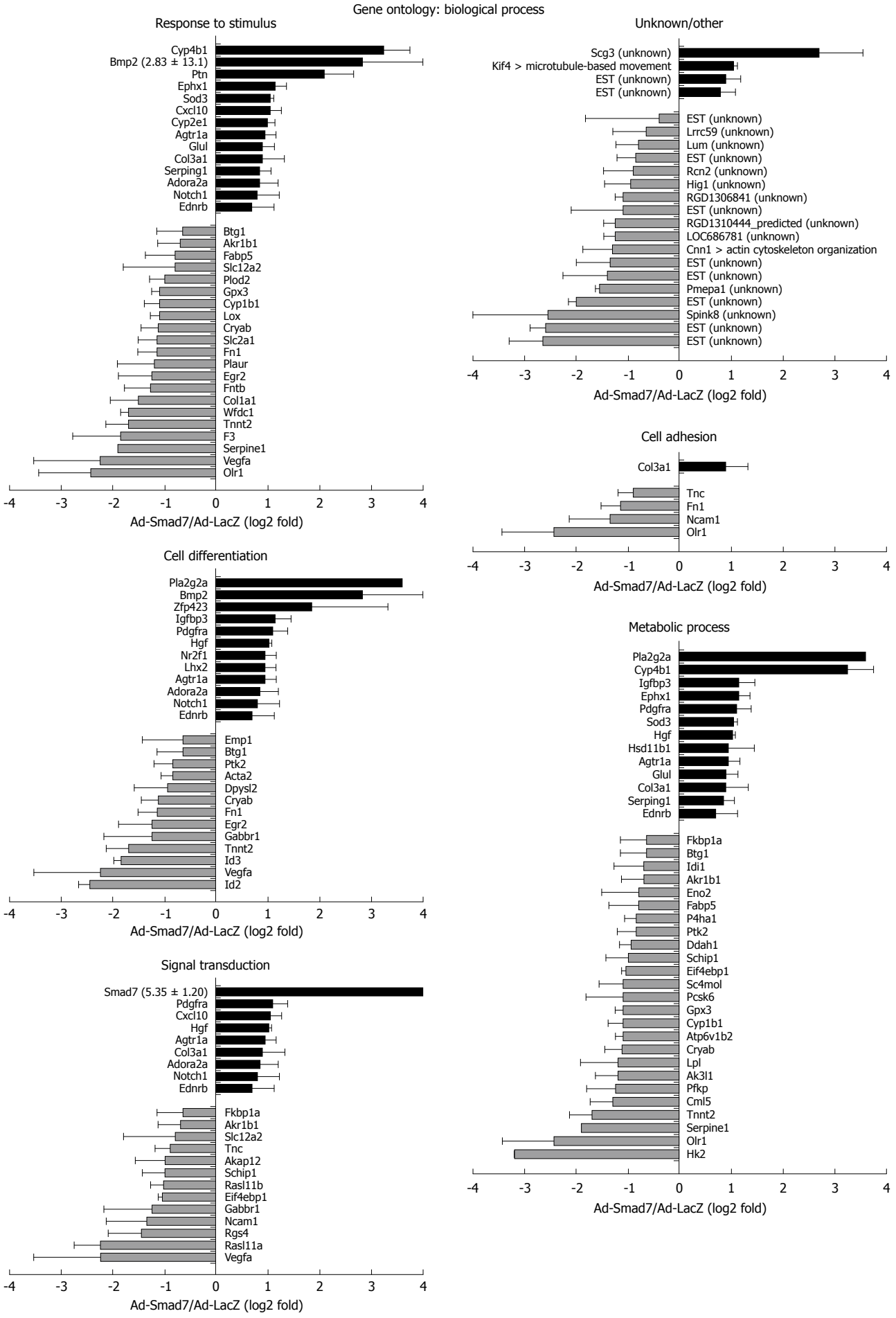
Using the Affymetrix Microarray approach, we systematically analyzed the effects of Smad7 overexpression during HSC transdifferentaition. About 100 genes were identified to be regulated upon Smad7 overexpression. For obvious reasons, only some of the regulated genes can be discussed below in detail. Nevertheless, all gene expression changes found constitute potential starting points for future research projects to unravel the process of liver fibrogenesis.

Tumor supressor genes were upregulated by Smad7 overexpression in HSCs

Pla2g2a and Cyp4B1 were strongly upregulated after Smad7 overexpression in HSCs. Pla2g2a participates in lipid metabolism/catabolism and was previously described as a tumor repressor in different cancer models, i.e. intestinal tumorigenesis, neuroblastoma, melanoma and colon cancer cell lines[17,18]. VEGF and Glut1, both known to be upregulated in tumor cells[19,20] were Smad7 dependently downregulated in HSCs. These results suggest an influence of Smad7 on tumor development and progression which is a long debated issue considering its regulative impact on ambiguous TGF-β signaling in tumorigenesis.

Cytochrome P450s are haem-thiolate proteins involved in oxidative degradation of particularly environmental toxins and mutagens and play a role in electron transport reactions. Additionally, they are key players in alcohol induced oxidative stress in liver, causing hepatocyte necrosis, apoptosis and liver fibrosis[21]. During steatosis, lipid peroxidation by Cyp2E1 is associated with inflammation and HSC activation including increased TGF-β production, possibly through up-regulation of KLF6[22]. Members of the Cyp P450 family are also upregulated during HSC activation[14].

Interestingly, overexpression of Smad7 increased the expression of some members of the Cytochrome P450 system through HSC activation, i.e. Cyp4B1 which is important in the metabolism of drugs, cholesterol, steroids and lipids, and Cyp2E1, while others are downregulated


Gene ontology: biological process
Response to stimulus
Cyp4b1
Bmp2 (2.83 ± 13.1)
Ptn
Ephx1
Sod3
Cxcl10
Cyp2e1
Agtr1a
Glul
Col3a1
Serping1
Adora2a
Notch1
Ednrb
Btg1
Akr1b1
Fabp5
Slc12a2
Plod2
Gpx3
Cyp1b1
Lox
Cryab
Slc2a1
Fn1
Plaur
Egr2
Fntb
Col1a1
Wfdc1
Tnnt2
F3
Serpine1
Vegfa
Olr1
-4 -3 -2 -1 0 1 2 3 4
Ad-Smad7/Ad-LacZ (log2 fold)
Unknown/other
Scg3 (unknown)
Kif4 > microtubule-based movement
EST (unknown)
EST (unknown)
EST (unknown)
Lrrc59 (unknown)
Lum (unknown)
EST (unknown)
Rcn2 (unknown)
Hig1 (unknown)
RGD1306841 (unknown)
EST (unknown)
RGD1310444_predicted (unknown)
LOC686781 (unknown)
Cnn1 > actin cytoskeleton organization
EST (unknown)
EST (unknown)
Pmepa1 (unknown)
EST (unknown)
Spink8 (unknown)
EST (unknown)
EST (unknown)
-4 -3 -2 -1 0 1 2 3 4
Ad-Smad7/Ad-LacZ (log2 fold)
Cell adhesion
Col3a1
Tnc
Fn1
Ncam1
Olr1
-4 -3 -2 -1 0 1 2 3 4
Ad-Smad7/Ad-LacZ (log2 fold)
Cell differentiation
Pla2g2a
Bmp2
Zfp423
Igfbp3
Pdgfra
Hgf
Nr2f1
Lhx2
Agtr1a
Adora2a
Notch1
Ednrb
Emp1
Btg1
Ptk2
Acta2
Dpysl2
Cryab
Fn1
Egr2
Gabbr1
Tnnt2
Id3
Vegfa
Id2
-4 -3 -2 -1 0 1 2 3 4
Ad-Smad7/Ad-LacZ (log2 fold)
Metabolic process
Pla2g2a
Cyp4b1
Igfbp3
Ephx1
Pdgfra
Sod3
Hgf
Hsd11b1
Agtr1a
Glul
Col3a1
Serping1
Ednrb
Fkbp1a
Btg1
Idi1
Akr1b1
Eno2
Fabp5
P4ha1
Ptk2
Ddah1
Schip1
Eif4ebp1
Sc4mol
Pcsk6
Gpx3
Cyp1b1
Atp6v1b2
Cryab
Lpl
Ak3l1
Pfkp
Cml5
Tnnt2
Serpine1
Olr1
Hk2
-4 -3 -2 -1 0 1 2 3 4
Ad-Smad7/Ad-LacZ (log2 fold)
Signal transduction
Smad7 (5.35 ± 1.20)
Pdgfra
Cxcl10
Hgf
Agtr1a
Col3a1
Adora2a
Notch1
Ednrb
Fkbp1a
Akr1b1
Slc12a2
Tnc
Akap12
Schip1
Rasl11b
Eif4ebp1
Gabbr1
Ncam1
Rgs4
Rasl11a
Vegfa
-4 -3 -2 -1 0 1 2 3 4
Ad-Smad7/Ad-LacZ (log2 fold)

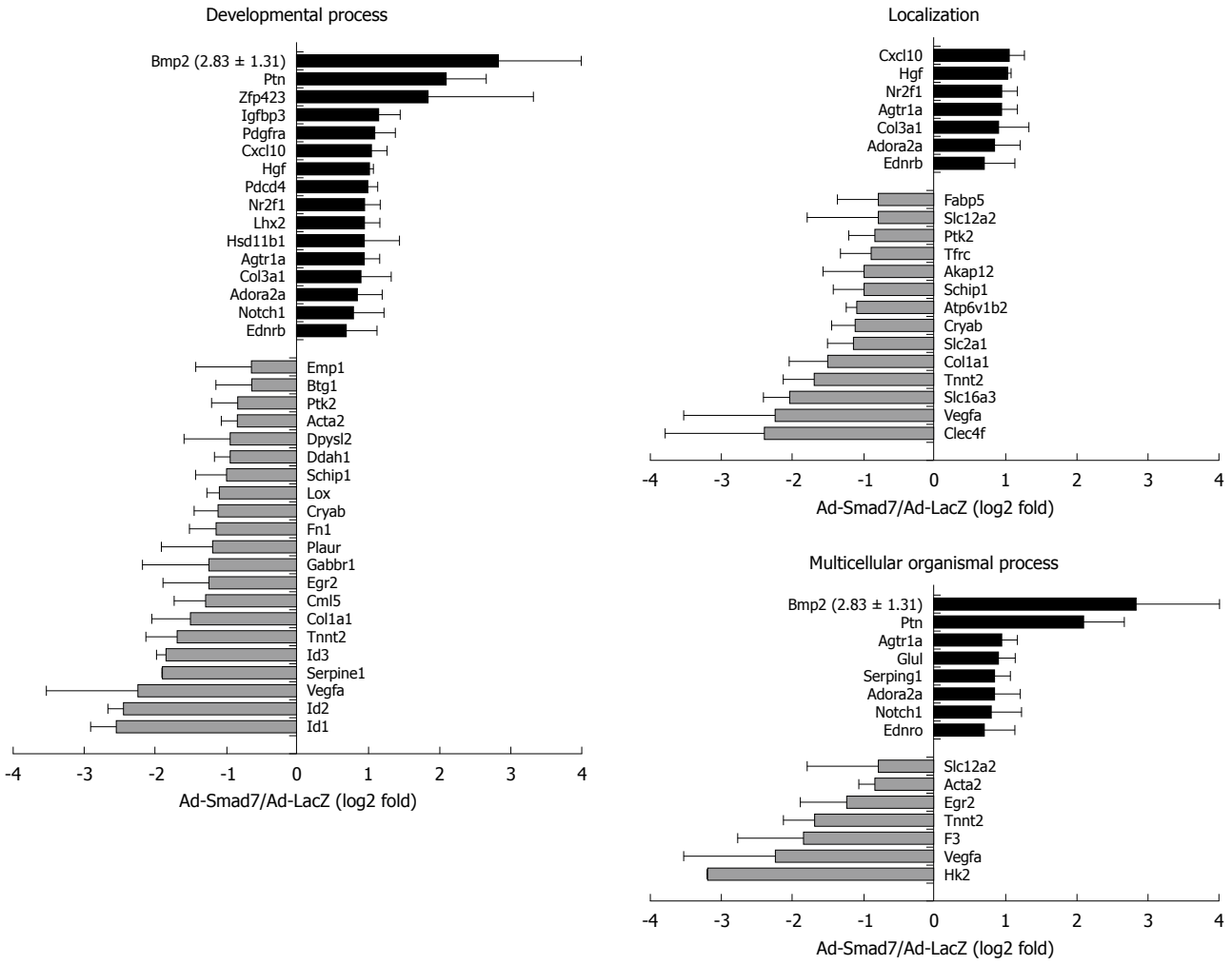
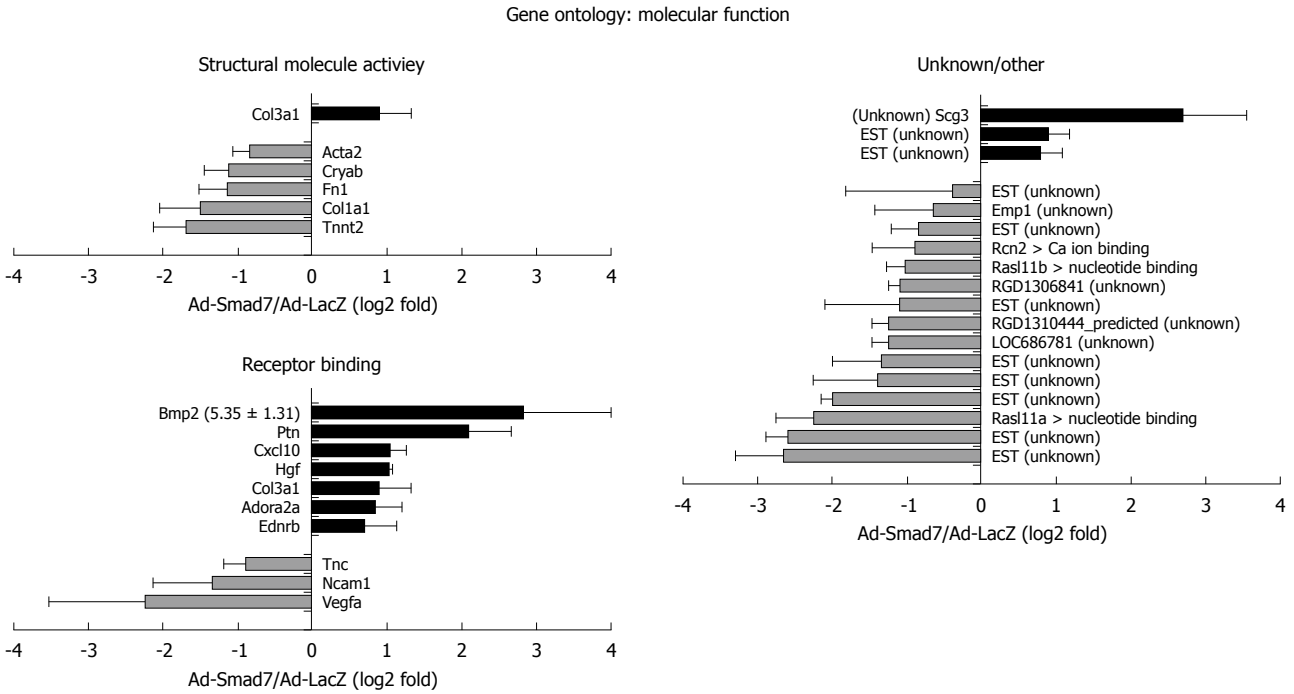


Figure 2 Genes with changed expression levels after overexpression of Smad7 are matched to 8 gene ontology terms of the biological process and to unknown/other. Unknown: Genes without annotation; Other: Genes with another annotation not assignable to the given annotations, details in brackets. Change of expression is given as log2 value of the fold factor with the SD. Black bars: Upregulated; Gray bars: Downregulated.



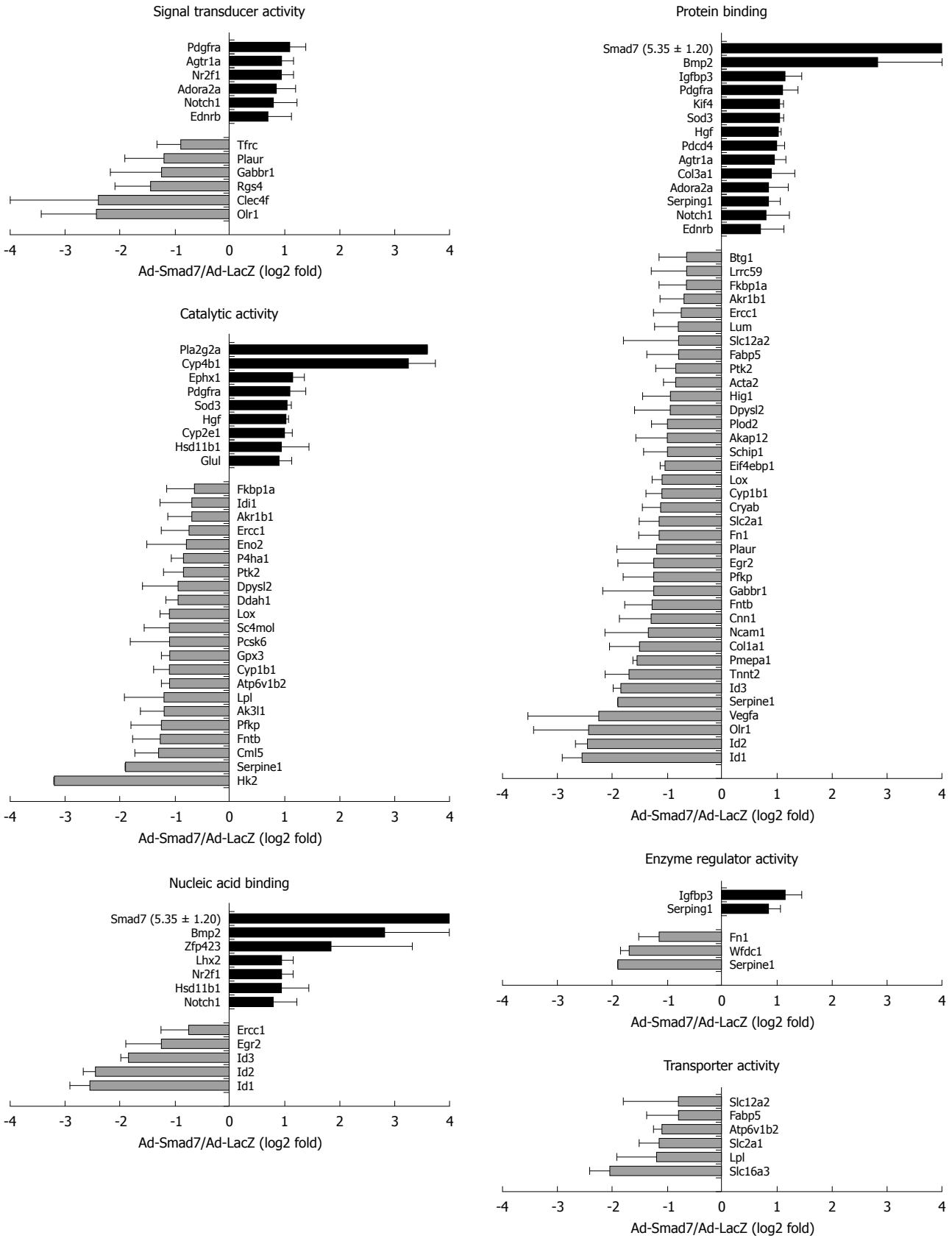


Figure 3 Genes whose expression levels were changed after overexpression of Smad7 are matched to 8 gene ontology terms of the molecular function and to unknown/other. Unknown: Genes without annotation; Other: Genes with another annotation not assignable to the given annotations, details in brackets. Change of expression is given as log2 value of the fold factor with the SD. The term nucleic acid binding includes the annotations nucleic acid binding, transcription factor activity, RNA binding, and transcription regulator activity. Black bars: Upregulated; Gray bars: Downregulated.

Table 3 Comparison of gene regulation in activated hepatic stellate cells *in vivo* (De Minicis *et al*[113])

Gene symbol	Smad7 overexpressing HSCs	*In vivo* activated untransformed HSCs
Acta2	↓	↑
BMP2[1]	↑	↓
Cnn1	↓	↑
Col1a1	↓	↑
Col3a1	↑	↑
Cryab	↓	↓
Cyp1b1	↓	↑
Ddah1	↓	↑
Ednrb	↑	↑[2]
Eno2	↓	↓
Ephx1	↑	↑[2]
Fn1	↓	↑
Gabbr1	↓	↓
Hgf	↑	↑[2]
Hk2[1]	↓	↑
Hsd11b1	↑	↑[2]
Id1	↓	↓
Igfbp3	↑	↑[2]
Kif4	↑	↑
Lox	↓	↑
Lpl	↓	↑[2]
Lum	↓	↑
Ncam1	↓	↑
P4ha1	↓	↑
Pdgfra	↑	↓
Pfkp	↓	↑
Plod2	↓	↑
Rasl11b	↓	↑
Serping1	↑	↑
Slc16a3	↓	↑
Slc2a1	↓	↑
Sod3	↑	↑
Tmepai_predicted	↓	↓[2]
Tnc	↓	↓[2]
Tnnt2	↓	↑
VEGFa[1]	↓	↑ (VEGFc)[1]
Wfdc1	↓	↑[2]

Gene expression profiles during hepatic stellate cell activation in culture and *in vivo* (*Gastroenterology* 2007; 132: 1937-1946[113]) and Smad7 overexpressing hepatic stellate cells (HSCs) (our study). [1]Genes regulated the strongest in our study are marked; [2]The regulation of *in vivo* activated HSCs which is different compared to culture activated HSCs in De Minicis study. In total 37 genes overlap in both studies, 22 of those (60%) are oppositely regulated indicating participation of transforming growth factor-β/Smad7 on the regulation of those genes *in vivo*.

upon Smad7 overexpression, e.g. Cyp1B1. These ambiguous effects probably reflect the complex control of oxidative metabolism in the cell.

Glucose metabolism and angiogenesis/vascularisation is downregulated by Smad7

Hk2 is a hexokinase, one of the best known enzymes of glycolysis, and is involved in cell cycle progression. According to the results of the microarray analysis it represents the most downregulated gene in AdSmad7 infected HSCs. One feature of activated HSCs is the ability to proliferate. TGF-β antagonizes proliferation in quiescent HSCs, whereas it has a growth promoting effect in transdifferentiated MFBs. Thus, Hk2 might be induced by TGF-β in HSCs during activation, subsequently stimulating HSC

Table 4 Comparison of gene regulation according to quantitative real-time polymerase chain reaction analysis and array analysis

Gene	Probe set ID	Array	RT-PCR analysis
Cyp4B1	M29853_at	Up	Up
Smad7	AF042499_at	Up	Up
BMP2	L02678_at/ rc_AA997410_s_at	Up	Up
SGIII	U02983_at	Up	Up
Zfp423	U92564_at/ U92564_g_at	Up	Up
Pla2g2a	x51529_at	Up	Up
EST189231	AA799734_at	Down	Down
Olr1	AB005900_at/ AB018104cds_s_at/ rc_AI071531_s_at	Down	Down
ID1	L23148_g_at	Down	Down
HK2	D26393exon_s_at	Down	3 d down/**7 d up**
Slc16a3	U87627_at	Down	3 d down/**7 d up**
VEGFa/ratVEGF.1 in RT-PCR	L20913_s_at/ M32167_g_at	Down	3 d down/**7 d up**

Different results in array and quantitative real-time polymerase chain reaction (RT-PCR) analysis are marked bold.

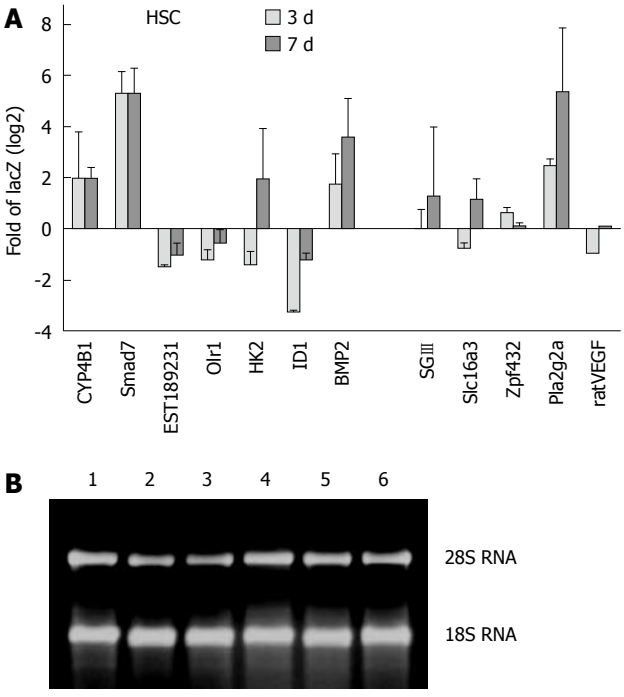


Figure 4 Validation of microarray results using quantitative real-time polymerase chain reaction. A: SYBR Green I-based real-time quantification to compare the mRNA expression patterns of 12 selected genes in hepatic stellate cell which were infected either with AdLacZ or AdSmad7. Transforming growth factor-β RI, not affected by Smad7 overexpression, served as a house-keeping gene. Results are given as relative expression of log2 fold of LacZ. 3 d (light grey column) and 7 d (dark grey column): 3 d and 7 d after adenoviral infection. Values are the mean of three measurements each performed in duplicates ± SD from independent experiments; B: Total RNA purity and integrity was verified by formaldehyde agarose gel electrophoresis. Lane 1: LacZ, 7 d; Lane 2: LacZ, 3 d; Lane 3: Smad7, 7 d; Lane 4: Smad 7, 3 d; Lane 5: Untreated control, 7 d; Lane 6: Untreated control, 3 d.

proliferation and thus providing at least part of the growth stimulatory effect of TGF-β. Although physiological ef-

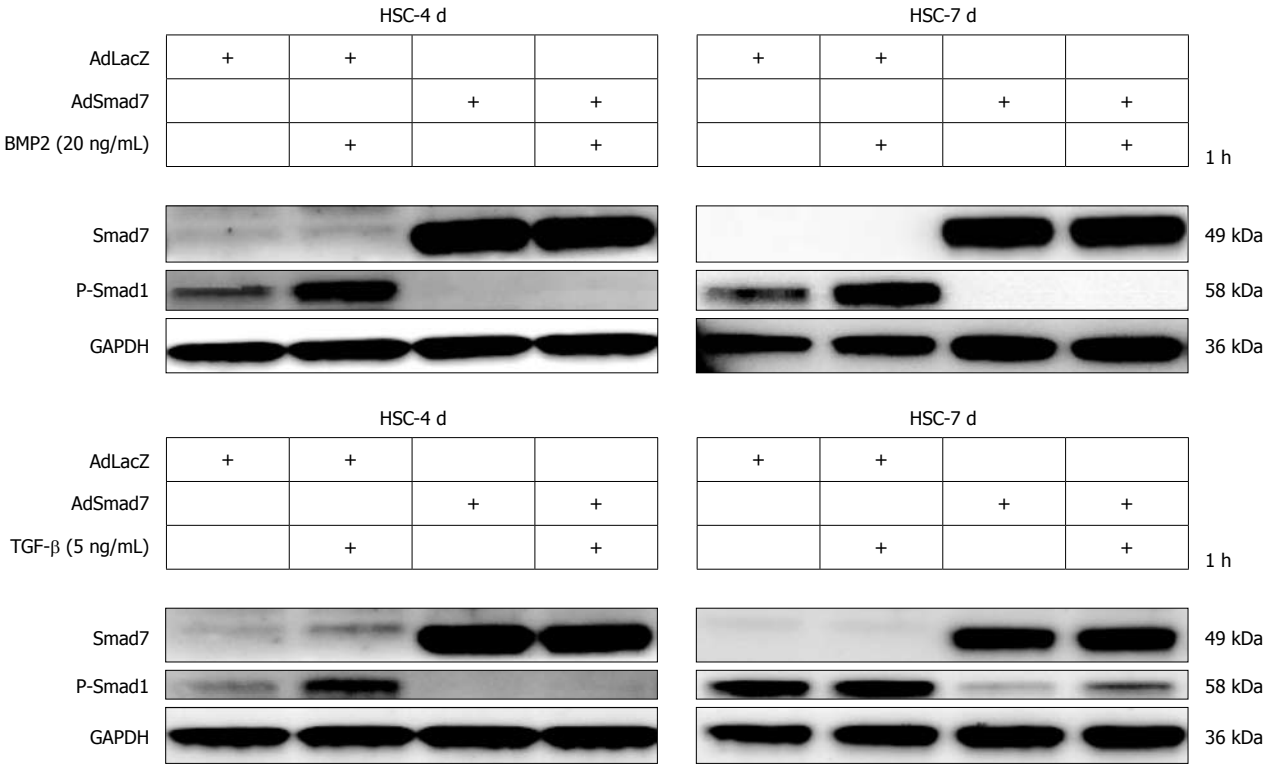


Figure 5 Smad7 overexpression inhibits BMP2 and transforming growth factor-β dependent Smad1 phosphorylation. Transdifferentiating (4 d old) and fully activated (7 d old) hepatic stellate cells (HSCs) infected or non-infected with either AdLacZ (control) or AdSmad7 were stimulated for 1 h with 20 ng/mL BMP2 or 5 ng/mL transforming growth factor (TGF)-β as indicated. Smad7 overexpression and Smad1 phosphorylation were analysed using Western blottings. GADPH served as a reference. While transdifferentiating HSCs are sensitive to both BMP2 and TGF-β stimulation, fully activated HSCs are only responsive to BMP2.

fects of glucose metabolism in the liver are traditionally associated to hepatocytes and provide a direct link to fibrogenesis *via* hyperglycemia and insulin resistance[23-25], one could speculate that activated HSCs need more energy and thus, glycolysis is upregulated TGF-β dependently in this cell type. In line, HSCs become sensitive to glucose signaling during activation, high glucose concentrations stimulate ROS production through PKC-dependent activation of NADPH oxidase and induce MAP kinase phosphorylation subsequent to proliferation and type I collagen production in this cell type[26] suggesting a crucial role of HSC-sugar metabolism in fibrogenesis.

Upregulation of Hk2 during activation of HSCs further suggests that glycolysis induction and increased levels of involved proteins may occur by other means than elevated blood glucose levels ([14], our study). This in turn indicates a direct connection between fibrosis and enhanced glycolysis independent of inducing external stimuli of either process.

Beside Hk2 regulation, other genes encoding enzymes of glycolysis (Eno2, PFKP) or related to glucose metabolism were downregulated Smad7 dependently, e.g. VEGFa, PAI-1 (Serpine1), F3, Slc2a1 (Glut1), FN1, EIF4ebp1 and PTK2 (Figure 6). A list of all references proving the relation of these genes to glucose metabolism will be provided to interested readers on request.

Glut1 is a glucose transporter protein which becomes upregulated in activated HSCs or upon HSC activation[14]. Since Smad7 decreases Glut1 expression levels and other proteins involved in glycolysis, TGF-β seems to enhance glucose metabolism and energy supply during HSC activation thus enabling the cells to proliferate and transdifferentiate towards activated myofibroblasts.

In contrast, decreased numbers of Glut1 molecules are reported in hepatocytes subjected to chronic alcohol consumption[27] resulting in a reduced availability of glucose for glycolysis in hepatocytes. The resulting energy deficiency has been shown to impair this cell type's ability to perform critical functions and to contribute therefore to cell death and alcoholic liver disease.

In line with our results, relations between glucose metabolism and fibroproliferative processes were identified in other organs. For example in human kidney[28,29] exposure of proximal tubule cells and cortical fibroblasts to high extracellular glucose concentrations results directly in altered cell growth and collagen synthesis.

IGFBP3 and Cyp2E1, known to participate in glucose metabolism were upregulated in AdSmad7 infected HSCs, indicating that they might be under negative control through TGF-β. However, upregulation of IGFBP3 in our study could be simply due to "culture" but not "*in vivo* activation" of HSCs (for term definition compare[14]) instead of being mechanistically important. Interestingly, Smad7 even seems to enhance the upregulation of IGFBP3, which already occurs upon HSC activation. If there is any pathologic relevance of this finding, it suggests a mechanism of regulation independent of canonical TGF-β/Smad7 signaling in HSC activation.

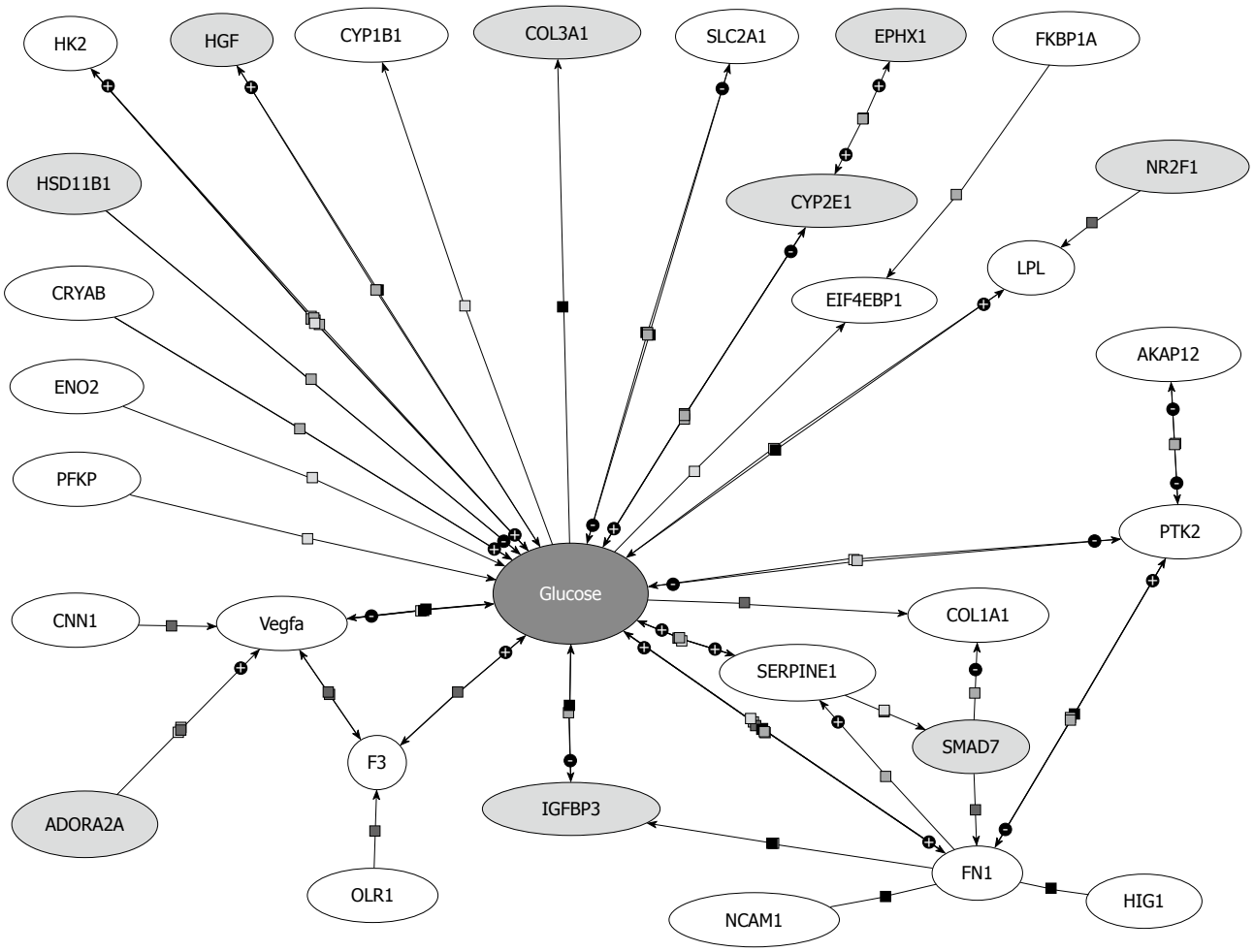


Figure 6 Biological interaction between glucose and genes up- (light grey) or down-regulated (white) in primary hepatic stellate cells after overexpression of Smad7. Genes linked to glucose by binding or regulatory interactions are depicted as interconnecting lines between glucose and the gene symbols. Pathway analysis was done with Pathway Architect software (Stratagene).

VEGFs are growth factors involved in angiogenesis, vasculogenesis and endothelial cell growth, promotion of cell migration, apoptosis inhibition and induction of blood vessel permeabilization. De Minicis *et al*[14] showed VEGFc to be upregulated[14], while in the present report VEGFa was downregulated after Smad7 overexpression in activated HSCs, indicating its induction as a response to profibrogenic TGF-β signaling. Previous investigation of hypoxia in a stellate cell line demonstrates upregulation of VEGF expression[30]. Hypoxia leads to cell dysfunction or death and occurs during liver damage and inflammation. HIF1, considered to be the major regulator of about 100 genes including VEGF and PAI-1, is also upregulated in that study. In contrast HIF1 did not display an altered expression in our study indicating that HIF1 expression in HSCs is TGF-β independent and that another TGF-β dependent route exists to induce PAI-1 (serpine1) and VEGFa expression.

Smad7 decreases cell adhesion regulators in HSCs

Expression of several proteins linked to cell adhesion, i.e. Olr1, Ncam1, Fn1 and Tnc was decreased after Smad7 overexpression in HSCs. Accordingly, Ncam1 and Fn1 were upregulated in activated HSCs[14]. Although for Olr1 no information about the regulation in activated HSCs is available so far, we assume from our results that Smad7 antagonizes cell adhesion features of activated HSCs. This suggests that profibrogenic TGF-β signaling improves cell adhesion for transdifferentiating HSCs. Nevertheless it should be noted that downregulation of Tnc in activated HSCs is supported by Smad7.

Smad7 induces BMP2 expression in HSCs

Generally, TGF-β signals *via* Smad2 and Smad3 but also induces the second canonical pathway *via* ALK1/Smad1/5/8. BMP2, another member of the TGF-β family, solely signals *via* Smads1/5/8 utilizing ALK3 and ALK6[12]. Here we show that (1) BMP2 was strongly upregulated in Smad7 expressing HSCs (Table 2, Figure 4A and B); and (2) Smad7 potently inhibited BMP2 dependent and TGF-β dependent Smad1 phosphorylation. Even in fully activated HSCs which are not responsive to TGF-β concerning Smad1 phosphorylation, BMP2 dependent Smad1 phosphorylation was abolished (Figure 5).

These results indicate a tight crosstalk between TGF-β and BMP signaling pathways in HSCs. It seems that HSCs

try to keep up a functional Smad1 signaling upon blocking TGF-β pathways with Smad7. Accordingly, BMP2 is capable of inducing Smad1 signaling in fully activated 7 d-HSCs which are no longer responsive to TGF-β stimulation. Thus BMP2 expression might be induced to overcome a lack of TGF-β/Smad1 signaling upon Smad7 expression or HSC activation using a corresponding autocrine loop. Although our *in vitro* experiments demonstrate that Smad7 is able to inhibit BMP2/Smad1 signaling effectively, Smad7 dependent induction of BMP2 expression in HSCs *in vivo* might be strong enough to sustain an active Smad1/5/8 signaling pathway. Further experiments could delineate whether BMP2 expression is directly induced by the recently described DNA binding activity of Smad7[31], if a running TGF-β signaling pathway has a negative regulatory role toward the BMP2 promoter or if other mechanisms are responsible for BMP2 induction in Smad7 overexpressing HSCs.

In summary we conclude that genes regulated contrarily during HSC activation[14] *vs* ectopic Smad7 expression (this study) most probably represent critical profibrogenic components. As Smad7 is able to blunt HSC transdifferentiation *in vitro* and *in vivo*[7] we assume glucose metabolism and the crosstalk between the TGF-β and the BMP2 pathways are critical components of HSC activation.

In general our study underlines the potential of top down systemic approaches to delineate effects of cell signaling regulation and opens the opportunity to find targets for drug development.

COMMENTS

Background

Activation of hepatic stellate cells (HSCs) as a consequence of liver damage includes proliferation and extracellular matrix (ECM) deposition and represents a major step in fibrogenesis. Transforming growth factor (TGF)-β is a master contributor and its signaling pathway is modulated during the HSC activation process, whereby its cytostatic action is lost and ECM producing effects become predominant. Smad7 is a powerful antagonist of TGF-β. Expression of Smad7 is transiently induced by the canonical TGF-β/R-Smad signaling cascade, thereby providing a negative feedback loop to regulate TGF-β signals. Smad7 is able to inhibit HSC transdifferentiation and attenuate the extent of fibrosis, suggesting Smad7 is a promising antifibrotic compound.

Research frontiers

The findings offer important new information about the process of HSC transdifferentiation and fibrogenesis as well as cell biology of signal transduction in the liver. Moreover, providing a list of genes previously not known as participants in HSC activation and fibrogenesis, members of the field may use these data as starting points to get new insight into mechanisms of HSC (patho)physiology. Thus, it will definitely be of interest to the scientific community, especially in the field of hepatology and gastroenterology.

Innovations and breakthroughs

In the present report, the authors systematically investigated transcriptional effects of Smad7 overexpression in cultured HSCs by microarray analysis. Using this powerful top down approach, the authors identified 100 target genes to be significantly regulated by Smad7 overexpression. These represent potential targets for delineating mechanisms of HSC activation and to set up therapeutic approaches. The results imply a crosstalk between TGF-β and BMP2 signaling pathways in HSCs and for the first time a significant involvement of glucose metabolism in the HSC transdifferentiation processes.

Applications

The results are of special interest for future attempts to understand the process of stellate cell activation and to set up TGF-β and/or Smad7 directed treatment approaches in chronic liver diseases, especially as they reflect the most powerful negative regulatory process of TGF-β signaling.

Terminology

Bone morphogenetic protein (BMP): BMPs are multi-functional growth factors belonging to the TGF-β superfamily. BMPs were originally discovered by their ability to induce the formation of bone and cartilage, but are now considered to constitute a group of pivotal morphogenetic signals, orchestrating tissue architecture throughout the body. BMP signals are mediated by type Ⅰ and Ⅱ BMP receptors and their downstream molecules Smad1, 5 and 8. Microarray: A method for profiling gene and protein expression in cells and tissues. A microarray consists of different nucleic acid/protein probes that are chemically attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. Hybridization of test samples to these probes can be measured by different means.

Peer review

This is a potentially interesting study aimed at clarifying Smad7-regulated gene expression during the transdifferentiation of hepatic stellate cells, a major profibrogenic cell type in the liver. Overall, the study is well-performed and the manuscript is well-written.

REFERENCES

1 **Arthur MJ**. Fibrogenesis II. Metalloproteinases and their inhibitors in liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G245-G249

2 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218

3 **Weng H**, Mertens PR, Gressner AM, Dooley S. IFN-gamma abrogates profibrogenic TGF-beta signaling in liver by targeting expression of inhibitory and receptor Smads. *J Hepatol* 2007; **46**: 295-303

4 **Weng HL**, Wang BE, Jia JD, Wu WF, Xian JZ, Mertens PR, Cai WM, Dooley S. Effect of interferon-gamma on hepatic fibrosis in chronic hepatitis B virus infection: a randomized controlled study. *Clin Gastroenterol Hepatol* 2005; **3**: 819-828

5 **Purps O**, Lahme B, Gressner AM, Meindl-Beinker NM, Dooley S. Loss of TGF-beta dependent growth control during HSC transdifferentiation. *Biochem Biophys Res Commun* 2007; **353**: 841-847

6 **Dooley S**, Said HM, Gressner AM, Floege J, En-Nia A, Mertens PR. Y-box protein-1 is the crucial mediator of antifibrotic interferon-gamma effects. *J Biol Chem* 2006; **281**: 1784-1795

7 **Dooley S**, Hamzavi J, Breitkopf K, Wiercinska E, Said HM, Lorenzen J, Ten Dijke P, Gressner AM. Smad7 prevents activation of hepatic stellate cells and liver fibrosis in rats. *Gastroenterology* 2003; **125**: 178-191

8 **Stopa M**, Anhuf D, Terstegen L, Gatsios P, Gressner AM, Dooley S. Participation of Smad2, Smad3, and Smad4 in transforming growth factor beta (TGF-beta)-induced activation of Smad7. THE TGF-beta response element of the promoter requires functional Smad binding element and E-box sequences for transcriptional regulation. *J Biol Chem* 2000; **275**: 29308-29317

9 **Knook DL**, DeLeeuw AM. Isolation and characterization of fat storing cells from the rat liver. In: Knook DL, Wisse E, editros. Sinusoidal liver cells. Rijswijk: Elsevier Biomedical Press, 1982: 45-52

10 **Schäfer S**, Zerbe O, Gressner AM. The synthesis of proteoglycans in fat-storing cells of rat liver. *Hepatology* 1987; **7**: 680-687

11 **Wickert L**, Steinkrüger S, Abiaka M, Bolkenius U, Purps O, Schnabel C, Gressner AM. Quantitative monitoring of the mRNA expression pattern of the TGF-beta-isoforms (beta 1, beta 2, beta 3) during transdifferentiation of hepatic stellate cells using a newly developed real-time SYBR Green PCR. *Biochem Biophys Res Commun* 2002; **295**: 330-335

12 **Wiercinska E**, Wickert L, Denecke B, Said HM, Hamzavi J, Gressner AM, Thorikay M, ten Dijke P, Mertens PR, Breitkopf

K, Dooley S. Id1 is a critical mediator in TGF-beta-induced transdifferentiation of rat hepatic stellate cells. *Hepatology* 2006; **43**: 1032-1041
13 **Zeeberg BR**, Feng W, Wang G, Wang MD, Fojo AT, Sunshine M, Narasimhan S, Kane DW, Reinhold WC, Lababidi S, Bussey KJ, Riss J, Barrett JC, Weinstein JN. GoMiner: a resource for biological interpretation of genomic and proteomic data. *Genome Biol* 2003; **4**: R28
14 **De Minicis S**, Seki E, Uchinami H, Kluwe J, Zhang Y, Brenner DA, Schwabe RF. Gene expression profiles during hepatic stellate cell activation in culture and in vivo. *Gastroenterology* 2007; **132**: 1937-1946
15 **Dooley S**, Delvoux B, Lahme B, Mangasser-Stephan K, Gressner AM. Modulation of transforming growth factor beta response and signaling during transdifferentiation of rat hepatic stellate cells to myofibroblasts. *Hepatology* 2000; **31**: 1094-1106
16 **Wickert L**, Abiaka M, Bolkenius U, Gressner AM. Corticosteroids stimulate selectively transforming growth factor (TGF)-beta receptor type III expression in transdifferentiating hepatic stellate cells. *J Hepatol* 2004; **40**: 69-76
17 **Cormier RT**, Hong KH, Halberg RB, Hawkins TL, Richardson P, Mulherkar R, Dove WF, Lander ES. Secretory phospholipase Pla2g2a confers resistance to intestinal tumorigenesis. *Nat Genet* 1997; **17**: 88-91
18 **Haluska FG**, Thiele C, Goldstein A, Tsao H, Benoit EP, Housman D. Lack of phospholipase A2 mutations in neuroblastoma, melanoma and colon-cancer cell lines. *Int J Cancer* 1997; **72**: 337-339
19 **Vaupel P**, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* 2007; **26**: 225-239
20 **Suganuma N**, Segade F, Matsuzu K, Bowden DW. Differential expression of facilitative glucose transporters in normal and tumour kidney tissues. *BJU Int* 2007; **99**: 1143-1149
21 **Albano E**. Alcohol, oxidative stress and free radical damage. *Proc Nutr Soc* 2006; **65**: 278-290
22 **Stärkel P**, Sempoux C, Leclercq I, Herin M, Deby C, Desager JP, Horsmans Y. Oxidative stress, KLF6 and transforming growth factor-beta up-regulation differentiate non-alcoholic steatohepatitis progressing to fibrosis from uncomplicated steatosis in rats. *J Hepatol* 2003; **39**: 538-546
23 **Canbay A**, Gieseler RK, Gores GJ, Gerken G. The relationship between apoptosis and non-alcoholic fatty liver disease: an evolutionary cornerstone turned pathogenic. *Z Gastroenterol* 2005; **43**: 211-217
24 **Parekh S**, Anania FA. Abnormal lipid and glucose metabolism in obesity: implications for nonalcoholic fatty liver disease. *Gastroenterology* 2007; **132**: 2191-2207
25 **Kmieć Z**. Cooperation of liver cells in health and disease. *Adv Anat Embryol Cell Biol* 2001; **161**: III-XIII, 1-151
26 **Sugimoto R**, Enjoji M, Kohjima M, Tsuruta S, Fukushima M, Iwao M, Sonta T, Kotoh K, Inoguchi T, Nakamuta M. High glucose stimulates hepatic stellate cells to proliferate and to produce collagen through free radical production and activation of mitogen-activated protein kinase. *Liver Int* 2005; **25**: 1018-1026
27 **Van Horn CG**, Ivester P, Cunningham CC. Chronic ethanol consumption and liver glycogen synthesis. *Arch Biochem Biophys* 2001; **392**: 145-152
28 **Jones SC**, Saunders HJ, Pollock CA. High glucose increases growth and collagen synthesis in cultured human tubulointerstitial cells. *Diabet Med* 1999; **16**: 932-938
29 **Gilbert RE**, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury? *Kidney Int* 1999; **56**: 1627-1637
30 **Shi YF**, Fong CC, Zhang Q, Cheung PY, Tzang CH, Wu RS, Yang M. Hypoxia induces the activation of human hepatic stellate cells LX-2 through TGF-beta signaling pathway. *FEBS Lett* 2007; **581**: 203-210
31 **Shi X**, Chen F, Yu J, Xu Y, Zhang S, Chen YG, Fang X. Study of interaction between Smad7 and DNA by single-molecule force spectroscopy. *Biochem Biophys Res Commun* 2008; **377**: 1284-1287

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BRIEF ARTICLE

Transient elastography: A non-invasive tool for assessing liver fibrosis in HIV/HCV patients

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Abstract

AIM: To assess the prevalence of advanced liver fibrosis (ALF) in human immunodeficiency virus (HIV), hepatitis C virus (HCV) and HIV/HCV patients using transient elastography, and to identify factors associated with ALF.

METHODS: Between September 2008 and October 2009, 71 HIV mono-infected, 57 HIV/HCV co-infected and 53 HCV mono-infected patients on regular follow-up at our Center were enrolled in this study. Alcohol intake, the main parameters of liver function, presence of HCV-RNA, HIV-RNA, duration of highly active anti-retroviral therapy (HAART) and CD4 cell count were recorded. ALF was defined as liver stiffness (LS) $\geqslant$ 9.5 kPa. To estimate liver fibrosis (LF) a further 2 reliable biochemical scores, aspartate aminotransferase platelet ratio index (APRI) and FIB-4, were also used.

RESULTS: LS values of co-infected patients were higher than in either HIV or HCV mono-infected patients (χ^2_{MH} = 4, P < 0.04). In fact, LS $\geqslant$ 9.5 was significantly higher in co-infected than in HIV and HCV mono-infected patients (χ^2 = 5, P < 0.03). Also APRI and the FIB-4 index showed more LF in co-infected than in HIV mono-infected patients (P < 0.0001), but not in HCV mono-infected patients. In HIV/HCV co-infected patients, the extent of LS was significantly associated with alcohol intake (P < 0.04) and lower CD4+ cell count (P < 0.02). In HCV patients, LS was correlated with alcohol intake (P < 0.001) and cholesterol levels (P < 0.03). Body mass index, diabetes, HCV- and HIV-viremia were not significantly correlated with LS. In addition, 20% of co-infected patients had virologically unsuccessful HAART; in 50% compliance was low, CD4+ levels were < 400 cells/mm^3 and LS was > 9.5 kPa. There was no significant correlation between extent of LF and HAART exposure or duration of HAART exposure, in particular with specific dideoxynucleoside analogues.

CONCLUSION: ALF was more frequent in co-infected than mono-infected patients. This result correlated with lower CD4 levels. Protective immunological effects of HAART on LF progression outweigh its hepatotoxic effects.

Key words: Liver fibrosis; Transient elastography; Aspartate aminotransferase platelet ratio index; FIB-4 test; Fibrosis evaluation; Human immunodeficiency virus infection; Hepatitis C virus infection

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INTRODUCTION

In the last few years, liver disease associated with hepatitis C virus (HCV) has emerged as a significant problem in human immunodeficiency virus (HIV)-infected patients, thanks to improved survival in the highly active antiretroviral therapy (HAART) era[1]. It has been reported that HIV and HCV co-infection leads to a more rapid progression of liver disease to cirrhosis[2,3]. Other factors such as severe immune suppression and alcohol consumption accelerate the progression of HCV-related fibrosis[4,5]. Virologically successful HAART slows the progression of liver fibrosis (LF) and reduces hepatic necroinflammatory activity in HIV/HCV co-infected patients[2,6]. In contrast, antiretroviral-related liver toxicity could contribute to liver damage in HIV- and HIV/HCV-infected patients[7]. Mitochondrial toxicity of nucleoside analogues[8], and glucose or lipid abnormalities, such as hyperglycemia and lipodystrophy, which are particularly common when using some protease inhibitors[9], may produce or enhance LF progression in HIV mono- and HIV/HCV co-infected patients. Currently, in this respect, a growing number of cases of cryptogenetic liver disease in symptomatic and asymptomatic HIV-infected patients has been reported[10,11].

Percutaneous liver biopsy is the gold standard for assessing LF. However, it may be associated with sampling variability[12], is an invasive technique with rates of morbidity of 3% and mortality of 0.03%[13,14], and as a consequence, is not suitable for repeated assessment, which is required when monitoring LF.

For these reasons, new non-invasive methods for the assessment of LF have been developed. Transient elastography (TE) (Fibro-Scan®; EchoSens, Paris, France) is a rapid, reliable and tolerable imaging technique for the assessment of LF by measuring liver stiffness (LS)[15,16].

On the other hand, many biochemical markers have been implemented to estimate LF, with the aim of reducing the number of liver biopsies[14].

The advent of TE and biochemical markers has been demonstrated to be very helpful in the non-invasive measurement of LF, particularly in asymptomatic HIV-infected patients in whom liver biopsy is not recommended[11]. TE has already been validated for the measurement of LF in HIV and HCV seropositive patients[17,18].

The aim of this study was to assess the prevalence of LF and cirrhosis in a group of HIV mono-infected, HCV mono-infected and HIV/HCV co-infected patients using TE and biochemical markers. In addition, we evaluated which of the factors studied correlated with advanced LF (ALF) and cirrhosis.

MATERIALS AND METHODS

Study population

Between September 2008 and October 2009 all consecutive HIV mono-infected and HIV/HCV co-infected patients on regular follow-up at the AIDS Center of the University of Palermo, as well as HCV mono-infected patients seen consecutively at the Outpatient Clinic of the Department of Clinical Medicine and Emerging Pathologies of the University of Palermo were enrolled in this study.

Information on age, gender, risk factors for HCV and HIV infections, cumulative exposure to non-nucleoside and nucleoside reverse-transcriptase inhibitors, protease inhibitors and specific antiretroviral drugs within each class were all recorded in a database designed for this study.

For all HIV-infected patients the absolute number of CD4+ T-cells and plasma HIV-RNA levels was assessed. In HCV-infected patients, HCV-genotype and plasma HCV-RNA levels were also recorded. In addition, at baseline, complete blood cell counts, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γGT), total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and glycemia were measured.

Alcohol intake > 20 g/d either at the time of the study or in the past was recorded through patient interviews. Diabetes or impaired fasting glucose (IFG) were defined according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus criteria[19].

Patients with acute liver decompensation, hepatocellular carcinoma or chronic hepatitis B were excluded.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Patients were enrolled after written informed consent was obtained.

Assessment of LF

LF was assessed by a single certified operator (trained by the manufacturer) using TE (FibroScan®; EchoSens, Paris, France). TE provides an assessment of LS expressed in kPa units. In brief, an ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissues. The speed of propagation of this vibration across the liver is directly related to tissue stiffness.

The tip of the probe transducer was placed in the intercostal spaces at the right lobe of the liver. Only patients with 10 valid elastometric measures, interquartile ranges > 30% and ⩾ 60% success rate (the number of validated

measurements divided by the total number of measurements) were considered to be reliable. ALF (severe fibrosis and cirrhosis) was defined as a median LS of 9.5 kPa. As previously published, this cut-off value is strongly correlated with a Metavir score of F3, both in HCV mono-infected and HCV/HIV co-infected patients[17,18].

LF was also assessed biologically using 2 different well-validated indices, the aspartate aminotransferase platelet ratio index (APRI) index and the FIB-4 index. The APRI was calculated as follows: AST/upper limit of normal × 100/platelet count (10^9/L)[20,21]. The FIB-4 index was calculated as follows: age × AST (IU/L)/{[platelet count (10^9/L)] × [ALT (IU/L)]$^{1/2}$}[22]. The prevalence of ALF was estimated using as a reference a FIB-4 index > 3.25 and an APRI index > 1.5[20,22].

Statistical analysis

When data distribution was Gaussian, values were expressed as mean ± SD and their differences were calculated using the Student *t*-test; otherwise, data were expressed as the median and range and analyzed using the Mann-Whitney *U* test. Fisher's exact and χ^2 tests, the χ^2 test of Mantel Haenszel, Spearman's rank correlations (ρ) and Pearson's correlation (*r*) were used where appropriate. Multiple linear regression analysis was used to study the association between increased values of LS and variables statistically significant at univariate analysis. All analyses were performed using the SPSS software package (version 13.0; Chicago, IL, USA).

RESULTS

Study population

A total of 201 patients on regular follow-up at both our Centers were enrolled in the study.

In 11 patients (4 HIV mono-infected, 6 HCV mono-infected and one co-infected) a valid elastometric assessment could not be obtained because of truncular obesity, therefore 190 patients were finally included in this study. There were 137 HIV-infected patients, including 71 HIV mono-infected and 66 HIV/HCV co-infected, and 53 HCV mono-infected patients. Patient characteristics at the time of LS measurement are summarized in Table 1.

HIV patients were significantly younger than HCV mono-infected individuals ($P < 0.002$). Body mass index (BMI) was significantly higher in HCV mono-infected patients than in HIV and HIV/HCV co-infected patients ($P < 0.002$). The most frequent risk factor of HCV contamination was intravenous drug use in co-infected *vs* mono-infected HCV patients ($P < 0.0001$), while it was transfusion of blood products in HCV mono-infected *vs* co-infected patients ($P < 0.0001$). Most HIV mono-infected (80.2%) and HIV/HCV co-infected patients (90.9%) were under HAART. However, only 62% of HIV mono-infected and 68% of HIV/HCV co-infected patients had an HIV-RNA load of < 47 copies/mL. In addition, 20% of co-infected patients had virologically unsuccessful HAART; in 50% compliance was low, and CD4+ levels were < 400 cells/mm^3. HIV/HCV co-infected patients were more often infected by HCV genotype 3 compared with HCV mono-infected patients ($P < 0.03$).

Table 1 Demographic characteristics of the study populations (mean ± SD) *n* (%)

	HIV (*n* = 71)	HCV (*n* = 53)	HIV/HCV (*n* = 66)
Age (yr)	43.3 ± 10.3[a]	49.7 ± 12.5	45.6 ± 12.5
Male gender	50 (70)	36 (67.9)	49 (74.2)
BMI (kg/m^2)	23.6 ± 3.4[a]	26.0 ± 3.6	22.8 ± 3.0[b]
Risk group			
Transfusion	-	17 (32.1)	4 (6.1)[b]
Homosexual men	21 (29.5)	-	1 (1.5)
Intravenous drug use	4 (5.6)	7 (13.2)[c]	41 (62.1)
Others	-	12 (22.6)	-
Unknown	9 (12.6)	16 (30.2)	8 (12.1)
HCV genotype 3:non3	-	7:46[d]	20:46
HAART	57 (80.2)	-	60 (90.9)
Alcohol	6 (8.4)	4 (7.5)	5 (7.5)
Diabetes + IFG	2 (2.8)	4 (7.5)[e]	11 (16.6)

[a]$P < 0.002$, [b]$P < 0.0001$ *vs* hepatitis C virus (HCV); [c]$P < 0.0001$, [d]$P < 0.03$, [e]$P < 0.001$ *vs* human immunodeficiency virus (HIV)/HCV. IFG: Impaired fasting glucose; BMI: Body mass index; HAART: Highly active anti-retroviral therapy.

Table 2 shows the main hematological and virological parameters in the 3 study groups. Serum ALT levels were significantly higher in HCV mono-infected patients than in HIV/HCV co-infected and HIV mono-infected patients ($P < 0.02$). Serum AST, ALT and γGT levels were significantly higher in HIV/HCV co-infected than in HIV mono-infected patients ($P < 0.0001$). Only γGT levels were more elevated in HIV/HCV co-infected patients than in HCV mono-infected patients ($P < 0.01$). TG levels were significantly higher in HIV mono-infected and HIV/HCV co-infected patients than in HCV mono-infected patients ($P < 0.004$).

Extent of LF

In the overall population LS measured by TE ranged from 3.2 to 48.8 kPa. In 9 HIV/HCV co-infected patients, HCV-RNA was undetectable and for this reason these patients were excluded from the analysis, which was thus carried out only in the remaining 57 co-infected patients. However, their LS was lower than in the remaining co-infected group (data not shown).

Table 3 shows the distribution of LS values measured in all 3 study groups. Co-infected patients had higher LS values than mono-infected patients ($\chi^2_{MH} = 4$, $P < 0.04$). The HIV/HCV-co-infected population had LS ≥ 9.5 kPa (50.9%) more often than HCV and HIV mono-infected patients (28.3%) ($\chi^2 = 5$, $P < 0.03$). In this respect 60% of co-infected patients under virologically unsuccessful HAART showed LS ≥ F3. The individual values of LS increased from HIV to HCV and to HIV/HCV infected patients (ρ = 0.5, $P < 0.0001$) (Figure 1).

Overall, by multiple linear regression analysis, the variables significantly associated with ALF were AST values (β = 0.47, $P < 0.0001$) and HIV/HCV co-infection (β = 0.25, $P < 0.002$). To better understand which variables

Table 2 Main hematological and virological parameters in the three study groups (mean ± SD)

	HIV (*n* = 71)	HCV (*n* = 53)	HIV/HCV (*n* = 66)
AST/ALT	1 ± 0.4	0.72 ± 0.28[a,j]	0.9 ± 0.4
ALT (U/L), mean (range)	22 (7-113)[f,h]	75 (16-551)	56.5 (9-280)[g]
AST (U/L), mean (range)	21 (11-56)[f,h]	52.5 (18-209)	42.5 (14-281)
γGT (IU/L), mean (range)	33 (9-461)[h]	40.5 (10-479)[i]	76.5 (11-479)
Mean platelet count (10^9/L)	208 ± 59	184 ± 60	173 ± 61[b]
T-Chol (mg/dL)	201 ± 46	175 ± 28[d]	171 ± 48[c]
HDL-Chol (mg/dL)	44 ± 16	47 ± 12	44 ± 15
LDL Chol (mg/dL)	123 ± 32	108 ± 37.5	97 ± 40[a]
TG (mg/dL), mean (range)	137 (36-615)	103 (32-354)[e,j]	118 (49-614)
HCV-RNA (IU/mL) > 700.000	-	29 (54.7)	44 (66.6)
HIV-RNA (copies/mL), mean (range)	6050 (50-700 000)	-	3300 (60-1 100 000)
HIV-RNA < 47 copies/mL (%)	44 (62)		45 (68.1)
CD4+ count (cells/μL), mean (range)	466.5 (17-1282)	-	446 (35-1445)
CD4+ < 200 cells/μL	38[1]	-	38[2]

[a]$P < 0.0001$, [b]$P < 0.002$, [c]$P < 0.001$, [d]$P < 0.004$, [e]$P < 0.02$ *vs* human immunodeficiency virus (HIV); [f]$P < 0.0001$, [g]$P < 0.02$ *vs* hepatitis C virus (HCV); [h]$P < 0.0001$, [i]$P < 0.01$, [j]$P < 0.05$ *vs* HIV/HCV. [1]Data available in 66 patients; [2]Data available in 56 patients. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: γ-glutamyl transferase; T-Chol: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; TG: Triglyceride.

Table 3 Estimates of liver fibrosis using transient elastography in the study population *n* (%)

LS	Metavir	HIV (*n* = 71)	HCV (*n* = 53)	HIV/HCV (*n* = 57)	
< 7.1	F0-F1	56 (78.8)	23 (43.4)	19 (33.3)	
7.1-9.4	F2	11 (15.4)	15 (28.3)	9 (15.8)	
9.5-12.4	F3	1 (1.4)	4 (7.5)	9 (15.8)	
≥ 12.5	F4	3 (4.2)	11 (20.7)	20 (35.1)	$\chi^2_{MH} = 4$, $P < 0.04$
≥ F3		4 (5.6)	15 (28.3)	29 (50.9)	$\chi^2 = 5$, $P < 0.03$

HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; LS: Liver stiffness.

Table 4 Estimates of liver fibrosis using biochemical markers and transient elastography, mean (range)

	HIV (*n* = 71)	HCV (*n* = 53)	HIV/HCV (*n* = 57)
APRI	0.31 (0.1-1.6)[a,b]	0.93 (0.28-6.07)	0.92 (0.17-19.7)
FIB-4	0.93 (0.39-4.28)[a]	1.62 (0.47-12.6)	1.63 (0.63-23.8)
LS	5.4 (3.2-26.6)[a,b]	7.3 (3.4-40.9)[c]	9.8 (4.3-48.8)

[a]$P < 0.0001$ *vs* hepatitis C virus (HCV); [b]$P < 0.0001$, [c]$P < 0.05$ *vs* human immunodeficiency virus (HIV)/HCV. LS: Liver stiffness; APRI: Aspartate aminotransferase platelet ratio index.

were associated with LS in patients with HIV mono- and co-infection, we also performed multiple linear regression analysis of these 2 groups and found that ALF correlated positively with AST serum levels (β = 0.34, $P < 0.0001$) and presence of HIV/HCV co-infection (β = 0.4, $P < 0.0001$) and negatively with lower CD4+ cell counts (β = -0.21, $P < 0.003$).

Median values of APRI and FIB-4 were significantly higher in HCV mono- and co-infected patients than in HIV mono-infected patients. There were no significant differences in APRI and FIB-4 medians in HCV mono- and HIV/HCV co-infected patients (Table 4). Median values of LS were significantly lower in HIV mono-infected than in HCV mono- and HIV/HCV co-infected patients ($P < 0.0001$). Median values of HIV/HCV co-infected patients were also significantly higher than in HCV mono-infected patients ($P < 0.05$).

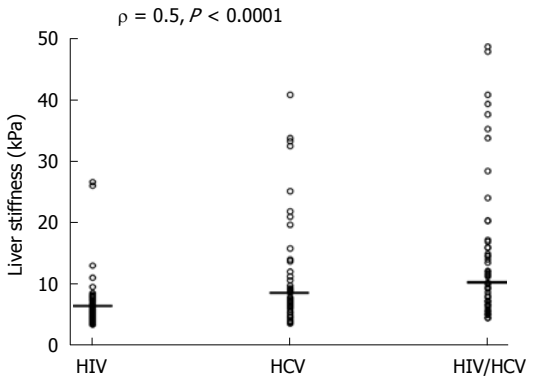

Figure 1 Median and distribution of liver stiffness in human immunodeficiency virus, hepatitis C virus, human immunodeficiency virus/hepatitis C virus patients. HIV: Human immunodeficiency virus; HCV: Hepatitis C virus.

Overall, correlations between LS and APRI values and LS and FIB-4 values were statistically significant ($r = 0.60$, $P < 0.0001$ and $r = 0.64$, $P < 0.0001$, respectively). However, when correlations were made according to the 3 cut-off values of LS, we found a significant correlation only for values of LS ≥ 9.5 ($r = 0.50$, $P < 0.0001$ *vs* APRI and $r = 0.53$, $P < 0.0001$ *vs* FIB-4) (Table 5).

Correlation between LS and risk factors studied

Table 6 shows the factors associated with LS in the 3 groups. No correlation between the studied parameters and LS was found in the HIV mono-infected group. In HCV mono-infected patients, LS positively correlated with alcohol intake > 20 g/d ($P < 0.001$) and AST serum level ($P < 0.0001$), while it negatively correlated with the

Table 5 Correlation between liver stiffness and aminotransferase platelet ratio index/FIB-4 values according to the liver stiffness cut-offs

	LS < 7.1 kPa (*n* = 98)		LS (7.1-9.4 kPa) (*n* = 35)		LS ≥ 9.5 kPa (*n* = 48)	
	r	*P*	*r*	*P*	*r*	*P*
APRI	0.01	NS	0.23	NS	0.50	< 0.0001
FIB-4	0.01	NS	0.25	NS	0.53	< 0.0001

APRI: Aminotransferase platelet ratio index; LS: Liver stiffness; NS: Not significant.

Table 6 Correlation (*r* or ρ) between liver stiffness and studied parameters

	LS		
	HIV	HCV	HIV/HCV
BMI	0.11	-0.1	0.02
HCV genotype 3/non 3	-	-0.1	-0.26
HCV-RNA	-	-	-0.26
HIV-RNA	0.21	-	0.18
Time HAART	-0.047	-	0.09
CD4 cells count	0.074	-	-0.32[d]
Alcohol	-0.096	0.70[a]	0.30[e]
T-Chol	-0.09	-0.32[b]	-0.18
TG	0.06	0.18	0.1
HDL-Chol	-0.1	0.05	-0.16
Diabetes + IFG	-0.01	0.19	0.14
Time HCV	-	0.24	-0.02
ALT	0.03	0.1	0.23
AST	0.18	0.56[c]	0.40[a]
Platelets	-0.02	-0.56[c]	-0.61[c]
APRI score	0.04	0.70[c]	0.50[c]
FIB-4 score	0.06	0.75[c]	0.60[c]

LS: Liver stiffness; HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; BMI: Body mass index; HAART: Highly active anti-retroviral therapy; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; T-Chol: Total cholesterol; HDL: High density lipoprotein; TG: Triglyceride; IFG: Impaired fasting glucose; APRI: Aminotransferase platelet ratio index. [a]$P < 0.001$; [b]$P < 0.03$; [c]$P < 0.0001$; [d]$P < 0.02$; [e]$P < 0.04$.

number of platelets ($P < 0.0001$) and serum cholesterol levels ($P < 0.03$). APRI and FIB-4 values were significantly associated with LS both in HCV and HIV/HCV co-infected patients ($P < 0.0001$).

In HIV/HCV-co-infected patients the extent of LS was significantly correlated with alcohol intake ($P < 0.04$) AST level ($P < 0.0001$) and lower CD4+ cells count ($P < 0.02$) and was negatively correlated with platelets ($P < 0.0001$).

BMI, presence of diabetes or IFG, HCV- and HIV-viremia were not significantly correlated with LS in any of the 3 study groups. In addition, there was no significant correlation between the extent of LF under HAART exposure and duration of HAART exposure. There was no significant correlation either between extent of LF and cumulative exposure to each class of antiretroviral drugs (non-nucleoside/nucleoside reverse-transcriptase inhibitors, protease inhibitors) and of specific dideoxynucleoside analogues (didanosine, stavudine, zidovudine).

DISCUSSION

Our results, in agreement with other studies, confirmed that LF is more severe in HIV/HCV co-infected patients than in HCV or HIV mono-infected patients[5,23,24]. In addition, ALF was significantly associated with a lower CD4+cell count in co-infected patients. There is convincing evidence that co-infection with HIV worsens the prognosis of HCV-related liver disease. It has been reported that in patients co-infected with HIV and HCV the risk of progressing to cirrhosis and liver failure is higher than in those infected with only HCV[25,26], especially in individuals with CD4 < 200 cells/μL and alcohol consumption[2].

In our study, 20% of HIV/HCV co-infected patients were under virologically unsuccessful HAART and in 50% CD4+ levels were below 400 cells/mm^3 suggesting, in agreement with former findings, that the less successful the response to HAART, the less marked is its clinical benefit. In fact, immune recovery under HAART has been associated with longer overall survival, slower progression of HCV-related liver damage in HIV co-infected patients and with lengthier survival times before death attributable to liver disease[27]. In the same way, Pineda *et al*[27] demonstrated that liver decompensation emerged earlier in patients who maintained an undetectable HIV viral load for a shorter period during follow-up. Nevertheless, the association between ALF and lower CD4 cell count suggests that the response to HAART, measured using CD4 cell gain and HIV viral load decline, determines the evolution of liver disease and that virologically and immunologically successful HAART may slow progression of LF in HIV/HCV co-infected patients.

On the other hand, antiretroviral-related liver toxicity could have further contributed to liver damage in our HIV population[7]. Mitochondrial toxicity of nucleoside analogues and glucose or lipid abnormalities, particularly common when using some protease inhibitors, may produce or enhance LF progression in HIV-seropositive patients. The correlation between use of antiretroviral drugs and LF in patients with HIV/HCV co-infection has been evaluated in different studies but with contradictory results[21,28-30].

Macías *et al*[21] reported that HAART regimens, including nevirapine, were associated with an increased degree of LF, while the use of protease inhibitor-based HAART was associated with less severe fibrosis and a slower progression of fibrosis in HIV/HCV co-infected patients. In contrast, Berenguer *et al*[28] found that exposure to NNRTI was associated with a reduction in LF progression. In addition, Halfon *et al*[29] showed that exposure to NNRTI was an independent factor in LF while Blanco *et al*[30] highlighted that exposure to dideoxynucleosides was an independent factor associated with ALF.

In our study, no correlation was found between HAART exposure, duration of HAART exposure or cumulative exposure to any class of antiretroviral drugs and LF. In addition, we analyzed the correlation between duration of

exposure to dideoxynucleosides (in particular didanosine, stavudine, zidovudine) and LF but also in this case no correlation was observed, suggesting that these drugs could not play any role in the progression of LF.

ALF was significantly higher in HIV/HCV co-infected patients than in HCV mono-infected patients when the subset of co-infected patients with undetectable HCV-RNA were excluded from the analysis. Overall, HIV/HCV co-infected patients with undetectable HCV-RNA had either no or only mild fibrosis (F0-F1) compared to the remaining co-infected patients, suggesting that the presence of HCV is important in conditioning the progression of LF and that anti-HCV therapy is mandatory in HIV/HCV co-infected patients in order to eradicate the virus. In fact, other authors have reported that achieving HCV clearance may reduce liver-related complications and mortality[31,32] and probably permits at least a partial regression of LF[33]. However, in HIV-positive patients liver cirrhosis may also occur without chronic viral hepatitis, and possible causes of hepatic steatosis in patients with HIV may be due to HIV itself, pathological alcohol use, diabetes mellitus, obesity or antiretroviral medications[34].

Evaluation of LF using the 2 biochemical scores (APRI and FIB-4) was not in full agreement with LS measurement. In fact, these 2 biochemical tests were in agreement with TE values only for high-grade LF, but not in low and moderate LF, suggesting that at least in these cases liver biopsy could be necessary to assess the precise degree of LF. In this respect, we are aware that the lack of liver biopsy, as a reference tool of LF, is a limitation of our study.

More consideration, perhaps, should be given to transaminase levels. In fact, the HCV mono-infected group had the highest levels of transaminases, which may have influenced LS values by increasing them. This result could further support our observation that co-infected patients are at highest risk of LF because of their high AST levels and the immune suppression associated with a low CD4 cell count.

Finally, also in our area, HCV genotype 3 was confirmed to be more associated with HIV-positive patients, because of their habits as drug abusers[35].

In conclusion, in our population, HIV/HCV co-infected patients had more ALF than HCV and HIV mono-infected patients. This result was not correlated with long-term exposure to HAART but with a lower CD4 cell count, suggesting that immunologically successful HAART may protect from progression of liver damage in HIV/HCV co-infected patients. In addition, the detection of unsuspected ALF in HIV mono-infected patients confirms that FibroScan® is very useful in this population. HCV infection, with its different pattern of cytolysis, may condition LS values, but viral eradication is mandatory to reduce fibrosis progression. Finally, the use of these non-invasive parameters of LF should be considered with caution. In fact, from our data it emerges that both TE and the biochemical scores may be suitable only for high grades of LF. In contrast, for mild/moderate degrees of fibrosis, they could not replace liver biopsy in the correct evaluation of LF.

COMMENTS

Background

Liver disease associated with hepatitis C virus (HCV) has emerged as a significant problem in human immunodeficiency virus (HIV) patients, thanks to improved survival in the highly active anti-retroviral therapy (HAART) era. Co-infection with HIV is known to lead to a more rapid progression of HCV liver disease to cirrhosis. Other factors such as severe immune suppression and alcohol consumption accelerate the progression of HCV-related fibrosis. In addition, successful HAART slows the progression of liver fibrosis (LF), but antiretroviral-liver toxicity could contributes to hepatic damage in co-infected patients. The advent of transient elastography (TE) has demonstrated to be very helpful for the non-invasive measurement of LF.

Research frontiers

Percutaneous liver biopsy is the gold standard for assessing LF but it is an invasive technique with risk of morbidity and mortality. For these reasons new non-invasive methods for the assessment of LF have recently been developed. TE (FibroScan®) and biochemical markers have demonstrated to be very helpful in the non-invasive measurement of LF. In this study, using these non-invasive tools, i.e. TE plus 2 biochemical tests, aminotransferase platelet ratio index (APRI) and FIB-4, we showed that advanced LF was significantly higher in HIV/HCV co-infected patients than in mono-infected patients and that it was significantly associated with lower CD4+ cells count. The APRI and FIB-4 tests correlated only with the highest values of TE, i.e. $\geqslant$ 9.5, suggesting that they are useful tools in diagnosing high grade liver disease, but in the case of a low or moderate degrees of LF liver biopsy remains the best means for correctly diagnosing the degree of fibrosis. Furthermore, the results showed that, overall, a greater number of HIV/HCV co-infected patients with undetectable HCV-RNA had either no or mild fibrosis (F0-F1) compared to the remaining co-infected patients with detectable HCV-RNA.

Innovations and breakthroughs

Several studies have been carried out to identify factors related to an accelerated progression of LF in HIV/HCV co-infected patients. Conflicting results have been reported in the literature about the role of antiretroviral therapy on the progression of LF. In the study, information on alcohol intake, duration of HCV infection and cumulative exposure to non-nucleoside and nucleoside reverse-transcriptase inhibitors, protease inhibitors and specific dideoxynucleoside analogues (didanosine, stavudine, zidovudine) was evaluated. The results showed that on univariate analysis liver stiffness (LS) was significantly associated with alcohol intake > 20 g/d in both HCV mono-infected and co-infected patients, but we did not find any correlations between LF and duration of HCV infection, HAART exposure, duration of HAART exposure or cumulative exposure to any class of antiretroviral drugs.

Applications

A good adherence to antiretroviral therapy, when it is indicated, is important to reduce the risk of progression of LF in co-infected patients. In addition, HCV mono- and co-infected patients should modify negative habits and lifestyles, such as alcohol consumption, which could accelerate the progression of LF. Important fields for further study could include the use and evaluation of the applicability of FibroScan® for repeated assessment in the monitoring of LF.

Terminology

Transient elastometry (Fibro-Scan®; EchoSens, Paris, France) is a rapid, reliable and well-tolerated imaging technique for the assessment of LF by measuring LS.

Peer review

The authors aimed to assess the prevalence of advanced LF (ALF) in HIV, HCV and HIV/HCV patients using TE and to identify factors associated with ALF. They concluded that HIV/HCV co-infected patients had ALF more frequently at TE than HCV and HIV mono-infected patients. The title reflects accurately the contents of the article, and the abstract delineates concisely the research.

REFERENCES

1 **Mocroft A**, Soriano V, Rockstroh J, Reiss P, Kirk O, de Wit S, Gatell J, Clotet B, Phillips AN, Lundgren JD. Is there evidence for an increase in the death rate from liver-related disease in patients with HIV? *AIDS* 2005; **19**: 2117-2125

2 **Benhamou Y**, Bochet M, Di Martino V, Charlotte F, Azria

F, Coutellier A, Vidaud M, Bricaire F, Opolon P, Katlama C, Poynard T. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. *Hepatology* 1999; **30**: 1054-1058
3 **Pineda JA**, Romero-Gómez M, Díaz-García F, Girón-González JA, Montero JL, Torre-Cisneros J, Andrade RJ, González-Serrano M, Aguilar J, Aguilar-Guisado M, Navarro JM, Salmerón J, Caballero-Granado FJ, García-García JA. HIV coinfection shortens the survival of patients with hepatitis C virus-related decompensated cirrhosis. *Hepatology* 2005; **41**: 779-789
4 **Martín-Carbonero L**, Benhamou Y, Puoti M, Berenguer J, Mallolas J, Quereda C, Arizcorreta A, Gonzalez A, Rockstroh J, Asensi V, Miralles P, Laguno M, Moreno L, Girón JA, Vogel M, García-Samaniego J, Nuñez M, Romero M, Moreno S, de la Cruz JJ, Soriano V. Incidence and predictors of severe liver fibrosis in human immunodeficiency virus-infected patients with chronic hepatitis C: a European collaborative study. *Clin Infect Dis* 2004; **38**: 128-133
5 **Mohsen AH**, Easterbrook PJ, Taylor C, Portmann B, Kulasegaram R, Murad S, Wiselka M, Norris S. Impact of human immunodeficiency virus (HIV) infection on the progression of liver fibrosis in hepatitis C virus infected patients. *Gut* 2003; **52**: 1035-1040
6 **Bräu N**, Salvatore M, Ríos-Bedoya CF, Fernández-Carbia A, Paronetto F, Rodríguez-Orengo JF, Rodríguez-Torres M. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. *J Hepatol* 2006; **44**: 47-55
7 **Núñez M**, Soriano V. Hepatotoxicity of antiretrovirals: incidence, mechanisms and management. *Drug Saf* 2005; **28**: 53-66
8 **John M**, Moore CB, James IR, Nolan D, Upton RP, McKinnon EJ, Mallal SA. Chronic hyperlactatemia in HIV-infected patients taking antiretroviral therapy. *AIDS* 2001; **15**: 717-723
9 **Carr A**, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, Cooper DA. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* 1998; **12**: F51-F58
10 **Dinh MH**, Stosor V, Rao SM, Miller FH, Green RM. Cryptogenic liver disease in HIV-seropositive men. *HIV Med* 2009; **10**: 447-453
11 **Castellares C**, Barreiro P, Martín-Carbonero L, Labarga P, Vispo ME, Casado R, Galindo L, García-Gascó P, García-Samaniego J, Soriano V. Liver cirrhosis in HIV-infected patients: prevalence, aetiology and clinical outcome. *J Viral Hepat* 2008; **15**: 165-172
12 **Poniachik J**, Bernstein DE, Reddy KR, Jeffers LJ, Coelho-Little ME, Civantos F, Schiff ER. The role of laparoscopy in the diagnosis of cirrhosis. *Gastrointest Endosc* 1996; **43**: 568-571
13 **Piccinino F**, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J Hepatol* 1986; **2**: 165-173
14 **Montalto G**, Soresi M, Carroccio A, Bascone F, Tripi S, Aragona F, Di Gaetano G, Notarbartolo A. Percutaneous liver biopsy: a safe outpatient procedure? *Digestion* 2001; **63**: 55-60
15 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713
16 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54
17 **de Lédinghen V**, Douvin C, Kettaneh A, Ziol M, Roulot D, Marcellin P, Dhumeaux D, Beaugrand M. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**: 175-179
18 **de Lédinghen V**, Barreiro P, Foucher J, Labarga P, Castéra L, Vispo ME, Bernard PH, Martin-Carbonero L, Neau D, García-Gascó P, Merrouche W, Soriano V. Liver fibrosis on account of chronic hepatitis C is more severe in HIV-positive than HIV-negative patients despite antiretroviral therapy. *J Viral Hepat* 2008; **15**: 427-433
19 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553
20 **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
21 **Macías J**, Girón-González JA, González-Serrano M, Merino D, Cano P, Mira JA, Arizcorreta-Yarza A, Ruíz-Morales J, Lomas-Cabeza JM, García-García JA, Corzo JE, Pineda JA. Prediction of liver fibrosis in human immunodeficiency virus/hepatitis C virus coinfected patients by simple noninvasive indexes. *Gut* 2006; **55**: 409-414
22 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325
23 **Martinez-Sierra C**, Arizcorreta A, Díaz F, Roldán R, Martín-Herrera L, Pérez-Guzmán E, Girón-González JA. Progression of chronic hepatitis C to liver fibrosis and cirrhosis in patients coinfected with hepatitis C virus and human immunodeficiency virus. *Clin Infect Dis* 2003; **36**: 491-498
24 **Thein HH**, Yi Q, Dore GJ, Krahn MD. Natural history of hepatitis C virus infection in HIV-infected individuals and the impact of HIV in the era of highly active antiretroviral therapy: a meta-analysis. *AIDS* 2008; **22**: 1979-1991
25 **Deng LP**, Gui XE, Zhang YX, Gao SC, Yang RR. Impact of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. *World J Gastroenterol* 2009; **15**: 996-1003
26 **Graham CS**, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, Koziel MJ. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. *Clin Infect Dis* 2001; **33**: 562-569
27 **Pineda JA**, García-García JA, Aguilar-Guisado M, Ríos-Villegas MJ, Ruiz-Morales J, Rivero A, del Valle J, Luque R, Rodríguez-Baño J, González-Serrano M, Camacho A, Macías J, Grilo I, Gómez-Mateos JM. Clinical progression of hepatitis C virus-related chronic liver disease in human immunodeficiency virus-infected patients undergoing highly active antiretroviral therapy. *Hepatology* 2007; **46**: 622-630
28 **Berenguer J**, Bellón JM, Miralles P, Alvarez E, Sánchez-Conde M, Cosín J, López JC, Alvarez F, Catalán P, Resino S. Identification of liver fibrosis in HIV/HCV-coinfected patients using a simple predictive model based on routine laboratory data. *J Viral Hepat* 2007; **14**: 859-869
29 **Halfon P**, Pénaranda G, Carrat F, Bedossa P, Bourlière M, Ouzan D, Renou C, Tran A, Rosenthal E, Wartelle C, Delasalle P, Cacoub P. Influence of insulin resistance on hepatic fibrosis and steatosis in hepatitis C virus (HCV) mono-infected compared with HIV-HCV co-infected patients. *Aliment Pharmacol Ther* 2009; **30**: 61-70
30 **Blanco F**, Barreiro P, Ryan P, Vispo E, Martín-Carbonero L, Tuma P, Labarga P, Medrano J, González-Lahoz J, Soriano V. Risk factors for advanced liver fibrosis in HIV-infected individuals: role of antiretroviral drugs and insulin resistance. *J Viral Hepat* 2010; Epub ahead of print
31 **Soriano V**, Maida I, Núñez M, García-Samaniego J, Barreiro P, Martín-Carbonero L, González-Lahoz J. Long-term follow-up of HIV-infected patients with chronic hepatitis C virus

infection treated with interferon-based therapies. *Antivir Ther* 2004; **9**: 987-992

32 **Berenguer J**, Alvarez-Pellicer J, Martín PM, López-Aldeguer J, Von-Wichmann MA, Quereda C, Mallolas J, Sanz J, Tural C, Bellón JM, González-García J. Sustained virological response to interferon plus ribavirin reduces liver-related complications and mortality in patients coinfected with human immunodeficiency virus and hepatitis C virus. *Hepatology* 2009; **50**: 407-413

33 **Soriano V**, Labarga P, Ruiz-Sancho A, Garcia-Samaniego J, Barreiro P. Regression of liver fibrosis in hepatitis C virus/HIV-co-infected patients after treatment with pegylated interferon plus ribavirin. *AIDS* 2006; **20**: 2225-2227

34 **Sterling RK**, Contos MJ, Smith PG, Stravitz RT, Luketic VA, Fuchs M, Shiffman ML, Sanyal AJ. Steatohepatitis: Risk factors and impact on disease severity in human immunodeficiency virus/hepatitis C virus coinfection. *Hepatology* 2008; **47**: 1118-1127

35 **Soriano V**, Mocroft A, Rockstroh J, Ledergerber B, Knysz B, Chaplinskas S, Peters L, Karlsson A, Katlama C, Toro C, Kupfer B, Vogel M, Lundgren J. Spontaneous viral clearance, viral load, and genotype distribution of hepatitis C virus (HCV) in HIV-infected patients with anti-HCV antibodies in Europe. *J Infect Dis* 2008; **198**: 1337-1344

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BRIEF ARTICLE

NKX2-3 and IRGM variants are associated with disease susceptibility to IBD in Eastern European patients

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Abstract

AIM: To investigate variants of immunity-related GTPase family M (IRGM) and NKX2-3 genes and genotype-phenotype in Eastern European patients with inflammatory bowel disease (IBD).

METHODS: We analyzed 1707 Hungarian and Czech subjects with Crohn's disease (CD) (n = 810, age: 37.1 ± 12.6 years, duration: 10.7 ± 8.4 years) and ulcerative colitis (UC) (n = 428, age: 43.7 ± 15.0 years, duration: 12.6 ± 9.9 years), as well as 469 healthy controls. IRGM rs13361189, NKX2-3 rs10883365 and ECM1 rs13294 polymorphisms were tested by LightCycler allele discrimination. Detailed clinical phenotypes were determined by reviewing the medical charts.

RESULTS: NKX2-3 rs10883365 variant allele was associated with increased risk for CD (P = 0.009, OR = 1.24, 95% CI = 1.06-1.48) and UC (P = 0.001, OR = 1.36, 95% CI = 1.13-1.63), whereas variant IRGM allele increased risk for CD (P = 0.029, OR = 1.36, 95% CI = 1.03-1.79). In contrast, ECM1 rs13294 was not associated with either CD or UC. In CD, the variant IRGM allele was associated with a colon-only location (P = 0.02, OR = 1.62, 95% CI = 1.07-2.44), whereas in UC, the ECM1 variant was associated with cutaneous manifestations (P = 0.002, OR = 3.36, 95% CI = 1.48-7.63). Variant alleles did not predict resistance to steroids or azathioprine, efficacy of infliximab, or need for surgery.

CONCLUSION: NKX2-3 and IRGM are susceptibility loci

for IBD in Eastern European patients. Further studies are needed to confirm the reported phenotype-genotype associations.

Key words: Crohn's disease; Ulcerative colitis; NKX2-3; Immunity-related GTPase family M; ECM1; Genotype; Phenotype; Pharmacogenetics

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INTRODUCTION

Inflammatory bowel diseases (IBDs) are multifactorial with both environmental and genetic components; the latter displaying heterogeneity in terms of disease presentation as well as response to treatment[1]. Crohn's disease (CD) has a strong genetic component, and to date, at least nine susceptibility loci have been identified[2]. The first, and most consistently replicated critical mutations have been found in the NOD2/CARD15 gene on chromosome 16 (IBD1). Other loci encode genes that are involved in a number of homeostatic mechanisms: innate pattern recognition receptors (including NOD2/CARD15, TLR4 and CARD9), differentiation of Th17-lymphocytes (IL-23R, JAK2, STAT3, CCR6 and ICOSLG), autophagy [ATG16L1, immunity-related GTPase family M (IRGM) and LRRK2], maintenance of epithelial barrier integrity (IBD5, DLG5, PTGER4, ITLN1, DMBT1 and XBP1), and orchestration of the secondary immune response (HLA-region, TNFSF15/TL1A, IRF5, PTPN2, PTPN22, NKX2-3, IL-12B, IL-18RAP and MST1)[3-6]. As the incidence of IBD is rapidly increasing in some parts of Eastern Europe[1], it is of great importance to study social and environmental, as well as host-genetic factors that might underlie this trend.

The IRGM gene is located on chromosome 5q33.1, and encodes an autophagy-inducing protein and belongs to the immunity-related guanosine triphosphatases (IRGs), also known as p47 guanosine triphosphatases. IRGs play an important role in host defense against intracellular pathogens[7]. Genetic association of the autophagy genes, autophagy-related 16-like 1 (ATG16L1) and IRGM has been suggested in adult-onset CD by genome-wide association scan studies (GWASs), but not in UC[5,6,8-10]. Although a meta-analysis of the three index GWASs has implicated a single nucleotide polymorphism (SNP; rs11747270) based on imputed data with an OR of 1.33[10], other studies including a new meta-analysis[11] have confirmed the IRGM signal on rs13361189 and rs4958847 immediately flanking IRGM.

Recently, the rs13361189 variant, upstream of IRGM, has been shown to be in perfect linkage disequilibrium with a 20-kb deletion polymorphism that affects the expression of IRGM (and cellular autophagy) in a tissue-specific manner[9]. These results suggest that the CD association at IRGM arises from an alteration in IRGM regulation that affects the efficacy of autophagy, and identifies rs13361189 and its strongly correlated neighbors at the 5' end of IRGM, including the 20-kb deletion polymorphism as a likely causal variant.

Association between the rs10883365 variant of NK2 transcription factor related, locus 3 (NKX2-3) gene on chromosome 10q24.2 and susceptibility of CD has been reported in Western Europe, including The Wellcome Trust Case Control Consortium (WTCCC) GWA and a replication study from The Netherlands, and in Japan, with an OR of 1.2-1.6[12-14]. In addition, a modest association ($P = 3.3 \times 10^{-4}$ in the ulcerative colitis panel and $P = 2.4 \times 10^{-6}$ using the expanded WTCCC control panel) has also been reported between rs10883365 and UC in a non-synonymous SNP scan by Fisher *et al*[15]. *NKX2-3*-deficient mice show severe defects in gut development; primarily in the epithelium of the small intestine[16]. In addition, the lymphoid organs of these mice, including the spleen and Peyer's patches, have abnormal tissue architecture and abnormalities in the migration and segregation of B and T cells[17], however, the exact mechanism is unknown.

ECM1, on chromosome 1q21.2, is also a plausible candidate gene for UC; it encodes extracellular matrix protein 1, a glycoprotein expressed in small and large intestine, and it interacts with the basement membrane and inhibits matrix metalloproteinase[18]. Notably, ECM1 strongly activates nuclear factor-κB signaling, a key immune regulator. Expression is upregulated in colorectal cancer and metastases, which implicates ECM1 in epithelial-stromal interaction[18]. Of note, the WTCCC observed modest association between these ECM1 SNPs and ankylosing spondylitis, a related inflammatory disorder ($P = 0.0041$ and 0.0044), respectively[13]. More recently, an association between the rs3737240 and rs13294 variants of ECM1 and UC susceptibility was reported in a GWAS by Fisher *et al*[15], with an OR of 1.3-1.4 for the homozygous carriage of the variant allele. rs3737240 and rs13294 encode substitutions T130M and G290S: Thr130, residing within a collagen IV binding domain, is conserved in primates, whereas Gly290 is not.

Finally, data about the pharmacogenetics of IBD are still limited. Resistance to steroids is associated with high expression of β-glucocorticoid receptors (hGRβ) and overexpression of MDR1 has been found in patients who fail steroid therapy and require surgery[19]. In contrast, according to our group's earlier results, the presence of the DLG5 Arg30Gln variant allele, but not variants in ABCG2 or MDR1 genes, predicted resistance to steroids[20,21]. Vermeire *et al*[22] have reported a lack of association between the

presence of NOD2 mutations and response to infliximab therapy, and later, the same group reported an association between the presence of Fas ligand -843C/T variant and response to infliximab[23].

In light of the lack of data in Eastern European countries, our aim was to investigate the prevalence of IRGM rs13361189, NKX2-3 rs10883365 and ECM1 rs13294 variants in large independent cohorts of Czech and Hungarian IBD patients. We also aimed to investigate a possible association between genotype and clinical phenotype, need for surgery, and response to medical therapy.

MATERIALS AND METHODS

Study population

One thousand seven hundred and seven unrelated IBD patients (CD: n = 810, age: 37.1 ± 12.6 years, duration: 10.7 ± 8.4 years and UC: n = 428, age: 43.7 ± 15.0 years, duration: 12.6 ± 9.9 years) and 469 healthy subjects (blood donors) from Hungary and the Czech Republic were investigated. The clinical data of the CD and UC patients are presented in Table 1. A detailed clinical phenotype was available in 789 CD and 422 UC patients.

The diagnosis was based on the Lennard-Jones criteria[24]. Age; age at onset; presence of extraintestinal manifestations [arthritis: peripheral and axial; ocular manifestations: conjunctivitis, uveitis and iridocyclitis; skin lesions: erythema nodosum and pyoderma gangrenosum; and hepatic manifestations: primary sclerosing cholangitis (PSC)]; frequency of flare-ups (frequent relapses: > 1 clinical relapse/year); therapeutic effectiveness (e.g. steroid and/or immunosuppressive use, steroid resistance); need for surgery (resections); the presence of familial IBD; smoking habits; and in CD, perianal involvement, were investigated by reviewing the medical charts by the physician and completing a questionnaire. The disease phenotype (age at onset, duration, location and behavior) was determined according to the Montreal classification[25].

The control group for mutation analysis consisted of 469 age- and sex-matched healthy blood donors (male/female: 251/218, age: 40.5 ± 11.5 years old). Control subjects did not have any gastrointestinal and/or liver diseases and were selected from consecutive blood donors in Budapest, Veszprem, Debrecen and Prague. The study protocol was approved by the Ethical and Science Committee of the Ministry of Health. Each patient was informed of the nature of the study and gave signed informed consent.

Genotyping and DNA isolation

Genomic DNA was isolated from whole blood according to the manufacturers' description with High Pure PCR Template preparation Kit (Roche, Budaors, Hungary).

Detection of IRGM (rs13361189,g.11386323T>C), ECM1 (rs13294, c.1243A>G, p.Ser415Gly, g.975342G>A) and NKX2-3 (rs1083365, g.20036290G>A) polymorphisms

Genotyping was performed using the LightCycler (Roche Diagnostics, Basel, Switzerland) allelic discrimination system. Amplification primers and hybridization probes were designed by the LightCycler Probe Design software (Roche Diagnostics). All oligonucleotides were synthesized by VBC Biotech (Vienna, Austria). The following amplification primers and hybridization probes were used for genotyping IRGM: IRGM-LCF: 5'-ATGGACAGTCAGTACCCTGCAC-3', IRGM-LCR: 5'-CTCTTTACCATTGTACTCCTTGTGCC-3', IRGM-ANC: 5'-LC Red 640-TGCTCAGCGGGTACAGTTTAGAAAGGGAA-Phosphate-3', IRGM-SENS: 5'-GAAAATCGGATGTATATTAGTAGACCC-Fluorescein-3'.

The amplification primers and hybridization probes used for genotyping of ECM1 were: ECM1-LCF: 5'-ACCCACCACCACTTGTGT-3', ECM1-LCR: 5'-TGCTTGGTGAGAACTCTTTGGTTT-3', ECM1-ANC: 5'-LC Red 640-TCAAGATGTCCCGGTCATAGTTGGGGTAAGGAG-Phosphate-3', ECM1-SENS: 5'-TGACTCGACCGATGTCAAT-Fluorescein-3'.

The amplification primers and hybridization probes used for genotyping of NKX2-3 were: NKX2-LCF:

Table 1 Clinical characteristics of patients with inflammatory bowel diseases

	CD (n = 810)	UC (n = 428)
Male/female (n)	434/376	202/226
Age (yr)	37.1 ± 12.6	43.7 ± 15.0
Age at presentation (yr)[1]	26.5 ± 10.6	31.3 ± 13.4
Duration (yr)[1]	10.7 ± 8.4	12.6 ± 9.9
Familial IBD[1]	11.8%	7.6%
Location in CD[1]		
L1	28.2%	
L2	18.9%	-
L3	51.2%	
L4 only	1.7%	
All L4	7.9%	
Maximum extent in UC[1]		
Proctitis	-	7.8%
Left-sided		52.3%
Extensive		39.9%
Behavior in CD[1]		
B1	34.9%	
B2	34.5%	-
B3	30.7%	
Perianal disease[1]	41.2%	-
Frequent relapse in CD/chronic continuous in UC[1]	39.9%	27.6%
Arthritis[1]	38.6%	27.1%
Cutaneous[1]	7.8%	5.9%
Occular[1]	7.7%	3.8%
PSC[1]	2.4%	3.8%
Steroid use/refractory[1]	81.1%/11.6%	66.2%/12.3%
Azathioprine use[1]	68.3%	29.3%
Anti-TNF use[1]	33.2%	10.4%
Surgery in CD/colectomy in UC[1]	50.9%	10.0%
Smoking habits[2]		
No	55.1%	71.6%
Yes	31.3%	12.4%
Previous	13.6%	16.0%

[1]Clinical phenotype available in 789 Crohn's disease (CD) and 422 ulcerative colitis (UC) patients; [2]Available in 691 CD and 388 UC patients. L1: Ileal; L2: Colon; L3: Ileocolonic; L4: Upper gastrointestinal; B1: Inflammatory; B2: Stenosing; B3: Penetrating. IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor.

5'-CCGCATAAGACGTTACTTAAACATGT-3', NKX2-LCR: 5'-GCTATCTACTCGAAACTGTCT-GC-3', NKX2-ANC-2: 5'-TCTCCCCGGGGGTCAC-GTTG-Fluorescein-3', NKX2-SENS-2: 5'-LC Red 610-ACAAACACCTTCAAACCGTC-Phosphate-3'.

Polymerase chain reaction (PCR) was performed by LightCycler 480 real-time PCR System (Roche), in a reaction volume of 20 μL with 50 ng genomic DNA, 10 μL 2 × PCR Master Mix (Promega), and 5 pmol of the respective labeled oligonucleotides (sensor and anchor). For IRGM and NKX2-3, an asymmetric multiplex system was used for simultaneous amplification of the two fragments with 3:10 pmol forward:reverse (F:R) amplification oligonucleotides and 10:3 F:R amounts for IRGM and NKX2-3 SNPs, respectively. Similarly, for ECM1, an asymmetric PCR was applied with a 10:3 pmol F:R primer mix for amplification in a separate reaction. Cycling conditions were as follows: initial denaturation at 95℃ for 3 min, followed by 35 cycles of denaturation at 95℃ for 20 s, annealing at 55℃ for 30 s, and extension at 72℃ for 45 s, with a ramping rate of 4.4℃/s.

After amplification, a melting curve analysis was performed by cooling the samples to 40℃, followed by gradual heating to 80℃ with a ramp rate of 0.04℃/s. The decline in fluorescence was continuously monitored. Melting curves were converted to melting peaks with wild-type and variant alleles showing distinct melting points. For the IRGM and NKX2-3 multiplex PCR, color compensation was performed to compensate for the fluorescence crosstalk between detection channels. Genotype calling was carried out by two independent investigators.

Statistical methods

Variables were tested for normality using Shapiro Wilk's *W* test. A *t* test with separate variance estimates, χ^2 test and χ^2 test with Yates correction were used to evaluate differences between IBD patients and controls, as well as within subgroups of IBD patients. Logistic regression was used to compare genetic and clinical data and results are expressed as OR with 95% CI. $P < 0.05$ was considered as significant. For the statistical analysis, SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used. Statistical analysis was performed by Lakatos PL with the assistance of a statistician.

RESULTS

Association between NKX2-3, IRGM and ECM1 and disease susceptibility

All investigated polymorphisms were in Hardy-Weinberg equilibrium (P = 0.38-0.95). The success rate of the genotyping assays was 98%-99%. The genotype and allele frequencies are presented in Table 2. NKX2-3 rs10883365 variant allele was associated with increased risk for CD (P = 0.018 after Bonferroni correction, OR = 1.24, 95% CI = 1.06-1.48) and UC (P = 0.003 after Bonferroni correction, OR = 1.36, 95% CI = 1.13-1.63), whereas the variant IRGM allele increased the risk of CD (P = 0.04 after Bonferroni correction, OR = 1.36, 95% CI = 1.03-1.79). The association between NKX2-3 rs10883365 variant and IBD was also significant in the genotypic and dominant models. A similar trend was noted between IRGM and CD. In contrast, ECM1 rs13294 was not associated with either CD or UC. The combination of IRGM carrier/NKX2-3 homozygote genotype was significantly higher in CD compared to the controls (7.2% *vs* 2.1%, $P < 0.0001$ after Bonferroni correction, OR = 3.55, 95% CI = 1.80-7.01).

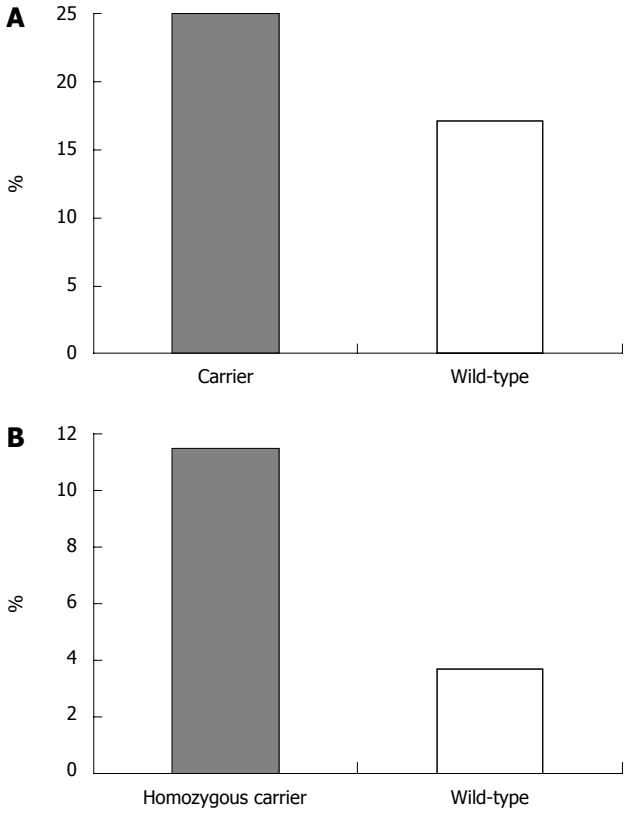

Figure 1 Association between immunity-related GTPase family, M and ECM1 and disease phenotype in patients with inflammatory bowel diseases. A: Association between the IRGM rs13361189 variant and disease location in Crohn's disease. P = 0.04, OR = 1.62, 95% CI: 1.07-2.44; B: Association between homozygous carriage of the ECM1 rs13294 variant allele and cutaneous manifestations in ulcerative colitis. P = 0.004, OR = 3.36, 95% CI: 1.48-7.63.

Role of NKX2-3, IRGM and ECM1 variants in predicting sex, familial disease, age at onset, disease location, phenotype or extraintestinal manifestations

In CD, presence of the variant IRGM allele was associated with colon-only location (in carriers: 25% *vs* wild-type: 17.1%, P = 0.04 after Bonferroni correction, OR = 1.62, 95% CI = 1.07-2.44, Figure 1A). In UC, homozygous carriage of the ECM1 variant allele was associated with cutaneous manifestations (11.5% in homozygotes *vs* 3.7% in patients with other genotypes, P = 0.004 after Bonferroni correction, OR = 3.36, 95% CI = 1.48-7.63, Figure 1B). No other significant associations were found in either CD or UC patients (data not shown).

Association between NKX2-3, IRGM and ECM1 and response to medical therapy or need for surgery

We also investigated the association between the NKX2-3, IRGM and ECM1 variants and the response to steroids,

Table 2 Association between NKX2-3, IRGM and ECM1 polymorphisms and disease susceptibility in patients with inflammatory bowel disease

	WT (%)	HET (%)	HOM (%)	*P* value	Carrier (%)	*P* value	OR (95%CI)	MAF	*P* value	OR (95%CI)
IRGM rs13361189										
All CD (*n* = 808)	636 (78.7)	156 (19.3)	16 (2.0)	0.060[1]	172 (21.3)	0.060[1]	-	188 (11.6)	0.029	1.36 (1.03-1.79)
Hungarian CD (*n* = 456)	355 (77.9)	89 (19.5)	12 (2.6)	NS[1,2]	101 (22.1)	NS[1,2]	-	113 (12.4)	NS[1,2]	-
Czech CD (*n* = 352)	281 (79.8)	67 (19.0)	4 (1.2)	NS[1,2]	71 (20.2)	NS[1,2]	-	75 (10.7)	NS[1,2]	-
All UC (*n* = 428)	351 (82.0)	76 (17.8)	1 (0.2)	NS[1,2]	77 (18.0)	NS[1,2]	-	78 (9.1)	NS[1,2]	-
Hungarian UC (*n* = 274)	221 (80.7)	52 (19.0)	1 (0.3)	NS[1,2]	53 (19.3)	NS[1,2]	-	54 (9.9)	NS[1,2]	-
Czech UC (*n* = 154)	130 (84.4)	24 (15.6)	0	NS[1,2]	24 (15.6)	NS[1,2]	-	24 (7.8)	NS[1,2]	-
All controls (*n* = 460)	382 (83.0)	75 (16.3)	3 (0.7)	-	78 (17.0)	-	-	81 (8.8)	-	-
Hungarian controls (*n* = 265)	217 (81.9)	46 (17.4)	2 (0.7)	-	48 (18.1)	-	-	50 (9.4)	-	-
Czech controls (*n* = 195)	165 (84.6)	29 (14.9)	1 (0.5)	-	30 (15.4)	-	-	31 (7.9)	-	-
NKX2 rs 10883365										
All CD (*n* = 810)	208 (25.7)	389 (48.1)	213 (26.2)	0.010[1]	602 (74.3)	NS[1,2]	-	815 (50.3)	0.009	1.24 (1.06-1.48)
Hungarian CD (*n* = 457)	115 (25.2)	220 (48.1)	122 (26.7)	NS[1,2]	342 (74.8)	NS[1,2]	-	464 (50.8)	NS[1,2]	-
Czech CD (*n* = 353)	93 (26.3)	169 (47.9)	91 (25.8)	0.070[1]	260 (73.7)	NS[1]	-	351 (49.7)	0.030[1]	1.31 (1.03-1.68)
All UC (*n* = 427)	90 (21.1)	226 (52.9)	111 (26.0)	0.004[1]	337 (78.9)	0.006[1]	1.52 (1.13-2.08)	448 (52.5)	0.001	1.36 (1.13-1.63)
Hungarian UC (*n* = 274)	56 (20.4)	152 (55.5)	66 (24.1)	NS[1,2]	218 (79.6)	0.070[1]		284 (51.8)	0.060	
Czech UC (*n* = 153)	34 (22.2)	74 (48.4)	45 (29.4)	0.017[1]	119 (77.8)	0.046[1]	1.63 (1.01-2.65)	164 (53.6)	0.005[1]	1.53 (1.14-2.07)
All controls (*n* = 469)	136 (29.0)	245 (52.2)	88 (18.8)	-	333 (71.0)	-	-	421 (44.9)	-	-
Hungarian controls (*n* = 271)	73 (26.9)	145 (53.5)	53 (19.6)	-	198 (73.1)	-	-	251 (46.3)	-	-
Czech controls (*n* = 198)	63 (31.8)	100 (50.5)	35 (17.7)	-	135 (68.2)	-	-	170 (42.9)	-	-
ECM1 rs13294										
All CD (*n* = 807)	139 (17.2)	392 (48.6)	276 (34.2)	NS[1,2]	688 (82.8)	NS[1,2]	-	944 (58.5)	NS[1,2]	-
Hungarian CD (*n* = 455)	89 (19.6)	228 (50.1)	138 (30.3)	NS[1,2]	366 (80.4)	NS[1,2]	-	504 (55.4)	NS[1,2]	-
Czech CD (*n* = 352)	50 (14.2)	164 (46.6)	138 (39.2)	NS[1,2]	302 (85.8)	NS[1,2]	-	440 (62.5)	NS[1,2]	-
All UC (*n* = 428)	77 (18.0)	225 (52.6)	126 (29.4)	NS[1,2]	351 (82.0)	NS[1,2]	-	477 (55.7)	NS[1,2]	-
Hungarian UC (*n* = 275)	44 (16.0)	156 (56.1)	75 (27.3)	NS[1,2]	231 (84.0)	NS[1,2]	-	306 (55.6)	NS[1,2]	-
Czech UC (*n* = 153)	33 (21.6)	69 (45.1)	51 (33.3)	NS[1,2]	120 (78.4)	NS[1,2]	-	171 (55.9)	NS[1,2]	-
All controls (*n* = 463)	90 (19.4)	222 (47.9)	151 (32.6)	-	373 (80.6)	-	-	524 (56.6)	-	-
Hungarian controls (*n* = 265)	56 (21.1)	124 (46.8)	85 (32.1)	-	209 (78.9)	-	-	294 (55.5)	-	-
Czech controls (*n* = 198)	34 (17.2)	98 (49.5)	66 (33.3)	-	164 (82.8)	-	-	230 (58.1)	-	-

[1]*P* value *vs* controls; [2]*P* value Crohn's disease (CD) *vs* ulcerative colitis (UC). WT: Wild-type; HET: Heterozygous carrier; HOM: Homozygous carrier; MAF: Minor allele frequency; NS: Not significant.

infliximab or azathioprine or the need for surgery in patients with CD. Two hundred and sixty-three unrelated CD patients were treated with anti-tumor necrosis factor (TNF): 259 received infliximab and four, adalimumab, two of whom were treated after secondary loss of response to infliximab therapy. Overall, there was no association between the presence of the above variants and short-term response (assessed at week 12) to infliximab induction therapy [5 mg/kg at weeks 0, 2 and 6; partial response: Crohn's disease activity index (CDAI) decreased by $\geqslant$ 70 points and/or $\geqslant$ 50% decrease in the number of draining fistulas; remission: CDAI < 150 or closure of all fistulas]; steroid use/resistance; azathioprine use; or need for surgery (data not shown). Similarly, no association was found between either of the variants and steroid use/resistance, azathioprine use or need for surgery in patients with UC (data not shown).

DISCUSSION

This is the first report on the prevalence of the IRGM rs13361189, NKX2-3 rs10883365 and ECM1 rs13294 variants, in large, independent IBD cohorts from Eastern Europe. The variant NKX2-3 allele conferred a risk in both UC and CD, whereas the IRGM rs13361189 variant allele was associated with increased risk for CD. In addition, the IRGM variant was associated with disease location in CD.

The genotype and allele frequencies for IRGM and NKX2-3 variants in CD, UC and controls reported in the present study were in line with most previous reports in Caucasian populations, whereas the frequency of the variant ECM1 rs13294 allele was approximately 10% higher in both IBD and controls, compared to that reported in previous studies[7,8,12,13,15,26].

We confirmed that the IRGM rs13361189 variant was associated with disease susceptibility in CD in Eastern European populations. The allelic OR of 1.36 (95% CI = 1.03-1.79) was in the range reported in Caucasian populations (OR = 1.38-1.56)[7,13] and in a recent meta-analysis (OR = 1.34)[10]. Similar genotype distributions were observed in both the Czech and Hungarian cohort, however, due to the low variant allele frequency, the difference became significant only in the combined analysis. A similar tendency (P = 0.06) was observed in the genotypic and dominant model. In contrast, in the present study we failed to replicate the weak association between IRGM and UC (rs13361189 P = 0.0069, pooled OR = 1.16; rs4958847 P = 0.014, pooled OR = 1.13) that was reported recently in a Spanish meta-analysis[10]. Of note, the present study was underpowered to detect such small

differences, however, there was even no trend for a difference between UC and controls.

In addition, in an Italian study[27], the rs4958847 polymorphism was associated with fistulizing behavior (P = 0.037, OR = 1.54, CI = 1.02-2.31) and perianal fistulas (P = 0.045, OR = 1.55, CI = 1.01-2.38) in a logistic regression analysis. This was later partially confirmed by the Leuven group. Henckaerts *et al*[28] have reported in a very elegant study a significant association between the IRGM rs4958847 variant, U7 gene desert rs12704036 T-allele, NOD2/CAR15 mutation, ileal involvement at diagnosis, male sex, and time to development of non-perianal fistulas in a Cox regression analysis. In a further study from New Zealand[29], rs13361189 variant increased the risk for ileal CD in 507 CD patients and 576 controls. The OR in ileal CD was 1.92 (95% CI = 1.27-2.96). Moreover, Peterson *et al*[30] have suggested an association between IRGM and pediatric disease onset (< 17 years) of CD in a North American cohort. However, only one of the two IRGM variants studied (rs13361189) was weakly associated with CD in their study (uncorrected P = 0.03). In the present study, we could not confirm the association between age at onset and the presence of the IRGM variant, in accordance with Canadian, Italian and Scottish results[29,31]. In contrast, we found an association between IRGM carriage and disease location in CD; colonic location was significantly more common in carriers of the variant allele (OR = 1.62, 95% CI = 1.07-2.44). Of note, however, the rs4958847 variant was not investigated in the present study.

The association between the rs10883365 variant of NKX2-3 gene and susceptibility of IBD was first reported in CD in Caucasian patients and in a Japanese study with an OR of 1.2-1.6[12-14]. In addition, using the UC panel and the expanded WTCCC control panel, Fisher *et al*[15] have reported a modest association ($P = 3.3 \times 10^{-4}$ and $P = 2.4 \times 10^{-6}$) between rs10883365 and UC. In the present study, we confirmed these findings; the rs10883365 variant was associated with UC and CD susceptibility in the allelic and genotypic models with an OR of 1.53 and 1.24, respectively. Based on our data, the association between NKX2-3 and IBD is stronger in UC compared to CD, at least in patients from Eastern Europe. In a recent Dutch study, the association between the rs10883365 variant of NKX2-3 and CD was linked to smoking status, with the risk being more pronounced in active and passive smokers[32]. In the present study, however, we did not find an association between NKX2-3 and smoking status, and similarly, no genotype-phenotype associations were found.

In addition, Weersma *et al*[13] have created genetic risk profiles by combining the presence of variant alleles in IL23R, ATG16L1, IRGM, NKX2-3, 1q24, 5p13, HERC2, CCNY, 10q21 and NOD2/CARD15, and the number of risk alleles was associated with gradually increased risk for CD. Similarly, in the present study, the combination of IRGM carrier/NKX2-3 homozygote genotype was associated with an increased risk for CD, with a much higher OR compared to either of the variant alleles alone.

Recently, an association between ECM1 rs3737240 and rs13294 variants and UC has been reported in a GWAS by Fisher *et al*[15], with an OR of 1.3-1.4 for the homozygous carriage of the variant allele. The association for the rs13294 variant has recently been confirmed in a Dutch study, with an OR of 1.24 in patients with UC[33]. In contrast, no association was found in CD[34], even though a different SNP (rs11205387) was investigated. Although the present study was powered to confirm a difference with an OR of 1.3-1.5 with adequate statistical power, we could not confirm an association between the ECM1 rs13294 variant and either UC or CD. The accidental association between homozygous carriage of the ECM1 variant allele and cutaneous manifestations (P = 0.002, OR = 3.36, 95% CI = 1.48-7.63, Figure 1B) requires further confirmation.

Theoretically, through influencing inflammatory responses, autophagy and epithelial-stromal interactions, polymorphisms in the above genes might be of potential importance in altering the efficacy of anti-inflammatory therapy and thereby the need for surgery. However, in the present study, none of the variant alleles was associated with the response to either steroid or infliximab therapy, or the need for surgery (resection in CD or colectomy in UC) in IBD.

In conclusion, NKX2-3 and IRGM are susceptibility loci for IBD in Eastern European patients. None of the variants investigated were associated with the need for surgery or efficacy of medical therapy. Further studies are needed to confirm the reported phenotype-genotype associations found in this study.

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COMMENTS

Background

Sequence variants in the autophagy gene immunity-related GTPase family M (IRGM) and NKX2-3 have been reported to contribute to Crohn's disease (CD) susceptibility, whereas ECM1 contributes to ulcerative colitis (UC) in genome-wide association scans in North America and Western Europe.

Research frontiers

There is a lack of data in Eastern European countries, therefore, our aim was to investigate the prevalence of IRGM rs13361189, NKX2-3 rs10883365 and ECM1 rs13294 variants. In addition, the possible association between genotype and clinical phenotype and the need for surgery is conflictive, and the association between the above genetic variants and response to medical therapy was not investigated.

Innovations and breakthroughs

In the present study, the authors showed in two well-characterized, independent

CD cohorts with strict clinical follow-up, that NKX2-3 rs10883365 variant allele was associated with increased susceptibility to CD and UC, whereas variant IRGM allele increased the risk for CD only. Our data suggest that the variant IRGM allele is associated with colon-only location, whereas in UC, the ECM1 variant was associated with cutaneous manifestations. None of the variants predicted resistance to steroids and azathioprine, efficacy of infliximab, or need for surgery.

Application

The new data presented from Eastern Europe might contribute to better understanding of genetic or environmental differences between populations and the association of those differences with disease susceptibility and phenotype.

Terminology

Vienna-Montreal classification: classification systems of disease phenotypes in CD. The classification assesses the age at presentation, disease location and disease behavior. The IRGM gene encodes an autophagy-inducing protein and plays an important role in host defense against intracellular pathogens. NKX2-3 is a member of the NKX family of homeodomain-containing transcription factors, which are implicated in many aspects of cell type specification and maintenance of differentiated tissue functions. ECM1 encodes extracellular matrix protein 1, a glycoprotein expressed in small and large intestine. Notably, ECM1 strongly activates nuclear-κB signaling, a key immune regulator.

Peer review

The need to extend studies performed in the other parts of the world to Eastern Europe is valid, and provides a point for understanding subtle genetic or environmental differences between populations and the association of those differences with disease.

REFERENCES

1 **Lakatos PL**. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World J Gastroenterol* 2006; **12**: 6102-6108

2 **Van Limbergen J**, Wilson DC, Satsangi J. The genetics of Crohn's disease. *Annu Rev Genomics Hum Genet* 2009; **10**: 89-116

3 **Lakatos PL**, Lakatos L, Szalay F, Willheim-Polli C, Osterreicher C, Tulassay Z, Molnar T, Reinisch W, Papp J, Mozsik G, Ferenci P. Toll-like receptor 4 and NOD2/CARD15 mutations in Hungarian patients with Crohn's disease: phenotype-genotype correlations. *World J Gastroenterol* 2005; **11**: 1489-1495

4 **Duerr RH**, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461-1463

5 **Hampe J**, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Günther S, Prescott NJ, Onnie CM, Häsler R, Sipos B, Fölsch UR, Lengauer T, Platzer M, Mathew CG, Krawczak M, Schreiber S. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; **39**: 207-211

6 **Lakatos PL**, Szamosi T, Szilvasi A, Molnar E, Lakatos L, Kovacs A, Molnar T, Altorjay I, Papp M, Tulassay Z, Miheller P, Papp J, Tordai A, Andrikovics H. ATG16L1 and IL23 receptor (IL23R) genes are associated with disease susceptibility in Hungarian CD patients. *Dig Liver Dis* 2008; **40**: 867-873

7 **Singh SB**, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006; **313**: 1438-1441

8 **Parkes M**, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, Roberts RG, Nimmo ER, Cummings FR, Soars D, Drummond H, Lees CW, Khawaja SA, Bagnall R, Burke DA, Todhunter CE, Ahmad T, Onnie CM, McArdle W, Strachan D, Bethel G, Bryan C, Lewis CM, Deloukas P, Forbes A, Sanderson J, Jewell DP, Satsangi J, Mansfield JC, Cardon L, Mathew CG. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet* 2007; **39**: 830-832

9 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962

10 **McCarroll SA**, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, Zody MC, Hall JL, Brant SR, Cho JH, Duerr RH, Silverberg MS, Taylor KD, Rioux JD, Altshuler D, Daly MJ, Xavier RJ. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet* 2008; **40**: 1107-1112

11 **Palomino-Morales RJ**, Oliver J, Gómez-García M, López-Nevot MA, Rodrigo L, Nieto A, Alizadeh BZ, Martín J. Association of ATG16L1 and IRGM genes polymorphisms with inflammatory bowel disease: a meta-analysis approach. *Genes Immun* 2009; **10**: 356-364

12 Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661-678

13 **Weersma RK**, Stokkers PC, Cleynen I, Wolfkamp SC, Henckaerts L, Schreiber S, Dijkstra G, Franke A, Nolte IM, Rutgeerts P, Wijmenga C, Vermeire S. Confirmation of multiple Crohn's disease susceptibility loci in a large Dutch-Belgian cohort. *Am J Gastroenterol* 2009; **104**: 630-638

14 **Yamazaki K**, Takahashi A, Takazoe M, Kubo M, Onouchi Y, Fujino A, Kamatani N, Nakamura Y, Hata A. Positive association of genetic variants in the upstream region of NKX2-3 with Crohn's disease in Japanese patients. *Gut* 2009; **58**: 228-232

15 **Fisher SA**, Tremelling M, Anderson CA, Gwilliam R, Bumpstead S, Prescott NJ, Nimmo ER, Massey D, Berzuini C, Johnson C, Barrett JC, Cummings FR, Drummond H, Lees CW, Onnie CM, Hanson CE, Blaszczyk K, Inouye M, Ewels P, Ravindrarajah R, Keniry A, Hunt S, Carter M, Watkins N, Ouwehand W, Lewis CM, Cardon L, Lobo A, Forbes A, Sanderson J, Jewell DP, Mansfield JC, Deloukas P, Mathew CG, Parkes M, Satsangi J. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nat Genet* 2008; **40**: 710-712

16 **Pabst O**, Zweigerdt R, Arnold HH. Targeted disruption of the homeobox transcription factor Nkx2-3 in mice results in postnatal lethality and abnormal development of small intestine and spleen. *Development* 1999; **126**: 2215-2225

17 **Pabst O**, Förster R, Lipp M, Engel H, Arnold HH. NKX2.3 is required for MAdCAM-1 expression and homing of lymphocytes in spleen and mucosa-associated lymphoid tissue. *EMBO J* 2000; **19**: 2015-2023

18 **Chan I**, Liu L, Hamada T, Sethuraman G, McGrath JA. The molecular basis of lipoid proteinosis: mutations in extracellular matrix protein 1. *Exp Dermatol* 2007; **16**: 881-890

19 **Farrell RJ**, Murphy A, Long A, Donnelly S, Cherikuri A, O'Toole D, Mahmud N, Keeling PW, Weir DG, Kelleher D. High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. *Gastroenterology* 2000; **118**: 279-288

20 **Fischer S**, Lakatos PL, Lakatos L, Kovacs A, Molnar T, Altorjay I, Papp M, Szilvasi A, Tulassay Z, Osztovits J, Papp J, Demeter P, Schwab R, Tordai A, Andrikovics H. ATP-binding cassette transporter ABCG2 (BCRP) and ABCB1 (MDR1) variants are not associated with disease susceptibility, disease phenotype response to medical therapy or need for surgeryin

Hungarian patients with inflammatory bowel diseases. *Scand J Gastroenterol* 2007; **42**: 726-733
21 **Lakatos PL**, Fischer S, Claes K, Kovacs A, Molnar T, Altorjay I, Demeter P, Tulassay Z, Palatka K, Papp M, Rutgeerts P, Szalay F, Papp J, Vermeire S, Lakatos L. DLG5 R30Q is not associated with IBD in Hungarian IBD patients but predicts clinical response to steroids in Crohn's disease. *Inflamm Bowel Dis* 2006; **12**: 362-368
22 **Vermeire S**, Louis E, Rutgeerts P, De Vos M, Van Gossum A, Belaiche J, Pescatore P, Fiasse R, Pelckmans P, Vlietinck R, Merlin F, Zouali H, Thomas G, Colombel JF, Hugot JP. NOD2/CARD15 does not influence response to infliximab in Crohn's disease. *Gastroenterology* 2002; **123**: 106-111
23 **Hlavaty T**, Pierik M, Henckaerts L, Ferrante M, Joossens S, van Schuerbeek N, Noman M, Rutgeerts P, Vermeire S. Polymorphisms in apoptosis genes predict response to infliximab therapy in luminal and fistulizing Crohn's disease. *Aliment Pharmacol Ther* 2005; **22**: 613-626
24 **Lennard-Jones JE.** Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-19
25 **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 Jr EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart 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: 5-36
26 **Franke A**, Balschun T, Karlsen TH, Hedderich J, May S, Lu T, Schuldt D, Nikolaus S, Rosenstiel P, Krawczak M, Schreiber S. Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. *Nat Genet* 2008; **40**: 713-715
27 **Latiano A**, Palmieri O, Cucchiara S, Castro M, D'Incà R, Guariso G, Dallapiccola B, Valvano MR, Latiano T, Andriulli A, Annese V. Polymorphism of the IRGM gene might predispose to fistulizing behavior in Crohn's disease. *Am J Gastroenterol* 2009; **104**: 110-116
28 **Henckaerts L**, Van Steen K, Verstreken I, Cleynen I, Franke A, Schreiber S, Rutgeerts P, Vermeire S. Genetic risk profiling and prediction of disease course in Crohn's disease patients. *Clin Gastroenterol Hepatol* 2009; **7**: 972-980.e2
29 **Roberts RL**, Hollis-Moffatt JE, Gearry RB, Kennedy MA, Barclay ML, Merriman TR. Confirmation of association of IRGM and NCF4 with ileal Crohn's disease in a population-based cohort. *Genes Immun* 2008; **9**: 561-565
30 **Peterson N**, Guthery S, Denson L, Lee J, Saeed S, Prahalad S, Biank V, Ehlert R, Tomer G, Grand R, Rudolph C, Kugathasan S. Genetic variants in the autophagy pathway contribute to paediatric Crohn's disease. *Gut* 2008; **57**: 1336-1337; author reply 1337
31 **Amre DK**, Mack DR, Morgan K, Krupoves A, Costea I, Lambrette P, Grimard G, Dong J, Feguery H, Bucionis V, Deslandres C, Levy E, Seidman EG. Autophagy gene ATG16L1 but not IRGM is associated with Crohn's disease in Canadian children. *Inflamm Bowel Dis* 2009; **15**: 501-507
32 **van der Heide F**, Nolte IM, Kleibeuker JH, Wijmenga C, Dijkstra G, Weersma RK. Differences in genetic background between active smokers, passive smokers, and non-smokers with Crohn's disease. *Am J Gastroenterol* 2010; **105**: 1165-1172
33 **Festen EA**, Stokkers PC, van Diemen CC, van Bodegraven AA, Boezen HM, Crusius BJ, Hommes DW, van der Woude CJ, Balschun T, Verspaget HW, Schreiber S, de Jong DJ, Franke A, Dijkstra G, Wijmenga C, Weersma RK. Genetic analysis in a Dutch study sample identifies more ulcerative colitis susceptibility loci and shows their additive role in disease risk. *Am J Gastroenterol* 2010; **105**: 395-402
34 **Anderson CA**, Massey DC, Barrett JC, Prescott NJ, Tremelling M, Fisher SA, Gwilliam R, Jacob J, Nimmo ER, Drummond H, Lees CW, Onnie CM, Hanson C, Blaszczyk K, Ravindrarajah R, Hunt S, Varma D, Hammond N, Lewis G, Attlesey H, Watkins N, Ouwehand W, Strachan D, McArdle W, Lewis CM, Lobo A, Sanderson J, Jewell DP, Deloukas P, Mansfield JC, Mathew CG, Satsangi J, Parkes M. Investigation of Crohn's disease risk loci in ulcerative colitis further defines their molecular relationship. *Gastroenterology* 2009; **136**: 523-529.e3

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BRIEF ARTICLE

Clinical analysis of high serum IgE in autoimmune pancreatitis

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Abstract

AIM: To clarify the clinical significance of high serum IgE in autoimmune pancreatitis (AIP).

METHODS: Forty-two AIP patients, whose IgE was measured before steroid treatment, were analyzed. To evaluate the relationship between IgE levels and the disease activity of AIP, we examined (1) Frequency of high IgE (> 170 IU/mL) and concomitant allergic diseases requiring treatment; (2) Correlations between IgG, IgG4, and IgE; (3) Relationship between the presence of extrapancreatic lesions and IgE; (4) Relationship between clinical relapse and IgE in patients treated with steroids, and (5) Transition of IgE before and after steroid treatment.

RESULTS: IgE was elevated in 36/42 (86%) patients. Concomitant allergic disease was observed in seven patients (allergic rhinitis in three, bronchial asthma in three, and urticaria in one). There were no significant correlations between IgG, IgG4, and IgE (r = -0.168 for IgG, and r = -0.188 for IgG4). There was no significant difference in IgE in the patients with and without extrapancreatic lesions (526 ± 531 IU/mL *vs* 819 ± 768 IU/mL, P = 0.163), with and without clinical relapse (457 ± 346 IU/mL *vs* 784 ± 786 IU/mL, P = 0.374). There was no significant difference in IgE between before and after steroid treatment (723 ± 744 IU/mL *vs* 673 ± 660 IU/mL, P = 0.633).

CONCLUSION: Although IgE does not necessarily reflect the disease activity, IgE might be useful for the diagnosis of AIP in an inactive stage.

Key words: IgE; IgG4; IgG; Autoimmune pancreatitis

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INTRODUCTION

Autoimmune pancreatitis (AIP) is a unique, benign pan-

creatic disease characterized by irregular narrowing of the pancreatic duct, swelling of the pancreas, lymphoplasmacytic infiltration and fibrosis, and favorable response to steroid therapy[1-8]. Serologically, elevation of IgG and IgG4 is the most remarkable characteristic in this disease[9-12]. A recent study showed that IgM and IgA were decreased in AIP[13]. There has been no detailed clinical analysis of IgE, although some clinicians have noted elevated serum IgE in AIP or IgG4-related diseases[14-17].

In most allergic diseases, total serum IgE levels do not reflect disease activity; however, in allergic bronchopulmonary aspergillosis, it is reported that IgE is a useful marker for therapeutic monitoring[18-21]. The expression of T-helper type 2 (Th2) cytokines [interleukin (IL)-4, IL-5, and IL-13] are upregulated in the affected tissues of AIP[22]. Both IgG4 and IgE production are dependent on help by Th2; therefore, all IgG4-inducing antigens are also efficient IgE inducers[23]. As IgG4 reflects the disease activity of AIP[12], it is reasonable to expect that IgE is also related to the disease activity of AIP and could become a clinically useful marker. Thus, we decided to clarify whether IgE is related to the disease activity of AIP from various viewpoints.

MATERIALS AND METHODS

Patients

Between 1997 and 2009, 67 patients were diagnosed as having AIP at the University of Tokyo hospital and affiliated hospitals. All the patients fulfilled the diagnostic criteria of AIP proposed by the Mayo Clinic[3] or the revised criteria by the Japan Pancreas Society[4]. Serum IgE was measured in 48 patients before steroid treatment. As the method of measurement was different in six patients, these patients were excluded. Thus, 42 patients whose IgE level was measured by the same method before steroid treatment were enrolled in this study. Of the 42 patients, 33 were men and nine were women. The mean age of onset was 65 years old. Thirty-seven patients received steroid treatment. Prednisolone at an initial dose of 30-40 mg/d was administered for 2-4 wk in most cases. It was then tapered by 5 mg every 2-6 wk until 10 mg/d, and 2.5-7.5 mg/d was continued as maintenance therapy in principle.

This retrospective study was approved by the review board of our institute.

Methods

Serum IgE was measured by fluorescence enzyme immunoassay. To evaluate the relationship between IgE levels and disease activity, we examined (1) frequency of high IgE (> 170 IU/mL) and concomitant allergic diseases requiring treatment; (2) correlations among IgG, IgG4, and IgE; (3) relationship between the presence of extrapancreatic lesions and IgE; (4) relationship between clinical relapse and IgE in patients treated with steroids; and (5) transition of IgE before and after steroid treatment.

With regard to allergic diseases, only diseases that required treatment during follow-up were counted. There are many extrapancreatic lesions in AIP; however, in this study, only representative and definite lesions, including sclerosing cholangitis[7,8,24], retroperitoneal fibrosis[8,25], sclerosing sialadenitis[17], interstitial pneumonia[26], and tubulointerstitial nephritis[27] were counted. With regard to the number of extrapancreatic lesions, we used the number of extrapancreatic lesions that were observed when IgE was measured. We do not regard intrapancreatic biliary stricture as an extrapancreatic lesion, because it is influenced by pancreatic edema[24]. We defined "clinical relapse" as AIP-related symptomatic unfavorable events; i.e. obstructive jaundice from distal bile duct stenosis due to exacerbated pancreatitis with pancreatic swelling, increased levels of biliary enzymes caused by sclerosing cholangitis (in which extrapancreatic biliary strictures were confirmed on imaging findings), retroperitoneal fibrosis, interstitial pneumonia, and interstitial nephritis (for which simple observation seemed very inadequate and remission induction therapy was introduced). Concerning clinical relapses and IgE, we analyzed patients whose follow-up after the initiation of steroid treatment was more than 6 mo. With regard to the transition of IgE, IgE measured before steroid treatment and during maintenance steroid treatment (2.5-7.5 mg prednisolone/d) were compared.

Statistical analysis

Categorical variables were compared by the χ^2 or Fisher exact test, where appropriate. Continuous variables were reported as mean ± SD and compared by the Student *t* test, Welch *t* test, or paired *t* test, where appropriate. A *P* value of < 0.05 was considered statistically significant. Statistical analyses were performed by the statistical software JMP 7.0.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Frequency of high IgE and concomitant allergic diseases

The clinical profiles of 42 patients with AIP are summarized in Table 1. Serum IgE was elevated in 36/42 (86%). The average value of IgE was 679 ± 675 IU/mL (range, 67-3000 IU/mL). No patient had concomitant parasitosis. Concomitant allergic diseases were observed in seven patients, comprising allergic rhinitis in three, bronchial asthma in three, and urticaria in one. There was no significant difference between the average IgE values of these seven patients and those of the other 35 patients (970 ± 775 IU/mL *vs* 621 ± 650 IU/mL, $P = 0.216$). The frequency of high IgE was 100% (7/7) in these patients, and 63% (29/35) in the others; however, this difference was not statistically significant ($P = 0.567$).

Correlations between IgG, IgG4, and IgE

The values of IgG and IgG4, which were measured at the same time as IgE before steroid treatment, were used in this analysis. Elevation of IgG and IgG4 were observed in 20 (47%) and 39 (93%) patients, respectively. The correlation coefficient of IgG and IgE was -0.168 (not significant, $P = 0.290$). The correlation coefficient of IgG4 and IgE was -0.188 (not significant, $P = 0.235$). The correla-

Table 1 Clinical profiles of 42 patients with autoimmune pancreatitis

Patient	Sex	Age	IgE (< 171 U/mL)	IgG (870-1800 mg/dL)	IgG4 (< 135 mg/dL)	Total bilirubin (0.3-1.3 mg/dL)	Concomitant allergic diseases	Extrapancreatic lesion associated with AIP
1	F	58	670	2542	592	0.6	AR	-
2	M	63	650	2055	691	3.0	AR	-
3	F	53	500	1527	143	7.9	AR	SA
4	M	64	490	2457	670	0.5	BA	-
5	M	56	1800	1712	436	0.5	BA	-
6	F	43	340	1036	223	0.9	BA	-
7	M	74	2339	1481	98	0.9	Urticaria	RF
8	F	70	480	2190	133	5.8	-	-
9	M	57	120	3793	1420	10.6	-	RF
10	M	55	480	1419	320	1.7	-	-
11	M	61	270	1878	410	0.4	-	SC
12	M	66	1200	1620	310	0.5	-	SA
13	M	79	170	1585	420	3.3	-	SC
14	M	73	290	1647	360	5.4	-	-
15	M	79	410	1404	554	0.7	-	-
16	M	76	1000	1728	65	14.1	-	SC
17	F	72	650	2384	1400	1.6	-	SA
18	F	65	190	1511	374	0.6	-	-
19	M	68	290	1656	253	2.0	-	SC
20	M	61	940	1448	578	1.6	-	-
21	F	61	640	2177	354	0.4	-	-
22	M	58	2289	1973	481	6.9	-	-
23	M	71	1915	2318	470	0.6	-	-
24	M	61	69	2215	974	0.5	-	SA
25	M	65	91	3032	1260	5.4	-	SC, RF
26	M	64	330	1730	361	0.4	-	SC
27	M	66	1330	1849	270	4.7	-	-
28	F	64	328	2898	456	12.8	-	SC
29	M	69	440	1875	270	8.7	-	-
30	M	73	70.3	2395	393	0.4	-	RF
31	M	67	660	1683	230	0.6	-	-
32	M	72	3000	1579	455	0.5	-	-
33	M	61	467	1301	331	2.9	-	-
34	M	40	320	1996	650	1.0	-	RF
35	M	62	480	1368	236	0.5	-	SC, SA
36	M	71	267	1827	458	1.3	-	-
37	M	59	625	1876	139	0.5	-	RF
38	M	73	302	2834	1800	0.5	-	SA
39	F	76	426	1458	543	0.9	-	RF
40	M	76	943	1840	431	0.7	-	SC
41	M	59	67	1338	140	0.7	-	-
42	M	64	198	1709	232	10.2	-	-

AIP: Autoimune pancreatitis; AR: Allergic rhinitis; BA: Bronchial asthma; SA: Sialadenitis; RF: Retroperitoneal fibrosis; SC: Sclerosing cholangitis.

Table 2 Correlations between IgG, IgG4, and IgE

	Correlation coefficient	*P* value
IgG and IgE	-0.168	0.290
IgG4 and IgE	-0.188	0.235
IgG and IgG4	0.698	< 0.0001

tion coefficient of IgG and IgG4 was 0.698, which was significant ($P < 0.0001$) (Table 2).

Relationship between the presence of extrapancreatic lesions and IgE

Extrapancreatic lesions were observed in 20 patients (48%). Two patients had two lesions, and 18 patients had one lesion. Sclerosing cholangitis, retroperitoneal fibrosis, and sclerosing sialadenitis, were observed in nine, seven, and six patients, respectively. The IgE levels of the patients with and without extrapancreatic lesions were compared, and the same analysis was performed for IgG and IgG4. The results are shown in Table 3. IgG tended to be related to the presence of extrapancreatic lesions, although statistical significance was not attained ($P = 0.093$). No such tendency existed for IgE.

Table 3 Comparison of IgE, IgG, and IgG4 between patients with and without extrapancreatic lesions

	Patients with extrapancreatic lesions (n = 20)	Patients without extrapancreatic lesions (n = 22)	*P* value
IgE (IU/mL)	526 ± 531	819 ± 768	0.163
IgG (mg/dL)	2065 ± 644	1775 ± 396	0.093
IgG4 (mg/dL)	588 ± 505	392 ± 161	0.110

Table 4 Comparison of IgE, IgG, and IgG4 between patients with and without clinical relapses

	Patients with clinical relapses (n = 5)	Patients without clinical relapses (n = 28)	P value
IgE (IU/mL)	457 ± 346	784 ± 786	0.374
IgG (mg/dL)	1898 ± 424	1915 ± 579	0.953
IgG4 (mg/dL)	266 ± 157	558 ± 429	0.148

Table 5 Transition of IgE, IgG, and IgG4 before and after steroid treatment

	Before	After	P value
IgE (IU/mL) (n = 29)	723 ± 744	673 ± 660	0.633
Proportion of high IgE	26/29	23/29	0.470
IgG (mg/dL) (n = 30)	1891 ± 566	1155 ± 315	< 0.0001
Proportion of high IgG	14/30	1/30	0.0002
IgG4 (mg/dL) (n = 28)	557 ± 429	229 ± 112	0.0002
Proportion of high IgG4	27/28	20/28	0.0248

Normal range, IgE: < 171 IU/mL, IgG: 870-1800 mg/dL, IgG: < 135 mg/dL.

Relationship between clinical relapse and IgE in the patients treated with steroids

There were 33 patients whose follow-up period was more than 6 mo. The mean follow-up period was 52 mo (range, 8-141 mo). Clinical relapse was observed in five patients. The style of clinical relapse was pancreatitis in two, interstitial pneumonia in two, and sclerosing cholangitis in one. Their relapses occurred 16 mo after the initiation of steroid therapy on average (range, 3-26 mo). The mean follow-up period was the same between the patients with and without clinical relapse (53.0 mo *vs* 51.8 mo, $P = 0.929$). IgE levels of the patients with and without clinical relapse were compared, and the same analysis was performed for IgG and IgG4. The results are shown in Table 4. Neither IgE, IgG nor IgG4, were related to later clinical relapses.

Transition of IgE before and after steroid treatment

IgE measured before steroid treatment and during maintenance therapy could be compared in 29 patients (Figure 1). IgE increased in 10, and decreased in 18. There was no significant difference in IgE between before and after steroid treatment (723 ± 744 IU/mL *vs* 673 ± 660 IU/mL, $P = 0.633$). Abnormally high IgE (> 170 IU/mL) was observed in 90% (26/29) before steroid treatment, and in 79% (23/29) after steroid treatment ($P = 0.470$). By contrast, IgG and IgG4 did show significant differences before and after steroid treatment (Table 5).

DISCUSSION

High IgE in AIP has been frequently documented[14-17], but its frequency and clinical significance were unknown. Kamisawa *et al*[14] reported that elevation of IgE was observed in 34% (12/35) of patients, and that all the patients with high IgE had present and/or past histories of allergic diseases, although none of the patients with normal IgE had such histories. In the present study, the

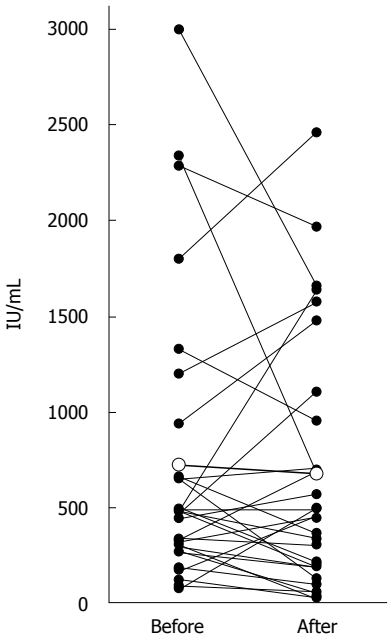

Figure 1 IgE levels measured before steroid treatment (Before) and during maintenance therapy (After) were compared in 29 patients. White circles show average values.

frequency of high IgE was surprisingly high at 86% (36/42), which might be equal to frequency of high IgG4 (73.3%-94.3%)[12]. On the other hand, unlike the previous report, there seemed no definite relationship between IgE and allergic diseases. It seemed unreasonable to count allergic diseases that occurred decades ago. In addition, it is difficult to accurately judge past histories of mild allergic diseases. Thus, we included only concomitant allergic diseases. For reference, there were at least eight patients who had past histories of allergic diseases, but no concomitant ones. Comparison between patients with (n = 15) and without (n = 27) present and/or past histories of allergic diseases showed no significant difference in mean IgE values (654 ± 605 IU/mL *vs* 693 ± 721 IU/mL, $P = 0.860$) and frequency of high IgE (93% *vs* 81%, $P = 0.395$); therefore, the presence of past allergic disease did not affect the results.

From the results shown in Table 3, IgE levels appear to be unrelated to disease activity from the viewpoint of extrapancreatic lesions. On the contrary, it is possible that high IgE is associated with lower disease activity, when considering the higher IgE levels in the group without extrapancreatic lesions, and the negative correlation coefficient of IgG (IgG4) and IgE. It is difficult to analyze the results shown in Table 4 because of the small number of patients with clinical relapses. Nevertheless, it is likely that high IgE is not a risk factor for later clinical relapses, especially considering the higher IgE levels in the group without clinical relapses. IgG4 seems a little high in the group without clinical relapses (Table 4), which is similar to previous reports[8,28].

It was of great interest whether IgE could become a useful marker for therapeutic monitoring in AIP, like IgG and IgG4. From the results shown in Table 5, we

cannot help but conclude that IgE is not a useful marker. However, this phenomenon is not strange in other allergic diseases. For example, Gunnar *et al*[18] reported that steroid treatment did not alter IgE levels in patients with atopic dermatitis. Kumar *et al*[19] showed that changes in serum IgE are not related to severity of asthma or allergic rhinitis. Exceptionally, in allergic bronchopulmonary aspergillosis, it is reported that the response of IgE (35% or more reduction) to steroid treatment is a sensitive marker in the management[20].

Although IgE does not seem to reflect disease activity, we speculate that this feature might be useful for the diagnosis of inactive AIP. Indeed, three patients in the present series showed low IgG4 (65, 98, and 133 mg/dL) at the diagnosis, but all of them showed high IgE (1000, 2339 and 480 IU/mL). When patients with a past history suggestive of AIP, such as voluntarily improved jaundice, do not show high IgG and IgG4, IgE should be measured. If IgE is also low, the possibility that their diagnosis is AIP will be low. If IgE is high, it might indicate AIP in an inactive stage.

In summary, the elevation of serum IgE is very frequent in AIP. It is also observed even in patients without other allergic diseases. IgE might not reflect the disease activity; however, it might be useful for the diagnosis of AIP in an inactive stage.

COMMENTS

Background

It is known that elevation of serum IgE is frequently observed in autoimmune pancreatitis (AIP). However, its clinical significance has not yet been clarified.

Research frontiers

This study demonstrated the frequency of high IgE in AIP, and investigated whether IgE is related to the presence of extrapancreatic lesions and later clinical relapses. In addition, the transition of IgE before and after steroid treatment was investigated to confirm whether IgE can become a marker for therapeutic monitoring.

Innovations and breakthroughs

This study confirmed the high frequency of elevated serum IgE in AIP, although IgE does not seem to be related to the disease activity.

Applications

Measuring IgE might be useful for the diagnosis of AIP especially in an inactive stage.

Peer review

These data about IgE were not positive, which means that IgE is not considered as a useful marker for AIP. However, the author revealed that many (86%) AIP patients have IgE elevation and some AIP patients with low IgG4 have a high level of IgE. IgE should be considered as one of the supportive parameters for diagnosis of AIP and the authors succeeded in clarifying that.

REFERENCES

1 **Shimosegawa T**, Kanno A. Autoimmune pancreatitis in Japan: overview and perspective. *J Gastroenterol* 2009; **44**: 503-517

2 **Okazaki K**, Uchida K, Fukui T. Recent advances in autoimmune pancreatitis: concept, diagnosis, and pathogenesis. *J Gastroenterol* 2008; 43: 409-418

3 **Chari ST**, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, Zhang L, Clain JE, Pearson RK, Petersen BT, Vege SS, Farnell MB. Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. *Clin Gastroenterol Hepatol* 2006; **4**: 1010-1016; quiz 934

4 **Okazaki K**, Kawa S, Kamisawa T, Naruse S, Tanaka S, Nishimori I, Ohara H, Ito T, Kiriyama S, Inui K, Shimosegawa T, Koizumi M, Suda K, Shiratori K, Yamaguchi K, Yamaguchi T, Sugiyama M, Otsuki M. Clinical diagnostic criteria of autoimmune pancreatitis: revised proposal. *J Gastroenterol* 2006; **41**: 626-631

5 **Kawaguchi K**, Koike M, Tsuruta K, Okamoto A, Tabata I, Fujita N. Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. *Hum Pathol* 1991; **22**: 387-395

6 **Hirano K**, Fukushima N, Tada M, Isayama H, Mizuno S, Yamamoto K, Yashima Y, Yagioka H, Sasaki T, Kogure H, Nakai Y, Sasahira N, Tsujino T, Kawabe T, Fukayama M, Omata M. Diagnostic utility of biopsy specimens for autoimmune pancreatitis. *J Gastroenterol* 2009; **44**: 765-773

7 **Hirano K**, Shiratori Y, Komatsu Y, Yamamoto N, Sasahira N, Toda N, Isayama H, Tada M, Tsujino T, Nakata R, Kawase T, Katamoto T, Kawabe T, Omata M. Involvement of the biliary system in autoimmune pancreatitis: a follow-up study. *Clin Gastroenterol Hepatol* 2003; **1**: 453-464

8 **Hirano K**, Tada M, Isayama H, Yagioka H, Sasaki T, Kogure H, Nakai Y, Sasahira N, Tsujino T, Yoshida H, Kawabe T, Omata M. Long-term prognosis of autoimmune pancreatitis with and without corticosteroid treatment. *Gut* 2007; **56**: 1719-1724

9 **Hamano H**, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, Fukushima M, Nikaido T, Nakayama K, Usuda N, Kiyosawa K. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001; **344**: 732-738

10 **Hirano K**, Komatsu Y, Yamamoto N, Nakai Y, Sasahira N, Toda N, Isayama H, Tada M, Kawabe T, Omata M. Pancreatic mass lesions associated with raised concentration of IgG4. *Am J Gastroenterol* 2004; **99**: 2038-2040

11 **Hirano K**, Kawabe T, Yamamoto N, Nakai Y, Sasahira N, Tsujino T, Toda N, Isayama H, Tada M, Omata M. Serum IgG4 concentrations in pancreatic and biliary diseases. *Clin Chim Acta* 2006; **367**: 181-184

12 **Morselli-Labate AM**, Pezzilli R. Usefulness of serum IgG4 in the diagnosis and follow up of autoimmune pancreatitis: A systematic literature review and meta-analysis. *J Gastroenterol Hepatol* 2009; **24**: 15-36

13 **Taguchi M**, Kihara Y, Nagashio Y, Yamamoto M, Otsuki M, Harada M. Decreased production of immunoglobulin M and A in autoimmune pancreatitis. *J Gastroenterol* 2009; **44**: 1133-1139

14 **Kamisawa T**, Anjiki H, Egawa N, Kubota N. Allergic manifestations in autoimmune pancreatitis. *Eur J Gastroenterol Hepatol* 2009; **21**: 1136-1139

15 **Miura H**, Miyachi Y. IgG4-related retroperitoneal fibrosis and sclerosing cholangitis independent of autoimmune pancreatitis. A recurrent case after a 5-year history of spontaneous remission. *JOP* 2009; **10**: 432-437

16 **Kasashima S**, Zen Y, Kawashima A, Endo M, Matsumoto Y, Kasashima F. A new clinicopathological entity of IgG4-related inflammatory abdominal aortic aneurysm. *J Vasc Surg* 2009; **49**: 1264-1271; discussion 1271

17 **Masaki Y**, Dong L, Kurose N, Kitagawa K, Morikawa Y, Yamamoto M, Takahashi H, Shinomura Y, Imai K, Saeki T, Azumi A, Nakada S, Sugiyama E, Matsui S, Origuchi T, Nishiyama S, Nishimori I, Nojima T, Yamada K, Kawano M, Zen Y, Kaneko M, Miyazaki K, Tsubota K, Eguchi K, Tomoda K, Sawaki T, Kawanami T, Tanaka M, Fukushima T, Sugai S, Umehara H. Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders. *Ann Rheum Dis* 2009; **68**: 1310-1315

18 **Gunnar S**, Johansson O, Juhlin L. Immunoglobulin E in "healed" atopic dermatitis and after treatment with corticosteroids and azathioprine. *Br J Dermatol* 1970; **82**: 10-13

19 **Kumar L**, Newcomb RW, Hornbrook M. A year-round study

of serum IgE levels in asthmatic children. *J Allergy Clin Immunol* 1971; **48**: 305-312
20 **Ricketti AJ**, Greenberger PA, Patterson R. Serum IgE as an important aid in management of allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1984; **74**: 68-71
21 **Pien GC**, Orange JS. Evaluation and clinical interpretation of hypergammaglobulinemia E: differentiating atopy from immunodeficiency. *Ann Allergy Asthma Immunol* 2008; **100**: 392-395
22 **Zen Y**, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, Nakanuma Y. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007; **45**: 1538-1546
23 **Aalberse RC**, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* 2009; **39**: 469-477
24 **Hirano K**, Tada M, Isayama H, Yamamoto K, Mizuno S, Yagioka H, Yashima Y, Sasaki T, Kogure H, Togawa O, Arizumi T, Matsubara S, Nakai Y, Sasahira N, Tsujino T, Kawabe T, Omata M. Endoscopic evaluation of factors contributing to intrapancreatic biliary stricture in autoimmune pancreatitis. *Gastrointest Endosc* 2010; **71**: 85-90
25 **Hamano H**, Kawa S, Ochi Y, Unno H, Shiba N, Wajiki M, Nakazawa K, Shimojo H, Kiyosawa K. Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis. *Lancet* 2002; **359**: 1403-1404
26 **Hirano K**, Kawabe T, Komatsu Y, Matsubara S, Togawa O, Arizumi T, Yamamoto N, Nakai Y, Sasahira N, Tsujino T, Toda N, Isayama H, Tada M, Omata M. High-rate pulmonary involvement in autoimmune pancreatitis. *Intern Med J* 2006; **36**: 58-61
27 **Nishi H**, Tojo A, Onozato ML, Jimbo R, Nangaku M, Uozaki H, Hirano K, Isayama H, Omata M, Kaname S, Fujita T. Anticarbonic anhydrase II antibody in autoimmune pancreatitis and tubulointerstitial nephritis. *Nephrol Dial Transplant* 2007; **22**: 1273-1275
28 **Naitoh I**, Nakazawa T, Ohara H, Ando T, Hayashi K, Tanaka H, Okumura F, Miyabe K, Yoshida M, Sano H, Takada H, Joh T. Clinical significance of extrapancreatic lesions in autoimmune pancreatitis. *Pancreas* 2010; **39**: e1-e5

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BRIEF ARTICLE

Segmental gastrectomy with radical lymph node dissection for early gastric cancer

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Abstract

AIM: To describe a new surgical technique and evaluate the early results of segmental gastrectomy (SG) with modified D2 lymph node (LN) dissection for early gastric cancer (EGC).

METHODS: Fourteen patients with EGC underwent SG with modified D2 dissection from 2006 to 2008. Their operative results and postoperative courses were compared with those of 17 patients who had distal gastrectomy (DG) for EGC during the same period.

RESULTS: Operating time, blood loss, and hospital stay were similar between the 2 groups. Postoperative complications developed significantly more frequently in the DG group than in the SG group. Mean number of dissected LNs per each station in the SG group was comparable with that in the DG group. Postoperative recovery of body weight was significantly better in the SG group than in the DG group. The incidence of reflux esophagitis and gastritis after surgery was less frequent in the SG group than in the DG group.

CONCLUSION: SG with modified D2 LN dissection may be a new function-preserving gastrectomy that is feasible for treatment of EGC with possible LN involvement.

Key words: Gastrectomy; Early diagnosis; Gastric cancer; Lymph node; Metastasis; Gastrointestinal surgical procedures

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INTRODUCTION

Less invasive surgery for early gastric cancer (EGC) has been more commonly employed in Japan[1-4]. Segmental gastrectomy (SG), which was originally applied for peptic ulcers, is one type of limited gastrectomy available for treating EGC[5]. SG for EGC is generally accompanied only by dissection of the lymph nodes (LNs) adjacent to tumors, although only a few studies regarding the application of SG in EGC have been reported[1-4]. To establish SG with adjacent LN dissection as a valid option for surgical treatment of EGC, it is necessary to demonstrate intraoperatively that there is no lymph node metastasis (N0). However, at present, it is difficult to determine the status of LN metastasis intraoperatively, even using the sentinel node (SN) navigation method[6-8].

The superiority of limited gastrectomy over conven-

tional D2 gastrectomy for EGC in terms of postoperative quality of life seems apparent, as previously reported[1,4,9,10]. In 1995, Sawai *et al*[11] developed pylorus-preserving gastrectomy (PPG) with D2 dissection by preservation of the infrapyloric artery as a function-preserving gastrectomy. Although this technique is considered to be applicable to submucosal or undifferentiated gastric cancer, there remains a risk of adverse events such as postoperative gastric stasis and reduced capacity of the remnant stomach.

We developed SG with modified D2 dissection and have applied it to EGC that do not meet endoscopic treatment criteria. We report the technique here and evaluate the early results of SG with modified D2 dissection for EGC.

MATERIALS AND METHODS

Between April 2006 and November 2008, 35 patients with EGC in the middle or lower third of the stomach which did not meet endoscopic treatment criteria underwent gastrectomy at our institution: 17 patients who underwent SG and 18 patients who underwent conventional distal gastrectomy (DG) with D2 dissection. The criteria for endoscopic treatment is EGC with differentiated histology, invasion limited to the mucosal layer, a diameter smaller than 2 cm, and no ulcer findings. In this study, SG was distinguished from PPG by defining that the remaining pyloric cuff needed to be more than 4 cm in SG. When the distance from the distal edge of the resection to the pylorus was < 4 cm, DG was performed instead of PPG.

A total of 14 patients received SG with modified D2 dissection. The remaining 3 patients who were excluded from this study received SG only with adjacent LN dissection because of intraoperative determination of apparent mucosal cancer with no LN metastasis.

In 3 out of 18 patients in the DG group, SG was converted to DG based on intraoperative findings including suspected T2 tumor or positive LN metastasis.

Patients were followed every 3 mo after surgery. The follow-ups also included an endoscopy and computed tomography 1 year after surgery. The operative and postoperative results were compared between the SG and DG groups.

Operative procedures

To make it possible to locate the tumor intraoperatively, proximal and distal margins of the tumor were marked by endoscopic clipping before surgery. After laparotomy, we explored the abdominal cavity and determined if the preoperative diagnosis was correct. If a T2 or T3 tumor was suspected or LN metastases were apparent, DG or total gastrectomy (TG) with D2 dissection was performed. Swollen and/or hard palpable LNs located adjacent to the tumor were excised and immediately examined by frozen section analysis. Infrapyloric LNs (station 6) and those along the common hepatic artery (CHA; station 8a) were also routinely subjected to frozen section analysis. DG or TG with D2 dissection was performed if LN metastasis was proved by frozen section analysis, however, if T1N0 was confirmed SG with modified D2 dissection was carried out.

The resection line of the stomach was made 2 cm and 5 cm away from the tumor margin with differentiated and undifferentiated histology, respectively. The greater omentum was preserved, and the gastrocolic ligament corresponding to the resection area of the stomach was dissected, after separation, at least 3 cm away from the gastroepiploic vessels. For dissection of LNs located in station 6 and along the greater curvature (station 4d), right gastroepiploic vessels were skeletonized, allowing excision of adjacent LNs, and divided at the distal resection line of the stomach (Figure 1A). The suprapyloric LNs (station 5) were dissected in the same manner. The right gastric artery was skeletonized and divided distally to the second or third branch (Figure 1B). The left halves of LNs along the proper hepatic artery (station 12a) were removed, and the hepatic and pyloric branches of vagus nerves were preserved. Right paracardial LNs (station 1), LNs along the lesser curvature (station 3), and the left gastric artery (LGA) (station 7) were dissected by dividing the LGA at its origin. The celiac branch of the vagus nerve was also dissected. Dissection of the upper portion of station 1 LNs was occasionally omitted in order to preserve the origin of the hepatic branch of the vagus nerve and the anterior branch of the Latarjet nerve. LNs along the CHA (station 8a, 8p), around the celiac axis (station 9), and along the proximal splenic artery (station 11p) were routinely dissected. LNs along the left gastroepiploic artery (LGEA) (station 4sb) were dissected by dividing the last few branches of the LGEA. After transection of the stomach, the surgical margins were subjected to intraoperative histological examination by frozen section analysis. Reconstruction was performed using hand-sewn end-to-end anastomosis (Figure 1C).

Statistical analysis

Statistical analysis was performed using either the χ^2 test or Student's *t*-test. A *P*-value < 0.05 was considered significant.

RESULTS

There were no differences between the 2 groups in terms of sex, age, and tumor characteristics, with the exception of tumor location (Table 1). In 1 patient of the DG group and 2 patients of the SG group, LN metastasis was proven to be positive.

Operative data are shown in Table 2. Operating time, blood loss, and hospital stay were similar between the two groups. However, postoperative complications developed significantly more frequently in the DG group than in the SG group. There were no instances of anastomotic leakage, hemorrhage, gastric stasis, wound infection or operative death in either group.

The mean number of dissected LNs per station was comparable between the 2 groups (Table 3).

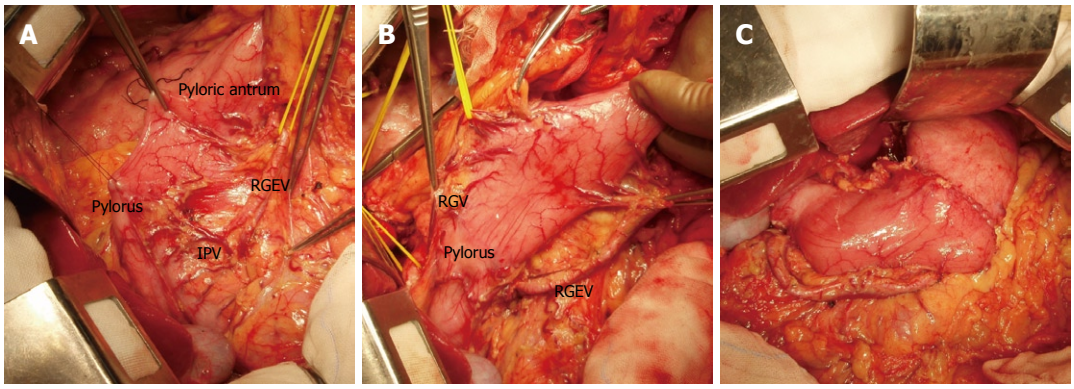

Figure 1 Photographs during surgery. A: The right gastroepiploic vessels were skeletonized allowing excision of adjacent lymph nodes (LNs) (stations 6 and 4 d). The surrounding nerve plexuses were preserved; B: For dissection of the suprapyloric LNs (station 5), the right gastric artery was skeletonized and divided distally to the 3rd branch; C: Reconstruction was performed using hand-sewn end-to-end anastomosis. RGEV: Right gastroepiploic vessels; IPV: Infrapyloric vessels; RGV: Right gastric vessels.

Table 1 Comparison of clinicopathological characteristics between the 2 groups

	SG (*n* = 14)	DG (*n* = 18)	*P*
Age (yr, mean ± SD)	63.9 ± 9.6	60.0 ± 12.9	0.204
Sex (M/F)	9/5	7/11	0.154
Location of tumor			0.002
Upper	0	0	
Middle	13	7	
Lower third of the stomach	1	11	
Depth of invasion			0.589
Mucosa	4	8	
Submucosa	8	9	
Muscularis propria	1	1	
Subserosa	1	0	
Histological type			
Differentiated	10	11	0.358
Undifferentiated	4	7	
Lymph node metastasis			0.401
No	12	17	
Yes	2	1	

SG: Segmental gastrectomy; DG: Distal gastrectomy.

Table 2 Operative results (mean ± SD)

	SG (*n* = 14)	DG (*n* = 18)	*P*
Operating time (min)	273 ± 65	262 ± 46	0.589
Blood loss (mL)	206 ± 124	179 ± 101	0.511
Hospital stay (d)	14.9 ± 2.6	16.7 ± 5.0	0.227
Complications	0	5	0.032
Wound infection	0	0	
Anastomotic leakage	0	0	
Hemorrhage	0	0	
Cholecystitis	0	2	
Pancreatitis	0	1	
Pancreatic fistula	0	1	
Adhesive ileus	0	1	
Operative death	0	0	1.000

SG: Segmental gastrectomy; DG: Distal gastrectomy.

Table 3 Numbers of dissected lymph nodes according to each station (mean ± SD)

Lymph node station	SG (*n* = 14)	DG (*n* = 18)	*P*
Right paracardial (1)	2.0 ± 1.3	3.1 ± 1.2	0.068
Left paracardial (2)	1.1 ± 1.5	0.7 ± 1.6	0.462
Along the lesser curvature (3)	8.7 ± 4.7	10.8 ± 7.3	0.362
Along the LGEA (4sb)	1.2 ± 2.1	1.4 ± 1.9	0.754
Along the RGEA (4d)	9.2 ± 4.9	11.7 ± 4.9	0.155
Suprapyloric (5)	0.3 ± 0.6	0.6 ± 0.9	0.286
Infrapyloric (6)	3.7 ± 3.7	5.6 ± 2.6	0.118
Along the LGA (7)	3.7 ± 2.4	4.3 ± 2.7	0.473
Along the CHA (8)	2.7 ± 1.2	3.3 ± 1.5	0.232
Along the celiac axis (9)	2.8 ± 1.6	3.3 ± 2.0	0.442
Along the proximal SA (11p)	1.5 ± 1.5	2.0 ± 1.7	0.412
Along the PHA (12a)	0.5 ± 1.0	0.2 ± 0.5	0.246
Along the SMV (14v)	-	1.2 ± 2.1	-

SG: Segmental gastrectomy; DG: Distal gastrectomy; LGEA: Left gastroepiploic artery; RGEA: Right gastroepiploic artery; LGA: Left gastric artery; CHA: Common hepatic artery; SA: Splenic artery; PHA: Proper hepatic artery; SMV: Superior mesenteric vein.

Table 4 Postoperative course (mean ± SD)

	SG (*n* = 14)	DG (*n* = 18)	*P*
Gastric stasis	0	0	1.000
Dumping syndrome	1	1	0.854
Body weight change ratio (%)	96.8 ± 6.1	90.4 ± 7.0	0.012
Laboratory data			
Lymphocytes (/mm^3)	2007 ± 437	1977 ± 646	0.885
Total protein (g/dL)	6.8 ± 0.3	7.0 ± 0.3	0.156
Total cholesterol (mg/dL)	215 ± 34	192 ± 32	0.063
Endoscopic examination			
Reflux gastritis	1	5	0.138
Reflux esophagitis	0	4	0.059

SG: Segmental gastrectomy; DG: Distal gastrectomy.

The postoperative course is summarized in Table 4. Postoperative recovery of body weight was significantly better in the SG group than in the DG group. The incidence of reflux esophagitis and gastritis after surgery was less frequent in the SG group than in the DG group.

No recurrence or death was observed in either group during a median follow-up period of 32.8 mo. One patient in the SG group developed colon cancer during the

follow-up period and subsequently underwent curative resection.

DISCUSSION

SG has not been previously used to treat EGC, since it was first reported by Wangensteen *et al*[5] as an operation for peptic ulcer. In 1999, Ohwada *et al*[2] modified the procedure and reported its use for treatment of EGC of the middle third of the stomach. However, previous studies concerning the use of SG for the treatment of EGC are limited, primarily due to difficulty in diagnosing LN metastasis accurately during surgery, and it being performed with very limited LN dissection. Deterioration of the radicality caused by omitting or limiting LN dissection should be avoided, because EGC can be treated with conventional D2 gastrectomy with excellent prognosis. Therefore, SG with limited LN dissection is not recommended unless T1N0 is confirmed.

On the other hand, a method for accurate intraoperative diagnosis of LN metastasis has yet to be established. Recently, SN biopsy has been used to assist intraoperative diagnosis of LN metastasis during surgical treatment of EGC. The SN concept seems feasible for treatment of EGC as described previously, however, several important issues including optimal tracer, method of injection, and false negative cases, still need to be resolved[6-8].

Our modified D2 dissection was considered to be comparable with conventional D2 dissection, because dissected stations in our SG corresponded with those in conventional D2 dissection. The mean number of dissected LNs of station 1 tended to be smaller in the SG group than in the DG group. This will be because dissection of the upper half of station 1 was occasionally omitted in order to preserve the anterior branches of the vagus nerve along the lesser curvature of the proximal remnant stomach, which may help maintain postoperative motility of this portion. The influence of limited dissection of station 1 on the oncological outcome is unclear, however, tumor recurrence resulting from incomplete dissection of station 1 has not been seen so far. Even in 3 patients whose LN metastasis was positive in the DG or SG group, no recurrence has been observed. In 1 of 2 patients whose LN metastasis was positive in the SG group, positive LN metastasis was proved after surgery in station 7 which belongs to the second tier. In the other 2 patients, LN metastasis was positive in station 3. These results may support the quality and effectiveness of our modified D2 dissection and the impact of D2 dissection.

In our procedures, 2-5 LNs were subjected to frozen section analysis. Station 8a was routinely examined by frozen section analysis. The frequency of LN metastasis in station 8a is relatively high even in EGC, and stations 7, 8a, and 11p are sometimes SN stations although they belong to the second tier[6,8,12-14]. Once the tumor advances to stage T2 or T3, lymphatic vessels can be obstructed by cancerous invasion, and metastasis in distant LNs can occur even in the absence of involvement of LNs adjacent to tumors. In the case where station 7 LN was proved positive after SG, intraoperative frozen section analysis detected no metastasis of stations 3 and 8a. Therefore, it is not recommended to limit LN dissection even if frozen section analysis reveals no LN involvement. Frozen section analysis with LN sampling was performed by us in order to rule out patients with positive LN metastasis rather than to confirm N0.

Unexpectedly, postoperative gastric stasis has not been observed in our patient population thus far. The precise mechanisms of development of gastric stasis after limited gastrectomy such as PPG remain unclear[15-18]. The main causes of gastric stasis are generally thought to include damage to the vagus nerve, insufficient blood supply to the pyloric region, or tonic and phasic contractions of the pylorus. In our procedure, not only the blood supply but also the venous drainage of the pyloric region is more sufficient than in PPG. These findings may have contributed to good maintenance of pyloric motility.

The present study demonstrated the safety and effectiveness of our procedures. This procedure could be a new function-preserving gastrectomy that is feasible for treatment of EGC with possible LN involvement. However, further studies including a larger number of patients and longer follow-up periods are essential for a more definitive conclusion.

COMMENTS

Background

Less invasive surgery for early gastric cancer (EGC) has been more commonly employed based on the background of an increase in EGC. Although it has been reported that pylorus-preserving gastrectomy can be performed with D2 dissection, there has been no report on segmental gastrectomy with D2 dissection.

Research frontiers

A sentinel node navigation method has been developed for accurate intraoperative determination of lymph node (LN) metastasis during surgery for EGC.

Innovations and breakthroughs

This is the first report to demonstrate that segmental gastrectomy can be performed with D2 dissection for treatment of EGC.

Applications

The superiority of function-preserving gastrectomy over conventional D2 gastrectomy for EGC in terms of postoperative quality of life is apparent. However, limited LN dissection accompanying function-preserving gastrectomy may reduce the radicality of gastrectomy for EGC. This procedure may be applicable to EGC with possible LN involvement.

Terminology

Segmental gastrectomy is one of the limited gastrectomies in which the central one third portion of the stomach is resected. The sentinel node is generally defined as the lymph node which receives the lymphatic flow first from the tumor.

Peer review

This is an interesting small series and I think it has value. I would like more information concerning the pathological outcomes before accepting the conclusions and suggestions in the discussion.

REFERENCES

1 **Furukawa H**, Hiratsuka M, Imaoka S, Ishikawa O, Kabuto T, Sasaki Y, Kameyama M, Ohigashi H, Nakano H, Yasuda T, Murata K. Phase II study of limited surgery for early gastric cancer: segmental gastric resection. *Ann Surg Oncol* 1999; **6**: 166-170

2 **Ohwada S**, Nakamura S, Ogawa T, Izumi M, Tanahashi Y, Sato Y, Ikeya T, Iino Y, Morishita Y. Segmental gastrectomy

for early cancer in the mid-stomach. Hepatogastroenterology 1999; **46**: 1229-1233
3 **Shinohara T**, Ohyama S, Muto T, Kato Y, Yanaga K, Yamaguchi T. Clinical outcome of high segmental gastrectomy for early gastric cancer in the upper third of the stomach. *Br J Surg* 2006; **93**: 975-980
4 **Ishikawa K**, Arita T, Ninomiya S, Bandoh T, Shiraishi N, Kitano S. Outcome of segmental gastrectomy versus distal gastrectomy for early gastric cancer. *World J Surg* 2007; **31**: 2204-2207
5 **Wangensteen OH**. Segmental gastric resection for peptic ulcer; method permitting restoration of anatomic continuity. *J Am Med Assoc* 1952; **149**: 18-23
6 **Ichikura T**, Chochi K, Sugasawa H, Yaguchi Y, Sakamoto N, Takahata R, Kosuda S, Mochizuki H. Individualized surgery for early gastric cancer guided by sentinel node biopsy. *Surgery* 2006; **139**: 501-507
7 **Tajima Y**, Yamazaki K, Masuda Y, Kato M, Yasuda D, Aoki T, Kato T, Murakami M, Miwa M, Kusano M. Sentinel node mapping guided by indocyanine green fluorescence imaging in gastric cancer. *Ann Surg* 2009; **249**: 58-62
8 **Ichikura T**, Sugasawa H, Sakamoto N, Yaguchi Y, Tsujimoto H, Ono S. Limited gastrectomy with dissection of sentinel node stations for early gastric cancer with negative sentinel node biopsy. *Ann Surg* 2009; **249**: 942-947
9 **Shimoyama S**, Seto Y, Yasuda H, Mafune K, Kaminishi M. Concepts, rationale, and current outcomes of less invasive surgical strategies for early gastric cancer: data from a quarter-century of experience in a single institution. *World J Surg* 2005; **29**: 58-65
10 **Morita S**, Katai H, Saka M, Fukagawa T, Sano T, Sasako M. Outcome of pylorus-preserving gastrectomy for early gastric cancer. *Br J Surg* 2008; **95**: 1131-1135
11 **Sawai K**, Takahashi T, Fujioka T, Minato H, Taniguchi H, Yamaguchi T. Pylorus-preserving gastrectomy with radical lymph node dissection based on anatomical variations of the infrapyloric artery. *Am J Surg* 1995; **170**: 285-288
12 **Xu YY**, Huang BJ, Sun Z, Lu C, Liu YP. Risk factors for lymph node metastasis and evaluation of reasonable surgery for early gastric cancer. *World J Gastroenterol* 2007; **13**: 5133-5138
13 **Kojima N**, Yonemura Y, Bando E, Morimoto K, Kawamura T, Yun HY, Ito I, Kameya T, Hayashi I. Optimal extent of lymph node dissection for T1 gastric cancer, with special reference to the distribution of micrometastasis, and accuracy of preoperative diagnosis for wall invasion. *Hepatogastroenterology* 2008; **55**: 1112-1117
14 **Tokunaga M**, Ohyama S, Hiki N, Fukunaga T, Yamada K, Sano T, Yamaguchi T. Investigation of the lymphatic stream of the stomach in gastric cancer with solitary lymph node metastasis. *World J Surg* 2009; **33**: 1235-1239
15 **Ohwada S**, Sato Y, Oriuchi N, Nakamura S, Tanahashi Y, Izumi M, Ogawa T, Takeyoshi I, Ikeya T, Iino Y, Morishita Y. Gastric emptying after segmental gastrectomy for early cancer in the middle part of the stomach. *Hepatogastroenterology* 1999; **46**: 2081-2085
16 **Nakabayashi T**, Mochiki E, Garcia M, Haga N, Suzuki T, Asao T, Kuwano H. Pyloric motility after pylorus-preserving gastrectomy with or without the pyloric branch of the vagus nerve. *World J Surg* 2002; **26**: 577-583
17 **Nunobe S**, Hiki N, Fukunaga T, Tokunaga M, Ohyama S, Seto Y, Yamaguchi T. Laparoscopy-assisted pylorus-preserving gastrectomy: preservation of vagus nerve and infrapyloric blood flow induces less stasis. *World J Surg* 2007; **31**: 2335-2340
18 **Tomita R**. Gastric emptying function in patients 5 years after pylorus-preserving distal gastrectomy with or without preserving pyloric and hepatic branches of the vagal nerve for early gastric cancer. *World J Surg* 2009; **33**: 2119-2126

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BRIEF ARTICLE

Predictive factors for lymph node metastasis in early gastric cancer

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Abstract

AIM: To analyze the predictive factors for lymph node metastasis (LNM) in early gastric cancer (EGC).

METHODS: Data from patients surgically treated for gastric cancers between January 1994 and December 2007 were retrospectively collected. Clinicopathological factors were analyzed to identify predictive factors for LNM.

RESULTS: Of the 2936 patients who underwent gastrectomy and lymph node dissection, 556 were diagnosed with EGC and included in this study. Among these, 4.1% of patients had mucosal tumors (T1a) with LNM while 24.3% of patients had submucosal tumors with LNM. Univariate analysis found that female gender, tumors ⩾ 2 cm, tumor invasion to the submucosa, vascular and lymphatic involvement were significantly associated with a higher rate of LNM. On multivariate analysis, tumor size, lymphatic involvement, and tumor with submucosal invasion were associated with LNM.

CONCLUSION: Tumor with submucosal invasion, size ⩾ 2 cm, and presence of lymphatic involvement are predictive factors for LNM in EGC.

Key words: Early gastric cancer; Lymph node metastasis; Endoscopic treatment; Endoscopic submucosa dissection; Depth of tumor invasion

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INTRODUCTION

The term early gastric cancer (EGC) describes gastric cancers involving the mucosa or submucosa irrespective of the presence of lymph node metastasis (LNM). Radical surgery including lymph node dissection has been the

standard treatment for early gastric cancer; however, LNM has only been associated with approximately 8% to 20% of EGC cases[1-3]. Unnecessary surgery could be avoided and endoscopic treatment might be a consideration in patients with EGC with negligible risk of LNM. Prior studies have demonstrated that the presence of LNM is the most significant factor for survival in patients with EGC[4,5] and usually constitutes the watershed between radical and endoscopic surgery. Identification of LNM cannot be achieved *via* endoscopic ultrasonography or computed tomography because the lymph node size is not a reliable parameter for detection of metastasis[1,2]. Many retrospective studies on EGC have established an indication for endoscopic treatment[6] and the probability of LNM in EGC has been estimated based on macroscopic-endoscopic tumor characteristics and histopathological findings.

According to the treatment guidelines for gastric cancer in Japan[7] the indication for endoscopic treatment such as endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) are patients with non-ulcerated tumors < 2 cm. Several investigators are currently attempting to extend the indication for endoscopic treatment to include: differentiated type, intramucosal cancer without ulcer, > 2 cm in size; differentiated type, intramucosal cancer, ≤ 3 cm in size if ulcerated; and, undifferentiated type, intramucosal tumor without ulcer, ≤ 2 cm in size (Table 1).

While endoscopic treatment for EGC is widely adapted in Japan and Korea using various criteria[4,8-10], it remains uncertain whether these guidelines for EMR/ESD are applicable to patients in areas outside of Japan and Korea. Japan and Korea have the highest gastric cancer rate in the world[11,12] and between 30% and 70% of all gastric cancers are diagnosed as EGCs. In other countries, EGCs account for only about 5% to 15% of all gastric cancers[13,14]. Japan and Korea have national screening programs in which the use of chromoendoscopy could increase the detection rate of EGC. Race, diet, and other factors such as pathologic diagnosis may explain differences in the diagnosis of EGC between Japan and other countries[15,16]; however, comparable data about EGC from other countries is rare or only involve a small case series[17]. The purpose of this study was to identify factors related to LNM in EGC and to elucidate which subgroup of EGC patients could be treated with EMR or ESD instead of radical surgery.

MATERIALS AND METHODS

Between January 1994 and December 2007, 2936 patients underwent gastrectomy for gastric cancer in the Department of Surgery, Chang Gung Memorial Hospital, Taipei, Taiwan, China. Clinicopathological data were obtained from a retrospectively constructed medical database, which had been reviewed by IRB, Chang Gung Memorial Hospital. In total, 556 of these patients were diagnosed with primary EGC and were included in this study. There were 330 men and 226 women with a median age of 62 years (range: 21-89 years).

Table 1 Extended indications for endoscopic mucosal resection/endoscopic submucosal dissection according to the treatment guidelines for gastric cancer in Japan

Differentiated type, mucosal cancer, ulcer (-), > 2 cm
Differentiated type, mucosal cancer, ulcer (+), ≤ 3 cm
Undifferentiated type, mucosal cancer, ulcer (-), ≤ 2 cm

Additional lymph node resection is not necessary when lymphovascular invasion is absent and also when it is not deeper than SM1 (-500 μm).

Poorly differentiated adenocarcinomas, signet ring cell carcinomas, and mucinous adenocarcinomas were classified as undifferentiated tumors. Well and moderately differentiated tubular adenocarcinoma and papillary adenocarcinoma were grouped together as differentiated tumors. Associations between the various clinicopathological factors and the presence of LNM were analyzed to identify risk factors of LNM. These factors included: gender; age (< 65 years or ≥ 65 years), carcinoembryonic antigen (CEA, < 5 ng/mL or ≥ 5 ng/mL), gross appearance, presence of an ulcer, histological type, depth of invasion (mucosa or submucosa), lymphatic involvement, and vascular involvement. Endoscopic macroscopic appearance was evaluated based on the Japanese Classification of Gastric Carcinoma established by the Japanese Research Society for Gastric Cancer[18]. Gross tumor findings were classified into five groups: type I (protruded); Ⅱa (superficially elevated); Ⅱb (flat); Ⅱc (superficially depressed); or Ⅲ (excavated). Macroscopic findings were defined as elevated types (type Ⅰ, Ⅱa, and combined Ⅰ or Ⅱa with Ⅱb), flat type (type Ⅱb), or depressed types (type Ⅱc, Ⅲ, and any combination of Ⅱb, Ⅱc or Ⅲ). When both elevated and depressed types were observed in one lesion, the lesion was defined as mixed type. An ulcer was identified based on the presence of an ulcer or ulcer scar defined endoscopically as converging folds and recognized histologically as a deformity of the muscularis propria or fibrosis in the submucosal layer[19].

Specimens were fixed in 5% formaldehyde and the tumor area and surrounding normal tissue were completely embedded in paraffin. The size (largest diameter) of each carcinoma was measured by the pathologist after fixation. From each block, 2 μm thick sections were cut and stained with hematoxylin and eosin. The depth of infiltration was measured at the deepest point of penetration of the cancer cells[20]. The pT1 category was confirmed as well as the subdivision in pT1a (limited to the mucosa or muscularis mucosa) and pT1b (submucosa).

All dissected lymph nodes were analyzed microscopically for metastatic disease. When necessary, additional lymph node sections were analyzed and special staining was applied. Immunohistochemistry for micrometastasis was not performed.

Statistical analysis

Calculations were performed using SPSS for Windows

Table 2 Clinicopathological features of 556 patients diagnosed with early gastric cancer and univariate analysis of potential risk factors of regional lymph node metastasis

Variables	Positive rate of lymph node metastasis (%)	*P* value
Age (yr)		0.876
< 65	40/288 (13.9)	
⩾ 65	36/268 (13.4)	
Gender		0.012
Male	35/330 (10.6)	
Female	41/226 (18.1)	
Size (cm)		< 0.001
< 2	34/352 (9.7)	
⩾ 2	42/204 (20.6)	
Endoscopic appearance		0.993
Elevated	9/65 (13.8)	
Depressed	43/307 (14.0)	
Flat	16/123 (13.0)	
Mixed	8/61 (13.1)	
Serum CEA (ng/mL)		0.152
< 5	43/336 (12.8)	
⩾ 5	7/32 (21.9)	
Depth of invasion		< 0.001
T1a	12/293 (4.1)	
T1b	64/263 (24.3)	
Histology differentiation		0.759
Differentiated	41/309 (13.3)	
Undifferentiated	35/247 (14.2)	
Vascular invasion		< 0.001
Absence	68/546 (12.5)	
Presence	8/10 (80.0)	
Lymphatic involvement		< 0.001
Absence	44/517 (8.5)	
Presence	32/39 (8.2)	
Ulcer		0.449
Absence	22/182 (12.1)	
Presence	54/374 (14.4)	

(version 11.5K, Chicago, Illinois). The χ^2 test was used to assess potential risk factors of LNM by bivariate comparisons of the categorical variables. Significant factors noted by univariate analysis were subsequently entered into a multivariate logistic regression model for analysis. *P* values < 0.05 were considered to be statistically significant.

RESULTS

EGCs were diagnosed in 18.9% of the gastric cancer cases (556 cases). Of these 556 diagnosed with EGC, 76 (13.7%) had LNM. As shown in Table 2, 293 (52.7%) were intramucosal tumors and 4.1% of these had LNM. In addition, 263 lesions (47.3%) penetrated the submucosa and 24.3% of submucosal tumors had LNM.

Univariate analysis identified that female gender, size ⩾ 2 cm, tumor invasion to the submucosa, presence of lymphatic involvement, and presence of vascular involvement were significantly associated with a higher rate of LNM (Table 2). Tumor size ⩾ 2 cm ($P < 0.008$), deep penetration into the submucosa ($P < 0.001$), and lymphatic involvement ($P < 0.001$) remained significant in multivariate analysis (Table 3).

Table 4 demonstrates the incidence of LNM of our patients fulfilled the criteria used for endoscopic treatment in EGD Japan[7]. In patients without lymphovascular invasion, 42 (tumor size ⩽ 2 cm) and 77 (any tumor size) patients with differentiated intramucosal cancers and no ulceration did not have LMN; intramucosal lesions with ulcer, size ⩽ 3 cm were found in 78 patients, who had no LNM; 3 of 35 patients with undifferentiated intramucosal tumors, no ulceration and size ⩽ 2 cm had LNM.

Table 3 Multivariate analysis of potential risk factors for regional lymph node metastasis

Variables	Odds ratio (95% CI)	*P* value
Gender (female/male)	1.49 (0.35-1.1)	0.163
Tumor size (⩾ 2 cm/< 2 cm)	2.28 (1.20-4.17)	0.008
Vascular invasion (yes/no)	1.86 (0.23-12.65)	0.598
Lymphatic invasion (yes/no)	27.2 (10.3-74.8)	< 0.001
Depth of invasion (T1b/T1a)	4.91 (2.44-9.89)	< 0.001

Table 4 Incidence of lymph node metastasis in our patients fulfilled the criteria used in endoscopic treatment for early gastric cancer in Japan

Criteria	Patient number with lymph node metastasis/total patient	Incidence (%)
Non-ulcerated, differentiated, intramucosal tumor without lymphovascular invasion, ⩽ 2 cm	0/42	0
Non-ulcerated, differentiated, intramucosal tumor without lymphovascular invasion, any size	0/77	0
Ulcerated, intramucosal tumor, without lymphovascular invasion, ⩽ 3 cm	0/78	0
Non-ulcerated, undifferentiated intramucosal tumor without lymphovascular invasion, ⩽ 2 cm	3/35	8.6

DISCUSSION

The incidence of LNM in EGC ranges from 2.6% to 4.8% in mucosal cancers and 16.5% to 23.6% in submucosal cancers[21,22]. In this study the positive rates of LNM in intramucosal and submucosal lesions were 4.1% and 24.3%, respectively, in line with the previous reports[21,22]. Endoscopic treatment for gastric cancer not only preserves gastric function but also helps maintain the patient's quality of life. For patients with EGC, "early" treatment is advocated as the best option for obtaining a complete cure. At present, a correct diagnosis of LNM is impossible during either EMR or ESD. This means that if LNM exists at the time of EMR or ESD, recurrence is very likely. For this reason, multiple patient- and tumor-related variables are currently under investigation as predictors of lymph node involvement[23], particularly in Japan and Korea. Many investigators have suggested possible extended criteria for local treatment[4].

In areas outside of Japan and Korea, the possibility

of using the above-described criteria remains problematic. Japan and Korea have the highest rate of gastric cancer rate in the world[11,12] and that in these countries, between 30% and 70% of all gastric cancers are diagnosed as EGCs. In other areas or countries, EGCs account for only about 5% to 15% of all gastric cancers[13,14,24].

National screening programs and chromoendoscopy could improve the detection rate of EGC; however, race, diet, and other factors such as pathological diagnosis may explain differences in the diagnosis of EGC between Japan and other countries. For example, there are some intestinal-type mucosal cancers in Japan that are not regarded as cancer in Western countries[15,16]. Hölscher *et al*[17] reported that the rate of LNM in mucosal cancer in one European series (6.5%) is higher than reported in Asian countries (2.7%). These differences are even greater if study data are limited to Japan and Korea; however, no difference in submucosal cancer exists (23.9% *vs* 22.1%). Therefore, whether the standard treatment employed in Japan and Korea can be used in other countries remains unknown. Compared with the data generated in this study, the rate of EGC was 18.9%, which is lower than that previously reported in Japan and Korea and similar to the reports from the United States and Europe. Our results revealed that the rate of LNM in intramucosal cancer was 4.1%, higher than the value published from Japan and Korea[17]. This might be explained due to the bias of histology criteria employed in our study (similar to those used in Western countries) and Japan and Korea.

In this study, various clinicopathological factors including gender, age, CEA levels, gross appearances, histological type, invasion depth, lymphatic involvement, and vascular involvement were analyzed for LNM in EGC. Our results showed that female gender was a significant factor in univariate analyses, but was not evident in multivariate analyses. Other research teams reported that being female was associated with LNM in both depressed EGCs and differentiated submucosally invasive EGC[10,25], possibly related to estrogen level[26]. At present, the precise link between gender and LNM remains unclear and further biologic studies are required to explain this effect.

Many studies suggest that serum CEA is an independent risk factor for hematogenous recurrence of gastric carcinoma[27]. Ikeda *et al*[28], for example, reported that stage Ⅱ and Ⅲ gastric cancer patients with higher preoperative CEA levels had frequent liver metastasis. This analysis, however, included all stages of gastric cancer. In the subgroup analysis of EGC in our studies, serum CEA levels were not a significant risk factor for LNM. Whether increased CEA levels have any impact on survival in patients with EGC needs further study.

Some studies have indicated that histological differentiation and ulceration had no significant association with LNM[25,26,29-31]. In contrast, Gotoda *et al*[4] performed a large study including 5265 patients with EGC. They reported that undifferentiated EGC and ulceration were independent factors. Moreover, Ye *et al*[32] assessed 591 patients with undifferentiated EGC and found that poorly differentiated EGCs had higher rates of LNM ($P < 0.001$). In the current study, histological differentiation was not a predictor of LNM, consistent with previous reports[25,26,29-31]. Nonetheless, our results showed that 3 of 35 cases (8.6%) with undifferentiated intramucosal tumors, which were not ulcerative and ⩽ 2 cm in size, had LNM. It is well accepted that in the presence of LNM, only radical gastrectomy provided a chance for curing patients with EGC. Therefore, we suggest that surgical resection with lymphadenectomy instead of endoscopic management should be the treatment choice for undifferentiated EGC.

Many researchers have accepted that depth of tumor invasion is the major factor relating to regional LNM[4,26,30,31] and endoscopic treatment is considered in EGC even if there is minimal submucosal invasion. Nonetheless, some of these researchers still advise against the use of extended criteria even if minimal submucosa invasion is present because rare cases had LNM that met the extended criteria for EMR/ESD[33].

In summary, tumor with submucosal invasion, size ⩾ 2 cm, and presence of lymphatic involvement appear to be significant factors for LNM. Endoscopic treatment might be an alternative in carefully selected EGC patients without predictors for LNM.

COMMENTS

Background

Endoscopic treatment for early gastric cancer (EGC) has been widely adapted in Japan and Korea to avoid unnecessary gastric resection. However, the presence of lymph node metastasis (LNM) which affects patient survival is considered a contraindication of endoscopic management in this situation. This study was designed to analyze the predictive factors for LNM in early gastric cancer.

Research frontiers

Studies have established an indication for endoscopic treatment for EGC and the probability of LNM in EGC is extremely low based on endoscopic and histopathological findings.

Innovations and breakthroughs

Guidelines for endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) remains uncertain in areas outside of Japan and Korea. The authors identify the predictive factors related to LNM in EGC and test the guideline.

Applications

Endoscopic treatment might be an alternative in carefully selected EGC patients without risk factors for LNM.

Terminology

EMR is an endoscopic technique of resection of a lesion that requires the separation of the submucosa using normal saline solution. ESD is a new method of resection, allowing the dissection of the lesion within the thickness of the submucosa or the interface between the submucosa and the muscularis propria.

Peer review

Excellent contribution highlighting the appropriate selection indicators for endoscopic treatment of early gastric cancer indicating that not all early cancers are the same.

REFERENCES

1 **Kikuchi S**, Katada N, Sakuramoto S, Kobayashi N, Shimao H, Watanabe M, Hiki Y. Survival after surgical treatment of early gastric cancer: surgical techniques and long-term survival. *Langenbecks Arch Surg* 2004; **389**: 69-74

2 **Borie F**, Plaisant N, Millat B, Hay JM, Fagniez PL. Appropriate gastric resection with lymph node dissection for early gastric cancer. *Ann Surg Oncol* 2004; **11**: 512-517

3 **Kunisaki C**, Akiyama H, Nomura M, Matsuda G, Otsuka Y, Ono H, Shimada H. Surgical outcomes for early gastric cancer in the upper third of the stomach. *J Am Coll Surg* 2005; **200**: 15-19

4 **Gotoda T**, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer* 2000; **3**: 219-225

5 **Gunji Y**, Suzuki T, Hori S, Hayashi H, Matsubara H, Shimada H, Ochiai T. Prognostic significance of the number of metastatic lymph nodes in early gastric cancer. *Dig Surg* 2003; **20**: 148-153

6 **Sano T**, Kobori O, Muto T. Lymph node metastasis from early gastric cancer: endoscopic resection of tumour. *Br J Surg* 1992; **79**: 241-244

7 **Japanese Gastric Cancer Association**. Treatment guidelines for gastric cancer in Japan (in Japanese). 2nd ed. Tokyo Kanehara, 2004

8 **Kim JH**, Lee YC, Kim H, Song KH, Lee SK, Cheon JH, Kim H, Hyung WJ, Noh SH, Kim CB, Chung JB. Endoscopic resection for undifferentiated early gastric cancer. *Gastrointest Endosc* 2009; **69**: e1-e9

9 **Chung IK**, Lee JH, Lee SH, Kim SJ, Cho JY, Cho WY, Hwangbo Y, Keum BR, Park JJ, Chun HJ, Kim HJ, Kim JJ, Ji SR, Seol SY. Therapeutic outcomes in 1000 cases of endoscopic submucosal dissection for early gastric neoplasms: Korean ESD Study Group multicenter study. *Gastrointest Endosc* 2009; **69**: 1228-1235

10 **Abe N**, Sugiyama M, Masaki T, Ueki H, Yanagida O, Mori T, Watanabe T, Atomi Y. Predictive factors for lymph node metastasis of differentiated submucosally invasive gastric cancer. *Gastrointest Endosc* 2004; **60**: 242-245

11 **Yamamoto S**. Stomach cancer incidence in the world. *Jpn J Clin Oncol* 2001; **31**: 471

12 **Ahn YO**, Park BJ, Yoo KY, Kim NK, Heo DS, Lee JK, Ahn HS, Kang DH, Kim H, Lee MS. Incidence estimation of stomach cancer among Koreans. *J Korean Med Sci* 1991; **6**: 7-14

13 **Siewert JR**. Gastric cancer: the dispute between East and West. *Gastric Cancer* 2005; **8**: 59-61

14 **Kim JP**, Hur YS, Yang HK. Lymph node metastasis as a significant prognostic factor in early gastric cancer: analysis of 1,136 early gastric cancers. *Ann Surg Oncol* 1995; **2**: 308-313

15 **Schlemper RJ**, Itabashi M, Kato Y, Lewin KJ, Riddell RH, Shimoda T, Sipponen P, Stolte M, Watanabe H, Takahashi H, Fujita R. Differences in diagnostic criteria for gastric carcinoma between Japanese and western pathologists. *Lancet* 1997; **349**: 1725-1729

16 **Schlemper RJ**, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, Dixon MF, Fenoglio-Preiser CM, Fléjou JF, Geboes K, Hattori T, Hirota T, Itabashi M, Iwafuchi M, Iwashita A, Kim YI, Kirchner T, Klimpfinger M, Koike M, Lauwers GY, Lewin KJ, Oberhuber G, Offner F, Price AB, Rubio CA, Shimizu M, Shimoda T, Sipponen P, Solcia E, Stolte M, Watanabe H, Yamabe H. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; **47**: 251-255

17 **Hölscher AH**, Drebber U, Mönig SP, Schulte C, Vallböhmer D, Bollschweiler E. Early gastric cancer: lymph node metastasis starts with deep mucosal infiltration. *Ann Surg* 2009; **250**: 791-797

18 **Japanese Gastric Cancer Association**. Japanese Classification of Gastric Carcinoma - 2nd English Edition. *Gastric Cancer* 1998; **1**: 10-24

19 **Abe N**, Watanabe T, Sugiyama M, Yanagida O, Masaki T, Mori T, Atomi Y. Endoscopic treatment or surgery for undifferentiated early gastric cancer? *Am J Surg* 2004; **188**: 181-184

20 **Ono H**, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S. Endoscopic mucosal resection for treatment of early gastric cancer. *Gut* 2001; **48**: 225-229

21 **Roviello F**, Rossi S, Marrelli D, Pedrazzani C, Corso G, Vindigni C, Morgagni P, Saragoni L, de Manzoni G, Tomezzoli A. Number of lymph node metastases and its prognostic significance in early gastric cancer: a multicenter Italian study. *J Surg Oncol* 2006; **94**: 275-280; discussion 274

22 **Kim DY**, Joo JK, Ryu SY, Kim YJ, Kim SK. Factors related to lymph node metastasis and surgical strategy used to treat early gastric carcinoma. *World J Gastroenterol* 2004; **10**: 737-740

23 **An JY**, Baik YH, Choi MG, Noh JH, Sohn TS, Kim S. Predictive factors for lymph node metastasis in early gastric cancer with submucosal invasion: analysis of a single institutional experience. *Ann Surg* 2007; **246**: 749-753

24 **Choi KW**, Kim WH, Song IS, Kim CY, Jung HC, Yoon JH, Lee DH, Lee KL, Lee JH, Kim BG, Hwang JH, Jeong JO, Lim YS, Lee DH, Jeong WT. Endoscopic mucosal resection (EMR) as a curative treatment of early gastric cancer. *Korean J Gastrointest Endosc* 1996; **16**: 928

25 **Yasuda K**, Shiraishi N, Suematsu T, Yamaguchi K, Adachi Y, Kitano S. Rate of detection of lymph node metastasis is correlated with the depth of submucosal invasion in early stage gastric carcinoma. *Cancer* 1999; **85**: 2119-2123

26 **Abe N**, Watanabe T, Suzuki K, Machida H, Toda H, Nakaya Y, Masaki T, Mori T, Sugiyama M, Atomi Y. Risk factors predictive of lymph node metastasis in depressed early gastric cancer. *Am J Surg* 2002; **183**: 168-172

27 **Marrelli D**, Roviello F, De Stefano A, Farnetani M, Garosi L, Messano A, Pinto E. Prognostic significance of CEA, CA 19-9 and CA 72-4 preoperative serum levels in gastric carcinoma. *Oncology* 1999; **57**: 55-62

28 **Ikeda Y**, Mori M, Kajiyama K, Kamakura T, Maehara Y, Haraguchi Y, Sugimachi K. Indicative value of carcinoembryonic antigen (CEA) for liver recurrence following curative resection of stage II and III gastric cancer. *Hepatogastroenterology* 1996; **43**: 1281-1287

29 **Kitamura K**, Yamaguchi T, Taniguchi H, Hagiwara A, Sawai K, Takahashi T. Analysis of lymph node metastasis in early gastric cancer: rationale of limited surgery. *J Surg Oncol* 1997; **64**: 42-47

30 **Park YD**, Chung YJ, Chung HY, Yu W, Bae HI, Jeon SW, Cho CM, Tak WY, Kweon YO. Factors related to lymph node metastasis and the feasibility of endoscopic mucosal resection for treating poorly differentiated adenocarcinoma of the stomach. *Endoscopy* 2008; **40**: 7-10

31 **Maehara Y**, Orita H, Okuyama T, Moriguchi S, Tsujitani S, Korenaga D, Sugimachi K. Predictors of lymph node metastasis in early gastric cancer. *Br J Surg* 1992; **79**: 245-247

32 **Ye BD**, Kim SG, Lee JY, Kim JS, Yang HK, Kim WH, Jung HC, Lee KU, Song IS. Predictive factors for lymph node metastasis and endoscopic treatment strategies for undifferentiated early gastric cancer. *J Gastroenterol Hepatol* 2008; **23**: 46-50

33 **Ishikawa S**, Togashi A, Inoue M, Honda S, Nozawa F, Toyama E, Miyanari N, Tabira Y, Baba H. Indications for EMR/ESD in cases of early gastric cancer: relationship between histological type, depth of wall invasion, and lymph node metastasis. *Gastric Cancer* 2007; **10**: 35-38

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BRIEF ARTICLE

Staging systems for predicting survival of patients with hepatocellular carcinoma after surgery

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Abstract

AIM: To compare the staging systems for stratifying and predicting the prognosis of patients with hepatocellular carcinoma (HCC) after partial hepatectomy (PH).

METHODS: Clinical data about 438 HCC patients who underwent PH from January 1991 to December 2004 at our hospital were retrospectively analyzed. Tumor stage was evaluated following the Chinese tumor node metastasis (TNM) and barcelona clinic liver cancer (BCLC) staging systems, respectively. Survival curves for the HCC patients were plotted using the Kaplan-Meier method and differences were compared by the log-rank test. The accuracy of each system for predicting death of HCC patients was evaluated by calculating the area under the receiver operating characteristic curve.

RESULTS: The HCC patients were classified into stages Ⅰ-Ⅲ, stages Ⅰ-Ⅳ and stages A-C, according to the 3 staging systems, respectively. Log-rank test showed that the cumulative survival rate was significantly different for the HCC patients at 3 Chinese system stages, TNM stages Ⅰ and Ⅱ, TNM stages Ⅲ and Ⅳ, and 3 BCLC stages ($P < 0.05$). However, no significant difference was found in the HCC patients at TNM stages Ⅱ and Ⅲ. The accuracy of the Chinese and BCLC staging systems was higher than that of the TNM staging system for predicting the survival rate of HCC patients.

CONCLUSION: The Chinese and BCLC staging systems are better for stratifying and predicting the prognosis of HCC patients after PH than the TNM staging system.

Key words: Hepatocellular carcinoma; Tumor staging; Prognosis; Survival; Hepatectomy

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INTRODUCTION

Hepatocellular carcinoma (HCC), one of the most common cancers in the world, especially in Eastern Asia, with a poor prognosis[1], is the first leading cause of cancer-related death in the southeast of China[2]. Treatment modalities for HCC are strongly dependent on tumor stage

and the underlying liver diseases. Surgical intervention is the only potentially curative modality for it at present. However, the long-term prognosis of HCC patients remains dismal even after radical excision or liver transplantation[3].

Staging systems are used to define the prognosis of HCC and its treatment[4]. Generally, a better clinical staging system can stratify patients according to their survival time, and is reliable and useful for comparing the curative effects on HCC. Several HCC staging systems have been proposed, such as the Okuda staging system[5], tumor node metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC)[6], cancer of the liver Italian program (CLIP) scoring system[7], Barcelona clinic liver cancer (BCLC) staging classification[8], Japan integrated staging (JIS) score[9], and Tokyo score[10]. However, no worldwide consensus has been reached on the use of any given HCC staging systems. Therefore, more accurate classification of HCC patients with a homogeneous prognosis would at minimum improve the application of currently available treatment modalities.

The Chinese staging system, established by the Chinese Society of Liver Cancer (CSLC) in 1999[11,12], combines the tumor-related factors and liver function reserve. However, its application in stratifying and predicting the prognosis of HCC patients remains to be defined. This study was to evaluate and compare the Chinese staging system, the AJCC TNM staging system (7th edition) and the BCLC staging system for stratifying and predicting the prognosis of a large cohort of Chinese HCC patients after partial hepatectomy (PH).

MATERIALS AND METHODS

Patients

Four hundred and thirty-eight patients with HCC who underwent PH from January 1991 to December 2004 at our hospital were included in this study. The diagnosis of HCC was pathologically confirmed. Clinicopathological features and survival rates of the patients were analyzed. The study was approved by the Ethical Committee of Sun Yat-Sen Memorial Hospital and in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from the patients or their guardians.

Tumor stage

HCC was divided into different stages following the criteria for the Chinese, TNM and BCLC staging systems, respectively. The detailed definition and criteria for the Chinese staging system are listed in Table 1.

Treatment

All patients underwent non-anatomical resection. According to the Couinaud's nomenclature for liver segmentation, minor hepatectomy (1-2 segments), major hepatectomy (≥ 3 segments), and wedge resection were performed in 179 (40.9%), 47 (10.9%), and 212 (48.2%) patients, respectively. Of the 438 patients, 24 (5.5%) with unresectable HCC underwent surgery after trans-catheter hepatic arterial chemoembolization (TACE, down-staged). Moreover, HCC patients with inoperable intrahepatic recurrence or extrahepatic metastases received combined therapies, including TACE, radiofrequency ablation (RFA), microwave coagulation therapy (MCT), percutaneous ethanol injection (PEI), and biotherapy or traditional Chinese therapy.

Table 1 Definition and criteria of Chinese staging system for hepatocellular carcinoma (1999)

Stage	Tumor	Thrombi	CLN metastasis	Distant metastasis	Child-Pugh score
Ⅰa	Solitary ≤ 3 cm	No	No	No	A
Ⅰb	Solitary or two ≤ 5 cm in single lobe	No	No	No	A
Ⅱa	Solitary or two ≤ 10 cm in single lobe, or two ≤ 5 cm in bilateral lobes	No	No	No	A
Ⅱb	Solitary or two > 10 cm in single lobe, or two > 5 cm in bilateral lobes	No	No	No	A
	Any	PV-branch, HV or BD	No	No	A
	Any	No	No	No	B
Ⅲa	Any	PV-trunk or IVC	Yes/No	Yes/No	A/B
	Any	Yes/No	Yes	Yes/No	A/B
	Any	Yes/No	Yes/No	Yes	A/B
Ⅲb	Any	Yes/No	Yes/No	Yes/No	C

CLN: Celiac lymph nodes; PV: Portal vein; HV: Hepatic vein; BD: Bile duct; IVC: Inferior vena cava.

Follow-up

During the first 6 mo after operation, the patients were re-examined every 1-2 mo followed by every 3-6 mo. The clinical, laboratory and radiological (abdominal computed tomography scan and chest X-ray) data were collected at each follow-up. Three hundred and ninety-two (89.5%) HCC patients were followed-up until the end of January 2005 or death, while 46 (10.5%) HCC patients were lost during the follow-up. The median follow-up time was 21 mo (range 1-156 mo).

Statistical analysis

Statistical analysis was conducted with the SPSS software package (version 13.0, SPSS, Chicago, IL). Quantitative data were presented as mean ± SE. Survival curves for the HCC patients were plotted using the Kaplan-Meier method and examined by the log-rank test. The accuracy of each system for predicting the 1-, 3-, and 5-year rates of HCC patients was evaluated by calculating the area under the receiver operating characteristic curve. Patients censored before 1, 3 and 5 years were excluded from the analysis. $P < 0.05$ was considered statistically significant.

Table 2 Characteristics of hepatocellular carcinoma patients enrolled in this study

Characteristics	*n* (%)
Sex	
Male	380 (86.8)
Female	58 (13.2)
Age (yr)	
≤ 50	235 (53.6)
> 50	203 (46.4)
Child-Pugh score	
Class A	391 (89.3)
Class B	47 (10.7)
Tumor number	
Single	374 (85.4)
Two	29 (6.6)
Multiple	35 (8)
Tumor size (cm)	
≤ 5	166 (37.9)
> 5	272 (62.1)
Tumor location	
Single lobe	389 (88.8)
Bilateral lobes	49 (11.2)
Capsular invasion	
With	346 (79)
Without	92 (21)
Vascular invasion	
With	84 (19.2)
Without	354 (80.8)
Lymph node metastasis	
With	11 (2.5)
Without	427 (97.5)
Extra-hepatic metastasis	
With	7 (1.6)
Without	431 (98.4)
Histological grade	
G1	129 (29.5)
G2	188 (42.9)
G3	121 (27.6)

RESULTS

Clinicopathological features and survival of HCC patients

The general characteristics of the 438 HCC patients are summarized in Table 2. The mean and median ages of the patients were 50.0 ± 0.6 years and 49.0 years (range: 35-68 years), respectively. By the end of follow up, 223 HCC patients (50.9%) died. The 1-, 3- and 5-year postoperative overall survival rates were 72.2%, 53.5% and 43.3%, respectively, for the patients after PH.

Prognosis stratification according to the three clinical staging systems

The 438 HCC patients were classified into stages Ⅰ-Ⅲ, stages Ⅰ-Ⅳ and stages A-C according to the Chinese, TNM and BCLC staging systems, respectively. The log-rank test showed that the cumulative survival rate was significantly different for the HCC patients at 3 Chinese system stages, TNM stages Ⅰ and Ⅱ, TNM stages Ⅲ and Ⅳ, and 3 BCLC stages (Table 3 and Figure 1, $P < 0.05$). However, no significant difference was found in the HCC patients at TNM stages Ⅱ and Ⅲ ($P > 0.05$).

Table 3 Overall survival rates of hepatocellular carcinoma patients according to their tumor stage

Tumor stage	*n*	Survival rate (%)		
		1-yr	3-yr	5-yr
CS				
Ⅰ	132	0.916	0.760	0.693
Ⅱ	265	0.622	0.426	0.281
Ⅲ	41	0.330	0.126	0.126
TNM				
Ⅰ	271	0.825	0.652	0.548
Ⅱ	67	0.521	0.354	0.216
Ⅲ	82	0.437	0.235	0.153
Ⅳ	18	0.278	/	/
BCLC				
A	178	0.870	0.678	0.602
B	165	0.756	0.574	0.402
C	95	0.375	0.182	0.142

CS: Chinese staging; TNM: Tumor node metastasis; BCLC: Barcelona clinic liver cancer.

Table 4 Accuracy of three staging systems for the death of hepatocellular carcinoma patients 1, 3 and 5 years after partial hepatectomy

System	AUC (95% CI)		
	1-yr death	3-yr death	5-yr death
CS	0.709 (0.658-0.761)	0.703 (0.645-0.761)	0.720 (0.648-0.791)
TNM	0.718 (0.662-0.774)	0.702 (0.646-0.759)	0.695 (0.629-0.760)
BCLC	0.730 (0.675-0.785)	0.701 (0.644-0.757)	0.710 (0.645-0.776)

AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; CS: Chinese staging; TNM: Tumor node metastasis; BCLC: Barcelona clinic liver cancer.

Accuracy of the three clinical staging systems for predicting survival rate of HCC patients

The accuracy of 3 staging systems for predicting the survival rate of HCC patients 1, 3 and 5 years after PH is summarized in Table 4. The accuracy of the Chinese and BCLC staging systems was higher than that of the TNM staging system for predicting the survival rates of HCC patients (Figure 2).

DISCUSSION

HCC is the second most lethal cancer after pancreatic ductal adenocarcinoma, leading to about 600 000 deaths every year worldwide, with nearly 55% of the deaths occurred in China alone[3]. The long term prognosis of HCC patients is extremely dismal and the incidence of HCC is continuously growing globally[1,13,14]. In the southeast of China, HCC is the leading cause of cancer-related death despite aggressive conventional therapies[2]. Surgery remains the most effective treatment of HCC with a curative potential[15,16]. However, HCC patients even after curative resection of the tumor often have a high rate of relapse.

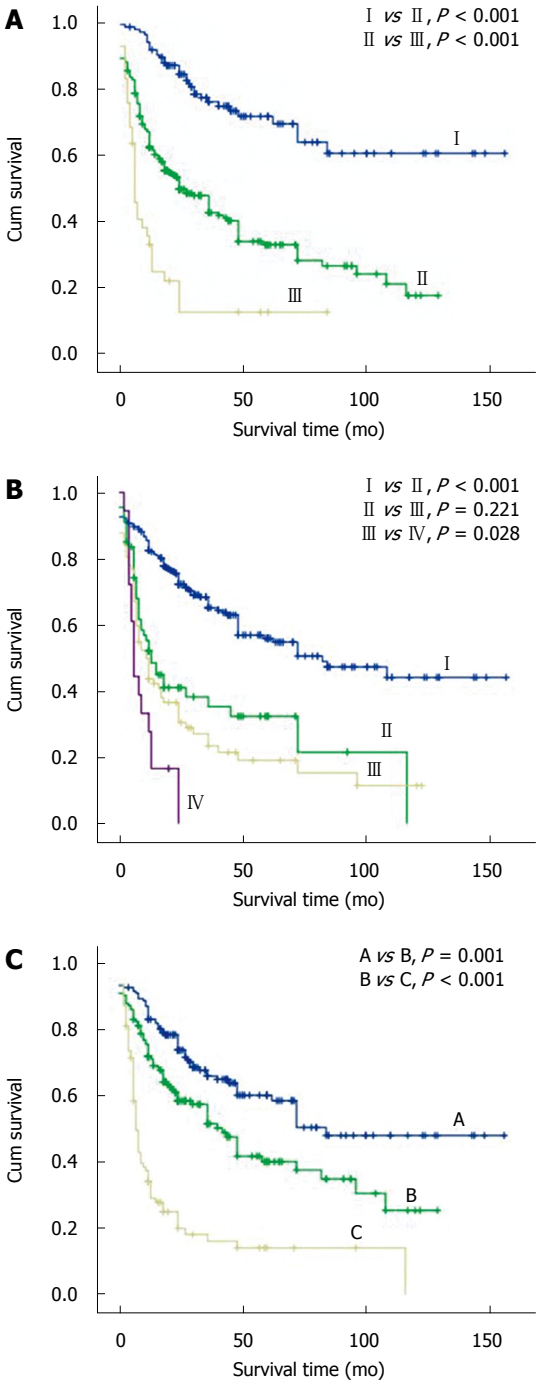

Figure 1 Survival curves for hepatocellular carcinoma patients according to the Chinese staging system (A), tumor node metastasis staging system (B), barcelona clinic liver cancer staging system (C).

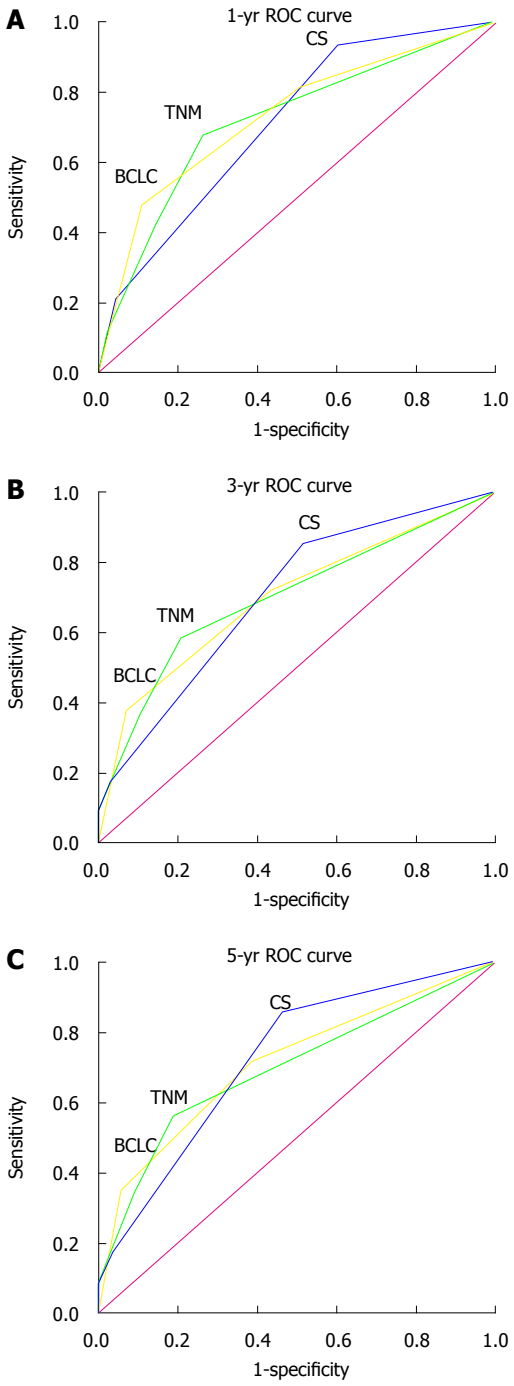

Figure 2 Accuracy of the three staging systems for predicting the 1-year (A), 3-year (B), 5-year (C) survival rates of hepatocellular carcinoma patients. ROC: Receiver operating characteristic curve; CS: Chinese staging; TNM: Tumor node metastasis; BCLC: Barcelona clinic liver cancer.

Therefore, tools that can be used to stratify the prognosis of HCC patients after therapy are urgently needed. Gene-expression analysis has led to the successful molecular classification of HCC according to its prognosis, aetiology and intra-hepatic recurrence[17]. However, it has not been widely accepted due to its high cost.

Staging systems are often used to select primary and adjunctive therapies and to assess their outcome. Generally, a well defined clinical staging system not only can classify HCC patients and predict their survival time, but also can be generally applied globally. Increasing evidence shows that liver function reserve and tumor-related factors can also significantly influence the prognosis of HCC patients[4]. Chen *et al*[18] reported that a better staging system can provide more valid tumor-related factors and liver function parameters. Therefore, both tumor-related features and liver function reserve should be included in the clinical staging systems for HCC[19].

Several clinical staging systems are available for pre-

dicting the prognosis of HCC patients after PH at present. However, no worldwide consensus has been reached on which staging system is the best for predicting the prognosis of HCC patients after surgery[20]. Although the Okuda staging system[5], CLIP scoring system[7], JIS score[9], and Tokyo score[10] are widely used, they have been mostly applied to unresectable HCC, and none is universally accepted[21]. The TNM staging system is the most widely used system worldwide at present[21]. However, the AJCC TNM staging system does not include any measurement of liver functions and is thus not widely used[20]. Llovet *et al*[22] reported that the AJCC TNM staging system fails to adequately stratify HCC patients and predict their prognosis. Lu *et al*[23] also reported that the TNM staging system provides inadequate information for the prognosis of HCC patients.

In the present study, application of the CS system in stratifying and predicting prognosis of a large cohort of Chinese HCC patients (n = 438) after PH was investigated and compared to that of the AJCC TNM and BCLC staging systems. The log-rank test showed that the cumulative survival rate was significantly different for HCC patients at Chinese system stages, TNM stages Ⅰ and Ⅱ, TNM stages Ⅲ and Ⅳ, and 3 BCLC stages, while no significant difference was found in the cumulative survival rate of HCC patients at TNM stages Ⅱ and stage Ⅲ. The accuracy of the Chinese and BCLC staging systems was higher than that of the TNM staging system for predicting the long-term survival (3- and 5-year) rates of HCC patients after PH, indicating that the Chinese and BCLC staging systems are better than the TNM staging system for stratifying and predicting the prognosis of HCC patients after PH. In our study, the AJCC TNM staging system could not accurately stratify the HCC patients with multiple nodules or vascular/peripheral invasion.

In conclusion, the Chinese and BCLC staging systems are better than the AJCC TNM staging system for stratifying and predicting the prognosis of HCC patients after PH. Further studies are needed to establish a single, worldwide staging system that can stratify and predict the prognosis of HCC patients after PH.

COMMENTS

Background

Staging systems are used to define the prognosis and treatment of hepatocellular carcinoma (HCC) patients. Several staging systems are available for predicting the prognosis of HCC patients, but no worldwide consensus has been reached on the use of any given HCC staging systems. Therefore, more accurate classification of HCC patients with homogeneous prognosis is urgently needed.

Research frontiers

Although the Okuda and cancer of the liver Italian program scoring systems, Japan integrated staging score, and Tokyo score are widely used, they have been mostly applied to unresectable HCC patients. The tumor node metastasis (TNM) staging system for HCC does not include any measurement of liver functions and is thus not widely used. The Chinese staging system for stratifying and predicting the prognosis of HCC patients remains to be defined.

Innovations and breakthroughs

The Chinese staging system was studied for stratifying and predicting the prognosis of HCC patients. Furthermore, the applicability of the Chinese staging system was compared with other staging systems for stratifying and predicting the prognosis of HCC patients after partial hepatectomy.

Applications

The results of this study demonstrate that the Chinese staging system can be used for predicting the prognosis of HCC patients after partial hepatectomy.

Peer review

The authors evaluated and compared the Chinese, American Joint Committee on Cancer (AJCC) TNM and Barcelona clinic liver cancer (BCLC) staging systems for stratifying and predicting the prognosis of a large cohort of Chinese HCC patients after partial hepatectomy. The results reveal that the Chinese and BCLC staging systems are better than AJCC TNM staging system for stratifying and predicting the prognosis of HCC patients after partial hepatectomy.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249
2 **Yuen MF**, Hou JL, Chutaputti A. Hepatocellular carcinoma in the Asia pacific region. *J Gastroenterol Hepatol* 2009; **24**: 346-353
3 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576
4 **Chung H**, Kudo M, Takahashi S, Hagiwara S, Sakaguchi Y, Inoue T, Minami Y, Ueshima K, Fukunaga T. Review of current staging systems for hepatocellular carcinoma. *Hepatol Res* 2007; **37** Suppl 2: S210-S215
5 **Okuda K**, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985; **56**: 918-928
6 **Edge SB**, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC cancer staging manual. 7th ed. New York, NY: Springer, 2010: 237-246
7 **Llovet JM**, Bruix J. Prospective validation of the Cancer of the Liver Italian Program (CLIP) score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. *Hepatology* 2000; **32**: 679-680
8 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338
9 **Kudo M**, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J Gastroenterol* 2003; **38**: 207-215
10 **Tateishi R**, Yoshida H, Shiina S, Imamura H, Hasegawa K, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Makuuchi M, Omata M. Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. *Gut* 2005; **54**: 419-425
11 **Yang BH**, Ren ZG, Tang ZY. The consideration and proponent of the liver cancer staging classification. *Zhonghua Gandan Waike Zazhi* 1999; **5**: 67-68
12 **Yan P**, Yan LN. Staging of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2003; **2**: 491-495
13 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917
14 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108
15 **Poon RT**, Fan ST, Tsang FH, Wong J. Locoregional therapies for hepatocellular carcinoma: a critical review from the surgeon's perspective. *Ann Surg* 2002; **235**: 466-486
16 **Rougier P**, Mitry E, Barbare JC, Taieb J. Hepatocellular carcinoma (HCC): an update. *Semin Oncol* 2007; **34**: S12-S20
17 **Farazi PA**, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer* 2006; **6**: 674-687
18 **Chen TW**, Chu CM, Yu JC, Chen CJ, Chan DC, Liu YC, Hsieh CB. Comparison of clinical staging systems in predicting survival of hepatocellular carcinoma patients receiv-

ing major or minor hepatectomy. *Eur J Surg Oncol* 2007; **33**: 480-487
19 **Varotti G**, Ramacciato G, Ercolani G, Grazi GL, Vetrone G, Cescon M, Del Gaudio M, Ravaioli M, Ziparo V, Lauro A, Pinna A. Comparison between the fifth and sixth editions of the AJCC/UICC TNM staging systems for hepatocellular carcinoma: multicentric study on 393 cirrhotic resected patients. *Eur J Surg Oncol* 2005; **31**: 760-767
20 **Yeh CN**, Chen MF, Lee WC, Jeng LB. Prognostic factors of hepatic resection for hepatocellular carcinoma with cirrhosis: univariate and multivariate analysis. *J Surg Oncol* 2002; **81**: 195-202
21 **Leung TW**, Tang AM, Zee B, Lau WY, Lai PB, Leung KL, Lau JT, Yu SC, Johnson PJ. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: a study based on 926 patients. *Cancer* 2002; **94**: 1760-1769
22 **Llovet JM**, Bruix J, Fuster J, Castells A, Garcia-Valdecasas JC, Grande L, Franca A, Brú C, Navasa M, Ayuso MC, Solé M, Real MI, Vilana R, Rimola A, Visa J, Rodés J. Liver transplantation for small hepatocellular carcinoma: the tumor-node-metastasis classification does not have prognostic power. *Hepatology* 1998; **27**: 1572-1577
23 **Lu W**, Dong J, Huang Z, Guo D, Liu Y, Shi S. Comparison of four current staging systems for Chinese patients with hepatocellular carcinoma undergoing curative resection: Okuda, CLIP, TNM and CUPI. *J Gastroenterol Hepatol* 2008; **23**: 1874-1878

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CASE REPORT

Primary malignant liver mesenchymal tumor: A case report

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Abstract

Primary malignant liver mesenchymal tumor is a rare condition defined as a tumor with vascular, fibrous, adipose, and other mesenchymal tissue differentiation. We report a case of primary malignant liver mesenchymal tumor in a 51-year-old male with anemia, weight loss and hepatomegaly. Finally unconventional liver biopsy and histological manifestation led to the definitive diagnosis.

Key words: Hepatic mesenchymal tumor; Liver biopsy; Hepatic leiomyosarcoma; Hepatic schwannoma

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INTRODUCTION

Primary malignant liver mesenchymal tumor is a very rare tumor, accounting for less than 1% of all hepatic malignancies[1]. Hepatic angiosarcoma, leiomyosarcoma, embryonal sarcoma, schwannoma and lymphoma are the more common mesenchymal tumors. The diagnosis is dependent on histological imaging. However, some cases of multiple nodular lesions can only be diagnosed by percutaneous liver biopsy (PLB) without surgery. We present a case of malignant liver mesenchymal tumor in an adult and its diagnosis and treatment were discussed.

CASE REPORT

A 51-year-old Chinese male was referred to our hospital with a 2-mo history of mild abdominal pain, an abdominal mass in the upper quadrant, fatigue and progressive weight loss. His symptoms gradually worsened with fatigue 1 wk after onset of the disease. He had no fever, cough or skin lesions in the past 2 mo and no family history of liver and autoimmune diseases. Physical examination revealed mild pale conjunctiva and skin, and enlargement of the liver without percussion pain.

His complete blood hemoglobin was 58 g/L. Stool occult blood test was negative. Liver functional test showed that his alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ glutamyl tranzpehtidaze, total bilirubin, direct bilirubin, and albumin were 81 U/L, 86 U/L, 224 U/L, 73 U/L, 23.55 μmol/L, 11.74 μmol/L, 30.5 g/L, respectively. Series hepatitis markers (hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, Epstein-Barr virus, and cucumber mosaic virus) were negative. α-fetoprotein, carcinoembryonic antigen and CA19-9 concentrations were also normal. Human immunodeficiency virus and syphilis antibodies were negative. Coagulation profile was normal.

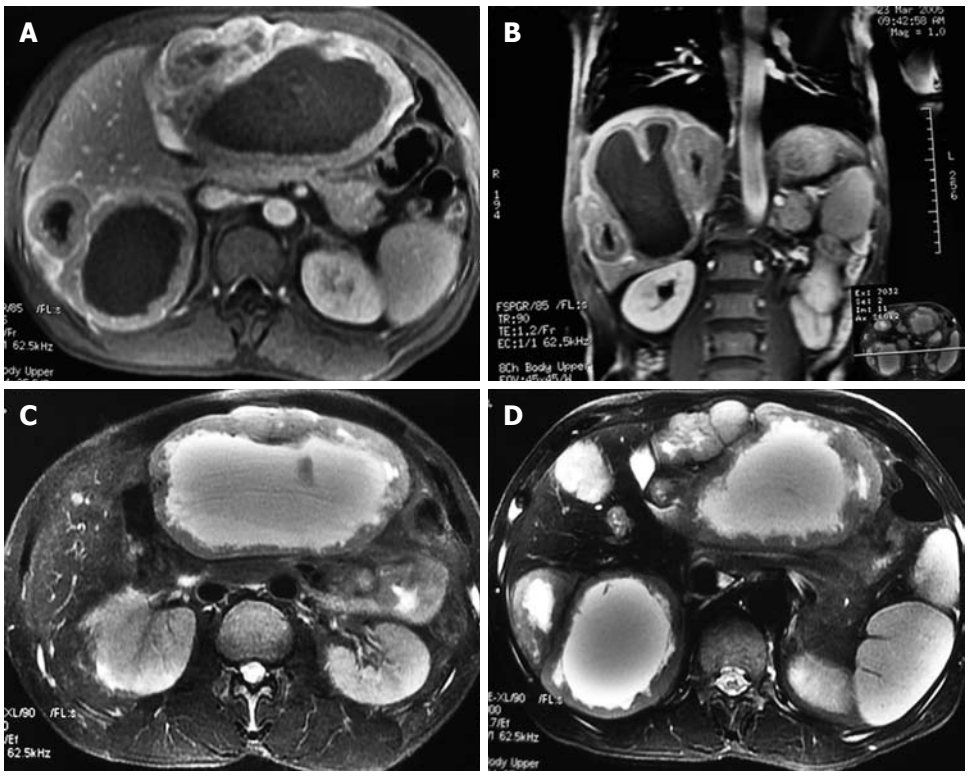

Figure 1 Axial fat-suppressed T2-weighted turbo spin echo magnetic resonance imaging showing two large lesions (A) and several small cyst-like lesions (B) in liver, and coronal and transverse T1-weighted imaging showing thickened wall of lesions with a ragged appearance (C) and enhancement (D) in liver.

Ultrasound and magnetic resonance imaging (MRI) were used as the initial diagnostic tools. Abdominal ultrasound revealed a solid mass in the liver with a low echo, a sharp border, a rich blood supply and liquefaction necrosis. The maximum diameter of the mass was 13.9 cm. Abdominal MRI showed multiple diffuse abnormal signals suggestive of a possible malignant tumor. Axial fat-suppressed T2-weighted turbo spin echo imaging showed two larger and several smaller well-margined cyst-like lesions in the liver (Figure 1A and B). Coronal and transverse T1-weighted imaging demonstrated thickened wall of lesions with a ragged appearance and enhancement (Figure 1C and D). Endoscopy showed duodenal ulcer (S2 stage), normal colon and rectum.

PLB was performed under ultrasonography (US) guidance using a Bard biopsy gun (18-gauge cut needle) to make a final diagnosis of the lesions in liver. During biopsy, bloody liquid was observed in the lesion. Microscopic examination confirmed that the bloody liquid contained a large number of erythrocytes. However, pathology of the liver only showed a spindle cell tumor. The possible reasons are as follows. First, the liver specimen was too small because the lesion contained only fluid and was surrounded by a very thin wall. Second, the location of tumor and its hardness limited the adjustable puncture angle.

To confirm the diagnosis of this patient, another unconventional liver biopsy was performed with gastroscopic biopsy forceps as follows.

At beginning of the procedure, a local anesthetic agent (5% lidocaine) was injected subcutaneously through a 25-gauge needle. Next, a 15 cm long 18 gauge puncture needle was passed into the cyst under US guidance. After the local skin and subcutaneous tissue were dilated, a PTC exchange stainless steel guide wire with a flexible tip was introduced into the cyst cavity through the needle, and then the needle was withdrawn. A 5-French catheter was plated into the cyst using the Seldinger technique. Finally, gastroscopic biopsy forceps was passed into the cyst through the catheter to get the liver tissue sample. The histological results indicated that spindle-shaped tumor cells were well-oriented, arranged in bundles and clustered in some part of the regional tumor. Nuclei with rare mitosis were elongated in a rod-like shape with different sizes and blunt ends. The final diagnosis was established as a low grade malignant liver mesenchymal tumor (Figure 2).

The patient was suggested to receive hepatic arterial chemoembolization. Selective digital subtraction angiography in the early and later phases showed lesions surrounded by abnormal tortuous tumor arteries (Figure 3A) and patchy enhancement (Figure 3B), respectively, in different areas. Then, 5-Fu (750 mg), mitomycin (5 mg), pyrazine imidacloprid Star (30 mg), and super-liquid iodized oil (5 mL) were infused into the tumor arteries. After treatment, liver function of the patient was improved and his hemoglobin level increased (Table 1). The patient died 2 years after treatment.

DISCUSSION

In this paper, we presented a case of multiple nodular cys-

Table 1 Changes in serum biochemical index before and after treatment

	WBC ($\times 10^9$/L)	HGB (g/L)	PLT ($\times 10^{12}$/L)	ALT (U/L)	AST (U/L)	TBIL (μmol/L)	ALB (g/L)
Diagnosis	10.44	58	294	81	86	23.55	30.5
Treatment	6.24	86	241	17	24	20.74	34.1

WBC: White blood cell count; HGB: Hemoglobin; PLT: Platelet count; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TBIL: Total bilirubin; ALB: Albumin.

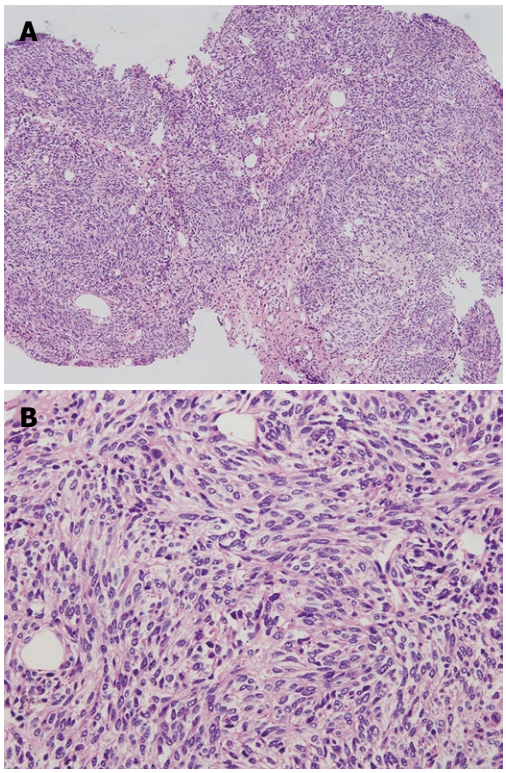

Figure 2 Hematoxylin and eosin staining showing a low grade malignant liver mesenchymal tumor under the magnification × 10 (A) and × 40 (B).

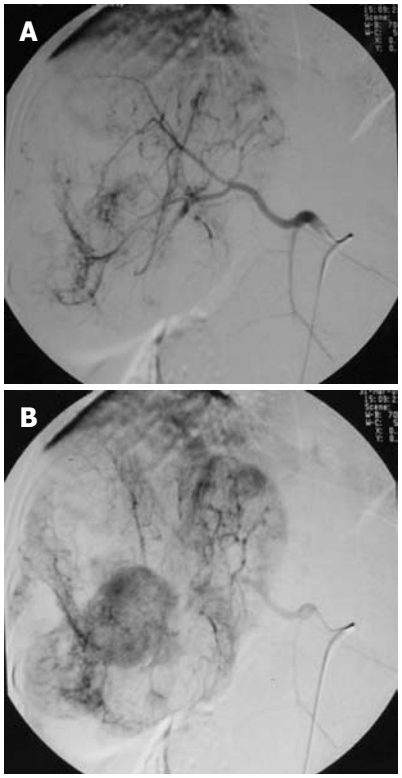

Figure 3 Selective digital subtraction angiography showing abnormal tortuous arteries in the early phase (A) and patchy enhancement in later phase (B) in different areas.

tic lesions in liver. The final diagnosis was established as a low grade malignant liver mesenchymal tumor. However, HE staining could not show the source of mesenchymal cells. Further immunohistochemistry staining with Vimentin, Desmin and α-SMA was performed, which could not sill show the source of mesenchymal cells.

Primary malignant liver leiomyosarcoma and schwannoma are both rare tumors in the liver. Primary liver leiomyosarcoma is a rare malignancy involving the liver, occurring as a primary liver sarcoma in patients without any underlying disorder, and its incidence increases as a primary tumor in immunodeficiency patients[2]. Its usual clinical presentation is painful hepatomegaly or epigastric mass[3]. Malignant liver schwannoma is the most common soft tissue sarcoma in adults, but primary liver schwannoma is extremely rare. Only 12, 8, and 1 cases of benign, malignant, and semimalignant liver schwannoma are available from the literature worldwide[4-12]. Hepatic schwannoma is usually associated with neurofibromatosis. However, two cases of malignant liver schwannoma without neurofibromatosis have been reported[9,11].

Unfortunately, it is difficult to make the diagnosis of malignant liver mesenchymal tumor because both its clinical presentation and imagine are nonspecific.

The common symptoms and signs of patients with malignant liver mesenchymal tumor include abdominal pain, weight loss, weakness, loss of appetite, vomiting, enlargement of the liver, ascites, and jaundice, which lack of specificity in differential diagnosis between benign and malignant mesenchymal tumors.

Furthermore, imaging studies, such as MRI scan, computed tomography (CT) scan and angio photography are the commonly used methods to identify the characteristics of liver tumor. However, imaging findings of liver mesenchymal tumors, including leiomyosarcoma and schwannoma, are nonspecific and infrequently reported[13-19]. It is quite difficult for MRI and CT scan to differentiate primary liver mesenchymal neoplasms from other liver malignancies, although they should be included in differential diagnosis when MRI or CT scan demonstrates hepatic lesions without characteristics of hepatocellular carcinoma,

especially in patients with no extrahepatic primary malignancies.

The diagnosis of malignant liver mesenchymal tumor depends on histological change in either needle or open biopsy, while metastatic status is facilitated by the presence of extrahepatic primary tumor.

Liver biopsy is an important diagnostic tool and helps make therapeutic decision for liver tumor. Open biopsy is a major surgical procedure for liver tumor. PLB under ultrasound or CT guidance is a safe and almost painless procedure for lesions with a soft tissue component or located close to vital structures[20]. Most reported cases of cystic liver mesenchymal tumor were diagnosed by open liver biopsy. In this case, uncommon PLB method was used instead of the routine PLB to make the final diagnosis, avoiding injury and complications of open biopsy. Minimally invasive treatment devices, such as gastroscopic biopsy forceps and catheters used in liver cancer intervention, make the new PLB method possible, which is safe and reliable and can thus be used in diagnosis of cystic liver lesions.

In conclusion, mesenchymal liver tumor is rare in adults and cross-sectional findings are varied, which can be diagnosed with the uncommon PLB method.

EFERENCES

1 **Weitz J**, Klimstra DS, Cymes K, Jarnagin WR, D'Angelica M, La Quaglia MP, Fong Y, Brennan MF, Blumgart LH, Dematteo RP. Management of primary liver sarcomas. *Cancer* 2007; **109**: 1391-1396

2 **Giuliante F**, Sarno G, Ardito F, Pierconti F. Primary hepatic leiomyosarcoma in a young man after Hodgkin's disease: diagnostic pitfalls and therapeutic challenge. *Tumori* 2009; **95**: 374-377

3 **Gates LK Jr**, Cameron AJ, Nagorney DM, Goellner JR, Farley DR. Primary leiomyosarcoma of the liver mimicking liver abscess. *Am J Gastroenterol* 1995; **90**: 649-652

4 **Tuder RM**, Moraes CF. Primary semimalignant Schwannoma of the liver. Light and electron microscopic studies. *Pathol Res Pract* 1984; **178**: 345-348

5 **Kim YC**, Park MS. Primary hepatic schwannoma mimicking malignancy on fluorine-18 2-fluoro-2-deoxy-D-glucose positron emission tomography-computed tomography. *Hepatology* 2010; **51**: 1080-1081

6 **Akin M**, Bozkirli B, Leventoglu S, Unal K, Kapucu LO, Akyurek N, Sare M. Liver schwannoma incidentally discovered in a patient with breast cancer. *Bratisl Lek Listy* 2009; **110**: 298-300

7 **Lee WH**, Kim TH, You SS, Choi SP, Min HJ, Kim HJ, Lee OJ, Ko GH. Benign schwannoma of the liver: a case report. *J Korean Med Sci* 2008; **23**: 727-730

8 **Momtahen AJ**, Akduman EI, Balci NC, Fattahi R, Havlioglu N. Liver schwannoma: findings on MRI. *Magn Reson Imaging* 2008; **26**: 1442-1445

9 **Kóbori L**, Nagy P, Máthé Z, Hartmann E, Doros A, Paku S, Dezso K, Sápi Z. Malignant peripheral nerve sheath tumor of the liver: a case report. *Pathol Oncol Res* 2008; **14**: 329-332

10 **Young SJ**. Primary malignant neurilemmona (schwannoma) of the liver in a case of neurofibromatosis. *J Pathol* 1975; **117**: 151-153

11 **Fiel MI**, Schwartz M, Min AD, Sung MW, Thung SN. Malignant schwannoma of the liver in a patient without neurofibromatosis: a case report and review of the literature. *Arch Pathol Lab Med* 1996; **120**: 1145-1147

12 **Morikawa Y**, Ishihara Y, Matsuura N, Miyamoto H, Kakudo K. Malignant schwannoma of the liver. *Dig Dis Sci* 1995; **40**: 1279-1282

13 **Ohtomo K**, Araki T, Itai Y, Monzawa S, Ohba H, Nogata Y, Hihara T, Koizumi K, Uchiyama G. MR imaging of malignant mesenchymal tumors of the liver. *Gastrointest Radiol* 1992; **17**: 58-62

14 **Danhaive O**, Ninane J, Sokal E, Latinne D, Philippe M, Gosseye S, Clapuyt P, Otte JB. Hepatic localization of a fibrosarcoma in a child with a liver transplant. *J Pediatr* 1992; **120**: 434-437

15 **Soyer P**, Blanc F, Vissuzaine C, Marmuse JP, Menu Y. Primary leiomyosarcoma of the liver MR findings. *Clin Imaging* 1996; **20**: 273-275

16 **Ferrozzi F**, Bova D, Zangrandi A, Garlaschi G. Primary liver leiomyosarcoma: CT appearance. *Abdom Imaging* 1996; **21**: 157-160

17 **McLeod AJ**, Zornoza J, Shirkhoda A. Leiomyosarcoma: computed tomographic findings. *Radiology* 1984; **152**: 133-136

18 **Noon MA**, Young SW, Castellino RA. Leiomyosarcoma metastatic to the liver: CT appearance. *J Comput Assist Tomogr* 1980; **4**: 527-530

19 **Soyer P**, Bluemke DA, Riopel M, Hruban RH, Fishman EK. Hepatic leiomyosarcomas: CT features with pathologic correlation. *Eur J Radiol* 1995; **19**: 177-182

20 **Cholongitas E**, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006; **125**: 710-721

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Meetings

Events Calendar 2010

January 25-26
Tamilnadu, India
International Conference on Medical Negligence and Litigation in Medical Practice

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 26-27
Dubai, United Arab Emirates
2nd Middle East Gastroenterology Conference

January 28-30
Hong Kong, China
The 1st International Congress on Abdominal Obesity

February 11-13
Fort Lauderdale, FL, United States
21th Annual International Colorectal Disease Symposium

February 26-28
Carolina, United States
First Symposium of GI Oncology at The Caribbean

March 04-06
Bethesda, MD, United States
8th International Symposium on Targeted Anticancer Therapies

March 05-07
Peshawar, Pakistan
26th Pakistan Society of Gastroenterology & Endoscopy Meeting

March 09-12
Brussels, Belgium
30th International Symposium on Intensive Care and Emergency Medicine

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian National Association for Study of the Liver

March 23-26
Cairo, Egypt
14th Pan Arab Conference on Diabetes PACD14

March 25-28
Beijing, China
The 20th Conference of the Asian Pacific Association for the Study of the Liver

March 27-28
San Diego, California, United States
25th Annual New Treatments in Chronic Liver Disease

April 07-09
Dubai, United Arab Emirates
The 6th Emirates Gastroenterology and Hepatology Conference, EGHC 2010

April 14-17
Landover, Maryland, United States
12th World Congress of Endoscopic Surgery

April 14-18
Vienna, Austria
The International Liver Congress™ 2010

April 28-May 01
Dubrovnik, Croatia
3rd Central European Congress of surgery and the 5th Croatian Congress of Surgery

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual Meeting

May 06-08
Munich, Germany
The Power of Programming: International Conference on Developmental Origins of Health and Disease

May 15-19
Minneapolis, MN, United States
American Society of Colon and Rectal Surgeons Annual Meeting

June 04-06
Chicago, IL, United States
American Society of Clinical Oncologists Annual Meeting

June 09-12
Singapore, Singapore
13th International Conference on Emergency Medicine

June 14
Kosice, Slovakia
Gastro-intestinal Models in the Research of Probiotics and Prebiotics-Scientific Symposium

June 16-19
Hong Kong, China
ILTS: International Liver Transplantation Society ILTS Annual International Congress

June 20-23
Mannheim, Germany
16th World Congress for Bronchoesophagology-WCBE

June 25-29
Orlando, FL, United States
70th ADA Diabetes Scientific Sessions

August 28-31
Boston, Massachusetts, United States
10th OESO World Congress on Diseases of the Oesophagus 2010

September 10-12
Montreal, Canada
International Liver Association's Fourth Annual Conference

September 11-12
La Jolla, CA, United States
New Advances in Inflammatory Bowel Disease

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference on Antimicrobial Agents and Chemotherapy Annual Meeting

September 16-18
Prague, Czech Republic
Prague Hepatology Meeting 2010

September 23-26
Prague, Czech Republic
The 1st World Congress on Controversies in Gastroenterology & Liver Diseases

October 07-09
Belgrade, Serbia
The 7th Biannual International Symposium of Society of Coloproctology

October 15-20
San Antonio, TX, United States
ACG 2010: American College of Gastroenterology Annual Scienitfic Meeting

October 23-27
Barcelona, Spain
18th United European Gastroenterology Week

October 29-November 02
Boston, Massachusetts, United States
The Liver Meeting® 2010--AASLD's 61st Annual Meeting

November 13-14
San Francisco, CA, United States
Case-Based Approach to the Management of Inflammatory Bowel Disease

December 02-04
San Francisco, CA, United States
The Medical Management of HIV/AIDS

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Instructions to authors

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There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

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Key words

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Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use

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Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

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Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixudiarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315223018.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

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