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REVIEW

Portal hypertensive enteropathy

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Abstract

Portal hypertensive enteropathy (PHE) is a condition that describes the pathologic changes and mucosal abnormalities observed in the small intestine of patients with portal hypertension. This entity is being increasingly recognized and better understood over the past decade due to increased accessibility of the small intestine made possible by the introduction of video capsule endoscopy and deep enteroscopy. Though challenged by its diverse endoscopic appearance, multiple scoring systems have been proposed to classify the endoscopic presentation and grade its severity. Endoscopic findings can be broadly categorized into vascular and non-vascular lesions with many subtypes of both categories. Clinical manifestations of PHE can range from asymptomatic incidental findings to fatal gastrointestinal hemorrhage. Classic endoscopic findings in the setting of portal hypertension may lead to a prompt diagnosis. Occasionally histopathology and cross sectional imaging like computed tomography or magnetic resonance imaging may be helpful in establishing a diagnosis. Management of overt bleeding requires multidisciplinary approach involving hepatologists, endoscopists, surgeons, and interventional radiologists. Adequate resuscitation, reduction of portal pressure, and endoscopic therapeutic intervention remain the main principles of the initial treatment. This article reviews the existing evidence on PHE with emphasis on its classification, diagnosis, clinical manifestations, endoscopic appearance, pathological findings, and clinical management. A new schematic management of ectopic variceal bleed is also proposed.

Key words: Portal hypertension; Enteropathy; Intestinal vasculopathy; Ectopic varices; Gastrointestinal bleeding

Core tip: Portal hypertensive enteropathy (PHE) is an under recognized complication of portal hypertension. It can present with a broad spectrum of clinical manifestations and endoscopic findings, making its diagnosis challenging. Video capsule endoscopy and deep enteroscopy are diagnostic tools of choice. PHE should be considered in patients with portal hypertension who present with occult or overt gastrointestinal bleeding, especially when portal hypertensive gastropathy and advanced cirrhosis are also present. Adequate resuscitation, reduction of portal pressure, and endoscopic therapeutic intervention remain the mainstay of initial treatment though definitive management may require a multidisciplinary approach involving hepatologists, endoscopists, surgeons, and interventional radiologists.

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INTRODUCTION

Portal hypertension is defined as increased pressure in portal circulation, as estimated with the measurement of hepatic venous pressure gradient (HVPG)[1] which is the difference between wedge hepatic venous pressure (hepatic sinusoidal pressure) and free hepatic venous pressure. HVPG of more than 6 mmHg is considered abnormal[1,2]. Clinically significant portal hypertension occurs when HVPG is more than 10 mmHg and it is considered severe portal hypertension when HVPG is more than 12 mmHg[3,4]. Cirrhosis with consequent increased hepatic vascular resistance and portal venous inflow remains the most common etiology of portal hypertension, though it can also occasionally be seen due to other pre and post hepatic conditions such as congestive heart failure, Budd-Chiari syndrome, portal vein thrombosis, *etc.* The major clinical manifestations of portal hypertension were first recognized in the late nineteenth century and portal hypertension was described as a clinical syndrome of ascites, splenomegaly, and esophageal hemorrhage by Gilbert and Carnot in 1902[5]. Rupture and consequent hemorrhage from esophageal varices remains the most lethal complication of portal hypertension and therefore guidelines now call for periodic endoscopy for surveillance of these varices. Upper and lower endoscopy have led to recognition of other mucosal changes of gastrointestinal tract which previously had been less well recognized.

Microcirculatory changes of gastric mucosa, as a result of portal hypertension was first described as congestive gastropathy in 1985 by McCormack *et al*[6]. The characteristic findings of mosaic-pattern mucosa (irregular, cleft-bordered polygonal reticulated area, or snake skin-like pattern), small flat red-point lesions, elevated large cherry red spots, and black-brown spots have been variously termed as inflammatory gastritis, mucosal vasculopathy, portal hypertensive mucosa, and portal hypertensive gastropathy[6-10]. Subsequent studies suggested that these changes may be found in other areas of gastrointestinal tract as well[11-14]. The term, portal hypertensive colopathy, was used to describe similar abnormalities found in the colon of patients with portal hypertension. Endoscopic findings of portal hypertensive colopathy include diffuse erythematous and edematous mucosa, inflammatory (colitis-like) lesions, angiodysplasia-like lesions, and ectopic or anorectal varices[15-24].

Once the gastric and colonic mucosal changes associated with portal hypertension were recognized, jejunal and ileal mucosal changes were also noted. Occasional reports suggested small bowel bleeding in cirrhotic patients which was presumed to be a consequence of portal hypertension. The introduction of video capsule endoscopy and deep enteroscopy has increased our ability to evaluate the small bowel completely with good quality imaging and has led to better recognition and understanding of these portal hypertensive changes in the small bowel. This article reviews the existing evidence on portal hypertension-related changes in the small intestine, also known as portal hypertensive enteropathy (PHE) with emphasis on its classification, clinical manifestations, endoscopic appearance, and clinical management.

DEFINITION AND CLASSIFICATION

PHE previously termed portal hypertensive intestinal vasculopathy[25], includes pathologic changes and mucosal abnormalities observed in the small intestine of patients with portal hypertension[26]. Like other portal hypertensive related changes, PHE has also been reported in non-cirrhotic etiologies of portal hypertension[27]. The definition and diagnosis of PHE have evolved over the past decade due to increased accessibility of the small intestine. Introduction of video capsule endoscopy (VCE) and deep enteroscopy has shed more light into this area of the gastrointestinal tract and redefined the disease. Multiple scoring systems have been proposed to classify PHE and to grade its severity[28-30].

De Palma *et al*[29] were among the first groups to study small intestinal changes in cirrhotic patients with portal hypertension using VCE[29]. They found that portal hypertensive gastropathy and portal hypertensive colopathy are significantly associated with PHE and suggested these changes across different parts of the gastrointestinal tract could be the regional manifestations of the same process in portal hypertension, rather than distinct entities. Other parameters that were associated with PHE in their study were grade 2+ or larger esophageal varices and Child-Pugh class C cirrhosis. They classified endoscopic findings of PHE into two categories: mucosal inflammatory-like abnormalities (edema, erythema, granularity, friability) and vascular lesions (cherry red spots, telangiectasias, or angiodysplasia-like lesions, varices)[29].

Abdelaal *et al*[28] classified PHE lesions into 4 subtypes: inflammatory-like lesions, red spots, angioectasia, and small bowel varices. They created a scoring system based on VCE findings, giving each type one point with an additional point for multiple (more than two) lesions. Using this PHE score, they confirmed the findings by De Palma *et al*[29] by redemonstrating the association between PHE and

Table 1 Endoscopy based classification systems for portal hypertensive enteropathy

	De Palma *et al*[29]	Abdelaal *et al*[28]	Kodama *et al*[30]
Classification	Inflammatory lesions Vascular lesions	Inflammatory-like lesions Red spots Angioectasia Small bowel varices	Villous abnormalities Edema Atrophy Erythema Vascular lesions angiodysplasia-like lesions dilated/proliferated vessels varices
Scoring system	None	One point for each type of lesion. An additional point for > 2 lesions	One point for each type of lesion
Clinical significance	Associated with PHG, PHC, large esophageal varices and Child-Pugh class C cirrhosis	Associated with PHG, large esophageal varices, Child-Pugh class C cirrhosis, and a history of EVL	Associated with ascites

PHG: Portal hypertensive gastropathy; PHC: Portal hypertensive colopathy; EVL: Endoscopic variceal ligation.

portal hypertensive gastropathy, large esophageal varices, Child-Pugh class C cirrhosis, and a history of prior endoscopic variceal injection sclerotherapy or banding ligation in a non-randomized, case-controlled, prospective study[28].

Kodama *et al*[30] proposed a PHE scoring system based on double balloon enteroscopy findings. They classified PHE lesions into 2 categories: villous abnormalities and vascular lesions. They further subclassified each category into 3 subtypes: edema, atrophy and reddening for villous abnormalities, and angiodysplasia-like lesions, dilated/proliferated vessels, and varices for vascular lesions. A single point is given for each type, resulting in a scoring system with a maximum of 6 points. This scoring system, however, was associated with only the presence of ascites, making its clinical significance unclear[30].

In summary, PHE lesions can be described based on VCE findings or optical endoscopic findings. They can be categorized into subtypes of vascular and non-vascular lesions. Despite multiple proposed scoring systems as described in Table 1, presently there is insufficient data to standardize or validate these systems. Besides ectopic varices and bleeding lesions, the clinical significance of other subtypes of mucosal changes remains unclear.

EPIDEMIOLOGY

There is great heterogeneity among reported prevalence of PHE ranging from 15% up to 82% in cirrhotic patients[5,27,29,31]. We believe that this heterogeneity is a result of a wide spectrum of clinical severity of portal hypertension in cirrhotic patients, together with increase in reported prevalence due to recent advances in small bowel imaging in the past decade. The prevalence of PHE ranges only 15%-25% in studies where traditional endoscopic modalities like duodenoscopy, push enteroscopy or colonoscopy with ileal intubation were used[14,20] but is much higher (40%-82%) in other studies when VCE was used[5,26,29,32-35].

Prevalence of each type of endoscopic finding also varies among studies. Red spots (22.2%-62.2%) and angiodysplasia-like lesions (24.3%-55.7%) seem to be more common than inflammatory-like lesions (5.6%-13%) or varices (8.1%-38.9%). Mixed lesions, especially multiple vascular lesions (varice, angiodysplasia-like, and red spots) can be seen in up to 22.3%[26,29,36]. Small bowel varices account for 12%-35% of all ectopic varices[37,38]. Portal hypertensive polypoid enteropathy is a rarer manifestation of PHE and polypoid lesions are less common in the small intestine (0.3%) compared to polypoid gastropathy (0.6%)[39].

Actively bleeding lesions are not uncommon and can be seen in up to 17.8% of all patients with PHE, suggesting their clinical significance as a possible culprit source of obscure overt or obscure occult gastrointestinal bleeding. The bleeding lesions are commonly from angiodysplasia-like lesions and varices, though occasionally can be associated with polypoid enteropathy as well[26,29].

CLINICAL PRESENTATION

PHE should be suspected when there is gastrointestinal bleeding or anemia not otherwise explained by more common etiologies, along with signs of portal hypertension such as ascites, splenomegaly, thrombocytopenia, or hepatic venous pressure gradient more than 8 mmHg[39,40]. PHE can present as anemia, melena, hematochezia, hematemesis or may be asymptomatic. Fatal and life threatening ectopic variceal hemorrhage in small intestine has also been reported[26,38-41]. Small intestinal variceal rupture can present with a classic triad of hematochezia (without hematemesis), portal hypertension, and previous intra-abdominal surgery[38,42,43]. The most common indication for diagnostic work up is occult gastrointestinal bleeding.

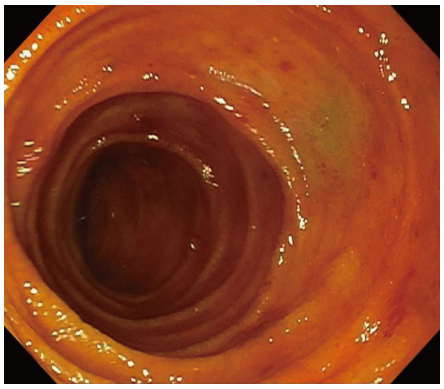

Figure 1 Mucosal red spots.

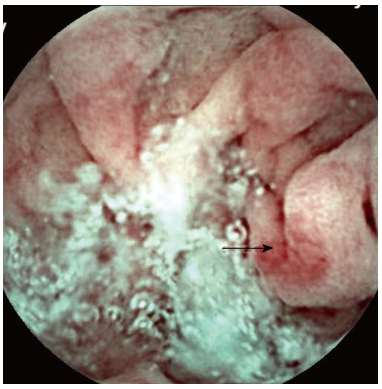

Figure 2 Angiodysplasia-like lesion.

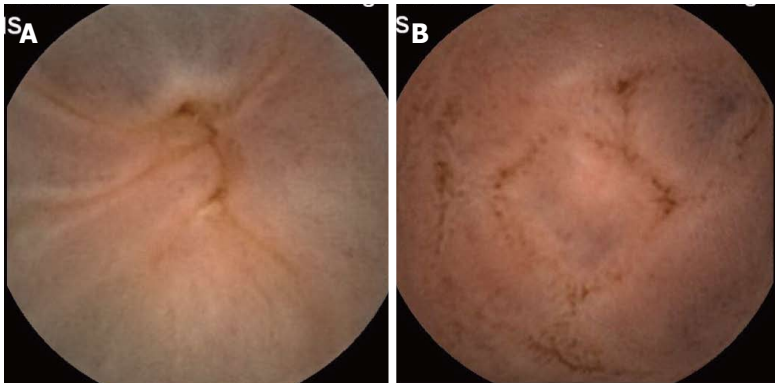

Figure 3 Ileal varices (A and B).

Certain clinical parameters have been shown to associate with PHE and can serve as clues to search for the disease. These parameters include large esophageal varices, history of endoscopic variceal injection sclerotherapy, history of endoscopic variceal ligation, portal hypertensive colopathy, portal hypertensive gastropathy, low hemoglobin, thrombocytopenia, splenomegaly, multiple signs of portal hypertension seen on CT scan, and Child-Pugh class C[26,28,29,36,44,45]. However, not all of these associations were seen in every study. Only advanced cirrhosis (Child-Pugh class C) and the presence of portal hypertensive gastropathy were consistently associated with PHE in most studies[28,29,36,44]. PHE has also been reported in non-cirrhotic etiologies of portal hypertension like polycystic liver disease, portal vein thrombosis, and Budd-Chiari syndrome[27,46-48]. PHE is usually diagnosed by a VCE or a deep enteroscopy[35].

ENDOSCOPIC FINDINGS

Endoscopically, PHE can be associated with a wide range of mucosal changes including the mucosal edema, congested rounded blunt villi giving a classic "herring-roe" appearance, loss of vascularization, friability, hyperrhemia, flat red spots, angiodysplasia-like lesions, pigmented black-brown spots, mucosal granularity, ulcers, reticulated mosaic-like pattern mucosa, protruding red bumps, inflammatory polyps, and varices[11,26,39,44,47,49,50]. Recognizing these findings through an optical endoscope or capsule endoscopy can lead to a prompt diagnosis and avoid further unnecessary and potentially harmful intervention. As mentioned above, these endoscopic findings can be classified into vascular and non-vascular lesions. They can be further categorized as inflammatory-like lesions, red spots, angioectasia, and small bowel varices[28]. Special attention should be paid to vascular lesions such as red spots, angioectasia-like lesions, and varices, which are more likely to cause clinically significant bleeding and are amenable to endoscopic intervention[28].

Red spots are small, symmetrical, uniformly erythematous, vascular areas on intestinal mucosa as shown in Figure 1. The lesions are usually flat. They are a very common manifestation of PHE and have been reported in up to 55% of cirrhotic patients with portal hypertension in one series[28].

Angioectasia are aberrant submucosal vascular lesions, characterized as small red patches with arborizing ectatic vessels as shown in Figure 2. Variceal lesions in the small bowel are described as tortuously enlarged veins that usually have serpiginous or nodular shape with overlying mosaic-like shining mucosa with bluish discoloration as shown in Figure 3A and B[26,27]. Endoscopic characters of these lesions should be promptly recognized to avoid potentially disastrous diagnostic biopsy attempts.

Duodenal varices are most commonly found in the duodenal bulb and the second portion of duo-

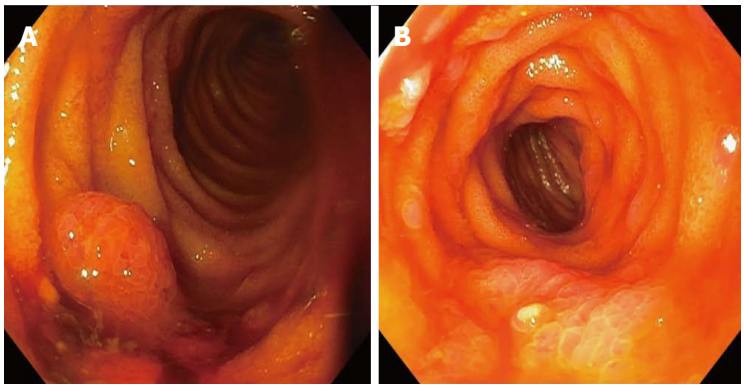

Figure 4 Portal hypertensive polypoid enteropathy (A and B).

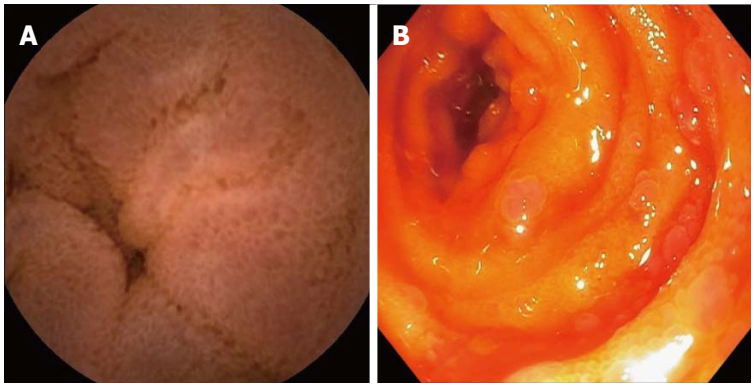

Figure 5 Herring roe appearance of small bowel mucosa (A and B).

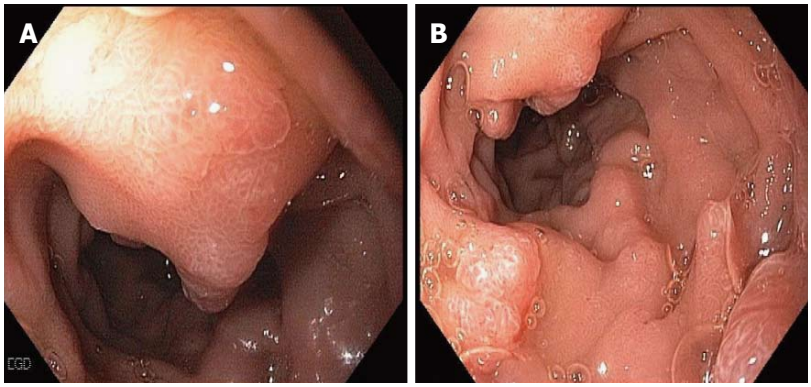

Figure 6 Mucosal edema with granularity of the small bowel (A and B).

denum[51-53]. Jejunal and ileal varices are more commonly seen in patients with history of abdominal surgery due to post surgical portosystemic collaterals related to bowel anastomosis and are more difficult to diagnose due to their deep location in the intestinal tract[38,42,43]. Small intestinal varices usually originate from portal venous trunk or superior mesenteric vein[48,54,55]. Polypoid enteropathy is a rare manifestation of PHE and can present in any part of the small intestine. Polypoid lesions can have varied endoscopic manifestations. They can be single or multiple, sessile or pedunculated, small or large and may occasionally mimic adenomatous polyps as shown in Figure 4A and B. They usually arise in the background of inflamed mucosa with classic mosaic or herring-roe pattern and have been associated with occult or overt gastrointestinal bleeding[39,49,50,56].

Other inflammatory lesions include mucosal edema, granularity, patchy erythematous mucosa, and herring roe appearance (rounded blunted villi on the background of congested mucosa with granularity) as shown in Figures 5 and 6. Clinical significance of these lesions is unclear but it is believed that they are less likely to cause overt gastrointestinal bleeding[26,28,44].

Some of these endoscopic findings are non-specific and can pose a diagnostic dilemma especially in a patient whose portal hypertensive status is unknown. Differential diagnosis includes inflammatory bowel disease, celiac disease, arteriovenous malformations, and familial adenomatous polyposis. Biopsy of non-vascular lesions can be performed with caution to confirm the diagnosis.

In portal hypertension when splanchnic blood flow cannot effectively return to the systemic circulation, consequently splanchnic vasodilation ensues and mucosa of small intestine becomes congested.

Therefore, histopathologic changes of PHE show evidence of congested mucosa and vascular ectasia. These histologic findings include capillary dilation in the lamina propria (mean vascular diameter of 380 micrometers), increased capillary wall thickness, fibromuscular proliferation, a decreased villous/crypt ratio, neovascularization, vascular ectasia, vessels containing fibrin thrombi, inflammatory lymphoplasmatocytic cells infiltration in the lamina propria, reactional nucleocytoplasmic atypia in the epithelial cells, and crenulated aspect of the glands[13,14,39,57-59]. However, these histopathological changes are non-specific and can be seen in patients without portal hypertension and in patients with normal endoscopic findings[12-14]. Therefore, the diagnosis of PHE should not be made on histopathology alone, but rather in conjunction with other clinical and endoscopic characteristics.

OTHER DIAGNOSTIC TESTS

Jeon *et al*[26] has evaluated the use of computerized tomography (CT) scan findings of portal hypertension such as esophageal varices, gastric varices, peri umbilical varices, portal hypertensive colopathy, portal hypertensive gastropathy, portal hypertensive cholecystopathy, splenomegaly, and ascites as the radiologic predictors of PHE. They have created a scoring system giving each CT finding one point with a maximum score of 6 points. A CT score of more than 3 was found to be significantly associated with PHE[26]. Cross sectional imaging by computed tomography angiography and magnetic resonance angiography can also aid in evaluation of vascular origin of the ectopic varices[55,60].

Abdelaal *et al*[28] have explored the use of a transient elastrography (FibroScan®), a novel non-invasive ultrasound-based technology using pulse-echo ultrasound signals to measure liver stiffness as a surrogate marker of severity of portal hypertension in cirrhotic patients[4,61]. They found that a high transient elastography score had a linear relation with a high PHE score (r^2 = 0.314, *P*-value 0.004), suggesting that higher severity of PHE may be associated with higher degree of liver disease and portal hypertension. Mean liver stiffness measurement in PHE group was 29 kPa, which is much higher than the portal hypertensive cut-off value of 13.6 kPa[4,28]. They concluded that transient elastography may be a new non-invasive method for detecting the presence and severity of PHE in cirrhotic patients[28].

MANAGEMENT

VCE is the preferred initial diagnostic modality in evaluating the small bowel due to its non-invasive nature[5,27,33,35]. It also serves as a road map for subsequent interventions. A deep enteroscopy is warranted when a therapeutically amenable lesion is found on VCE in patients with obscure gastrointestinal bleeding[27]. Different endoscopic findings may require different therapeutic interventions.

Due to its rarity and insufficient evidence, there are no standardized therapeutic guidelines for symptomatic PHE[40]. Clinical significance and the need for any intervention for inflammatory lesions and red spots remain unclear. Argon plasma coagulation is generally used for angioectasia[27] while multiple approaches have been used for small bowel varices and polypoid enteropathy[39,55,56,62,63].

Treatment of portal hypertensive polypoid enteropathy depends on number of polyps and endoscopic accessibility. A polypectomy can be safely performed if the polyp is accessible and amenable for endoscopic removal. Endoclip can be used at the stalk to achieve complete hemostasis[56]. Argon plasma coagulation can be used on the inflamed surface of bleeding polyp to achieve hemostasis but recurrent bleeding has been reported[39]. Non-selective beta blocker, transjugular intrahepatic portosystemic shunt (TIPS), surgical small bowel resection, and liver transplantation have all been reported anecdotally to be successful treatment for portal hypertensive polypoid enteropathy[39,56].

Bleeding small bowel varices occurs in 0.4% of patients with portal hypertension and account for up to 5% of all variceal bleeding. Similar to esophagogastric variceal hemorrhage, it usually presents as massive life-threatening hemorrhage with mortality rate as high as 40%[38,40,51,54]. Available therapeutic options are endoscopic treatment, surgical interventions, and interventional radiological approaches. Similar to polypoidal lesions, management of small bowel varices depends largely on endoscopic accessibility, patient's surgical risk, available therapy and local expertise.

Adequate resuscitation with intravenous fluids, blood product transfusion, close monitoring, and airway protection remain the main principles of initial management. The benefit of medical management has not been extensively studied in ectopic varices or other manifestations of PHE, but given its established role in esophageal and gastric variceal management[64], it is reasonable to consider the use of vasoactive agents such as octreotide and non-selective beta blockers to reduce the splanchnic and portal pressure in both primary and secondary prophylaxis of ectopic varices[40].

Despite the technical challenges, endoscopic band ligation and endoscopic variceal obturation with tissue glue monomer such as N-butyl-2-cyanoacrylate are endoscopic interventions of choice for hemostasis in ectopic variceal rupture. However, the achieved hemostasis is temporary and re-bleeding is a major concern[52,53]. Endoscopic variceal obturation has shown better success and lower re-bleeding rate compared to endoscopic variceal banding[38,65-68]. Endoscopic sclerotherapy and band

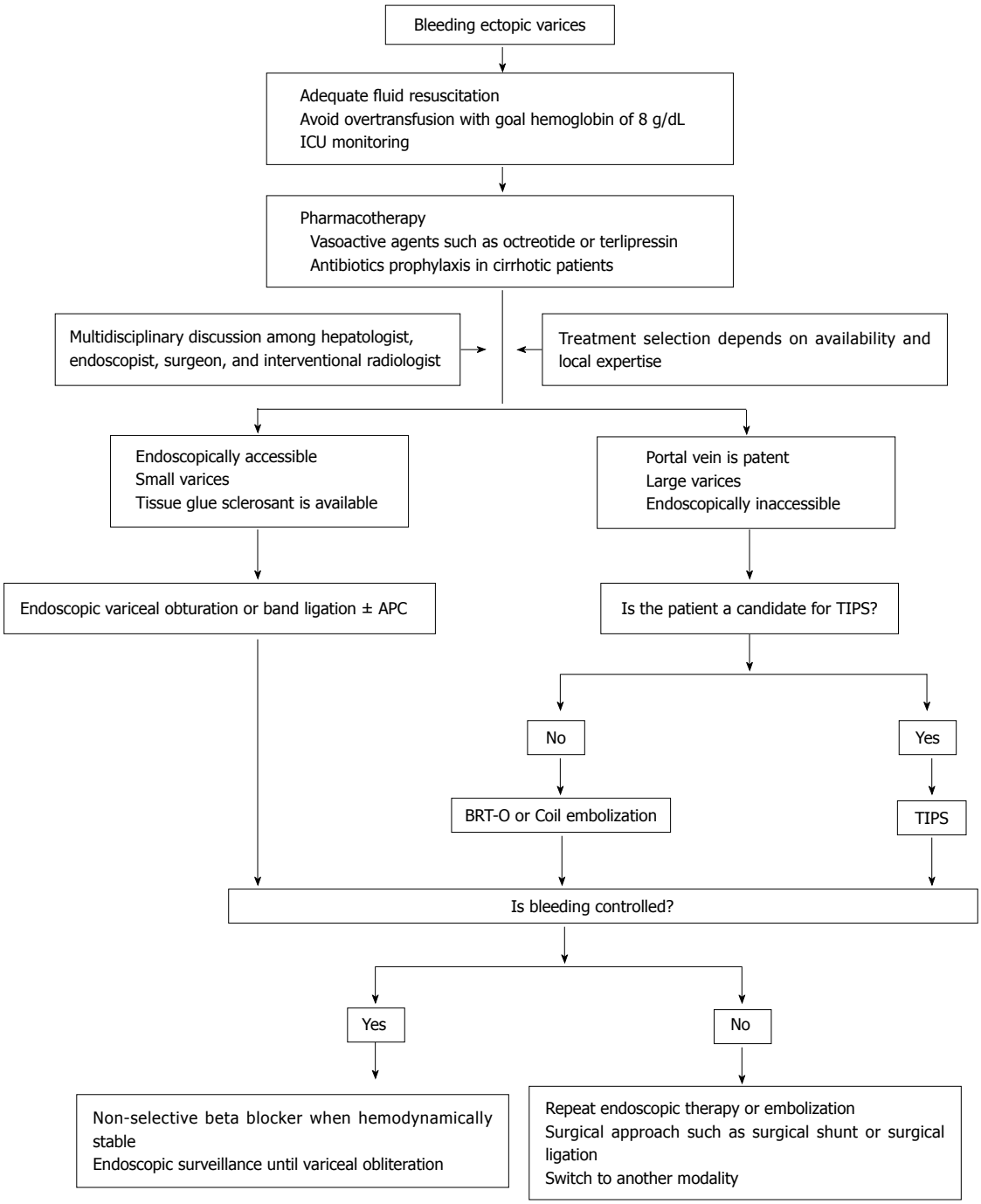

Figure 7 Management of bleeding ectopic varices. TIPS: Transjugular intrahepatic portosystemic shunt; APC: Argon plasma coagulation; BRTO: Balloon-occluded retrograde transvenous obliteration.

ligation are not recommended for large varices, especially the varices with larger diameter than the endoscope itself. This is because incomplete banding can lead to mucosal defect in the remaining varix causing recurrent hemorrhage while excessive dilution of injected sclerosant in large varices decreases the success rate of the sclerotherapy[40,63]. Limited evidence suggests supplemental use of argon plasma coagulation after variceal band ligation as a successful intervention in prevention of rebleeding in esophageal and ileal varices and can be considered in endoscopic management of ectopic varices as well[69-71].

Interventional radiology approaches are effective modalities especially for large varices, lesions that are not endoscopically accessible, and for patients with poor overall general condition who are poor surgical candidates[38,63,72]. These interventions include TIPS, balloon-occluded retrograde transvenous obliteration (B-RTO), and percutaneous coil embolization[38,40,55,62].

Even though TIPS is relatively safe procedure,

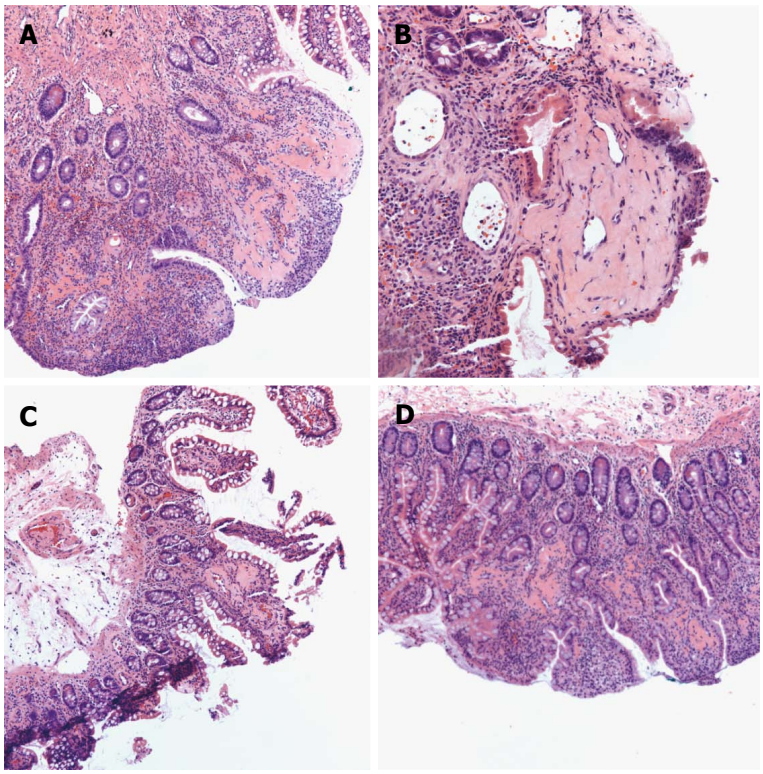

Figure 8 Histopathological changes of portal hypertensive enteropathy (A-D).

widely available, and with good success rate, it has limitations in patients with hepatic encephalopathy, high MELD score, severely decompensated cirrhosis, and severe hepatic atrophy[73,74]. It, however, remains an effective intervention to control the variceal bleeding and prevent recurrence, especially in those who have failed endoscopic and medical management. The American Association for the Study of Liver Diseases (AASLD) has recommended TIPS as the preferred approach for the prevention of rebleeding of ectopic varices (including intestinal, stomal, and anorectal varices)[75].

Percutaneous transhepatic and transjugular coil embolization are other radiological interventions that may offer a focal occlusion of the feeding vein of the varices. They can be safely performed even in a large varix, and have good short term results. However, as the portal system is not decompressed, they carry a very high recurrent bleeding rate[76-81]. A retrospective study by Macedo *et al*[76] showed that even though an immediate bleeding control can be achieved in 75% of patients receiving coil embolization, the rebleeding rate was 67% with a mean bleeding-free interval of 7.8 mo. This result was similar to previous studies that demonstrated a rebleeding rate as high as 55% at 6 mo and 92% at 4 years[76,82]. A combined approach of TIPS and percutaneous embolization in the setting of persistent bleeding after TIPS is preferred[76].

B-RTO is a non-surgical therapy that can occlude not only the varices, but also the feeding afferent and efferent vessel. It is particularly useful in patients who bleed at lower portal pressure, have non-patent portal vein, or are not candidates for TIPS. The success of B-RTO in treating small bowel ectopic varices has been reported, especially in the Japanese literature[83-86]. However, B-RTO can cause significant elevation of porto-systemic pressure gradient and subsequent variceal formation has also been reported[55,87].

Surgical approaches, such as portosystemic surgical shunt, segmental small bowel resection and surgical variceal ligation, can be performed but are usually reserved for patients who are refractory to other therapy[38,41,88,89]. Surgical shunt such as distal splenorenal shunt has equal efficacy as TIPS with no difference in survival or hepatic encephalopathy rate but is less cost-effective approach compared to TIPS[75]. Other surgical approaches such as duodenotomy with simple oversewing of the varix and duodenal dearterialization with stapling have been reported[90,91].

Similar to what Helmy *et al*[40] have proposed, management of bleeding ectopic varices should focus on adequate initial resuscitation and use a multimodality approach depending on availability and local expertise of each institution. We suggest an algorithm for management of bleeding ectopic varices in Figure 7. As mentioned above, we also recommend individually tailored therapies for non variceal bleeding depending on the clinical situation and local expertise.

CONCLUSION

PHE-associated small bowel mucosal changes are

increasingly being recognized due to introduction of VCE and deep enteroscopy of VCE and deep enteroscopy which enables a more thorough small bowel evaluation than previously possible. Endoscopically, PHE can have myriad presentations ranging from mild mucosal inflammatory changes to angioectasias, inflammatory appearing polyps, and occasionally as large ectopic varices. Due to the non-specific endoscopic and histopathological findings, a high index of suspicion is required to recognize and accurately diagnose this condition. VCE and/or a deep enteroscopy are the current preferred modalities for establishing the diagnosis. PHE has been known to cause significant life-threatening overt gastrointestinal bleeding or be a source of occult gastrointestinal blood loss. However, due to low prevalence and lack of large studies, its prognostic value and clinical significance on morbidity and mortality remain unclear. Management of PHE has not yet been standardized and should be individualized based on acuity and severity of the hemorrhage, endoscopic accessibility of the lesion, surgical risk of the patient, patency of portal vein, available therapy and expertise of each institution.

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REFERENCES

1 **Burroughs AK**, Groszmann R, Bosch J, Grace N, Garcia-Tsao G, Patch D, Garcia-Pagan JC, Dagher L. Assessment of therapeutic benefit of antiviral therapy in chronic hepatitis C: is hepatic venous pressure gradient a better end point? *Gut* 2002; **50**: 425-427 [PMID: 11839726 DOI: 10.1136/gut.50.3.425]

2 **Sanyal AJ**, Bosch J, Blei A, Arroyo V. Portal hypertension and its complications. *Gastroenterology* 2008; **134**: 1715-1728 [PMID: 18471549 DOI: 10.1053/j.gastro.2008.03.007]

3 **Ripoll C**, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS, Bosch J. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; **133**: 481-488 [PMID: 17681169 DOI: 10.1053/j.gastro.2007.05.024]

4 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971 DOI: 10.1002/hep.21665]

5 **Goulas S**, Triantafyllidou K, Karagiannis S, Nicolaou P, Galanis P, Vafiadis I, Tzivras M, Mavrogiannis C. Capsule endoscopy in the investigation of patients with portal hypertension and anemia. *Can J Gastroenterol* 2008; **22**: 469-474 [PMID: 18478132]

6 **McCormack TT**, Sims J, Eyre-Brook I, Kennedy H, Goepel J, Johnson AG, Triger DR. Gastric lesions in portal hypertension: inflammatory gastritis or congestive gastropathy? *Gut* 1985; **26**: 1226-1232 [PMID: 3877665 DOI: 10.1136/gut.26.11.1226]

7 **Tarnawski AS**, Sarfeh IJ, Stachura J, Hajduczek A, Bui HX, Dabros W, Gergely H. Microvascular abnormalities of the portal hypertensive gastric mucosa. *Hepatology* 1988; **8**: 1488-1494 [PMID: 3192161 DOI: 10.1002/hep.1840080604]

8 **Sarfeh IJ**, Tarnawski A. Gastric mucosal vasculopathy in portal hypertension. *Gastroenterology* 1987; **93**: 1129-1131 [PMID: 3498660]

9 **Carpinelli L**, Primignani M, Preatoni P, Angeli P, Battaglia G, Beretta L, Bortoli A, Capria A, Cestari R, Cosentino F, Crotta S, Gerunda G, Lorenzini I, Maiolo P, Merighi A, Rossi A, Sangiovanni A, de Franchis R. Portal hypertensive gastropathy: reproducibility of a classification, prevalence of elementary lesions, sensitivity and specificity in the diagnosis of cirrhosis of the liver. A NIEC multicentre study. New Italian Endoscopic Club. *Ital J Gastroenterol Hepatol* 1997; **29**: 533-540 [PMID: 9513828]

10 **Primignani M**, Carpinelli L, Preatoni P, Battaglia G, Carta A, Prada A, Cestari R, Angeli P, Gatta A, Rossi A, Spinzi G, De Franchis R. Natural history of portal hypertensive gastropathy in patients with liver cirrhosis. The New Italian Endoscopic Club for the study and treatment of esophageal varices (NIEC). *Gastroenterology* 2000; **119**: 181-187 [PMID: 10889167 DOI: 10.1053/gast.2000.8555]

11 **Thiruvengadam R**, Gostout CJ. Congestive gastroenteropathy--an extension of nonvariceal upper gastrointestinal bleeding in portal hypertension. *Gastrointest Endosc* 1989; **35**: 504-507 [PMID: 2599293 DOI: 10.1016/S0016-5107(89)72898-4]

12 **Nagral AS**, Joshi AS, Bhatia SJ, Abraham P, Mistry FP, Vora IM. Congestive jejunopathy in portal hypertension. *Gut* 1993; **34**: 694-697 [PMID: 8504973 DOI: 10.1136/gut.34.5.694]

13 **Misra V**, Misra SP, Dwivedi M, Gupta SC. Histomorphometric study of portal hypertensive enteropathy. *Am J Clin Pathol* 1997; **108**: 652-657 [PMID: 9384446]

14 **Desai N**, Desai D, Pethe V, Deodhar KP, Sawant P, Nanivadekar S. Portal hypertensive jejunopathy: a case control study. *Indian J Gastroenterol* 2004; **23**: 99-101 [PMID: 15250567]

15 **Kozarek RA**, Botoman VA, Bredfeldt JE, Roach JM, Patterson DJ, Ball TJ. Portal colopathy: prospective study of colonoscopy in patients with portal hypertension. *Gastroenterology* 1991; **101**: 1192-1197 [PMID: 1936789]

16 **Hosking SW**, Smart HL, Johnson AG, Triger DR. Anorectal varices, haemorrhoids, and portal hypertension. *Lancet* 1989; **1**: 349-352 [PMID: 2563507 DOI: 10.1016/S0140-6736(89)91724-8]

17 **Ganguly S**, Sarin SK, Bhatia V, Lahoti D. The prevalence and spectrum of colonic lesions in patients with cirrhotic and noncirrhotic portal hypertension. *Hepatology* 1995; **21**: 1226-1231 [PMID: 7737627]

18 **Misra SP**, Dwivedi M, Misra V. Prevalence and factors influencing hemorrhoids, anorectal varices, and colopathy in patients with portal hypertension. *Endoscopy* 1996; **28**: 340-345 [PMID: 8813499 DOI: 10.1055/s-2007-1005477]

19 **Misra SP**, Misra V, Dwivedi M. Effect of esophageal variceal sclerotherapy on hemorrhoids, anorectal varices and portal colopathy. *Endoscopy* 1999; **31**: 741-744 [PMID: 10604617 DOI: 10.1055/s-1999-77]

20 **Misra SP**, Dwivedi M, Misra V, Gupta M. Ileal varices and portal hypertensive ileopathy in patients with cirrhosis and portal hypertension. *Gastrointest Endosc* 2004; **60**: 778-783 [PMID: 15557954 DOI: 10.1016/S0016-5107(04)02049-8]

21 **Ito K**, Shiraki K, Sakai T, Yoshimura H, Nakano T. Portal hypertensive colopathy in patients with liver cirrhosis. *World J Gastroenterol* 2005; **11**: 3127-3130 [PMID: 15918202]

22 **Ghoshal UC**, Biswas PK, Roy G, Pal BB, Dhar K, Banerjee PK. Colonic mucosal changes in portal hypertension. *Trop Gastroenterol* 2001; **22**: 25-27 [PMID: 11398241]

23 **Eleftheriadis E**, Kotzampassi K, Karkavelas G, Tzioufa V, Papadimitriou K, Aletras H. Portal Hypertensive Colopathy-Endoscopic, Hernodynamic and Morphometric Study. *Digestive Endoscopy* 1993; **5**: 224-230 [DOI: 10.1111/j.1443-1661.1993.tb00622.x]

24 **Tam TN**, NG WW, Lee SD. Colonic mucosal changes in patients with liver cirrhosis. *Gastrointest Endosc* 1995; **42**: 408-412 [PMID: 8566628 DOI: 10.1016/S0016-5107(95)70040-4]

25 **Viggiano TR**, Gostout CJ. Portal hypertensive intestinal vasculopathy:

a review of the clinical, endoscopic, and histopathologic features. *Am J Gastroenterol* 1992; **87**: 944-954 [PMID: 1642217]
26 **Jeon SR**, Kim JO, Kim JB, Ye BD, Chang DK, Shim KN, Cheung DY, Kim JS, Choi MG, Song HJ, Lim YJ, Park SJ, Kim JH, Moon JS, Jeen YT. Portal hypertensive enteropathy diagnosed by capsule endoscopy in cirrhotic patients: a nationwide multicenter study. *Dig Dis Sci* 2014; **59**: 1036-1041 [PMID: 24493093 DOI: 10.1007/s10620-014-3036-3]
27 **Figueiredo P**, Almeida N, Lérias C, Lopes S, Gouveia H, Leitão MC, Freitas D. Effect of portal hypertension in the small bowel: an endoscopic approach. *Dig Dis Sci* 2008; **53**: 2144-2150 [PMID: 18026837 DOI: 10.1007/s10620-007-0111-z]
28 **Abdelaal UM**, Morita E, Nouda S, Kuramoto T, Miyaji K, Fukui H, Tsuda Y, Fukuda A, Murano M, Tokioka S, Arafa UA, Kassem AM, Umegaki E, Higuchi K. Evaluation of portal hypertensive enteropathy by scoring with capsule endoscopy: is transient elastography of clinical impact? *J Clin Biochem Nutr* 2010; **47**: 37-44 [PMID: 20664729 DOI: 10.3164/jcbn.10-14]
29 **De Palma GD**, Rega M, Masone S, Persico F, Siciliano S, Patrone F, Matantuono L, Persico G. Mucosal abnormalities of the small bowel in patients with cirrhosis and portal hypertension: a capsule endoscopy study. *Gastrointest Endosc* 2005; **62**: 529-534 [PMID: 16185966 DOI: 10.1016/S0016-5107(05)01588-9]
30 **Kodama M**, Uto H, Numata M, Hori T, Murayama T, Sasaki F, Tsubouchi N, Ido A, Shimoda K, Tsubouchi H. Endoscopic characterization of the small bowel in patients with portal hypertension evaluated by double balloon endoscopy. *J Gastroenterol* 2008; **43**: 589-596 [PMID: 18709480 DOI: 10.1007/s00535-008-2198-1]
31 **Akyuz F**, Pinarbasi B, Ermis F, Uyanikoglu A, Demir K, Ozdil S, Besisik F, Kaymakoglu S, Boztas G, Mungan Z. Is portal hypertensive enteropathy an important additional cause of blood loss in portal hypertensive patients? *Scand J Gastroenterol* 2010; **45**: 1497-1502 [PMID: 20695721 DOI: 10.3109/00365521.2010.510568]
32 **Leighton JA**, Triester SL, Sharma VK. Capsule endoscopy: a meta-analysis for use with obscure gastrointestinal bleeding and Crohn's disease. *Gastrointest Endosc Clin N Am* 2006; **16**: 229-250 [PMID: 16644453 DOI: 10.1016/j.giec.2006.03.004]
33 **Triester SL**, Leighton JA, Leontiadis GI, Fleischer DE, Hara AK, Heigh RI, Shiff AD, Sharma VK. A meta-analysis of the yield of capsule endoscopy compared to other diagnostic modalities in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2005; **100**: 2407-2418 [PMID: 16279893 DOI: 10.1111/j.1572-0241.2005.00274.x]
34 **Tang SJ**, Zanati S, Dubcenco E, Cirocco M, Christodoulou D, Kandel G, Haber GB, Kortan P, Marcon NE. Diagnosis of small-bowel varices by capsule endoscopy. *Gastrointest Endosc* 2004; **60**: 129-135 [PMID: 15229446 DOI: 10.1016/S0016-5107(04)01458-0]
35 **Rondonotti E**, Villa F, Dell' Era A, Tontini GE, de Franchis R. Capsule endoscopy in portal hypertension. *Clin Liver Dis* 2010; **14**: 209-220 [PMID: 20682230 DOI: 10.1016/j.cld.2010.03.004]
36 **Rondonotti E**, Villa F, Signorelli C, de Franchis R. Portal hypertensive enteropathy. *Gastrointest Endosc Clin N Am* 2006; **16**: 277-286 [PMID: 16644456 DOI: 10.1016/j.giec.2006.01.019]
37 **Lebrec D**, Benhamou JP. Ectopic varices in portal hypertension. *Clin Gastroenterol* 1985; **14**: 105-121 [PMID: 3872747]
38 **Sato T**, Akaike J, Toyota J, Karino Y, Ohmura T. Clinico-pathological features and treatment of ectopic varices with portal hypertension. *Int J Hepatol* 2011; **2011**: 960720 [PMID: 21994879 DOI: 10.4061/2011/960720]
39 **Lemmers A**, Evrard S, Demetter P, Verset G, Gossum AV, Adler M, Devière J, Moine OL. Gastrointestinal polypoid lesions: a poorly known endoscopic feature of portal hypertension. *United European Gastroenterol J* 2014; **2**: 189-196 [PMID: 25360302 DOI: 10.1177/2050640614529108]
40 **Helmy A**, Al Kahtani K, Al Fadda M. Updates in the pathogenesis, diagnosis and management of ectopic varices. *Hepatol Int* 2008; **2**: 322-334 [PMID: 19669261 DOI: 10.1007/s12072-008-9074-1]
41 **Kastanakis M**, Anyfantakis D, Katsougris N, Bobolakis E. Massive gastrointestinal bleeding due to isolated jejunal varices in a patient without portal hypertension. *Int J Surg Case Rep* 2013; **4**: 439-441 [PMID: 23528981 DOI: 10.1016/j.ijscr.2013.01.029]
42 **Cappell MS**, Price JB. Characterization of the syndrome of small and large intestinal variceal bleeding. *Dig Dis Sci* 1987; **32**: 422-427 [PMID: 3549204 DOI: 10.1007/BF01296297]
43 **Yuki N**, Kubo M, Noro Y, Kasahara A, Hayashi N, Fusamoto H, Ito T, Kamada T. Jejunal varices as a cause of massive gastrointestinal bleeding. *Am J Gastroenterol* 1992; **87**: 514-517 [PMID: 1553940]
44 **Higaki N**, Matsui H, Imaoka H, Ikeda Y, Murakami H, Hiasa Y, Matsuura B, Onji M. Characteristic endoscopic features of portal hypertensive enteropathy. *J Gastroenterol* 2008; **43**: 327-331 [PMID: 18592149 DOI: 10.1007/s00535-008-2166-9]
45 **Repici A,** Pennazio M, Ottobrelli A, Barbon V, De Angelis C, De Lio A. Endoscopic capsule in cirrhotic patients with portal hypertension: spectrum and prevalence of small bowel lesions. *Endoscopy* 2005; **37** (Suppl 1): A72
46 **Gentilucci UV**, Gallo P, Perrone G, Del Vescovo R, Galati G, Spataro S, Mazzarelli C, Pellicelli A, Afeltra A, Picardi A. Non-cirrhotic portal hypertension with large regenerative nodules: a diagnostic challenge. *World J Gastroenterol* 2011; **17**: 2580-2584 [PMID: 21633664 DOI: 10.3748/wjg.v17.i20.2580]
47 **Tang SJ**, Jensen DM, Gralnek IM, Roth BE. Portal hypertensive enteropathy in a patient with polycystic liver disease: a unique endoscopic finding. *Gastrointest Endosc* 2002; **56**: 924-926 [PMID: 12447315 DOI: 10.1016/S0016-5107(02)70377-5]
48 **Amin R**, Alexis R, Korzis J. Fatal ruptured duodenal varix: a case report and review of literature. *Am J Gastroenterol* 1985; **80**: 13-18 [PMID: 3871304]
49 **Sawada K**, Ohtake T, Ueno N, Ishikawa C, Abe M, Miyoshi S, Suzuki Y, Tokusashi Y, Fujiya M, Kohgo Y. Multiple portal hypertensive polyps of the jejunum accompanied by anemia of unknown origin. *Gastrointest Endosc* 2011; **73**: 179-182 [PMID: 20869709 DOI: 10.1016/j.gie.2010.07.011]
50 **Oluyemi A**, Amole A. Portal hypertensive duodenopathy manifesting as "kissing" duodenal ulcers in a nigerian with alcoholic cirrhosis: a case report and brief review of the literature. *Case Rep Med* 2012; **2012**: 618729 [PMID: 23118766 DOI: 10.1155/2012/618729]
51 **Jonnalagadda SS**, Quiason S, Smith OJ. Successful therapy of bleeding duodenal varices by TIPS after failure of sclerotherapy. *Am J Gastroenterol* 1998; **93**: 272-274 [PMID: 9468260 DOI: 10.1111/j.1572-0241.1998.270_3.x]
52 **Shiraishi M**, Hiroyasu S, Higa T, Oshiro S, Muto Y. Successful management of ruptured duodenal varices by means of endoscopic variceal ligation: report of a case. *Gastrointest Endosc* 1999; **49**: 255-257 [PMID: 9925710 DOI: 10.1016/S0016-5107(99)70498-0]
53 **Yoshida Y**, Imai Y, Nishikawa M, Nakatukasa M, Kurokawa M, Shibata K, Shimomukai H, Shimano T, Tokunaga K, Yonezawa T. Successful endoscopic injection sclerotherapy with N-butyl-2-cyanoacrylate following the recurrence of bleeding soon after endoscopic ligation for ruptured duodenal varices. *Am J Gastroenterol* 1997; **92**: 1227-1229 [PMID: 9219810]
54 **Khouqeer F**, Morrow C, Jordan P. Duodenal varices as a cause of massive upper gastrointestinal bleeding. *Surgery* 1987; **102**: 548-552 [PMID: 3498234]
55 **Koo SM**, Jeong SW, Jang JY, Lee TH, Jeon SR, Kim HG, Kim JO, Kim YJ. Jejunal variceal bleeding successfully treated with percutaneous coil embolization. *J Korean Med Sci* 2012; **27**: 321-324 [PMID: 22379346 DOI: 10.3346/jkms.2012.27.3.321]
56 **Zeitoun JD**, Chryssostalis A, Terris B, Prat F, Gaudric M, Chaussade S. Portal hypertensive duodenal polyp: a case report. *World J Gastroenterol* 2007; **13**: 1451-1452 [PMID: 17457981 DOI: 10.3748/wjg.v13.i9.1451]
57 **El-Khayat HR**, El Khattib A, Nosseir M, Ezz MA, El-Amin H, Fouad YM. Portal hypertensive enteropathy before and after variceal obliteration: an endoscopic, histopathologic and immunohistochemical study. *J Gastrointestin Liver Dis* 2010; **19**: 175-179 [PMID: 20593051]
58 **Tang S.** Portal Hypertensive Gastropathy, Enteropathy, and Colopathy. *VJGIEN* 2013; **1**: 269-271 [DOI: 10.1016/S2212-0971(13)70117-1]

59 **Shudo R**, Yazaki Y, Sakurai S, Uenishi H, Yamada H, Sugawara K. Duodenal erosions, a common and distinctive feature of portal hypertensive duodenopathy. *Am J Gastroenterol* 2002; **97**: 867-873 [PMID: 12003421 DOI: 10.1111/j.1572-0241.2002.05602.x]

60 **Lim LG**, Lee YM, Tan L, Chang S, Lim SG. Percutaneous paraumbilical embolization as an unconventional and successful treatment for bleeding jejunal varices. *World J Gastroenterol* 2009; **15**: 3823-3826 [PMID: 19673028 DOI: 10.3748/wjg.15.3823]

61 **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 [PMID: 14698338 DOI: 10.1016/j.ultrasmedbio.2003.07.001]

62 **Haskal ZJ**, Scott M, Rubin RA, Cope C. Intestinal varices: treatment with the transjugular intrahepatic portosystemic shunt. *Radiology* 1994; **191**: 183-187 [PMID: 8134568 DOI: 10.1148/radiology.191.1.8134568]

63 **Norton ID**, Andrews JC, Kamath PS. Management of ectopic varices. *Hepatology* 1998; **28**: 1154-1158 [PMID: 9755256 DOI: 10.1002/hep.510280434]

64 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]

65 **Brechmann T**, Schmiegel W, Nicolas V, Reiser M. Gastrointestinal bleeding 30 years after a complicated cholecystectomy. *World J Gastroenterol* 2010; **16**: 4747-4750 [PMID: 20872978 DOI: 10.3748/wjg.v16.i37.4747]

66 **Albert J.** Endoscopic Therapy of Variceal Bleeding from the Small Bowel. Video *VJGIEN* 2013; **1:** 226-227 [DOI: 10.1016/S2212-0971(13)70096-7]

67 **Gertsch P**, Blumgart LH. Cure of a bleeding duodenal varix by sclerotherapy. *Br J Surg* 1988; **75**: 717 [PMID: 3262004 DOI: 10.1002/bjs.1800750731]

68 **Barbish AW**, Ehrinpreis MN. Successful endoscopic injection sclerotherapy of a bleeding duodenal varix. *Am J Gastroenterol* 1993; **88**: 90-92 [PMID: 8420280]

69 **Cipolletta L**, Bianco MA, Rotondano G, Piscopo R, Prisco A, Garofano ML. Prospective comparison of argon plasma coagulator and heater probe in the endoscopic treatment of major peptic ulcer bleeding. *Gastrointest Endosc* 1998; **48**: 191-195 [PMID: 9717787 DOI: 10.1016/S0016-5107(98)70163-4]

70 **Furukawa K**, Aoyagi Y, Harada T, Enomoto H. The usefulness of prevention consolidation therapy of esophageal varices using an argon plasma coagulation technique. *Hepatol Res* 2002; **23**: 220-225 [PMID: 12076718 DOI: 10.1016/S1386-6346(01)00179-6]

71 **Nakamura S**, Mitsunaga A, Murata Y, Suzuki S, Hayashi N. Endoscopic induction of mucosal fibrosis by argon plasma coagulation (APC) for esophageal varices: A prospective randomized trial of ligation plus APC vs. ligation alone. *Endoscopy* 2001; **33**: 210-215 [PMID: 11293751 DOI: 10.1055/s-2001-12804]

72 **Guth E**, Katz MD, Hanks SE, Teitelbaum GP, Ralls P, Korula J. Recurrent bleeding from ileal varices treated by transjugular intrahepatic portosystemic shunt: value of Doppler ultrasonography in diagnosis and follow-up. *J Ultrasound Med* 1996; **15**: 67-69 [PMID: 8667487]

73 **Vidal V**, Joly L, Perreault P, Bouchard L, Lafortune M, Pomier-Layrargues G. Usefulness of transjugular intrahepatic portosystemic shunt in the management of bleeding ectopic varices in cirrhotic patients. *Cardiovasc Intervent Radiol* 2006; **29**: 216-219 [PMID: 16284702 DOI: 10.1007/s00270-004-0346-4]

74 **Vangeli M**, Patch D, Terreni N, Tibballs J, Watkinson A, Davies N, Burroughs AK. Bleeding ectopic varices--treatment with transjugular intrahepatic porto-systemic shunt (TIPS) and embolisation. *J Hepatol* 2004; **41**: 560-566 [PMID: 15464235 DOI: 10.1016/j.jhep.2004.06.024]

75 **Boyer TD**, Haskal ZJ. The Role of Transjugular Intrahepatic Portosystemic Shunt (TIPS) in the Management of Portal Hypertension: update 2009. *Hepatology* 2010; **51**: 306 [PMID: 19902484 DOI: 10.1002/hep.23383]

76 **Macedo TA**, Andrews JC, Kamath PS. Ectopic varices in the gastrointestinal tract: short- and long-term outcomes of percutaneous therapy. *Cardiovasc Intervent Radiol* 2005; **28**: 178-184 [PMID: 15719183 DOI: 10.1007/s00270-004-0148-8]

77 **Ozaki CK**, Hansen M, Kadir S. Transhepatic embolization of superior mesenteric varices in portal hypertension. *Surgery* 1989; **105**: 446-448 [PMID: 2922679]

78 **Samaraweera RN**, Feldman L, Widrich WC, Waltman A, Steinberg F, Greenfield A, Srinivasan M, Robbins AH, Johnson WC. Stomal varices: percutaneous transhepatic embolization. *Radiology* 1989; **170**: 779-782 [PMID: 2783784 DOI: 10.1148/radiology.170.3.2783784]

79 **Smith-Laing G**, Scott J, Long RG, Dick R, Sherlock S. Role of percutaneous transhepatic obliteration of varices in the management of hemorrhage from gastroesophageal varices. *Gastroenterology* 1981; **80**: 1031-1036 [PMID: 7009311]

80 **Toumeh KK**, Girardot JD, Choo IW, Andrews JC, Cho KJ. Percutaneous transhepatic embolization as treatment for bleeding ileostomy varices. *Cardiovasc Intervent Radiol* 1995; **18**: 179-182 [PMID: 7648595 DOI: 10.1007/BF00204146]

81 **Viamonte M**, Pereiras R, Russell E, Le Page J, Hutson D. Transhepatic obliteration of gastroesophageal varices: results in acute and nonacute bleeders. *AJR Am J Roentgenol* 1977; **129**: 237-241 [PMID: 409156 DOI: 10.2214/ajr.129.2.237]

82 **L'Herminé C**, Chastanet P, Delemazure O, Bonnière PL, Durieu JP, Paris JC. Percutaneous transhepatic embolization of gastroesophageal varices: results in 400 patients. *AJR Am J Roentgenol* 1989; **152**: 755-760 [PMID: 2784259 DOI: 10.2214/ajr.152.4.755]

83 **Haruta I**, Isobe Y, Ueno E, Toda J, Mitsunaga A, Noguchi S, Kimura T, Shimizu K, Yamauchi K, Hayashi N. Balloon-occluded retrograde transvenous obliteration (BRTO), a promising nonsurgical therapy for ectopic varices: a case report of successful treatment of duodenal varices by BRTO. *Am J Gastroenterol* 1996; **91**: 2594-2597 [PMID: 8946993]

84 **Sonomura T**, Horihata K, Yamahara K, Dozaiku T, Toyonaga T, Hiroka T, Sato M. Ruptured duodenal varices successfully treated with balloon-occluded retrograde transvenous obliteration: usefulness of microcatheters. *AJR Am J Roentgenol* 2003; **181**: 725-727 [PMID: 12933468 DOI: 10.2214/ajr.181.3.1810725]

85 **Akazawa Y**, Murata I, Yamao T, Yamakawa M, Kawano Y, Nomura N, Isomoto H, Mizuta Y, Murase K, Kohno S. Successful management of bleeding duodenal varices by endoscopic variceal ligation and balloon-occluded retrograde transvenous obliteration. *Gastrointest Endosc* 2003; **58**: 794-797 [PMID: 14595327 DOI: 10.1016/S0016-5107(03)02008-X]

86 **Ohta M**, Yasumori K, Saku M, Saitsu H, Muranaka T, Yoshida K. Successful treatment of bleeding duodenal varices by balloon-occluded retrograde transvenous obliteration: a transjugular venous approach. *Surgery* 1999; **126**: 581-583 [PMID: 10486613 DOI: 10.1016/S0039-6060(99)70102-9]

87 **Tanihata H**, Minamiguchi H, Sato M, Kawai N, Sonomura T, Takasaka I, Nakai M, Sahara S, Nakata K, Shirai S. Changes in portal systemic pressure gradient after balloon-occluded retrograde transvenous obliteration of gastric varices and aggravation of esophageal varices. *Cardiovasc Intervent Radiol* 2009; **32**: 1209-1216 [PMID: 19688368 DOI: 10.1007/s00270-009-9679-3]

88 **Yoshida H**, Mamada Y, Taniai N, Yoshioka M, Hirakata A, Kawano Y, Mizuguchi Y, Shimizu T, Ueda J, Uchida E. Treatment modalities for bleeding esophagogastric varices. *J Nippon Med Sch* 2012; **79**: 19-30 [PMID: 22398787 DOI: 10.1272/jnms.79.19]

89 **Ueda J**, Yoshida H, Mamada Y, Taniai N, Mizuguchi Y, Shimizu T, Matsumoto S, Kakinuma D, Ishikawa Y, Kanda T, Akimaru K, Teranishi N, Naito Z, Tajiri T. Successful emergency enterectomy for bleeding ileal varices in a patient with liver cirrhosis. *J Nippon Med Sch* 2006; **73**: 221-225 [PMID: 16936448 DOI: 10.1272/jnms.73.221]

90 **Hidajat N**, Stobbe H, Hosten N, Schroeder RJ, Fauth M, Vogl T, Felix R. Transjugular intrahepatic portosystemic shunt and transjugular embolization of bleeding rectal varices in portal

hypertension. *AJR Am J Roentgenol* 2002; **178**: 362-363 [PMID: 11804893 DOI: 10.2214/ajr.178.2.1780362]
91 **McAlister VC**, Al-Saleh NA. Duodenal dearterialization and stapling for severe hemorrhage from duodenal varices with portal vein thrombosis. *Am J Surg* 2005; **189**: 49-52 [PMID: 15701491 DOI: 10.1016/j.amjsurg.2004.04.011]

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REVIEW

Current biomarkers for hepatocellular carcinoma: Surveillance, diagnosis and prediction of prognosis

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Abstract

Biomarkers for surveillance, diagnosis and prediction of prognosis in patients with hepatocellular carcinoma (HCC) are currently not ready for introduction into clinical practice because of limited sensitivity and specificity. Especially for the early detection of small HCC novel biomarkers are needed to improve the current effectiveness of screening performed by ultrasound. The use of high-throughput technologies in hepatocellular research allows to identify molecules involved in the complex pathways in hepatocarcinogenesis. Several invasive and non-invasive biomarkers have been identified already and have been evaluated in different clinical settings. Gene signatures with prognostic potential have been identified by gene expression profiling from tumor tissue. However, a single "all-in-one" biomarker that fits all-surveillance, diagnosis, prediction of prognosis-has not been found so far. The future of biomarkers most probably lies in a combination of non-invasive biomarkers, imaging and clinical parameters in a surveillance setting. Molecular profiling of tumorous and non-tumorous liver tissue may allow a prediction of prognosis for the individual patient and hopefully clear the way for individual treatment approaches. This article gives an overview on current developments in biomarker research in HCC with a focus on currently available and novel biomarkers, in particular on microRNA.

Key words: Hepatocellular carcinoma; Biomarker; Diagnosis; Prognosis; MicroRNA

Core tip: The aim of this review is to provide an overview on current invasive and non-invasive biomarkers in hepatocellular carcinoma (HCC) with respect to their use in surveillance, diagnosis and prediction of prognosis. We also give an outlook on the future development of HCC biomarker research with a focus on microRNA.

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INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is rising throughout the world as a consequence of a rising prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection and an increase in prevalence of (non-alcoholic) fatty liver disease due to the metabolic syndrome[1-4]. The number of patients that are at risk to develop HCC and need to undergo structured surveillance is therefore constantly rising in parallel.

Transabdominal ultrasound currently is the only recommended tool for surveillance in the Western world which has been shown to be cost-effective. Its sensitivity is dependent on many factors, including the quality of the ultrasound machine, the experience of the examiner and also the patient. Especially in obese patients with NASH cirrhosis liver ultrasonography may be difficult and therefore not always appropriate to rule out HCC. In patients with liver cirrhosis regenerative nodules may be hard to distinguish from HCC on ultrasound, and the sensitivity of ultrasound to detect early HCC lies in a range of 32% to 65%[5,6]. On the other hand, surveillance by contrast-enhanced computed tomography or contrast-enhanced magnetic resonance tomography is rather expensive and not cost-effective. In addition, they are associated with the additional exposure to radiation with an increased risk of tumor development and/or contrast-media related deterioration of kidney function. In developing countries, where even the availability of ultrasound surveillance is quite low, serological markers for surveillance are of special interest[7].

Biomarkers in blood, other body fluids or tissue for screening, prediction of prognosis and monitoring of response to a therapy would be an important contribution to the management of patients with HCC.

Early detection of HCC is the most important factor to offer the patient the chance of cure. α-fetoprotein (AFP) is the most widely used and broadly known biomarker for HCC, but the measurement of serum AFP levels has been dropped from current surveillance guidelines in Europe and the United States because of low sensitivity and specificity. This is based on the knowledge that almost 80% of small HCCs do not show increased levels of AFP, and the sensitivity decreases to 25% in tumors smaller than 3 cm[8]. Nonetheless, serum AFP measurement is still combined with ultrasound by many physicians worldwide to reduce the risk of missing small lesions in the cirrhotic liver that have not been detected by ultrasound. Alternative or additional biomarkers may be useful tools for surveillance or as a decisional tool in clinical practice to identify patients that will benefit from advanced imaging methods in a surveillance setting to augment the proportion of patients with HCC diagnosed in an early tumor stage.

Apart from their role as a surveillance tool, biomarkers may play a role as diagnostic tool once a suspicious lesion in a patient with liver cirrhosis has been detected. In the past, a significant concentration of AFP in the serum of a patient with liver cirrhosis and a suspicious mass in the liver larger than 2 cm was sufficient to diagnose HCC[9]. However, diagnostic algorithms endorsed by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) nowadays strictly rely on typical radiological hallmarks in dynamic contrast-enhanced imaging apart from biomarkers[9-11].

Once the diagnosis of HCC is confirmed, molecular biomarkers could potentially be used for prediction of prognosis of the individual patient and also for the guidance of therapeutic decisions. Currently, early studies on predictive biomarkers are on their way to make a step towards a personalized and individualized therapy of patients with HCC.

This review gives a concise overview on current clinical-translational knowledge on biomarkers in surveillance, diagnosis and prediction of prognosis with a focus on miRNA.

ROLE OF BIOMARKERS IN SURVEILLANCE AND DIAGNOSIS

Geographical differences in tumor prevalence, tumor biology and resources have resulted in differences in current guidelines with respect to screening recommendations. The main pillar of surveillance in high risk populations is repeated transabdominal ultrasound with small differences with respect to the definition of target populations in various guidelines. Throughout the world three serum biomarkers are suggested as tools to determine the risk of liver cancer in high risk populations: AFP, the proportion of the fucosylated isoform of AFP, AFP-L3, and des-gamma-carboxy-prothrombin (DCP). These markers are FDA approved for this indication, but not a part of the surveillance guidelines published by the AASLD and the EASL[10,11]. Current expert opinion from Western countries has been rather critical on these biomarkers regarding their clinical value[12]. On the other hand, the Asia Pacific guideline recommends to combine ultrasound with the measurement of AFP levels in the serum and the Japanese society even recommends to apply all three mentioned biomarkers[13,14] for surveillance.

However, most studies on the performance of biomarkers in HCC detection have not been performed in a surveillance setting but compared levels of predefined biomarkers in patients with HCC with a comparator group, in most cases in patients with chronic liver disease.

A randomized controlled study performed in a high-risk population in China showed that screening by AFP measurement led to earlier diagnosis of HCC but had no impact on mortality[15]. On the

Table 1 Diagnostic performance of alpha-fetoprotein serum levels in selected studies

Ref.	Year	*n*	Comparator	Cut-off-level	Sensitivity	Specificity	AUC
Marrero *et al*[20]	2009	836 total (419 HCC)	Liver cirrhosis	20 ng/mL	59%	90%	0.8
Mao *et al*[21]	2010	4217 total (789 HCC)	Amongst others healthy controls, HBV carriers, liver cirrhosis	35 ng/mL	58.20%	85.30%	
Farinati *et al*[22]	2006	1158 HCC	No control	400 ng/mL	18%		0.59
Lok *et al*[23]	2010	39 HCC, 77 matched controls	Hepatitis C	20 ng/mL	61%	81%	0.79

AUC: Area under the cure; HCC: Hepatocellular carcinoma.

other hand, semiannual screening for HCC by AFP measurement in a population-based study in Alaska was effective in detecting HCC at early stages and significantly prolonged survival rates[16].

As the discussion on the rise and fall of AFP as biomarker in HCC surveillance and diagnosis has been intense and sometimes even emotional during the last decade[17-19], data on the most important diagnostic studies referred to in this discussion are summarized in Table 1.

A recent meta-analysis on the performance of AFP in diagnosis of HCC included seven studies and revealed a pooled sensitivity of 66% with a specificity of 86% and an area under the cure (AUC) of 0.87[24]. In a further meta-analysis including ten studies the pooled sensitivity of AFP for the diagnosis of HCC was 51.9% at a specificity of 94% (AUC = 0.81)[25]. It is a major drawback of AFP as surveillance tool that its serum levels are influenced by the activity of the underlying liver disease and therefore increased in patients with elevated ALT levels even in the absence of HCC as shown in the HALT-C trial[26]. Additionally, only a proportion of patients with HCC exerts elevated AFP serum levels leading to low sensitivity of the marker. The heterogeneity of tumor biology in HCC therefore results in a necessity to find better or complementary markers to close this diagnostic gap.

The clinical utility of high-sensitivity AFP-L3 (hs-AFP-L3) in early prediction of HCC development in patients with chronic HBV or HCV infection was recently evaluated in a large Japanese study. Even at low AFP levels and in absence of suspicious ultrasound findings an elevation of hs-AFP-L3 was an early predictor of HCC development with an elevation in 34.3% of patients one year prior to diagnosis of HCC[27]. In patients with low AFP levels (< 20 ng/mL), the diagnostic sensitivity for hs-AFP-L 3% at a cut-off of 5% was 41.5% with a specificity of 85.1%[28].

Numerous studies have investigated the performance of alternative markers or combinations of already established markers. New candidate markers include squamous cell carcinoma antigen-immunoglobulin M complex (SCCA-IGM), α-l-fucosidase[29], glypican-3 (GPC-3), insulin-like growth factor (IGF)[30], vascular endothelial growth factor (VEGF), or Dickkopf-1 (DKK1)[31].

Three further biomarkers have intensively been studied for their potential use in screening for HCC, namely Golgi protein 73 (GP73), interleukin-6 (IL-6) and squamous cell carcinoma antigen (SCCA) and were addressed in a recent meta-analysis[32]. The transmembrane glycoprotein GP73 has a sensitivity of 62% with a specificity of 88% at a cut-off of 10 relative units in a study comparing 144 patients with HCC to 152 patients with cirrhosis and 56 healthy controls[33]. A further study including 4217 subjects of whom 789 were patients with HCC revealed a sensitivity of 74.6% with a specificity of 97.4% at a cut-off of 8.5 relative units[21]. Two smaller studies were identified in the meta-analysis studying the cytokine IL-6. Using different cut-off-values, sensitivity for HCC ranged from 46% to 73% with a specificity of 87% to 95%[32,34,35]. The largest study on the role of the serine protease inhibitor SCCA included 961 patients and resulted in a sensitivity of 42% at specificity of 83% using a cut-off of 3.8 ng/mL[32,36].

Seven well-designed studies on the diagnostic performance of osteopontin, an integrin-binding glycophosphoprotein, were published and recently summarized in a meta-analysis[24]. Osteopontin is expressed by transformed malignant cells and has been evaluated also in colon and pancreatic cancer. All of the reported studies were retrospective in design and included a range of 30 to 179 patients with HCC. The pooled sensitivity of osteopontin for HCC was 86% with a specificity of 86% resulting in a diagnostic accuracy comparable to that of AFP in the included studies. The authors of the meta-analysis conclude that further validation studies are needed before the marker could be suggested for the use in daily clinical routine.

By combining two or more biomarkers the diagnostic performance of a single non-invasive test can be optimized. This has been investigated for the three best established non-invasive biomarkers in HCC, AFP, AFP-L3 and DCP.

When comparing 164 European patients with HCC to 422 controls with chronic liver disease a significant increase in AFP serum levels was mainly shown in patients with advanced stages of HCC and in patients suffering from viral hepatitis while DCP was more frequently elevated in patients with early-stage and NASH associated HCC. Taken alone,

Table 2 Diagnostic performance of novel non-invasive biomarkers

Ref.	Year	Marker	*n*	Comparator	Cut-off-level	Sensitivity	Specifity	AUC
Toyoda *et al*[31]	2011	hs-AFP-L3%	666	Chronic liver disease and AFP < 20 ng/mL	5%	41.50%	85.10%	0.707
Ertle *et al*[37]	2013	DCP	586	Chronic liver disease	5 ng/mL	45.80%	95%	0.87
Wan *et al*[24]	2014	Osteopontin	Meta-analysis (7 studies)	mixed		Pooled: 86%	Pooled: 86%	0.92
Hsia *et al*[35]	2007	IL-6	128	Mixed, including chronic liver disease and healthy controls	3 pg/mL	46%	95%	
Mao *et al*[21]	2010	GP73	4217	Mixed, including chronic liver disease and healthy controls	8.5 rel. units	74.60%	97.40%	0.94
Giannelli *et al*[36]	2007	SCCA	961	Liver cirrhosis	3.8 ng/mL	41.90%	82.60%	0.656
Ertle *et al*[37]	2013	AFP combined with DCP	586	Chronic liver disease	DCP 5 ng/mL AFP 10 ng/mL	78%	89.30%	0.91
Johnson *et al*[40]	2014	GALAD-score	670	Chronic liver disease		93%	89%	

hs-AFP: High-sensitivity α-fetoprotein; DCP: Des-gamma-carboxy-prothrombin; SCCA: Squamous cell carcinoma antigen; GP73: Golgi protein 73; IL-6: Interleukin-6; AUC: Area under the cure.

neither of the two parameters could detect more than one third of HCC patients independently of stage or etiology but by combination of AFP with DCP a sensitivity of 55% for early stage HCC and 78% for all stages (cut-off for AFP 10 ng/mL and for DCP 5 ng/mL) was reached[37]. The addition of AFP-L3% to this combination, led to a further gain in sensitivity (84%) in another European study[38].

The incorporation of clinical variables like age and gender into models based on a combination of biomarkers for HCC detection further improve the predictive performance of these models[39]. A model using a combination of age, gender, AFP, AFP-L3 and DCP estimates the probability to suffer from HCC in an individual patient with chronic liver disease with a sensitivity of 86% for HCC in BCLC stage 0 or A and a sensitivity of 94% for later tumor stages[40]. The diagnostic performance of novel circulating biomarkers and scores is summarized in Table 2.

Although the complex process of hepatocarcinogenesis is still not fully understood, several signal transduction pathways have been identified as critical players in the pathophysiology of HCC, including the Wnt/β-Catenin pathway, the p53 pathway, the tumor suppressor retinoblastoma protein pRb1 pathway, the mitogen-activated protein kinase pathway, the Ras pathway, JAK/STAT signaling, mechanisms of cellular stress response like heat shock proteins and epidermal growth factor receptor and transforming growth factor-β signaling[41]. As a consequence of different risk factors causing HCC in the individual patient, the alterations in these pathways differ in different settings which is probably the cause of insufficient sensitivity of single biomarkers. Genetic and epigenetic alterations occur in these pathways and mediate cell proliferation. The possibility to perform proteomic profiling and whole genome sequencing in combination with systems biology has led to a new era in biomarker development that will hopefully help to understand the complex interactions in hepatocarcinogenesis of multiple proteins, genes and transcription factors. First examples of this approach have successfully been evaluated in clinical studies, but none of the signatures has been validated in large prospective studies.

In patients with chronic HBV infection and liver cirrhosis, proteomic analyses in the plasma identified a cluster of 11 proteins that is able to identify patients at high risk for HCC development (OR = 4.83, 95%CI: 1.26-18.56)[42].

Gene expression profiling of peripheral blood mononuclear cells in HCC patients using microarrays and bioinformatics-driven analysis of the data has identified a blood-based signature of three genes, namely Chemokine (C-X-C motif) receptor 2 (CXCR2), C-C chemokine receptor type 2 (CCR2) and E1A-Binding Protein P400 (EP400), that predicts HCC with an AUC of 0.96 yielding at a sensitivity of 93% with a specificity of 89%[43].

High-throughput metabolomics technologies with the comprehensive analysis of small molecular metabolites may additionally identify serum metabolic profiles that can be used as diagnostic biomarkers. First steps into this direction have also already been taken[44,45].

To distinguish dysplastic nodules from well-differentiated HCC is a challenge, not only for the radiologist on imaging, but also for the pathologist on tissue samples.

Molecular signatures derived from gene expression profiling have been identified that are helpful to answer this critical question that is decisive for the further management of the patient.

Characteristic genomic changes during hepatocarcinogenesis have been identified. Specific gene signatures accurately reflect the pathological progression of disease from cirrhosis to dysplasia to early and advanced HCC in patients with HCV infection in Asian and Western patients[46,47].

A three gene set in the tissue including glypican

3 (GPC3; 18-fold increase in HCC, $P = 0.01$), LYVE1 (12-fold decrease in HCC, $P = 0.0001$), and survivin (2.2-fold increase in HCC, $P = 0.02$) has an accuracy of 94% to discriminate dysplastic nodules from early HCC in HCV cirrhosis. Especially immunostaining for GPC3 is highly discriminative[48].

Heat shock protein 70 and cyclase-associated protein 2 are further examples for tissue biomarkers identified in comprehensive approaches that found their way into clinical testing and application[49,50].

MICRORNAS AS NOVEL BIOMARKERS

With respect to novel potential biomarkers, non-coding RNA and specifically microRNA (miRNA) have received the greatest attention over the past years[51]. MiRNAs are small non-coding and evolutionary conserved RNA molecules that serve as posttranscriptional regulators of mRNA expression and interfere with translation to protein. Following several common modifications steps, miRNA become a part of the so called RISC (RNA silencing complex) to be functionally active[52]. MiRNAs can either preserve their function intracellularly by regulating the expression of a target population of molecules, or can be released from the cell bound to other proteins and also as a free molecule[53,54]. As part of the released vesicles, specific miRNAs can further preserve their functional activity locally or be transported in blood or probably other specimens to other tissues or organs[55].

The most exiting advantages of miRNA over various other molecules are their stability against degradation, cell-type specific miRNA expression patterns and detectability in all types of human specimens such as blood, feces, saliva, *etc.*[56-59]. While mRNA or various proteins are relatively sensitive to extracellular enzymes, miRNA expression levels remain, as long as they are preserved in a natural milieu, relatively resistant to RNA digestions, heating, storage, drying, formalin fixation, *etc*. For detailed information regarding the biogenesis of miRNA as well as regarding the current knowledge on molecular function we refer to the several excellent reviews from the field[51,60,61].

Shortly after definite recognition of miRNA, several groups have provided seminal evidence for differences in miRNA expression patterns between different tissues and malignant conditions including HCC[62,63]. High quality analyses using deep sequencing have recently provided an important view in microRNAome in liver tissue and HCC[64]. Interestingly, about 86% of the miRNA were expressed in very low concentrations and only about 1% were expressed abundantly. Three of those miRNAs, namely miR-122, miR-192 and miR-199a/b-3p, were responsible for 74% of all miRNA in normal liver tissue with miR-122 accounting for almost 52% suggesting that those miRNAs are the most important ones in liver biology[64]. Many recent reports have shown a broad spectrum of changes in microRNAoma in HCC[64-67]. Therefore, miRNAs may have the potential to become valid biomarkers in HCC.

MIRNAS AS NON-INVASIVE DIAGNOSTIC BIOMARKERS FOR HCC

The biggest effort from miRNA-based biomarker research has been made to improve the diagnostic utility in HCC. In parallel with the dominant etiological factors in HCC development, the largest body of data comes from Asian populations and virus-related HCC cohorts. In one of the first profiling studies, Li *et al*[68] performed deep sequencing in pooled samples from chronic HBV virus patients, HCC patients and controls with and without cancer. They identified a pattern of 21 miRNA that show differential expression in cHBV patients and 6 miRNA differentially expressed in HCC patients. Following subsequent testing and validation, 13 miRNA, including miR-122, miR-375, miR-92a, miR-10a and let-7c, were identified as a biomarker for patients with HBV (acute and chronic) and HCV virus infection. Furthermore, using only 3 miRNAs (miR-25, miR-375, let7f) the authors could reach an AUC of 99.7% with a 97.9% sensitivity and a 99.1% specificity to discriminate controls from HCC patients. Most interestingly for HCC diagnosis, the comparison of the two cohorts with chronic HBV and HBV-associated HCC lead to identification of two miRNAs (miR-10a and miR-125b) that could separate the HCC cohort with an AUC of 99.2% (sensitivity 98.5% and specificity 98.5)[68]. Recently, another large scale study studied plasma samples from 934 patients with various conditions including healthy subjects, patients with chronic HBV, liver cirrhosis and HBV-related HCC[69]. Following discovery and training phases, the authors identified a panel of 7 microRNAs (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801) that provided the highest diagnostic accuracy for the identification of HBV-related HCC. Using an independent validation cohort of 390 samples, the area under the curve value was comparable with the training data and reached 0.888 with a sensitivity of 81.8% and a specificity of 83.5%. Interestingly, the diagnostic accuracy was independent of disease stage and was comparable to healthy subjects, patients with chronic hepatitis or livers cirrhosis. At present, this study is one of the largest to evaluate the biomarker potential of miRNAs in cancer. Notably, the expression of selected miRNA was analyzed using RT-PCR which may be critical for clinical translation of the results. Whether miR-1228 is the optimal normalizer needs further evaluation[69].

Besides profiling studies, a candidate-based

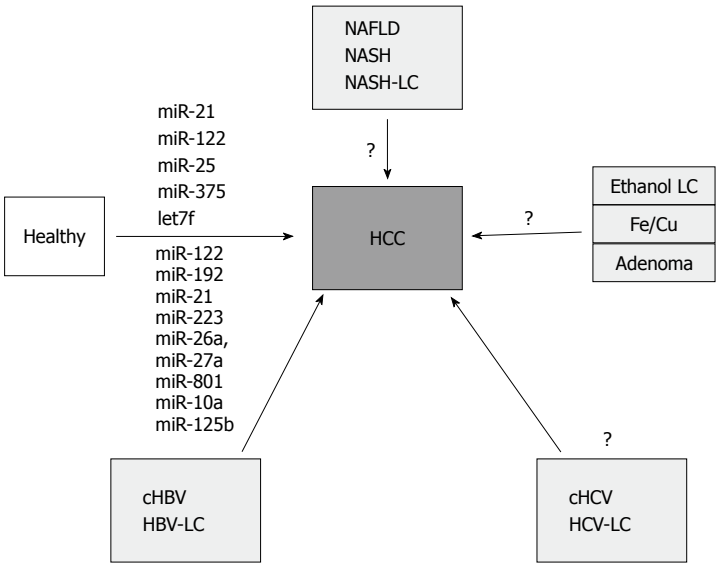

Figure 1 Schematic presentation of the potential microRNAs in diagnosis of hepatocellular carcinoma in relation to etiology. HBV-LC: Hepatitis B virus-liver cirrhosis; HCC: Hepatocellular carcinoma; NAFLD: Non-alcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; cHCV: Chronic hepatitis C virus; cHBV: Chronic hepatitis B-virus; Fe: Hemochromatosis; Cu: M. Wilson; miR: microRNA.

approach has been also used to evaluate the expression differences of liver and tumor-related miRNA. In particular miR-21, which is most frequently deregulated miRNA in cancer, was found at higher level both in sera and plasma from HCC patients[70,71], while other showed no significant expression differences[72,73]. In similar fashion, miR-122, the most abundant miRNA of the liver, was also found at high level in sera from HCC patients[71,72]. However, the level of circulating miR-122 may be strongly influenced by inflammation or apoptosis of hepatocytes in such conditions as acute or chronic hepatitis or nonalcoholic fatty liver disease, suggesting that background condition of the liver inflammation may strongly influence the miR-122 level[74]. Nevertheless, the data from candidate-based studies correlate with the data from Link *et al*[54] at least for both miRNAs miR-21 and miR-122[69]. There are several other studies that have identified additional miRNA, however, at present no independent validation has been performed (Figure 1). It is further more important to mention that the potential of miRNA as biomarker has not been equally analyzed in all HCC-related risk conditions. Systemic analyses for alcohol, NASH or HCV-related conditions are pending.

NON-INVASIVE PROGNOSTIC BIOMARKERS IN HCC

The molecular heterogeneity of HCC results in differences in outcome of affected patients. Clinically, main factors that have an impact on patient survival have been identified. These include tumor related factors like number and size of nodules, vascular invasion, existence of extrahepatic metastases, liver function and patient related factors. The Barcelona Classification summarizes these factors in a comprehensive algorithm and is endorsed by current guidelines[10,11]. However, within the defined tumor stages the survival of patients is still heterogeneous, and some patients that are treated in curative intent or even undergo liver transplantation show early recurrence of disease. Knowledge on high-risk profiles would therefore be important to guide individualized treatment.

Several of the non-invasive biomarkers that have been evaluated for their diagnostic power in HCC have also been studied for their prognostic significance.

High expression of AFP in serum correlates with high cell proliferation, high angiogenesis and low apoptosis and is associated with poor prognosis[75,76]. The fraction of AFP-L3 is another prognostic biomarker for survival after resection of HCC[77,78]. Patients that have undergone resection of HCC and had elevated levels of AFP, AFP-L3 and DCP at baseline had a worse prognosis than those patients that are positive for just one or two of the markers before surgery[79]. The combination of AFP, the percentage of AFP-L3 and DCP combined with the concentrations of bilirubin and albumin, summarized in the BALAD score, is prognostic for survival of patients with HCC in an Asian population[80]. Recently, a modification of this model, the BALAD-2 score, was validated in an international setting and confirmed to reliably predict the prognosis of patients with HCC[81].

Other circulating biomarkers that mirror current knowledge on pathways involved in hepatocarcinogenesis and shown to be of prognostic value are, amongst others, IGF1[82], DKK1[83,84], GPC-3 and HSP 70[85] although prospective validation studies are still to come. In patients with advanced HCC, baseline angiopoietin 2 (Ang2), and VEGF concentration in the plasma also independently predict survival[76].

MIRNAS AS NON-INVASIVE PROGNOSTIC BIOMARKERS FOR HCC

In addition to their diagnostic potential, miRNAs may be helpful in prediction of the prognosis of HCC. Li

Table 3 Genetic signatures from tumor tissue and their prognostic significance

Ref.	Year	Correlation with	No. of genes in signature	AUC	*P*-value
Nault *et al*[95]	2013	Disease-free survival after resection	5 (tumor)	0.8	< 0.0001
Lim *et al*[96]	2013	Disease-free survival after resection	25 (tumor)		0.002
Kurokawa *et al*[97]	2004	Tumor recurrence after resection	20 (tumor)		0.001
Yoshioka *et al*[98]	2009	Tumor recurrence after resection	172 (tumor)		< 0.0001
Woo *et al*[99]	2008	Recurrence free survival	628 (tumor)		< 0.01

AUC: Area under the cure.

et al[86] studied the expression of several miRNAs in sera from 46 HCC patients and 20 controls. Specifically, miR-221 was fond in high concentration in HCC sera samples, which correlated with tumor size, cirrhosis and tumor stage. Kaplan-Meier survival analyses revealed an inverse correlation between miR-221 expression and survival rates. In another study, Tomimaru *et al*[70] analyzed miR-21 expression in plasma from 126 HCC patients. MiR-21 expression was high in HCC and diminished after surgical treatment. Most importantly, high miR-21 expression level in plasma correlated with shorter cumulative survival following treatment. Köberle *et al*[87] analyzed the performance of miR-1 and miR-122 in European HCC patients. Higher miR-1 and miR-122 serum levels were associated with longer overall survival compared to low expression of those miRNAs. However, miR-122, but not miR-1, showed a correlation with hepatic inflammation, liver function and synthetic capacity. The authors conclude that miR-1 may be a liver function independent predictive biomarker of HCC. There is also growing evidence that miRNA signature profiling can be useful in prognostic stratification[88]. A signature of 31-miRNA correlates with stage of disease[89]. A distinct 20-miRNA signature associated with metastases of HCC has also been identified[90].

Despite of the promising potential, there are several pitfalls in utility and implementation of miRNA-based biomarkers in clinical practice. First, the majority of data comes from Asian populations with predominantly virus-related HCC (Figure 1)[54]. However, in European or American populations the incidence of virus-related HCC is dropping and increases for NAFLD-related conditions therefore the data in these patients may probably be different. Second, the complexity of the miRNA alterations in the background of liver pathology (ex. chronic hepatitis with early fibrosis or cirrhosis) may impact the pattern of miRNA expression with increasing expression in one of the conditions and decreasing level in another. Furthermore, the ideal biomarker is probably the one that is expressed in HCC tissue with increasing concentration during progression of the disease. A combination of miRNA with the established-although not ideal-biomarker AFP may probably be beneficial[70].

In the above section, we provided a brief insight into the growing field of miRNA-based biomarker research for HCC. Before this approach may be further utilized for clinical testing there are also critical technical questions that need to be answered. What is the best non-invasive specimen for the early diagnosis of HCC: plasma or serum? What normalizer is the best for the analyses? What is the best method for translational testing? Indeed, array-based analyses may be probably too expensive to apply, therefore, a candidate-based approach will need to be standardized for effective implementation. Those are only few reasons why the currently available data are so heterogeneous[54,67]. Nevertheless, this miRNA-based approach may provide an additional value in personal-based management by prediction and application of new therapeutic targets[91].

INVASIVE BIOMARKERS AS PROGNOSTIC TOOLS FOR HCC

After curative treatment of HCC the prognosis of the patient depends on the characteristics of the resected cancer but in addition on the risk of carcinogenesis due to the underlying etiology and inflammatory activity of chronic liver disease which persist after surgical resection or ablation. In a landmark study Hoshida *et al*[92] demonstrated that gene-expression profiling can be performed in frozen as well as in formalin-fixed paraffin-embedded tissues and identified a gene-expression signature in liver tissue adjacent to tumor in patients who underwent resection of HCC that correlated with survival.

Since then, a large number of gene-expression profile studies has been performed in HCC with the aim to distinguish molecular subtypes. A validated and commonly accepted molecular classification has not been identified so far. Based on a meta-analysis of gene expression profiles from eight European cohorts of patients with HCC, a classification framework for HCC based on gene expression profiles was proposed that distinguishes three HCC subclasses, each correlated with clinical parameters such as tumor size, extent of cellular differentiation, and serum α-fetoprotein levels[50,93]. The results of a

selection of recent studies with respect to prognostic gene expression profiles are summarized in Table 3. In a large validation study gene expression profiles of tumor and adjacent tissue were evaluated for their prognostic significance and a composite prognostic model was developed[94]. A further validation of this model is pending before it may be considered for clinical use.

CONCLUSION

High-throughput technologies allow the identification of new molecules involved in complex pathways and their interaction in hepatocarcinogenesis. A single perfect biomarker has not been found so far to accomplish with the clinical demand for optimal HCC patient care. A combination of serological biomarkers may offer a better risk stratification of patients belonging to high-risk populations in the future. The combination of clinical characteristics and morphological signatures of tumor and the surrounding tissue will most likely be the best option for risk stratification and prediction of prognosis in patients with HCC in the future. Individualized treatment approaches that take into account the patient's own cancer genetic profile need to be addressed in further research[12].

However, the critical step for translational research is to move the identified candidate signatures or single biomarkers from bench to bedside. There is a great hope that new molecular biomarkers can support clinicians in their daily routine and improve the care of patients with HCC. However, analyses tools need to be standardized and simplified in order to be useful, reliable and widely available.

REFERENCES

1 **Njei B,** Rotman Y, Ditah I, Lim JK. Emerging trends in hepatocellular carcinoma incidence and mortality. *Hepatology* 2014; **61**: 191-199[PMID: 25142309 DOI: 10.1002/hep.27388]

2 **El-Serag HB**, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: Where are we? Where do we go? *Hepatology* 2014; **60**: 1767-1775 [PMID: 24839253 DOI: 10.1002/hep.27222]

3 **Mittal S**, El-Serag HB. Epidemiology of hepatocellular carcinoma: consider the population. *J Clin Gastroenterol* 2013; **47** Suppl: S2-S6 [PMID: 23632345 DOI: 10.1097/MCG.0b013e3182872f29]

4 **Schütte K**, Kipper M, Kahl S, Bornschein J, Götze T, Adolf D, Arend J, Seidensticker R, Lippert H, Ricke J, Malfertheiner P. Clinical characteristics and time trends in etiology of hepatocellular cancer in Germany. *Digestion* 2013; **87**: 147-159 [PMID: 23548687 DOI: 10.1159/000346743]

5 **Singal A**, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, Marrero JA. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009; **30**: 37-47 [PMID: 19392863 DOI: 10.1111/j.1365-2036.2009.04014.x]

6 **Singal AG**, Conjeevaram HS, Volk ML, Fu S, Fontana RJ, Askari F, Su GL, Lok AS, Marrero JA. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 793-799 [PMID: 22374994 DOI: 10.1158/1055-9965.EPI-11-1005]

7 **Kim do Y**, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. *Liver Cancer* 2012; **1**: 2-14 [PMID: 24159567 DOI: 10.1159/000339016]

8 **Saffroy R**, Pham P, Reffas M, Takka M, Lemoine A, Debuire B. New perspectives and strategy research biomarkers for hepatocellular carcinoma. *Clin Chem Lab Med* 2007; **45**: 1169-1179 [PMID: 17635075 DOI: 10.1515/CCLM.2007.262]

9 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

10 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

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

12 **Bruix J**, Gores GJ, Mazzaferro V. Hepatocellular carcinoma: clinical frontiers and perspectives. *Gut* 2014; **63**: 844-855 [PMID: 24531850 DOI: 10.1136/gutjnl-2013-306627]

13 **Omata M**, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, Kudo M, Lee JM, Choi BI, Poon RT, Shiina S, Cheng AL, Jia JD, Obi S, Han KH, Jafri W, Chow P, Lim SG, Chawla YK, Budihusodo U, Gani RA, Lesmana CR, Putranto TA, Liaw YF, Sarin SK. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010; **4**: 439-474 [PMID: 20827404 DOI: 10.1007/s12072-010-9165-7]

14 **Makuuchi M**, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, Saida Y, Takayama T, Yamaoka Y. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res* 2008; **38**: 37-51 [PMID: 18039202 DOI: 10.1111/j.1872-034X.2007.00216.x]

15 **Chen JG**, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, Zhu YR. Screening for liver cancer: results of a randomised controlled trial in Qidong, China. *J Med Screen* 2003; **10**: 204-209 [PMID: 14738659 DOI: 10.1258/096914103771773320]

16 **McMahon BJ**, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, Dunaway E, Williams J. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. *Hepatology* 2000; **32**: 842-846 [PMID: 11003632 DOI: 10.1053/jhep.2000.17914]

17 **Sherman M**. Serological surveillance for hepatocellular carcinoma: time to quit. *J Hepatol* 2010; **52**: 614-615 [PMID: 20185193 DOI: 10.1016/j.jhep.2009.11.026]

18 **Forner A**, Reig M, Bruix J. Alpha-fetoprotein for hepatocellular carcinoma diagnosis: the demise of a brilliant star. *Gastroenterology* 2009; **137**: 26-29 [PMID: 19482098 DOI: 10.1053/j.gastro.2009.05.014]

19 **Giannini EG**, Trevisani F. Surveillance for hepatocellular carcinoma: just do it! *Am J Gastroenterol* 2013; **108**: 1013-1014 [PMID: 23735924 DOI: 10.1038/ajg.2013.98]

20 **Marrero JA**, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, Reddy KR, Harnois D, Llovet JM, Normolle D, Dalhgren J, Chia D, Lok AS, Wagner PD, Srivastava S, Schwartz M. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 110-118 [PMID: 19362088 DOI: 10.1053/j.gastro.2009.04.005]

21 **Mao Y**, Yang H, Xu H, Lu X, Sang X, Du S, Zhao H, Chen W, Xu Y, Chi T, Yang Z, Cai J, Li H, Chen J, Zhong S, Mohanti SR, Lopez-Soler R, Millis JM, Huang J, Zhang H. Golgi protein 73 (GOLPH2) is a valuable serum marker for hepatocellular carcinoma. *Gut* 2010; **59**: 1687-1693 [PMID: 20876776 DOI: 10.1136/gut.2010.214916]

22 **Farinati F**, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, Rapaccini G, Del Poggio P, Di Nolfo MA, Benvegnù L, Zoli M, Borzio F, Bernardi M, Trevisani F. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol* 2006; **101**: 524-532 [PMID: 16542289 DOI: 10.1111/j.1572-0241.2006.00443.x]

23 **Lok AS**, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di

Bisceglie AM, Morgan TR, Kim HY, Lee WM, Bonkovsky HL, Dienstag JL. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010; **138**: 493-502 [PMID: 19852963 DOI: 10.1053/j.gastro.2009.10.031]

24 **Wan H,** Xu H, Gu Y, Wang H, Xu W, Zu M. Comparison osteopontin vs AFP for the diagnosis of HCC: A meta-analysis. *Clin Res Hepatol Gastroenterol* 2014; **38**: 706-714 [PMID: 25034355 DOI: 10.1016/j.clinre.2014.06.008]

25 **Xu C**, Yan Z, Zhou L, Wang Y. A comparison of glypican-3 with alpha-fetoprotein as a serum marker for hepatocellular carcinoma: a meta-analysis. *J Cancer Res Clin Oncol* 2013; **139**: 1417-1424 [PMID: 23743582 DOI: 10.1007/s00432-013-1458-5]

26 **Richardson P**, Duan Z, Kramer J, Davila JA, Tyson GL, El-Serag HB. Determinants of serum alpha-fetoprotein levels in hepatitis C-infected patients. *Clin Gastroenterol Hepatol* 2012; **10**: 428-433 [PMID: 22155556 DOI: 10.1016/j.cgh.2011.11.025]

27 **Mossad NA**, Mahmoud EH, Osman EA, Mahmoud SH, Shousha HI. Evaluation of squamous cell carcinoma antigen-immunoglobulin M complex (SCCA-IGM) and alpha-L-fucosidase (AFU) as novel diagnostic biomarkers for hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 11559-11564 [PMID: 25129443 DOI: 10.1007/s13277-014-2467-y]

28 **Bertino G**, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellualar carcinoma serum markers. *Semin Oncol* 2012; **39**: 410-433 [PMID: 22846859 DOI: 10.1053/j.seminoncol.2012.05.001]

29 **Zhang J,** Zhao Y, Yang Q. Sensitivity and specificity of Dickkopf-1 protein in serum for diagnosing hepatocellular carcinoma: a meta-analysis. *Int J Biol Markers* 2014; **29**: e403-e410 [PMID: 24980448 DOI: 10.5301/jbm.5000101]

30 **Kumada T**, Toyoda H, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Tanaka J, Kagebayashi C, Satomura S. High-sensitivity Lens culinaris agglutinin-reactive alpha-fetoprotein assay predicts early detection of hepatocellular carcinoma. *J Gastroenterol* 2014; **49**: 555-563 [PMID: 24057163 DOI: 10.1007/s00535-013-0883-1]

31 **Toyoda H**, Kumada T, Tada T, Kaneoka Y, Maeda A, Kanke F, Satomura S. Clinical utility of highly sensitive Lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein & lt; 20 ng/mL. *Cancer Sci* 2011; **102**: 1025-1031 [PMID: 21244578 DOI: 10.1111/j.1349-7006.2011.01875.x]

32 **Witjes CD,** van Aalten SM, Steyerberg EW, Borsboom GJ, de Man RA, Verhoef C, Ijzermans JN. Recently introduced biomarkers for screening of hepatocellular carcinoma: a systematic review and meta-analysis. *Hepatol Int* 2013; **7**: 59-64 [PMID: 23519638 DOI: 10.1007/s12072-012-9374-3]

33 **Marrero JA**, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, Comunale MA, D'Amelio A, Lok AS, Block TM. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. *J Hepatol* 2005; **43**: 1007-1012 [PMID: 16137783 DOI: 10.1016/j.jhep.2005.05.028]

34 **Porta C**, De Amici M, Quaglini S, Paglino C, Tagliani F, Boncimino A, Moratti R, Corazza GR. Circulating interleukin-6 as a tumor marker for hepatocellular carcinoma. *Ann Oncol* 2008; **19**: 353-358 [PMID: 17962206 DOI: 10.1093/annonc/mdm448]

35 **Hsia CY**, Huo TI, Chiang SY, Lu MF, Sun CL, Wu JC, Lee PC, Chi CW, Lui WY, Lee SD. Evaluation of interleukin-6, interleukin-10 and human hepatocyte growth factor as tumor markers for hepatocellular carcinoma. *Eur J Surg Oncol* 2007; **33**: 208-212 [PMID: 17140760 DOI: 10.1016/j.ejso.2006.10.036]

36 **Giannelli G**, Fransvea E, Trerotoli P, Beaugrand M, Marinosci F, Lupo L, Nkontchou G, Dentico P, Antonaci S. Clinical validation of combined serological biomarkers for improved hepatocellular carcinoma diagnosis in 961 patients. *Clin Chim Acta* 2007; **383**: 147-152 [PMID: 17582392 DOI: 10.1016/j.cca.2007.05.014]

37 **Ertle JM**, Heider D, Wichert M, Keller B, Kueper R, Hilgard P, Gerken G, Schlaak JF. A combination of α-fetoprotein and des-γ-carboxy prothrombin is superior in detection of hepatocellular carcinoma. *Digestion* 2013; **87**: 121-131 [PMID: 23406785 DOI: 10.1159/000346080]

38 **Hadziyannis E**, Sialevris K, Georgiou A, Koskinas J. Analysis of serum α-fetoprotein-L3% and des-γ carboxyprothrombin markers in cases with misleading hepatocellular carcinoma total α-fetoprotein levels. *Oncol Rep* 2013; **29**: 835-839 [PMID: 23174906 DOI: 10.3892/or.2012.2147]

39 **Wang M**, Mehta A, Block TM, Marrero J, Di Bisceglie AM, Devarajan K. A comparison of statistical methods for the detection of hepatocellular carcinoma based on serum biomarkers and clinical variables. *BMC Med Genomics* 2013; **6** Suppl 3: S9 [PMID: 24564861 DOI: 10.1186/1755-8794-6-S3-S9]

40 **Johnson PJ**, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, Morse J, Hull D, Patman G, Kagebayashi C, Hussain S, Graham J, Reeves H, Satomura S. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 144-153 [PMID: 24220911 DOI: 10.1158/1055-9965.EPI-13-0870]

41 **Aravalli RN**, Steer CJ, Cressman EN. Molecular mechanisms of hepatocellular carcinoma. *Hepatology* 2008; **48**: 2047-2063 [PMID: 19003900 DOI: 10.1002/hep.22580]

42 **Liu CC**, Wang YH, Chuang EY, Tsai MH, Chuang YH, Lin CL, Liu CJ, Hsiao BY, Lin SM, Liu LY, Yu MW. Identification of a liver cirrhosis signature in plasma for predicting hepatocellular carcinoma risk in a population-based cohort of hepatitis B carriers. *Mol Carcinog* 2014; **53**: 58-66 [PMID: 22911910 DOI: 10.1002/mc.21952]

43 **Shi M**, Chen MS, Sekar K, Tan CK, Ooi LL, Hui KM. A blood-based three-gene signature for the non-invasive detection of early human hepatocellular carcinoma. *Eur J Cancer* 2014; **50**: 928-936 [PMID: 24332572 DOI: 10.1016/j.ejca.2013.11.026]

44 **Wang X**, Zhang A, Sun H. Power of metabolomics in diagnosis and biomarker discovery of hepatocellular carcinoma. *Hepatology* 2013; **57**: 2072-2077 [PMID: 23150189 DOI: 10.1002/hep.26130]

45 **Chen F**, Xue J, Zhou L, Wu S, Chen Z. Identification of serum biomarkers of hepatocarcinoma through liquid chromatography/mass spectrometry-based metabonomic method. *Anal Bioanal Chem* 2011; **401**: 1899-1904 [PMID: 21833635 DOI: 10.1007/s00216-011-5245-3]

46 **Wurmbach E**, Chen YB, Khitrov G, Zhang W, Roayaie S, Schwartz M, Fiel I, Thung S, Mazzaferro V, Bruix J, Bottinger E, Friedman S, Waxman S, Llovet JM. Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. *Hepatology* 2007; **45**: 938-947 [PMID: 17393520 DOI: 10.1002/hep.21622]

47 **Nam SW**, Park JY, Ramasamy A, Shevade S, Islam A, Long PM, Park CK, Park SE, Kim SY, Lee SH, Park WS, Yoo NJ, Liu ET, Miller LD, Lee JY. Molecular changes from dysplastic nodule to hepatocellular carcinoma through gene expression profiling. *Hepatology* 2005; **42**: 809-818 [PMID: 16175600 DOI: 10.1002/hep.20878]

48 **Llovet JM**, Chen Y, Wurmbach E, Roayaie S, Fiel MI, Schwartz M, Thung SN, Khitrov G, Zhang W, Villanueva A, Battiston C, Mazzaferro V, Bruix J, Waxman S, Friedman SL. A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. *Gastroenterology* 2006; **131**: 1758-1767 [PMID: 17087938 DOI: 10.1053/j.gastro.2006.09.014]

49 **Sakamoto M**, Mori T, Masugi Y, Effendi K, Rie I, Du W. Candidate molecular markers for histological diagnosis of early hepatocellular carcinoma. *Intervirology* 2008; **51** Suppl 1: 42-45 [PMID: 18544947 DOI: 10.1159/000122603]

50 **Mínguez B**, Lachenmayer A. Diagnostic and prognostic molecular markers in hepatocellular carcinoma. *Dis Markers* 2011; **31**: 181-190 [PMID: 22045404 DOI: 10.3233/DMA-2011-0841]

51 **Berindan-Neagoe I**, Monroig Pdel C, Pasculli B, Calin GA. MicroRNAome genome: a treasure for cancer diagnosis and therapy. *CA Cancer J Clin* 2014; **64**: 311-336 [PMID: 25104502 DOI: 10.3322/caac.21244]

52 **Link A**, Kupcinskas J, Wex T, Malfertheiner P. Macro-role of

microRNA in gastric cancer. *Dig Dis* 2012; **30**: 255-267 [PMID: 22722550 DOI: 10.1159/000336919]

53 **Arroyo JD**, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF, Pogosova-Agadjanyan EL, Stirewalt DL, Tait JF, Tewari M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci USA* 2011; **108**: 5003-5008 [PMID: 21383194 DOI: 10.1073/pnas.1019055108]

54 **Link A**, Goel A. MicroRNA in gastrointestinal cancer: a step closer to reality. *Adv Clin Chem* 2013; **62**: 221-268 [PMID: 24772669 DOI: 10.1016/B978-0-12-800096-0.00006-8]

55 **Redis RS**, Calin S, Yang Y, You MJ, Calin GA. Cell-to-cell miRNA transfer: from body homeostasis to therapy. *Pharmacol Ther* 2012; **136**: 169-174 [PMID: 22903157 DOI: 10.1016/j.pharmthera.2012.08.003]

56 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]

57 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]

58 **Weber JA**, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The microRNA spectrum in 12 body fluids. *Clin Chem* 2010; **56**: 1733-1741 [PMID: 20847327 DOI: 10.1373/clinchem.2010.147405]

59 **Link A**, Balaguer F, Shen Y, Nagasaka T, Lozano JJ, Boland CR, Goel A. Fecal MicroRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1766-1774 [PMID: 20551304 DOI: 10.1158/1055-9965.EPI-10-0027]

60 **Goel A**, Boland CR. Epigenetics of colorectal cancer. *Gastroenterology* 2012; **143**: 1442-1460.e1 [PMID: 23000599 DOI: 10.1053/j.gastro.2012.09.032]

61 **Ha M**, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 2014; **15**: 509-524 [PMID: 25027649 DOI: 10.1038/nrm3838]

62 **Lu J**, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]

63 **Volinia S**, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006; **103**: 2257-2261 [PMID: 16461460 DOI: 10.1073/pnas.0510565103]

64 **Hou J**, Lin L, Zhou W, Wang Z, Ding G, Dong Q, Qin L, Wu X, Zheng Y, Yang Y, Tian W, Zhang Q, Wang C, Zhang Q, Zhuang SM, Zheng L, Liang A, Tao W, Cao X. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011; **19**: 232-243 [PMID: 21316602 DOI: 10.1016/j.ccr.2011.01.001]

65 **Murakami Y**, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* 2006; **25**: 2537-2545 [PMID: 16331254 DOI: 10.1038/sj.onc.1209283]

66 **Toffanin S**, Hoshida Y, Lachenmayer A, Villanueva A, Cabellos L, Minguez B, Savic R, Ward SC, Thung S, Chiang DY, Alsinet C, Tovar V, Roayaie S, Schwartz M, Bruix J, Waxman S, Friedman SL, Golub T, Mazzaferro V, Llovet JM. MicroRNA-based classification of hepatocellular carcinoma and oncogenic role of miR-517a. *Gastroenterology* 2011; **140**: 1618-1628.e16 [PMID: 21324318 DOI: 10.1053/j.gastro.2011.02.009]

67 **Giordano S**, Columbano A. MicroRNAs: new tools for diagnosis, prognosis, and therapy in hepatocellular carcinoma? *Hepatology* 2013; **57**: 840-847 [PMID: 23081718 DOI: 10.1002/hep.26095]

68 **Li LM**, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, Shen HB, Zhang CY, Zen K. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010; **70**: 9798-9807 [PMID: 21098710 DOI: 10.1158/0008-5472.CAN-10-1001]

69 **Zhou J**, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang JF, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H, Fan J. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 4781-4788 [PMID: 22105822 DOI: 10.1200/JCO.2011.38.2697]

70 **Tomimaru Y**, Eguchi H, Nagano H, Wada H, Kobayashi S, Marubashi S, Tanemura M, Tomokuni A, Takemasa I, Umeshita K, Kanto T, Doki Y, Mori M. Circulating microRNA-21 as a novel biomarker for hepatocellular carcinoma. *J Hepatol* 2012; **56**: 167-175 [PMID: 21749846 DOI: 10.1016/j.jhep.2011.04.026]

71 **Xu J**, Wu C, Che X, Wang L, Yu D, Zhang T, Huang L, Li H, Tan W, Wang C, Lin D. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol Carcinog* 2011; **50**: 136-142 [PMID: 21229610 DOI: 10.1002/mc.20712]

72 **Qi P**, Cheng SQ, Wang H, Li N, Chen YF, Gao CF. Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. *PLoS One* 2011; **6**: e28486 [PMID: 22174818 DOI: 10.1371/journal.pone.0028486]

73 **Bihrer V**, Waidmann O, Friedrich-Rust M, Forestier N, Susser S, Haupenthal J, Welker M, Shi Y, Peveling-Oberhag J, Polta A, von Wagner M, Radeke HH, Sarrazin C, Trojan J, Zeuzem S, Kronenberger B, Piiper A. Serum microRNA-21 as marker for necroinflammation in hepatitis C patients with and without hepatocellular carcinoma. *PLoS One* 2011; **6**: e26971 [PMID: 22066022 DOI: 10.1371/journal.pone.0026971]

74 **Tan Y**, Ge G, Pan T, Wen D, Gan J. A pilot study of serum microRNAs panel as potential biomarkers for diagnosis of nonalcoholic fatty liver disease. *PLoS One* 2014; **9**: e105192 [PMID: 25141008 DOI: 10.1371/journal.pone.0105192]

75 **Mitsuhashi N**, Kobayashi S, Doki T, Kimura F, Shimizu H, Yoshidome H, Ohtsuka M, Kato A, Yoshitomi H, Nozawa S, Furukawa K, Takeuchi D, Suda K, Miura S, Miyazaki M. Clinical significance of alpha-fetoprotein: involvement in proliferation, angiogenesis, and apoptosis of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2008; **23**: e189-e197 [PMID: 18466288 DOI: 10.1111/j.1440-1746.2008.05340.x]

76 **Llovet JM**, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2290-2300 [PMID: 22374331 DOI: 10.1158/1078-0432.CCR-11-2175]

77 **Matsuda M**, Asakawa M, Amemiya H, Fujii H. Lens culinaris agglutinin-reactive fraction of AFP is a useful prognostic biomarker for survival after repeat hepatic resection for HCC. *J Gastroenterol Hepatol* 2011; **26**: 731-738 [PMID: 21155886 DOI: 10.1111/j.1440-1746.2010.06532.x]

78 **Saito Y**, Shimada M, Utsunomiya T, Morine Y, Imura S, Ikemoto T, Mori H, Hanaoka J, Yamada S, Asanoma M. Prediction of recurrence of hepatocellular carcinoma after curative hepatectomy using preoperative Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein. *Hepatol Res* 2012; **42**: 887-894 [PMID: 22524419 DOI: 10.1111/j.1872-034X.2012.01004.x]

79 **Kiriyama S**, Uchiyama K, Ueno M, Ozawa S, Hayami S, Tani M, Yamaue H. Triple positive tumor markers for hepatocellular carcinoma are useful predictors of poor survival. *Ann Surg* 2011; **254**: 984-991 [PMID: 21606837 DOI: 10.1097/SLA.0b013e3182215016]

80 **Toyoda H**, Kumada T, Osaki Y, Oka H, Urano F, Kudo M, Matsunaga T. Staging hepatocellular carcinoma by a novel scoring system (BALAD score) based on serum markers. *Clin Gastroenterol*

Hepatol 2006; **4**: 1528-1536 [PMID: 17162244 DOI: 10.1016/j.cgh.2006.09.021]

81 **Fox R**, Berhane S, Teng M, Cox T, Tada T, Toyoda H, Kumada T, Kagebayashi C, Satomura S, Johnson PJ. Biomarker-based prognosis in hepatocellular carcinoma: validation and extension of the BALAD model. *Br J Cancer* 2014; **110**: 2090-2098 [PMID: 24691419 DOI: 10.1038/bjc.2014.130]

82 **Kaseb AO**, Morris JS, Hassan MM, Siddiqui AM, Lin E, Xiao L, Abdalla EK, Vauthey JN, Aloia TA, Krishnan S, Abbruzzese JL. Clinical and prognostic implications of plasma insulin-like growth factor-1 and vascular endothelial growth factor in patients with hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 3892-3899 [PMID: 21911725 DOI: 10.1200/JCO.2011.36.0636]

83 **Huang Y**, Yang X, Zhao F, Shen Q, Wang Z, Lv X, Hu B, Yu B, Fan J, Qin W. Overexpression of Dickkopf-1 predicts poor prognosis for patients with hepatocellular carcinoma after orthotopic liver transplantation by promoting cancer metastasis and recurrence. *Med Oncol* 2014; **31**: 966 [PMID: 24878698 DOI: 10.1007/s12032-014-0966-8]

84 **Tung EK**, Mak CK, Fatima S, Lo RC, Zhao H, Zhang C, Dai H, Poon RT, Yuen MF, Lai CL, Li JJ, Luk JM, Ng IO. Clinicopathological and prognostic significance of serum and tissue Dickkopf-1 levels in human hepatocellular carcinoma. *Liver Int* 2011; **31**: 1494-1504 [PMID: 21955977 DOI: 10.1111/j.1478-3231.2011.02597.x]

85 **Wang L**, Yao M, Dong Z, Zhang Y, Yao D. Circulating specific biomarkers in diagnosis of hepatocellular carcinoma and its metastasis monitoring. *Tumour Biol* 2014; **35**: 9-20 [PMID: 24006223 DOI: 10.1007/s13277-013-1141-0]

86 **Li J**, Wang Y, Yu W, Chen J, Luo J. Expression of serum miR-221 in human hepatocellular carcinoma and its prognostic significance. *Biochem Biophys Res Commun* 2011; **406**: 70-73 [PMID: 21295551 DOI: 10.1016/j.bbrc.2011.01.111]

87 **Köberle V**, Kronenberger B, Pleli T, Trojan J, Imelmann E, Peveling-Oberhag J, Welker MW, Elhendawy M, Zeuzem S, Piiper A, Waidmann O. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. *Eur J Cancer* 2013; **49**: 3442-3449 [PMID: 23810247 DOI: 10.1016/j.ejca.2013.06.002]

88 **Li X**, Yang W, Lou L, Chen Y, Wu S, Ding G. microRNA: a promising diagnostic biomarker and therapeutic target for hepatocellular carcinoma. *Dig Dis Sci* 2014; **59**: 1099-1107 [PMID: 24390674 DOI: 10.1007/s10620-013-3006-1]

89 **Ura S**, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Sunakozaka H, Sakai Y, Horimoto K, Kaneko S. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology* 2009; **49**: 1098-1112 [PMID: 19173277 DOI: 10.1002/hep.22749]

90 **Budhu A**, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, Zanetti KA, Ye QH, Qin LX, Croce CM, Tang ZY, Wang XW. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology* 2008; **47**: 897-907 [PMID: 18176954 DOI: 10.1002/hep.22160]

91 **Janssen HL**, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, van der Meer AJ, Patick AK, Chen A, Zhou Y, Persson R, King BD, Kauppinen S, Levin AA, Hodges MR. Treatment of HCV infection by targeting microRNA. *N Engl J Med* 2013; **368**: 1685-1694 [PMID: 23534542 DOI: 10.1056/NEJMoa1209026]

92 **Hoshida Y**, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 1995-2004 [PMID: 18923165 DOI: 10.1056/NEJMoa0804525]

93 **Hoshida Y**, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM, Golub TR. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009; **69**: 7385-7392 [PMID: 19723656 DOI: 10.1158/0008-5472.CAN-09-1089]

94 **Villanueva A**, Hoshida Y, Battiston C, Tovar V, Sia D, Alsinet C, Cornella H, Liberzon A, Kobayashi M, Kumada H, Thung SN, Bruix J, Newell P, April C, Fan JB, Roayaie S, Mazzaferro V, Schwartz ME, Llovet JM. Combining clinical, pathology, and gene expression data to predict recurrence of hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 1501-15122.e2 [PMID: 21320499 DOI: 10.1053/j.gastro.2011.02.006]

95 **Nault JC**, De Reyniès A, Villanueva A, Calderaro J, Rebouissou S, Couchy G, Decaens T, Franco D, Imbeaud S, Rousseau F, Azoulay D, Saric J, Blanc JF, Balabaud C, Bioulac-Sage P, Laurent A, Laurent-Puig P, Llovet JM, Zucman-Rossi J. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. *Gastroenterology* 2013; **145**: 176-187 [PMID: 23567350 DOI: 10.1053/j.gastro.2013.03.051]

96 **Lim HY**, Sohn I, Deng S, Lee J, Jung SH, Mao M, Xu J, Wang K, Shi S, Joh JW, Choi YL, Park CK. Prediction of disease-free survival in hepatocellular carcinoma by gene expression profiling. *Ann Surg Oncol* 2013; **20**: 3747-3753 [PMID: 23800896 DOI: 10.1245/s10434-013-3070-y]

97 **Kurokawa Y**, Matoba R, Takemasa I, Nagano H, Dono K, Nakamori S, Umeshita K, Sakon M, Ueno N, Oba S, Ishii S, Kato K, Monden M. Molecular-based prediction of early recurrence in hepatocellular carcinoma. *J Hepatol* 2004; **41**: 284-291 [PMID: 15288478]

98 **Yoshioka S**, Takemasa I, Nagano H, Kittaka N, Noda T, Wada H, Kobayashi S, Marubashi S, Takeda Y, Umeshita K, Dono K, Matsubara K, Monden M. Molecular prediction of early recurrence after resection of hepatocellular carcinoma. *Eur J Cancer* 2009; **45**: 881-889 [PMID: 19167881]

99 **Woo HG,** Park ES, Cheon JH, Kim JH, Lee JS, Park BJ, Kim W, Park SC, Chung YJ, Kim BG, Yoon JH, Lee HS, Kim CY, Yi NJ, Suh KS, Lee KU, Chu IS, Roskams T, Thorgeirsson SS, Kim YJ. Gene expression-based recurrence prediction of hepatitis B virus-related human hepatocellular carcinoma. *Clin Cancer Res* 2008; **14**: 2056-64 [PMID: 18381945 DOI: 10.1158/1078-0432.CCR-07-1473]

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REVIEW

Gene therapeutic approaches to inhibit hepatitis B virus replication

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Abstract

Acute and chronic hepatitis B virus (HBV) infections remain to present a major global health problem. The infection can be associated with acute symptomatic or asymptomatic hepatitis which can cause chronic inflammation of the liver and over years this can lead to cirrhosis and the development of hepatocellular carcinomas. Currently available therapeutics for chronically infected individuals aim at reducing viral replication and to slow down or stop the progression of the disease. Therefore, novel treatment options are needed to efficiently combat and eradicate this disease. Here we provide a state of the art overview of gene therapeutic approaches to inhibit HBV replication. We discuss non-viral and viral approaches which were explored to deliver therapeutic nucleic acids aiming at reducing HBV replication. Types of delivered therapeutic nucleic acids which were studied since many years include antisense oligodeoxynucleotides and antisense RNA, ribozymes and DNAzymes, RNA interference, and external guide sequences. More recently designer nucleases gained increased attention and were exploited to destroy the HBV genome. In addition we mention other strategies to reduce HBV replication based on delivery of DNA encoding dominant negative mutants and DNA vaccination. In combination with available cell culture and animal models for HBV infection, *in vitro* and *in vivo* studies can be performed to test efficacy of gene therapeutic approaches. Recent progress but also challenges will be specified and future perspectives will be discussed. This is an exciting time to explore such approaches because recent successes of gene therapeutic strategies in the clinic to treat genetic diseases raise hope to find alternative treatment options for patients chronically infected with HBV.

Key words: Gene therapy; Hepatitis B virus; Antisense nucleic acid; RNA interference; Designer nuclease; Ribozyme; DNAzyme; Dominant negative mutant; External guide sequence; DNA vaccination

Core tip: With various successful clinical trials ongoing, gene therapeutic approaches gained increasing

attention in the community over the recent years. Here we introduce gene therapy as a versatile platform for treatment of hepatitis B (HBV) virus infection. Newest delivery methods based on non-viral and viral techniques combined with most advanced technologies for inhibition of HBV replication based on DNA, RNA and designer nucleases are discussed. An overview of various gene therapeutic systems which were explored *in vitro* and *in vivo* is provided. Advantages but also limitations of the different strategies to inhibit HBV replication are mentioned.

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INTRODUCTION

Hepatitis B virus

The hepatitis B virus (HBV) is an enveloped, partially double-stranded DNA virus which replicates through an RNA intermediate. Upon cell entry the DNA containing core particle is transported to the nucleus where the DNA is released. Next the partial double-stranded DNA is repaired by host enzymes to form the covalently closed circular DNA (cccDNA). The cccDNA serves as a template for transcription of viral proteins and reverse transcription of new viral genomes by the viral polymerase. The large 3.5 kb HBV transcript represents the pregenomic RNA serving as a template for virus replication. Furthermore, there are three additional mRNAs with a length of 2.4, 2.1, and 0.9 kb. The HBV genome is 3.2 kb in size and contains four overlapping major open reading frames tightly arranged that encode polymerase, surface (HBsAg), core (HBcAg) and X proteins (HBx)[1,2]. In addition, especially early during infection the HBV early antigen (HBeAg) can be detected which is a proteolytic product of the pre-core protein.

HBV infection counts as a major global health problem since more than two billion people show evidence of a past or present infection with the virus. This hepatotropic virus can cause acute and chronic infection of the liver. Fortunately, for most people the infection proceeds nearly without symptoms when taking an acute course of disease and complete recovery is likely. However, 240 million people suffer from chronic HBV infection and more than 780000 people die every year because of hepatitis B related secondary diseases. Mostly newborns and infants are prone to develop the chronic type of the infection[3] and so far no treatment is available that reliable cures those patients.

Current therapeutics for chronic HBV infection are intended to reduce viral replication and slow down or stop the progression of the disease. To date there are seven Food and Drug Administration (FDA) approved compounds for the treatment of chronic hepatitis B. These include interferon alpha and pegylated interferon alpha, nucleoside analogues (lamivudine, entecavir and telbivudine) and nucleotide analogues (adefovir, dipivoxil and tenofovir)[4].

Interferon alpha has an antiviral effect by inhibiting the synthesis of viral DNA and activating antiviral enzymes and additionally, acts in an immunomodulatory way by enhancing the cellular immune response against infected cells[5]. It has to be administered daily or three times a week as unmodified version and once in a week in the pegylated form. The main disadvantages of interferon alpha are the parenteral administration causing discomfort to the patients and potential adverse effects such as flu-like symptoms in the beginning of treatment and later on for instance fatigue and low blood counts. It is only given to selected patients because under certain conditions administration of interferon alpha is contraindicated[4]. In a long-term follow-up study of HBeAg-positive patients, 11% lost HBsAg after treatment with interferon alpha[6] which is considered as a cure of the disease.

Nucleos(t)ide analogues interfere with the HBV replication primarily by targeting the HBV polymerase functions such as reverse transcriptase and DNA polymerase activity[5]. These drugs are administered orally as a daily dose. The major limitation associated with nucleos(t)ide analogues is the emergence of antiviral drug resistance and that life-long treatment can be indicated in the presence of chronic infection. In this context failure of medication adherence is another problem, because viral relapse is common when ending the treatment. Another prognostic marker of HBV infection is the presence of HBeAg which correlates with high viral replication rates. However, HBeAg seroconversion can be achieved with nucleos(t)ide treatment. In addition, HBV DNA levels can be decreased to an undetectable level but at the same time HBsAg is not lost[4]. These features demonstrate another peculiarity of the hepatitis B virus. After entry into a cell the cccDNA is maintained as an episomally maintained template in the nucleus. It is not attacked by nucleos(t)ide analogues nor by interfon in general, so that it is able to serve as a reservoir from which previously cleared or treated infections can recur[4]. The clinical management of chronic hepatitis B infection is reviewed in detail by Santantonio and Fasano[7].

The viral reservoir and potential reactivation of the virus represents a major problem when developing novel HBV treatments options and this is a challenge that could be faced by gene therapy. Researchers seek to inhibit viral replication in a long-lasting manner without the need for continuous drug administration through gene therapeutic approaches.

The more ambitious goal is to completely eradicate the viral cccDNA depot and hence find a true cure for chronic hepatitis B virus infection. Here we discuss gene therapeutic approaches as a versatile platform to combat HBV infection.

Gene therapy

Gene therapy is a strategy to transfer therapeutic nucleic acids into the desired target cell for treatment of a variety of different diseases. To efficiently deliver the genetic payload, multiple gene transfection techniques were explored which can be subdivided into two major groups: virus-based and non-viral vector systems for delivery of respective therapeutic nucleic acid. Both delivery techniques were also utilized in gene therapeutic approaches to treat chronic infectious diseases such as HBV infection. Since HBV infection resides in liver, the majority of gene transfer approaches were focused on targeting hepatocytes.

Non-viral vectors are based on delivery of naked RNA or DNA which in combination with chemical and physical means can result in efficient delivery of the nucleic acid into the respective target cell[8,9]. Chemical methods in the context of non-viral vector delivery rely on various chemical formulations such as cationic lipids[10] and polymers including polyamidoamine dendrimers and polyethylenimine (PEI)[11]. All chemical reagents were explored in different approaches and there are several commercially available transfection reagents which are commonly used for transfection of DNA and RNA resulting in sufficient transfer efficiencies in many cell lines *in vitro*. Major constraints of these methods are transfection reagent-associated toxicity and the difficulty to cross the nuclear membrane. In addition to chemical transfer methods, physical transfer techniques were explored involving needle injection[12], gene gun[13], electroporation[14], sonoporation[15], magnetofection[16], and hydrodynamic gene transfer[17]. These methods directly deliver therapeutic nucleic acid into the cytosol of the target cell and compared to chemical methods these techniques harbor a reduced risk of transfection-mediated side effects due to dispersion of the transfection reagent. However, limitations of these methods are exposed by the difficulty to cross the nuclear membrane, potential cellular damage caused by the transfection method, and the requirement of costly instruments.

Virus-based transfection techniques were utilized in numerous pre-clinical and clinical gene therapeutic applications. Predominantly used viral vectors can be attributed to three viruses which were converted into viral vectors by deletion of essential viral genes: adenovirus, adeno-associated virus (AAV) and retrovirus. All viral vector systems display advantages and disadvantages which were discussed in more detail in previous reviews[18]. Adenoviruses combine a large transgene capacity of up to 36 kilo bases (kb), an episomal nature of the adenoviral genome reducing the risk of genotoxicity, the possibility to produce high viral titers and the ability to transduce dividing and non-dividing cells at high efficiencies *in vitro* and *in vivo*[19]. However, one major obstacle for *in vivo* applications are the innate and the adaptive immune responses induced by the incoming adenoviral particle. AAV vectors were explored in clinical trials, are non-pathogenic, lead to a reduced immune response and predominantly exist as extrachromosomal vector genomes in the transduced cell[20]. One major disadvantage, however, is the small transgene capacity which is below 5 kb. Lentiviral vectors[21] were broadly explored in clinical trials to treat rare genetic diseases in *ex vivo* gene therapeutic approaches. Various generations of lentiviral vectors are available which carry a transgene capacity of up to 8 kb. Although commonly used lentiviral vectors integrate their genetic cargo into the host genome, newest versions of these vectors can circumvent side effects associated with somatic integration by changing their integration profile.

Various non-viral and viral transfer techniques were exploited to combat HBV infection *in vitro* and *in vivo* which will be discussed in the following paragraphs.

GENE THERAPEUTIC APPROACHES AGAINST HBV

Various gene therapeutic approaches to treat HBV infection were studied in cell culture models and in animal models for HBV infection. Within the viral life cycle in an infected cell there are various points of attack which can serve as targets in gene therapeutic approaches to inhibit HBV replication. Figure 1 schematically shows the life cycle of HBV infection and indicates points of attack when considering a gene therapeutic treatment.

As shown in Figure 1 the mechanisms of viral inhibition in gene therapeutic approaches can be on the level of RNA (HBV derived transcripts), DNA (cccDNA) and proteins. On the RNA level antisense oligodeoxynucleotides and antisense RNA, catalytic nucleic acids such as ribozymes and DNAzymes, RNA interference and external guide sequences (EGS) can be considered. On the level of DNA (cccDNA) as a potential target designer nuclease such as zinc finger nuclease (ZFN), transcription activator-like effector nucleases (TALEN) and the *clustered regularly interspaced short palindromic repeats* (CRISPR)/Cas9 technology can be used. On the level of proteins, dominant negative HBV mutants and a strategy based on capsid-targeted viral inactivation (CTVI) were studied. Another technology

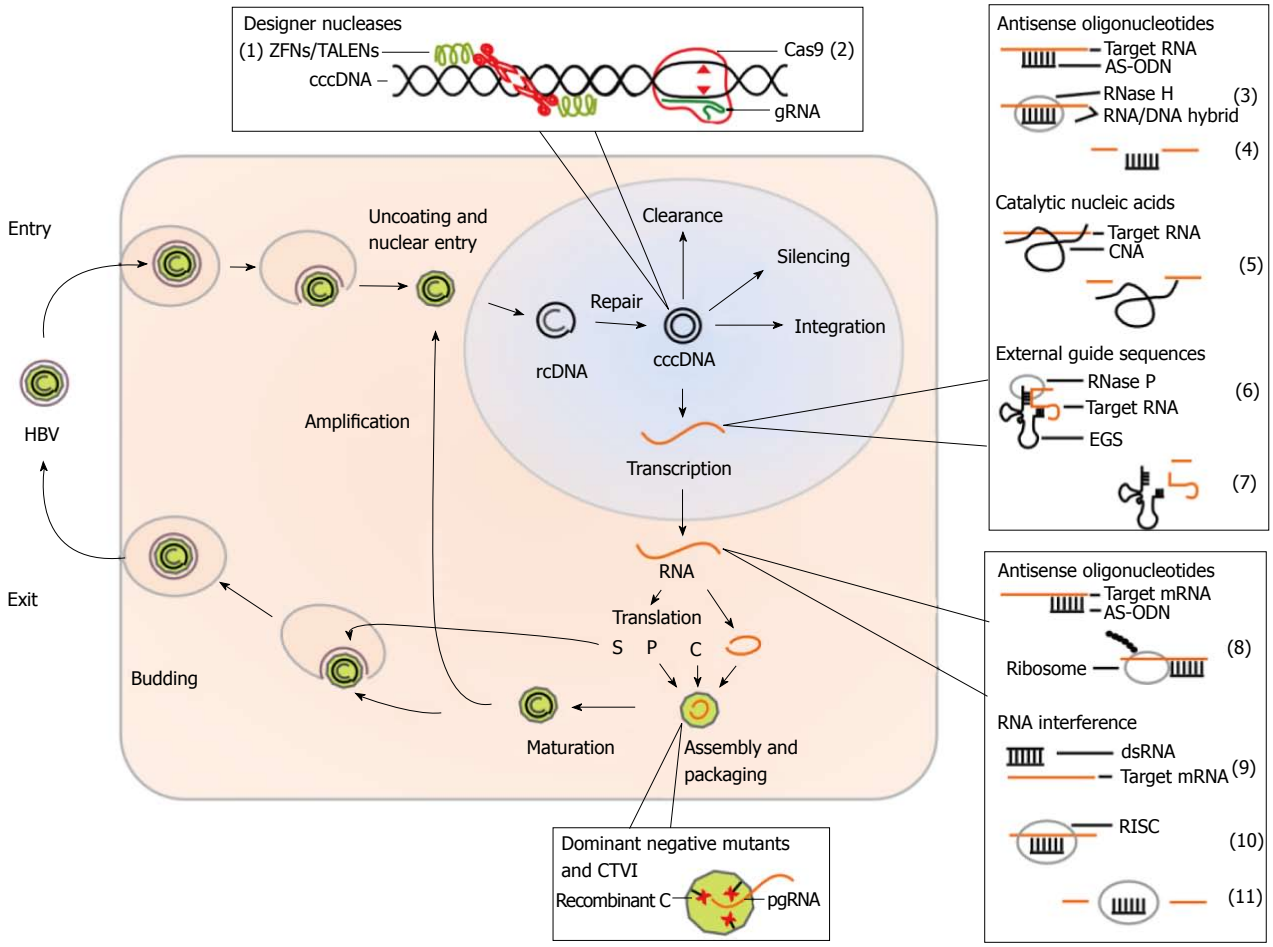

Figure 1 Hepatitis B virus replication cycle and gene therapeutic strategies. Enveloped virions of hepatitis B virus (HBV) infect liver cells *via* attachment to the cell membrane and endocytosis. The capsid with the relaxed circular (rc) DNA is released into the cytoplasm and the DNA is uncoated upon nuclear entry. In the nucleus the rcDNA is repaired to form the covalently closed circular (ccc) DNA. The cccDNA can be eventually cleared out, silenced or integrated into the host genome. Predominantly, it persists as an episome in the nucleus and is transcribed and translated by the host cell machinery. One of the transcripts forms the pregenomic (pg) RNA which is encapsulated together with the translated viral polymerase (P) by the translated viral capsid proteins (C). In the newly assembled nucleocapsid the pgRNA serves as a template for the viral polymerase which synthesizes the rcDNA. The nucleocapsid either migrates back to the nucleus to increase the pool of cccDNA or is internalized by the endoplasmatic reticulum (ER). In the latter process it is enveloped with ER-membrane that harbors translated viral surface proteins (S) and finally released from the cell. Gene therapeutic strategies act on several steps of the viral replication cycle. Designer nucleases are intended to promote disruption of the cccDNA. Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (1) use protein-based DNA-binding modules, while the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 nuclease system (2) is directed by a guide RNA (gRNA) to the target site. Antisense oligonucleotides (AS-ODNs) act inhibitory on the viral replication in two ways. In the nucleus they recruit RNase H (3) after the formation of RNA/DNA hybrids, which cleaves the viral RNA (4). In the cytosol they bind to the viral RNA which leads to a steric blockade of subsequent processes (8). Catalytic nucleotides (CNA) as ribozymes and DNAzymes are able to cleave targeted RNA by themselves (5). External guide sequences (EGS) are designed in a way that they resemble precursor tRNAs (6) when they bind to their target RNA and trigger cleavage by RNase P (7). RNA interference can be induced by different dsRNA species (9). Longer dsRNAs can be delivered exogenously or expressed in the cells as shRNAs. The RNAs are processed to approximately 21 nucleotide long dsRNAs termed siRNAs which are used to form the large RNA-induced silencing complex (RISC) (10) which degrades target messenger RNAs (mRNAs) (11). Dominant negative mutants of capsid proteins form a hindrance for proper packaging of viral progenitor RNA. In contrast, capsid-targeted viral inactivation (CTVI) is an approach where the capsid proteins are additionally fused to a destructive compound.

for prevention of infection but also for a potential treatment option of chronically infected patients, DNA vaccination can be considered as an attractive alternative. Although the majority of the described strategies for inhibition of HBV replication were not translated into the clinic so far, we believe that gene therapy may represent a valuable alternative in the future. Studies describing milestones of the various gene therapeutic approaches are listed in Table 1 and are described in more detail in the following sections.

Antisense nucleic acids

The beginning of gene therapy against HBV can be linked to the first tests of antisense oligodeoxynucleotides (AS-ODNs) directed against the HBV genome[22-24]. With respect to antisense nucleic acids post-transcriptional inhibition is achieved by blockade of ribosomal access, inhibition of ribosomal assembly and induction of RNase H cleavage[25].

First *in vivo* studies showed applicability of this approach in duck-HBV-infected Peking ducklings. Infected animals were treated daily by intravenous

Table 1 Advances in hepatitis B virus infection gene therapy in chronological order

Year	Strategy	Milestone	Ref.
1990	AS-ODN	First *in vitro* application	[22]
1992	AS-ODN	First non-viral transfection (targeted polycation peptide complex)	[28]
	Ribozyme	First *in vitro* application	[44]
1993	AS-ODN	First *in vivo* application	[26]
	DNA vaccination		[75]
1994	Dominant negative mutants	First *in vitro* application	[65]
1997	AS-ODN	First viral transduction (retroviral)	[39]
	Ribozyme		[46]
1998	DNAzyme	First *in vitro* application	[60]
	EGS		[87]
2001	CTVI		[69]
2003	RNAi	First *in vitro* application	[93]
		First *in vivo* application	[94]
2004	Ribozyme	First *in vivo* application	[56]
	DNA vaccination	First clinical trial in chronic HBV carriers	[81]
2008	CTVI	First *in vivo* application	[72]
2010	ZFN	First *in vitro* application	[138]
2011	RNAi	First clinical trial in chronic HBV carriers	[117]
2013	EGS	First *in vivo* application	[89]
	TALEN	First *in vitro*/*vivo* application	[146]
2014	CRISPR/Cas9	First *in vitro*/*vivo* application	[148]

AS-ODN: Antisense oligonucleotides; HBV: Hepatitis B virus; EGS: External guide sequence; CTVI: Capsid-targeted viral inactivation; RNAi: RNA interference; ZFN: Zinc finger nuclease; TALEN: Transcription activator-like effector nuclease; CRISPR: Clustered regularly interspaced short palindromic repeats; Cas9: Cas9 nuclease.

injection of AS-ODNs for ten days. The treatment resulted in nearly complete inhibition of viral replication which was assessed by liver DNA analysis for DNA replicative intermediates and blockade of viral gene expression as demonstrated by disappearance of surface antigen in serum and core antigen in liver[26]. AS-ODNs proved to be most effective when directed against the initiation site of the HBsAg-gene[22] or at the encapsidation signal[27]. Except for a study published by Wu *et al*[28] in which already targeted DNA complexes were used, delivery of AS-ODNs was initially restricted to simple cellular uptake and binding of the unmodified antisense DNA to its target sites. Soon the system was improved by enhancing stability of the respective nucleic acids and by increasing uptake of AS-ODNs by the chosen target cell[29-32]. In other studies AS-ODNs were conjugated to ribonuclease H or manganese porphyrin which after binding to the target site can lead to cleavage of the desired target sequences[33,34]. Furthermore, DNA carrier systems were used and also enhanced by making them targetable to hepatocytes[28,35,36].

Other oligonucleotide based approaches include antisense RNA delivered by episomally replicating expression vectors[37,38] or retroviral vectors[39,40]. The advantage of these approaches is the fact that antisense RNAs can be expressed in the cells, whereas AS-ODNs have to be exogenously delivered. This allows for experimental settings with long-term effects. For instance efficacies lasting longer than ten months were observed after stable transfection of antisense RNA expression vectors[37]. Antisense RNA-mediated inhibition functions preferentially through destabilization of the sense RNA by targeting the antisense/sense-RNA duplex to dsRNase[41].

A completely different idea unrelated to complementary antisense RNA was introduced by Hafkemeyer *et al*[42] and is based on so-called "antisense-toxin-RNA". In this approach the authors took advantage of the HBV reverse transcriptase in infected cells to selectively kill those cells through Pseudomonas exotoxin expression from reverse transcribed antisense-toxin-RNA.

Site-specifically cleaving nucleic acids

There are two types of catalytically active nucleic acids, ribozymes and DNAzymes. Ribozymes are naturally occurring RNA molecules that can execute enzymatic activity on itself in the absence of proteins (in *cis*) or on extrinsic targets (in *trans*). DNAzymes were generated by *in vitro* evolution. They resemble ribozymes and do not exist in nature. The substrate for both species is RNA. There are various types of ribozymes known, hammerhead and hairpin ribozymes being the most popular ones. All catalytic nucleic acids have in common that they consist of an antisense sequence recognizing the target site and a catalytic domain mediating cleavage[43]. Scientists were able to manipulate the recognition of target sites, rendering such nucleic acids very attractive for enhanced transient knockdown of gene expression.

The first experiments with ribozymes targeting the HBV genome were performed in 1992 using a triple ribozyme construct. In this study three hammerhead ribozymes were encoded on a single

DNA template and it was shown that they were simultaneously active *in vitro*. However, cleavage kinetics were similar to single ribozyme constructs. Nonetheless, this approach may still be favorable because it enables facing high target variability and emergence of viral resistance[44]. However, first studies performed in a cellular context disclosed a first drawback of the hammerhead ribozymes because they were active after *in vitro* transcription and in Mg^{2+}-supplemented cell extracts, but not in intact cells. The authors suggested some non-viral block, or inappropriate target site selection being responsible for the lack of intracellular activity[45].

However, to overcome these problems novel strategies were pursued using other types of ribozymes. Welch and colleagues[46] designed several hairpin ribozymes against different conserved regions of the HBV genome and tested them in a human hepatoma cell line (Huh7) transfected with full-length HBV genomes. The ribozymes were transduced into target cells using a retroviral vector system. The HBV production could be inhibited to up to 83% assayed through an endogenous polymerase assay. In the following years further *in vitro* studies were performed using different types of modified ribozymes[47-51]. These were predominantly delivered *via* transfection of an expression plasmid into various cell lines which were additionally transfected with a HBV genome-containing plasmid resulting in varying effects on inhibition of HBV replication[52-55].

In 2004 the first *in vivo* experiment in a transgenic mouse model was conducted by Pan *et al*[56]. For this study a self-processing triple-ribozyme cassette was used, with ribozymes acting in *cis* and in *trans.* The constructs were packaged in liposomes that where targeted to hepatocytes in the presence of asialofetuin. Quantitative PCR analysis for quantification of HBV genome copy numbers in murine liver showed a more than 80% decrease of HBV genome copy numbers and immunohistochemistry revealed a robust reduction in the number of hepatocytes staining positive for HBV core antigen. This was the first proof of concept demonstrating *in vivo* feasibility of viral RNA degradation mediated by ribozymes.

Next, recombinant hepatitis D virus (HDV)[57] as well as lentiviral vectors were utilized as delivery vehicles for respective ribozymes which achieved effective reduction of HBV mRNA levels over four months[58]. Furthermore, HDV-derived ribozymes were also utilized which were delivered by a pseudotyped retroviral vector (Moloney murine leukemia virus)[59]. According to the authors the main advantages of these ribozymes are the natural activity of HDV ribozymes in human cells even at physiological Mg^{2+}-ion concentrations and the comparably highest cleavage rates among all known ribozymes. Their results revealed significant reduction in the intracellular HBV DNA concentration in HepG2.2.15 cells, which secrete infectious HBV virions. Furthermore, decreased extracellular HBsAg and HBeAg levels were observed after treatment with HDV ribozymes in comparison to the negative control. The conclusion was that using this strategy, HBV can be effectively inhibited at post-transcription and replication levels.

DNAzymes cleave RNA substrates based on a similar mechanism also used by ribozymes. However, they may be superior to ribozymes because their production is comparably straight forward and DNAzymes are less sensitive to chemical and enzymatic degradation. The first DNAzyme directed against HBV mRNA was created by Asahina *et al*[60]. They targeted the direct repeat 1 (DR1) and polyadenylation signal regions of HBV. In this study the authors used stabilized forms of DNAzymes that on the one hand lost some degree of activity compared to unmodified versions but on the other hand the degradation level was less pronounced. The DNAzyme was tested in Huh7 cells on an HBV-luciferase fusion reporter system where it exhibited 48% suppression compared to untreated control groups. Further *in vitro* studies[61-64], however, revealed a major disadvantage of this system because intracellular expression of these DNA species is not feasible. DNAzymes have to be transfected directly because they act on post-transcriptional level which is clearly disadvantageous if long-term administration for instance in clinical applications is required.

Dominant negative mutants and CTVI

Dominant negative mutants of viral proteins are able to inhibit viral replication by interfering with the function of the wild type protein. The first study on the molecular effects of dominant negative mutants on the HBV replication was conducted in 1994 by Scaglioni *et al*[65] They mutated the core protein and observed an inhibition of viral replication by 90%-95%[65] and it was concluded that this was the result of the disruption of the viral nucleocapsid assembly process. In a follow-up study the authors provided a delivery system using retroviral and adenoviral expression vectors[66]. von Weizsäcker *et al*[67] showed that carboxy-terminal, but not amino-terminal core mutants inhibit viral replication. Furthermore, it was discussed that rather the packaging of the viral pre-genome and the reverse transcription reaction within the particles than the nucleocapsid formation itself is restrained by dominant negative core mutants[68]. However, it was also shown that some mixed particles retain replication competency suggesting a possible mechanism of viral escape.

Using the principle of CTVI may represent an interesting variant of capsid modification, as the goal is to introduce a destructive element into the virus. The first time this technology was pursued

was by fusing a Ca^{2+}-dependent nuclease from *Staphylococcus aureus* to the HBV core protein. This led to inhibition of proper synthesis of viral DNA inside the capsid and rapid viral DNA degradation[69]. Later human eosinophil-derived neurotoxin was fused to the HBV core protein by another group and thoroughly studied also *in vivo*[70-72].

Therapeutic DNA vaccination

Initial considerations to use vaccination in a therapeutic manner against HBV infection were made in 1993 by a group around Christian Bréchot[73,74]. This approach was based on classical vaccination compounds. Also in 1993 the first genetic immunization with expression vectors for HBsAg was introduced in mice[75]. In a further step Mancini *et al*[76] proved that a combination of both strategies is feasible and they showed efficacy in a HBsAg transgenic mouse as a model for chronic HBV infection. A single intramuscular injection of HBV envelope encoding plasmid DNA resulted in the elimination of serum HBV antigen levels as early as 4 wk after administration of the therapeutic DNA in some mice. Notably no liver cell injury was detected. Soon genetic immunization employing retroviral delivery was tested first in rhesus monkeys[77] and then in chimpanzees chronically infected with HBV[78]. The first time a DNA vaccine encoding HBsAg delivered with a gene gun was tested in healthy humans was in 1999 and it turned out to be ineffective at the applied dosage (0.25 μg)[79]. However, the vaccine was well tolerated. A subsequent study used higher dosages (1, 2, or 4 μg) and this time antigen-specific $CD8^+$ T cells, T helper cells, and protective levels of antibodies could be induced[80]. Therapeutic DNA vaccination for chronic HBV infection was first tested in 2004 and in other subsequent studies[81-83]. It was shown that HBV DNA vaccination was safe and immunologically effective because T-cell responses were activated in some chronic HBV carriers who did not respond to current antiviral therapies. These results indicated that in combination with conventional therapy, DNA vaccination may have a synergic effect leading to complete recovery from HBV infection.

EGS and RNA interference

The recruitment of ribonuclease P (RNase P) by EGS represents another interesting gene interference strategy. RNase P is an enzyme that removes the leader sequence of tRNA precursors by recognizing a common structure shared amongst all tRNAs. Using a custom-designed EGS that hybridizes with an mRNA to form a tRNA-like structure, RNase P can be recruited to cleave the target mRNA[84,85]. With respect to HBV infection, this strategy was first proposed by Werner *et al*[86] in 1997. They took advantage of EGS designed to target HBV sequences as proof-of-concept for the use of EGS as anti-viral or anti-cancer therapeutics[87,88]. The EGS technology gained more attraction in 2013, when EGS was considered as a sufficient method to antagonize HBV replication. One study was conducted in cell culture and in mice using *Salmonella*-mediated delivery. Oral inoculation of attenuated *Salmonella* carrying the EGS construct led to up to approximately 95% inhibition of HBV gene expression levels and a approximately 200000-fold reduction of viral DNA levels in the livers and sera of the treated mice transfected with a HBV plasmid[89]. In a follow-up study efficacy could be improved about 50-fold by modifying the EGS derived from natural tRNA through *in vitro* selection[90].

RNA interference (RNAi) is a natural intracellular antiviral immune response mechanism triggered by double-stranded RNA (dsRNA). After conversion of dsRNA into guide molecules (siRNA), dsRNA induces the degradation of RNA *via* the RNA-induced silencing complex (RISC). Synthetic siRNA can be delivered exogenously or produced endogenously in the form of precursor small hairpin RNAs (shRNA) from plasmid DNA or viral vectors[91]. When RNAi was first discovered[92] it released a wave of multiple studies and applications. Also in HBV therapy it gained attention. In 2003 several studies were published[93-97] and marked the beginning of the most intense exploitation of a gene therapy method as a valuable treatment option for HBV infection.

Shlomai *et al*[93] were the first to publish the use of RNAi in an *in vitro* cell culture model of HBV replication. They found that HBV gene suppression was achieved with different efficacies for different gene targets. Analysis of HBV transcripts revealed a reduction of about 68% on the level of all viral transcripts for RNAi targeting HBx protein. However, RNAi against two different targets on the same HBcAg open reading frame, which is exclusively encoded by the large 3.5 kb transcript, resulted in about 13% and 50% reduction on the level of the 3.5 kb transcript, respectively. This led to the conclusion, that RNAi target sequences have to be evaluated carefully.

In the next study McCaffrey *et al*[94] tested the RNAi-based approach *in vivo* in immunocompetent and immunodeficient mice. DNAs containing a HBV expression plasmid and a shRNA expression plasmid were co-transfected into mouse liver by hydrodynamic plasmid delivery. The authors could show that RNAi can inhibit all the steps of HBV replication that occur in cell culture and in mice. This was indicated by reduced secreted HBsAg levels in the supernatant of transfected cells and in mouse serum, reduced HBV RNAs levels in mouse liver, reduction of HBV genomic DNA to undetectable levels in mouse liver, and decreased numbers of cells stained positive for HBcAg.

The first viral vectors used to transduce RNAi sequence expression cassettes were either based on a prototype foamy virus (PFV) or an adeno-associated virus (AAV)[98]. The vectors expressing the respective RNAi molecule were assessed in 293T.HBs cells, a cell line stably expressing HBsAg and HepG2.2.15 cells. In 293T.HBs cells HBsAg was knocked down by approximately 90% if directly compared to controls cells. HBsAg expression was also inhibited in HepG2.2.15 cells even in the presence of HBV replication. Uprichard *et al*[99] introduced recombinant adenovirus vectors for the delivery of shRNAs. Additional, they used for the first time HBV-transgenic mice to show that ongoing HBV replication *in vivo* can be cleared by RNAi-targeted suppression of viral RNA for at least 26 d. Other viral vectors used included retroviral vectors[100,101], AAV-7, -8 and -9 pseudotyped vectors[102-104], recombinant human foamy virus[105], gene-deleted adenoviral vectors[106,107], lentiviral vectors[108,109], and recombinant baculovirus[110].

Further studies of the RNAi system included lipid-encapsulation of chemically modified siRNAs for non-viral delivery[111] and the assessment of efficacy and pharmacodynamic properties of different RNAi target sequences and constructs, including methyl-modified siRNAs and plasmid based DNA vectors[112]. Moreover, high-throughput generation and screening of siRNAs was established[113] and expression systems introducing multiple siRNAs were developed[105,114-117]. Ely *et al*[116] found that a Pol Ⅱ promoter may be advantageous compared to a Pol Ⅲ promoter, which was traditionally used for shRNA expression. The Pol Ⅲ promoter can result in shRNA overexpression and saturation of the endogenous microRNA pathway leading to serious toxic effect *in vivo*[118]. This could be restricted with the Pol Ⅱ promoter, which provides the possibility to control the production of RNAi activators[119].

Another non-viral vector system which was used in shRNA approaches is the episomal replicating plasmid vector pEPI-1. Herein, the transcription unit is linked to a scaffold/matrix attachment region (S/MAR) which ensures that the vector is mitotically stable in transfected cells. It was shown that it provides long-term expression of shRNAs which resulted in suppression of HBV gene expression, intracellular HBV DNA replication and release of progeny HBV over 8 mo[120].

Besides therapeutic DNA vaccination, siRNA is the only gene therapeutic approach that was translated into clinical trials. In 2006 a Phase Ib, first-in-human safety and tolerability study of an RNAi-based therapy (NUC B1000) in patients with mild to moderate chronic HBV infection was conducted[117]. NUC B1000 is composed out of four expressed shRNAs on one plasmid carried on a nanoparticle (cholesteryl spermine complex) and administered through intravenous infusion. The results revealed elevated cytokines and no HBV DNA or HBsAg decrease in the patients. However, the safety profile of RNAi therapy conducted among patients with HBV was considered as reasonable. A second compound, ARC-520, a liver-tropic cholesterol-conjugated siRNA (chol-siRNA), transported by the proprietary Dynamic Polyconjugate delivery system is just reaching a Phase II clinical study[121,122].

In summary the RNAi system was thoroughly exploited for treatment of chronic HBV infection in the past and research will be ongoing on this topic to overcome major hurdles like evocation of immune responses and maintenance of long-term suppression. Long-term suppression is required because it was shown that the HBV cccDNA depot in the host cells is not affected by this approach[110]. Interestingly, another limitation might be the manipulation of the host RNAi defense by the HBx protein that potentially functions as a RNA-silencing suppressor (RSS)[123].

Designer nucleases

For sequence-specific DNA targeting designer nucleases such as zinc finger nucleases (ZFNs)[124-126], transcription activator-like effector nucleases (TALENs)[127,128] and the clustered, regularly inter-spaced, short palindromic repeats (CRISPR)-CRISPR-associated protein (Cas) system[129-131] can be applied. They combine customizable DNA binding molecules for sequence-specific DNA-binding and a nuclease for introduction of doubled-strand DNA (dsDNA) breaks. The induced dsDNA breaks activate different cellular DNA repair pathways. The two most exploited pathways in gene therapy are homologous recombination (HR) and nonhomologous end-joining (NHEJ). In the presence of a respective homologous donor DNA, cells are able to repair the dsDNA break *via* HR by exchanging the respective sequence[132]. Without any homologous donor DNA cells repair dsDNA breaks *via* NHEJ. This error-prone repair mechanism can lead to insertions or deletions of one or several base pairs and may cause specific knockout of a gene[132]. Therefore, designer nucleases are valuable tools to specifically introduce knock out mutations at a desired DNA locus.

The DNA binding domains (DBD) of ZFNs commonly contains 3-4 zinc fingers. Each zinc finger consists of 30 amino acids and forms two β-sheets and one α-helix. Upon DNA-binding the α-helix is placed in the major groove of the dsDNA and directs contact with a certain base pair triplet. Depending on the amino acids within the α-helix and the number of zinc fingers, ZFNs can be designed specifically to target defined stretches of DNA triplets with high affinity[133-135]. The DBD is connected to a sequence independent cleavage domain of the type ⅡS restriction enzyme *Fok*I which causes double strand breaks after dimerization[136]. Therefore two ZFN monomers are necessary to create the desired dsDNA break in the spacer between the binding sites of

the ZFNs. The double strand breaks will be repaired *via* NHEJ, causing insertion and deletion mutations (indels) of several base pairs within the sequence. These indels can lead to frame shifts within the open reading frame or translation abortion by newly formed stop codons. Both options result in dysfunctional proteins.

There are some approaches using zinc fingers to target HBV cccDNA. Zimmerman *et al*[137] were the first to use zinc fingers in conjunction with HBV although they did not yet use ZFNs. Instead, they created several zinc finger proteins (ZFPs) targeting the enhancer region of duck HBV (DHBV), which probably form a steric hindrance for the RNA polymerase. After screening the candidates for binding efficiency, the two most efficient ZFPs were expressed in a special DHBV tissue culture system *via* transfection and both the transcription of viral genomic RNA and viral protein production was assessed *via* quantitative PCR and western blot analysis. They showed that both ZFPs significantly reduce transcription from cccDNA compared to controls. The authors concluded that ZFPs designed to target HBV DNA are able to substantially reduce viral transcription and interfere with viral replication of cccDNA.

In another study, Cradick *et al*[138] showed that ZFNs can mediate inhibition viral replication *in vitro*. They generated ZFN pairs targeting several conserved regions of HBV genomic DNA and chose the most robust pairs for further studies. Huh7 cells were transfected with plasmids encoding ZFNs under the control of a cytomegalovirus (CMV) immediate early promoter and the target plasmid pTHBV2. This target plasmid contains a 1.3-fold HBV genome which is capable of full transcription of the viral RNAs, translation of the proteins and production of infectious virus. Three days post transfection, Southern blotting analyses revealed about 26% linearized DNA and about 10% cleaved target plasmids being rejoined in a tail to tail orientation. Both DNA species indicate that ZFN-specific cleavage at the intended ZFN target site occurred. Linear genomes are the result of direct cleavage without any subsequent repair while concatamer formation is caused by NHEJ after ZFN cleavage. To investigate if NHEJ led to indels in recircularized genomes or head-to-tail concatamers, a *Xba*I site was inserted within the spacer region of the ZFN dimer. After NHEJ occurred this site should be destroyed. Target sites of treated samples were amplified by PCR, digested with *Xba*I and resistant amplicons were sequenced. 13 of 16 samples showed a frameshift which would lead to dysfunctional proteins. Furthermore this study indicated a 29% reduction of pregenomic RNA in northern blot analysis compared to controls. Besides these results it could also be demonstrated that the ZFN pairs caused only moderate toxicity. In conclusion, Cradick *et al*[138] revealed that specifically designed ZFNs targeting regions which are conserved among many different HBV serotypes can significantly reduce viral replication which is associated with negligible toxicity.

A third study by Weber *et al*[139] deals with the delivery of functional ZFNs into target cells *via* a viral vector system. Here, in contrast to the latter study, obligate heterodimeric ZFNs were designed, which are not able to form homodimers. This measure minimizes off-target effects because ZFNs that are able to form homodimers might cleave at unintended genomic loci by tolerating some mismatches. The three designed ZFN pairs targeted the open reading frames of HBx, HBcAg and polymerase. For delivery a self-complementary AAV-vector (scAAV) was used, which shows higher transduction efficiencies compared to single stranded AAV vectors[140]. Since scAAV vectors have a reduced transgene capacity each ZFN of a pair was delivered individually in co-transduction experiments. Transduction of each ZFN pair into HepAD38 cells, a model cell line for controllable HBV replication, revealed mutation rates ranging from 9.8% to 34%. Transduction of all three ZFN pairs simultaneously resulted in mutation rates of 8% to 20%. Off-target mutagenesis for seven potential off-target sites was investigated using single molecule real time (SMRT) sequencing which detected indels (> 1 nt) in four sequencing reads out of 9290 filtered reads. It is of note that indels of only one nucleotide were not taken into account because they could not be distinguished from sequencing artefacts. In order to test if ZFN treatment has an effect on HBV replication, ZFNs were transduced into HepAD38 cells in which HBV replication was shut down. After turning HBV replication on, the controls showed a 30-fold and 323-fold increase in HBV marker levels in cells and in the supernatant, respectively. In ZFNs treated cells no significant increase of cellular or supernatant HBV marker levels could be detected, leading to the conclusion that ZFN-mediated cleavage resulted in replication-deficient HBV. In summary, it was demonstrated that HBV specific ZFN can be delivered successfully *via* AAV vectors into target cells *in vitro*, resulting in an efficient inhibition of HBV replication.

TALENs are a promising new class of designer nucleases that can be specifically designed to bind DNA sequences of interest and to introduce dsDNA breaks. Comparable to ZFNs, TALENs are chimeric proteins consisting of a N-terminal nuclear localization signal, a central DNA binding domain and a C-terminal *Fok*I nuclease domain. The DBD originates from transcription activator like effectors (TALEs) of bacterial plant pathogens of the genus *Xanthomonas*, which secrete these proteins to regulate gene expression within their host cells[141]. The DBD is comprised of a repeat region consisting of a number of incomplete tandem repeats containing repeat-variable diresidues (RVD)[127]. The

RVD of each repeat is specific for binding a corresponding nucleotide in their contiguous target DNA sequence[127,141]. Similar to ZFNs, the TALEN-DNA binding domain is combined with a non-specific endonuclease activity which is dependent on dimerization of two *Fok*I-cleavage domains. Binding of the DBDs upstream and downstream of a DNA target brings the *Fok*I nuclease domains of a TALEN pair in close proximity and dimerization of the *Fok*I monomers introduces dsDNA breaks. Note that the spacer between the binding sites of a pair needs to be considered in the TALEN design to ensure optimal cleavage[127,128]. Up to now several techniques are available to specifically design[127,142] and to assemble TALENs for the desired application[127,143-145]. Assembly can be performed without highly complex screening procedures or the need for special equipment, making it cheap, simple and fast and thereby with that respect superior to the ZFN technology.

Bloom *et al*[146] designed TALENs with target sequences in the HBsAg or HBcAg expressing region of the HBV genome. TALEN efficacy was determined after co-transfection of Huh7 cells with TALEN expression plasmids together with the pCH-9/3091 HBV replication-competent plasmid. They observed that the HBsAg production was diminished in cells expressing TALENs. In a more stringent experimental model of HBV replication, the HepG2.2.15 cell line was transfected with TALEN expression plasmids. After three subsequent transfections and culturing cells under hypothermic conditions, HBsAg-specific TALEN expression resulted in disruption of cccDNA molecules with efficiencies of approximately 31%. This was confirmed in a T7E1 mutation detection assay and correlated with a decrease of HBsAg secretion of these cells. Expression of the HBcAg-specific TALEN pair only mutated 12% of cccDNA molecules which was not sufficient to inhibit HBsAg secretion. After hydrodynamic tail vein injection of replication-competent HBV DNA together with HBsAg-specific TALEN expression plasmids, serum HBsAg levels were decreased by more than 90% and circulating viral particle equivalents (VPEs) were decreased by approximately 70%. The T7E1 assay demonstrated mutation rates of 58%-87% in HBV-DNA extracted from livers of TALEN treated mice.

Chen *et al*[147] used three TALEN pairs targeting regions of HBV genomic DNA conserved among HBV genotypes A-D. Huh7 cells were transfected with the monomeric linear full-length HBV DNA of subgroups A, B, C, or D, respectively and plasmids expressing one of the respective TALEN pairs. Cells containing HBV DNA simulated the complete HBV replication cycle, including the nuclear generation of cccDNA. Suppression of HBeAg and HBsAg production by the TALEN expression was observed for all four HBV genotypes. Additionally HBcAg–RNA as well as pregenomic HBV RNA levels were decreased in TALEN expressing cells. Furthermore T7E1 mutation detection assay confirmed that mutations were successfully induced at the respective TALEN target site within the HBV genome leading to a 10%-50% decrease of cccDNA levels. In combination with interferon alpha treatment TALENs expression led to synergistic effects further increasing the inhibition of HBV transcription in Huh7 cells. After delivery of monomeric linear full-length HBV DNA and TALEN expression plasmids into C3H/HeN mice by hydrodynamic tail vein injection, serum levels of HBeAg, HBsAg and cccDNA as well as liver pregenomic HBV RNA significantly decreased compared to control animals. In summary, HBV-targeting TALENs were shown to be active in cell culture models as well as in *in vivo* models and are capable of introducing mutations at their target sites reducing HBV gene expression levels and cccDNA genome numbers.

The most recent approach exploited the CRISPR/Cas9 system for genome engineering[131]. Because sequence specificity is achieved by a guide RNA (gRNA) which can be produced from an adaptable expression cassette, this system is easier to manipulate in comparison to ZFNs and TALENs where the DBD is based on a protein sequence. Lin *et al*[148] were the first to apply this system for achieving HBV genome degradation[148]. They co-expressed the Cas9 nuclease together with eight HBV specific guide RNAs individually and in addition combined two of them on one expression vector. They tested all constructs in Huh7 cells which were transfected with a HBV-expression vector. Inhibition varied among the different guide sequences. A maximum inhibition of 70% of intracellular HBsAg expression was reached after using one guide RNA and Cas9 nuclease expressed from individual vectors and up to 96% inhibition was reached when they were combined on one expression vector. Next, they multiplexed the two most effective gRNAs, an approach which proved to be even more effective. Finally, the expression vectors that contained expression cassettes for one gRNA and the Cas9 nuclease were tested in the HBV-hydrodynamic mouse model. Serum HBsAg levels were significantly reduced two days post-injection but increased again on day seven. However, Southern blot analyses revealed a reduction of intrahepatic HBV-expression levels of 20% to 60%. In conclusion the RNA-guided Cas9 nuclease system may be a useful technique for achieving inhibition of HBV replication.

CONCLUSION

Numerous and highly diverse gene therapeutic approaches were pursued to combat chronic HBV infection. Although strategies that solely rely on nucleic acids like antisense oligonucleotides and catalytic nucleic acids have a great advantage in

their simplicity, these technologies are also limited due to their instability and imprecision. RNAi was most adopted and thoroughly investigated not only in the field of HBV therapy and this generated a deep knowledge regarding this technique. However, especially in the case of HBV, RNAi may be disadvantageous, because it does not affect the HBV cccDNA. It can rather be considered as an alternative to nucleos(t)ide analogues based therapy potentially associated with an improved ability to also respond to escape mutants. In addition, if long-term expression is required, RNAi-based approaches are superior compared to conventional therapy based on daily administration required for nucleos(t)ide analogues.

We believe that the most recent technology to combat HBV infection based on designer nucleases may be one of the most promising approaches to be explored in the future. This strategy bears the potential to actually eradicate cccDNA species in infected cells. Especially in combination with compounds that inhibit the HBV replication cycle this could be an attractive therapeutic option. However, major obstacles are the production and delivery of the designer nucleases and for translation of this approach into the clinic, this methodology needs to be further improved. The most promising results to date were obtained with genetic vaccines which were also pursued in the clinic. Also this strategy may hold great potential for eradicating chronic hepatitis B infection.

REFERENCES

1 **Ganem D**, Varmus HE. The molecular biology of the hepatitis B viruses. *Annu Rev Biochem* 1987; **56**: 651-693 [PMID: 3039907 DOI: 10.1146/annurev.bi.56.070187.003251]

2 **Glebe D**, Bremer CM. The molecular virology of hepatitis B virus. *Semin Liver Dis* 2013; **33**: 103-112 [PMID: 23749666 DOI: 10.1055/s-0033-1345717]

3 WHO. Hepatitis B Fact Sheet No. 204. Available from: URL: http://www.who.int/mediacentre/factsheets/fs204/en/

4 **Kwon H**, Lok AS. Hepatitis B therapy. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 275-284 [PMID: 21423260 DOI: 10.1038/nrgastro.2011.33]

5 **Thomas H**, Foster G, Platis D. Mechanisms of action of interferon and nucleoside analogues. *J Hepatol* 2003; **39** Suppl 1: S93-S98 [PMID: 14708685 DOI: 10.1016/s0168-8278(03)00207-1]

6 **Buster EH**, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, So TM, Feinman SV, Mach T, Akarca US, Schutten M, Tielemans W, van Vuuren AJ, Hansen BE, Janssen HL. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. *Gastroenterology* 2008; **135**: 459-467 [PMID: 18585385 DOI: 10.1053/j.gastro.2008.05.031]

7 **Santantonio TA**, Fasano M. Chronic hepatitis B: Advances in treatment. *World J Hepatol* 2014; **6**: 284-292 [PMID: 24868322 DOI: 10.4254/wjh.v6.i5.284]

8 **Jin L**, Zeng X, Liu M, Deng Y, He N. Current progress in gene delivery technology based on chemical methods and nano-carriers. *Theranostics* 2014; **4**: 240-255 [PMID: 24505233 DOI: 10.7150/thno.6914]

9 **Wang W**, Li W, Ma N, Steinhoff G. Non-viral gene delivery methods. *Curr Pharm Biotechnol* 2013; **14**: 46-60 [PMID: 23437936 DOI: 10.2174/1389201011314010008]

10 **Pezzoli D**, Kajaste-Rudnitski A, Chiesa R, Candiani G. Lipid-based nanoparticles as nonviral gene delivery vectors. *Methods Mol Biol* 2013; **1025**: 269-279 [PMID: 23918345 DOI: 10.1007/978-1-62703-462-3_21]

11 **Demeneix B**, Behr JP. Polyethylenimine (PEI). *Adv Genet* 2005; **53**: 217-230 [PMID: 16240995 DOI: 10.1016/S0065-2660(05)53008-6]

12 **Davis BR**, Brown DB, Prokopishyn NL, Yannariello-Brown J. Micro-injection-mediated hematopoietic stem cell gene therapy. *Curr Opin Mol Ther* 2000; **2**: 412-419 [PMID: 11249771]

13 **Johnston SA**, Tang DC. Gene gun transfection of animal cells and genetic immunization. *Methods Cell Biol* 1994; **43** Pt A: 353-365 [PMID: 7823871]

14 **Murakami T**, Sunada Y. Plasmid DNA gene therapy by electroporation: principles and recent advances. *Curr Gene Ther* 2011; **11**: 447-456 [PMID: 22023474 DOI: 10.2174/156652311798192860]

15 **Escoffre JM**, Zeghimi A, Novell A, Bouakaz A. In-vivo gene delivery by sonoporation: recent progress and prospects. *Curr Gene Ther* 2013; **13**: 2-14 [PMID: 23157546 DOI: 10.2174/1566523138048060 6]

16 **Schwerdt JI**, Goya GF, Calatayud MP, Hereñú CB, Reggiani PC, Goya RG. Magnetic field-assisted gene delivery: achievements and therapeutic potential. *Curr Gene Ther* 2012; **12**: 116-126 [PMID: 22348552 DOI: 10.2174/156652312800099616]

17 **Hodges BL**, Scheule RK. Hydrodynamic delivery of DNA. *Expert Opin Biol Ther* 2003; **3**: 911-918 [PMID: 12943450 DOI: 10.1517/14712598.3.6.911]

18 **Thomas CE**, Ehrhardt A, Kay MA. Progress and problems with the use of viral vectors for gene therapy. *Nat Rev Genet* 2003; **4**: 346-358 [PMID: 12728277 DOI: 10.1038/nrg1066]

19 **Cots D**, Bosch A, Chillón M. Helper dependent adenovirus vectors: progress and future prospects. *Curr Gene Ther* 2013; **13**: 370-381 [PMID: 24369061 DOI: 10.2174/15665232130513121212 5338]

20 **Mingozzi F**, High KA. Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. *Nat Rev Genet* 2011; **12**: 341-355 [PMID: 21499295 DOI: 10.1038/nrg2988]

21 **Naldini L**, Blömer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM, Trono D. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 1996; **272**: 263-267 [PMID: 8602510 DOI: 10.1126/science.272.5259.263]

22 **Goodarzi G**, Gross SC, Tewari A, Watabe K. Antisense oligodeoxyribonucleotides inhibit the expression of the gene for hepatitis B virus surface antigen. *J Gen Virol* 1990; **71** (Pt 12): 3021-3025 [PMID: 2177093 DOI: 10.1099/0022-1317-71-12-3021]

23 **Blum HE**, Galun E, von Weizsäcker F, Wands JR. Inhibition of hepatitis B virus by antisense oligodeoxynucleotides. *Lancet* 1991; **337**: 1230 [PMID: 1673773 DOI: 10.1016/0140-6736(91)92907-J]

24 **Reinis M**, Reinisová M, Korec E, Hlozánek I. Inhibition of hepatitis B virus surface gene expression by antisense oligodeoxynucleotides in a human hepatoma cell line. *Folia Biol* (Praha) 1993; **39**: 262-269 [PMID: 8206172]

25 **Zhang YC**, Taylor MM, Samson WK, Phillips MI. Antisense inhibition: oligonucleotides, ribozymes, and siRNAs. *Methods Mol Med* 2005; **106**: 11-34 [PMID: 15375310]

26 **Offensperger WB**, Offensperger S, Walter E, Teubner K, Igloi G, Blum HE, Gerok W. In vivo inhibition of duck hepatitis B virus replication and gene expression by phosphorothioate modified antisense oligodeoxynucleotides. *EMBO J* 1993; **12**: 1257-1262 [PMID: 8458338]

27 **Korba BE**, Gerin JL. Antisense oligonucleotides are effective inhibitors of hepatitis B virus replication in vitro. *Antiviral Res* 1995; **28**: 225-242 [PMID: 8629815 DOI: 10.1016/0166-3542(95)00050-V]

28 **Wu GY**, Wu CH. Specific inhibition of hepatitis B viral gene expression in vitro by targeted antisense oligonucleotides. *J Biol Chem* 1992; **267**: 12436-12439 [PMID: 1618751]

29 **Matsukura M**, Koike K, Zon G. Antisense phosphorothioates as antivirals against human immunodeficiency virus (HIV) and hepatitis B virus (HBV). *Toxicol Lett* 1995; **82-83**: 435-438 [PMID:

8597090 DOI: 10.1016/0378-4274(95)03574-5]
30 **Yao ZQ**, Zhou YX, Wang AL, Bai XF, Yang WS. Inhibition of hepatitis B viral gene expression by antisense phosphorothioate oligodeoxynucleotides. *J Viral Hepat* 1995; **2**: 85-89 [PMID: 7493302 DOI: 10.1111/j.1365-2893.1995.tb00011.x]
31 **Robaczewska M**, Narayan R, Seigneres B, Schorr O, Thermet A, Podhajska AJ, Trepo C, Zoulim F, Nielsen PE, Cova L. Sequence-specific inhibition of duck hepatitis B virus reverse transcription by peptide nucleic acids (PNA). *J Hepatol* 2005; **42**: 180-187 [PMID: 15664242 DOI: 10.1016/j.jhep.2004.10.010]
32 **Sun Z**, Xiang W, Guo Y, Chen Z, Liu W, Lu D. Inhibition of hepatitis B virus (HBV) by LNA-mediated nuclear interference with HBV DNA transcription. *Biochem Biophys Res Commun* 2011; **409**: 430-435 [PMID: 21596023 DOI: 10.1016/j.bbrc.2011.05.016]
33 **Walton CM**, Wu CH, Wu GY. A ribonuclease H-oligo DNA conjugate that specifically cleaves hepatitis B viral messenger RNA. *Bioconjug Chem* 2001; **12**: 770-775 [PMID: 11562195 DOI: 10.1021/bc010018e]
34 **Guang L**, Yuan F, Xi M, Zhao C, Liu L, Wen E, Ai Y. Combination and cleavage of HBV DNA fragments by triple helix-forming oligonucleotides modified with manganese porphyrin in vitro. *Chin Med J* (Engl) 2003; **116**: 1248-1252 [PMID: 12935421]
35 **Madon J**, Blum HE. Receptor-mediated delivery of hepatitis B virus DNA and antisense oligodeoxynucleotides to avian liver cells. *Hepatology* 1996; **24**: 474-481 [PMID: 8781310 DOI: 10.1002/hep.510240302]
36 **Nakazono K**, Ito Y, Wu CH, Wu GY. Inhibition of hepatitis B virus replication by targeted pretreatment of complexed antisense DNA in vitro. *Hepatology* 1996; **23**: 1297-1303 [PMID: 8675142 DOI: 10.1002/hep.510230601]
37 **Wu J**, Gerber MA. The inhibitory effects of antisense RNA on hepatitis B virus surface antigen synthesis. *J Gen Virol* 1997; **78** (Pt 3): 641-647 [PMID: 9049416]
38 **Zhang J**, Chen F, Zhong S, Tang K, Shi X, Wang M, Peng J. Anti-HBV effect of targeted antisense RNA against HBV C gene. *Zhonghua Ganzangbing Zazhi* 2000; **8**: 169-170 [PMID: 10880169]
39 **Ji W**, St CW. Inhibition of hepatitis B virus by retroviral vectors expressing antisense RNA. *J Viral Hepat* 1997; **4**: 167-173 [PMID: 9181525 DOI: 10.1046/j.1365-2893.1997.00144.x]
40 **Tung FY**, Bowen SW. Targeted inhibition of hepatitis B virus gene expression: a gene therapy approach. *Front Biosci* 1998; **3**: a11-a15 [PMID: 9450988]
41 **Brantl S**. Antisense-RNA regulation and RNA interference. *Biochim Biophys Acta* 2002; **1575**: 15-25 [PMID: 12020814 DOI: 10.1016/S0167-4781(02)00280-4]
42 **Hafkemeyer P**, Brinkmann U, Brinkmann E, Pastan I, Blum HE, Baumert TF. Pseudomonas exotoxin antisense RNA selectively kills hepatitis B virus infected cells. *World J Gastroenterol* 2008; **14**: 2810-2817 [PMID: 18473403 DOI: 10.3748/wjg.14.2810]
43 **Schubert S**, Kurreck J. Ribozyme- and deoxyribozyme-strategies for medical applications. *Curr Drug Targets* 2004; **5**: 667-681 [PMID: 15578948 DOI: 10.2174/1389450043345092]
44 **von Weizsäcker F**, Blum HE, Wands JR. Cleavage of hepatitis B virus RNA by three ribozymes transcribed from a single DNA template. *Biochem Biophys Res Commun* 1992; **189**: 743-748 [PMID: 1472046 DOI: 10.1016/0006-291X(92)92264-X]
45 **Beck J**, Nassal M. Efficient hammerhead ribozyme-mediated cleavage of the structured hepatitis B virus encapsidation signal in vitro and in cell extracts, but not in intact cells. *Nucleic Acids Res* 1995; **23**: 4954-4962 [PMID: 8559651 DOI: 10.1093/nar/23.24.4954]
46 **Welch PJ**, Tritz R, Yei S, Barber J, Yu M. Intracellular application of hairpin ribozyme genes against hepatitis B virus. *Gene Ther* 1997; **4**: 736-743 [PMID: 9282175 DOI: 10.1038/sj.gt.3300441]
47 **Zheng WC**, Qi GR. tRNA-embedded Hammerhead Ribozymes Mediate Destruction of HBV (Subtype adr) in vitro. *Shengwuhuaxue Yu Shengwuwuli Xuebao* (Shanghai) 1998; **30**: 432-438 [PMID: 12168009]
48 **Bergeron LJ**, Perreault JP. Development and comparison of procedures for the selection of delta ribozyme cleavage sites within the hepatitis B virus. *Nucleic Acids Res* 2002; **30**: 4682-4691 [PMID: 12409459 DOI: 10.1093/nar/gkf598]
49 **Tan TM**, Zhou L, Houssais S, Seet BL, Jaenicke S, Peter F, Lim SG. Intracellular inhibition of hepatitis B virus S gene expression by chimeric DNA-RNA phosphorothioate minimized ribozyme. *Antisense Nucleic Acid Drug Dev* 2002; **12**: 257-264 [PMID: 12238814 DOI: 10.1089/108729002320351575]
50 **Hoeprich S**, Zhou Q, Guo S, Shu D, Qi G, Wang Y, Guo P. Bacterial virus phi29 pRNA as a hammerhead ribozyme escort to destroy hepatitis B virus. *Gene Ther* 2003; **10**: 1258-1267 [PMID: 12858191 DOI: 10.1038/sj.gt.3302002]
51 **Goila R**, Banerjea AC. Sequence-specific cleavage of hepatitis X RNA in cis and trans by novel monotarget and multitarget hammerhead motif-containing ribozymes. *Oligonucleotides* 2004; **14**: 249-262 [PMID: 15665593 DOI: 10.1089/oli.2004.14.249]
52 **Ruiz J**, Wu CH, Ito Y, Wu GY. Design and preparation of a multimeric self-cleaving hammerhead ribozyme. *Biotechniques* 1997; **22**: 338-345 [PMID: 9043708]
53 **zu Putlitz J**, Yu Q, Burke JM, Wands JR. Combinatorial screening and intracellular antiviral activity of hairpin ribozymes directed against hepatitis B virus. *J Virol* 1999; **73**: 5381-5387 [PMID: 10364285]
54 **Passman M**, Weinberg M, Kew M, Arbuthnot P. In situ demonstration of inhibitory effects of hammerhead ribozymes that are targeted to the hepatitis Bx sequence in cultured cells. *Biochem Biophys Res Commun* 2000; **268**: 728-733 [PMID: 10679273 DOI: 10.1006/bbrc.2000.2209]
55 **Zheng WC**, Lu CD, Kong YY, Wang Y, Qi GR. Hammerhead Ribozymes Suppress HBV(adr) in HepG2 Cells. *Shengwuhuaxue Yu Shengwuwuli Xuebao* (Shanghai) 2001; **33**: 25-29 [PMID: 12053184]
56 **Pan WH**, Xin P, Morrey JD, Clawson GA. A self-processing ribozyme cassette: utility against human papillomavirus 11 E6/E7 mRNA and hepatitis B virus. *Mol Ther* 2004; **9**: 596-606 [PMID: 15093190 DOI: 10.1016/j.ymthe.2003.12.013]
57 **Li X**, Kuang E, Dai W, Zhou B, Yang F. Efficient inhibition of hepatitis B virus replication by hammerhead ribozymes delivered by hepatitis delta virus. *Virus Res* 2005; **114**: 126-132 [PMID: 16054262 DOI: 10.1016/j.virusres.2005.06.005]
58 **Nash KL**, Alexander GJ, Lever AM. Inhibition of hepatitis B virus by lentiviral vector delivered antisense RNA and hammerhead ribozymes. *J Viral Hepat* 2005; **12**: 346-356 [PMID: 15985004 DOI: 10.1111/j.1365-2893.2005.00612.x]
59 **Wang CX**, Lu YQ, Qi P, Chen LH, Han JX. Efficient inhibition of hepatitis B virus replication by hepatitis delta virus ribozymes delivered by targeting retrovirus. *Virol J* 2010; **7**: 61 [PMID: 20236514 DOI: 10.1186/1743-422X-7-61]
60 **Asahina Y**, Ito Y, Wu CH, Wu GY. DNA ribonucleases that are active against intracellular hepatitis B viral RNA targets. *Hepatology* 1998; **28**: 547-554 [PMID: 9696023 DOI: 10.1002/hep.510280236]
61 **Goila R**, Banerjea AC. Inhibition of hepatitis B virus X gene expression by novel DNA enzymes. *Biochem J* 2001; **353**: 701-708 [PMID: 11171068 DOI: 10.1042/0264-6021: 3530701]
62 **Hou W**, Wo JE, Li MW, Liu KZ. In vitro cleavage of hepatitis B virus C mRNA by 10-23 DNA enzyme. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 573-576 [PMID: 16286265]
63 **Wo JE**, Wu XL, Zhou LF, Yao HP, Chen LW, Dennin RH. Effective inhibition of expression of hepatitis B virus genes by DNAzymes. *World J Gastroenterol* 2005; **11**: 3504-3507 [PMID: 15962363]
64 **Hou W**, Ni Q, Wo J, Li M, Liu K, Chen L, Hu Z, Liu R, Hu M. Inhibition of hepatitis B virus X gene expression by 10-23 DNAzymes. *Antiviral Res* 2006; **72**: 190-196 [PMID: 16930733 DOI: 10.1016/j.antiviral.2006.07.001]
65 **Scaglioni PP**, Melegari M, Wands JR. Characterization of hepatitis B virus core mutants that inhibit viral replication. *Virology* 1994; **205**: 112-120 [PMID: 7975206 DOI: 10.1006/viro.1994.1625]
66 **Scaglioni P**, Melegari M, Takahashi M, Chowdhury JR, Wands J. Use of dominant negative mutants of the hepadnaviral core protein as antiviral agents. *Hepatology* 1996; **24**: 1010-1017 [PMID:

8903368 DOI: 10.1002/hep.510240506]
67 **von Weizsäcker F**, Wieland S, Köck J, Offensperger WB, Offensperger S, Moradpour D, Blum HE. Gene therapy for chronic viral hepatitis: ribozymes, antisense oligonucleotides, and dominant negative mutants. *Hepatology* 1997; **26**: 251-255 [PMID: 9252130 DOI: 10.1002/hep.510260237]
68 **von Weizsäcker F**, Köck J, Wieland S, Offensperger WB, Blum HE. Dominant negative mutants of the duck hepatitis B virus core protein interfere with RNA pregenome packaging and viral DNA synthesis. *Hepatology* 1999; **30**: 308-315 [PMID: 10385672 DOI: 10.1002/hep.510300139]
69 **Beterams G**, Nassal M. Significant interference with hepatitis B virus replication by a core-nuclease fusion protein. *J Biol Chem* 2001; **276**: 8875-8883 [PMID: 11124940 DOI: 10.1074/jbc.M006335200]
70 **Liu J**, Li YH, Xue CF, Ding J, Gong WD, Zhao Y, Huang YX. Targeted ribonuclease can inhibit replication of hepatitis B virus. *World J Gastroenterol* 2003; **9**: 295-299 [PMID: 12532452]
71 **Liu J**, Li YH, Ding J, Gong WD, Xue CF, Zhao Y, Huang YX. Quantifying anti-HBV effect of targeted ribonuclease by real-time fluorescent PCR. *World J Gastroenterol* 2004; **10**: 2883-2885 [PMID: 15334692]
72 **Zhao Y**, Li Y, Liu J, Liu Z, Huang Y, Lei J, Li S, Xue C. Adenoviral-vector mediated transfer of HBV-targeted ribonuclease can inhibit HBV replication in vivo. *Biochem Biophys Res Commun* 2008; **371**: 541-545 [PMID: 18455508 DOI: 10.1016/j.bbrc.2008.04.121]
73 **Pol S**, Driss F, Carnot F, Michel ML, Berthelot P, Brechot C. [Efficacy of immunotherapy with vaccination against hepatitis B virus on virus B multiplication]. *C R Acad Sci III* 1993; **316**: 688-691 [PMID: 8019891]
74 **Pol S**. Immunotherapy of chronic hepatitis B by anti HBV vaccine. *Biomed Pharmacother* 1995; **49**: 105-109 [PMID: 7647280 DOI: 10.1016/0753-3322(96)82602-3]
75 **Davis HL**, Michel ML, Whalen RG. DNA-based immunization induces continuous secretion of hepatitis B surface antigen and high levels of circulating antibody. *Hum Mol Genet* 1993; **2**: 1847-1851 [PMID: 8281146 DOI: 10.1093/hmg/2.11.1847]
76 **Mancini M**, Hadchouel M, Davis HL, Whalen RG, Tiollais P, Michel ML. DNA-mediated immunization in a transgenic mouse model of the hepatitis B surface antigen chronic carrier state. *Proc Natl Acad Sci USA* 1996; **93**: 12496-12501 [PMID: 8901610 DOI: 10.1073/pnas.93.22.12496]
77 **Townsend K**, Sällberg M, O'Dea J, Banks T, Driver D, Sauter S, Chang SM, Jolly DJ, Mento SJ, Milich DR, Lee WT. Characterization of CD8+ cytotoxic T-lymphocyte responses after genetic immunization with retrovirus vectors expressing different forms of the hepatitis B virus core and e antigens. *J Virol* 1997; **71**: 3365-3374 [PMID: 9094605]
78 **Sällberg M**, Hughes J, Javadian A, Ronlov G, Hultgren C, Townsend K, Anderson CG, O'Dea J, Alfonso J, Eason R, Murthy KK, Jolly DJ, Chang SM, Mento SJ, Milich D, Lee WT. Genetic immunization of chimpanzees chronically infected with the hepatitis B virus, using a recombinant retroviral vector encoding the hepatitis B virus core antigen. *Hum Gene Ther* 1998; **9**: 1719-1729 [PMID: 9721082 DOI: 10.1089/hum.1998.9.12-1719]
79 **Tacket CO**, Roy MJ, Widera G, Swain WF, Broome S, Edelman R. Phase 1 safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. *Vaccine* 1999; **17**: 2826-2829 [PMID: 10438052 DOI: 10.1016/S0264-410X(99)00094-8]
80 **Roy MJ**, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S, Culp J, Burkholder JK, Swain WF, Dixon RM, Widera G, Vessey R, King A, Ogg G, Gallimore A, Haynes JR, Heydenburg Fuller D. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. *Vaccine* 2000; **19**: 764-778 [PMID: 11115698 DOI: 10.1016/S0264-410X(00)00302-9]
81 **Mancini-Bourgine M**, Fontaine H, Scott-Algara D, Pol S, Bréchot C, Michel ML. Induction or expansion of T-cell responses by a hepatitis B DNA vaccine administered to chronic HBV carriers. *Hepatology* 2004; **40**: 874-882 [PMID: 15382173 DOI: 10.1002/hep.20408]
82 **Mancini-Bourgine M**, Fontaine H, Bréchot C, Pol S, Michel ML. Immunogenicity of a hepatitis B DNA vaccine administered to chronic HBV carriers. *Vaccine* 2006; **24**: 4482-4489 [PMID: 16310901 DOI: 10.1016/j.vaccine.2005.08.013]
83 **Yang FQ**, Yu YY, Wang GQ, Chen J, Li JH, Li YQ, Rao GR, Mo GY, Luo XR, Chen GM. A pilot randomized controlled trial of dual-plasmid HBV DNA vaccine mediated by in vivo electroporation in chronic hepatitis B patients under lamivudine chemotherapy. *J Viral Hepat* 2012; **19**: 581-593 [PMID: 22762143 DOI: 10.1111/j.1365-2893.2012.01589.x]
84 **Forster AC**, Altman S. External guide sequences for an RNA enzyme. *Science* 1990; **249**: 783-786 [PMID: 1697102 DOI: 10.1126/science.1697102]
85 **Yuan Y**, Hwang ES, Altman S. Targeted cleavage of mRNA by human RNase P. *Proc Natl Acad Sci USA* 1992; **89**: 8006-8010 [PMID: 1381505 DOI: 10.1073/pnas.89.17.8006]
86 **Werner M**, Rosa E, George ST. Design of short external guide sequences (EGSs) for cleavage of target molecules with RNase P. *Nucleic Acids Symp Ser* 1997; **(36)**: 19-21 [PMID: 9478194]
87 **Werner M**, Rosa E, Nordstrom JL, Goldberg AR, George ST. Short oligonucleotides as external guide sequences for site-specific cleavage of RNA molecules with human RNase P. *RNA* 1998; **4**: 847-855 [PMID: 9671057 DOI: 10.1017/S1355838298980323]
88 **Werner M**, Rosa E, Al Emran O, Goldberg AR, George ST. Targeted cleavage of RNA molecules by human RNase P using minimized external guide sequences. *Antisense Nucleic Acid Drug Dev* 1999; **9**: 81-88 [PMID: 10192292 DOI: 10.1089/oli.1.1999.9.81]
89 **Xia C**, Chen YC, Gong H, Zeng W, Vu GP, Trang P, Lu S, Wu J, Liu F. Inhibition of hepatitis B virus gene expression and replication by ribonuclease P. *Mol Ther* 2013; **21**: 995-1003 [PMID: 23481322 DOI: 10.1038/mt.2013.37]
90 **Zhang Z**, Vu GP, Gong H, Xia C, Chen YC, Liu F, Wu J, Lu S. Engineered external guide sequences are highly effective in inhibiting gene expression and replication of hepatitis B virus in cultured cells. *PLoS One* 2013; **8**: e65268 [PMID: 23776459 DOI: 10.1371/journal.pone.0065268]
91 **Hannon GJ**. RNA interference. *Nature* 2002; **418**: 244-251 [PMID: 12110901 DOI: 10.1038/418244a]
92 **Fire A**, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 1998; **391**: 806-811 [PMID: 9486653 DOI: 10.1038/35888]
93 **Shlomai A**, Shaul Y. Inhibition of hepatitis B virus expression and replication by RNA interference. *Hepatology* 2003; **37**: 764-770 [PMID: 12668968 DOI: 10.1053/jhep.2003.50146]
94 **McCaffrey AP**, Nakai H, Pandey K, Huang Z, Salazar FH, Xu H, Wieland SF, Marion PL, Kay MA. Inhibition of hepatitis B virus in mice by RNA interference. *Nat Biotechnol* 2003; **21**: 639-644 [PMID: 12740585 DOI: 10.1038/nbt824]
95 **Klein C**, Bock CT, Wedemeyer H, Wüstefeld T, Locarnini S, Dienes HP, Kubicka S, Manns MP, Trautwein C. Inhibition of hepatitis B virus replication in vivo by nucleoside analogues and siRNA. *Gastroenterology* 2003; **125**: 9-18 [PMID: 12851866 DOI: 10.1016/S0016-5085(03)00720-0]
96 **Giladi H**, Ketzinel-Gilad M, Rivkin L, Felig Y, Nussbaum O, Galun E. Small interfering RNA inhibits hepatitis B virus replication in mice. *Mol Ther* 2003; **8**: 769-776 [PMID: 14599810 DOI: 10.1016/S1525-0016(03)00244-2]
97 **Hamasaki K**, Nakao K, Matsumoto K, Ichikawa T, Ishikawa H, Eguchi K. Short interfering RNA-directed inhibition of hepatitis B virus replication. *FEBS Lett* 2003; **543**: 51-54 [PMID: 12753904 DOI: 10.1016/s0014-5793(03)00400-9]
98 **Moore MD**, McGarvey MJ, Russell RA, Cullen BR, McClure MO. Stable inhibition of hepatitis B virus proteins by small interfering RNA expressed from viral vectors. *J Gene Med* 2005; **7**: 918-925 [PMID: 15756649 DOI: 10.1002/jgm.739]

99 **Uprichard SL**, Boyd B, Althage A, Chisari FV. Clearance of hepatitis B virus from the liver of transgenic mice by short hairpin RNAs. *Proc Natl Acad Sci USA* 2005; **102**: 773-778 [PMID: 15640346 DOI: 10.1073/pnas.0409028102]

100 **Jia F**, Zhang YZ, Liu CM. A retrovirus-based system to stably silence hepatitis B virus genes by RNA interference. *Biotechnol Lett* 2006; **28**: 1679-1685 [PMID: 16900331 DOI: 10.1007/s10529-006-9138-z]

101 **Jia F**, Zhang YZ, Liu CM. Stable inhibition of hepatitis B virus expression and replication in HepG2.2.15 cells by RNA interference based on retrovirus delivery. *J Biotechnol* 2007; **128**: 32-40 [PMID: 17049658 DOI: 10.1016/j.jbiotec.2006.09.007]

102 **Chen CC**, Ko TM, Ma HI, Wu HL, Xiao X, Li J, Chang CM, Wu PY, Chen CH, Han JM, Yu CP, Jeng KS, Hu CP, Tao MH. Long-term inhibition of hepatitis B virus in transgenic mice by double-stranded adeno-associated virus 8-delivered short hairpin RNA. *Gene Ther* 2007; **14**: 11-19 [PMID: 16929350 DOI: 10.1038/sj.gt.3302846]

103 **Giering JC**, Grimm D, Storm TA, Kay MA. Expression of shRNA from a tissue-specific pol II promoter is an effective and safe RNAi therapeutic. *Mol Ther* 2008; **16**: 1630-1636 [PMID: 18665161 DOI: 10.1038/mt.2008.144]

104 **Chen CC**, Sun CP, Ma HI, Fang CC, Wu PY, Xiao X, Tao MH. Comparative study of anti-hepatitis B virus RNA interference by double-stranded adeno-associated virus serotypes 7, 8, and 9. *Mol Ther* 2009; **17**: 352-359 [PMID: 19066602 DOI: 10.1038/mt.2008.245]

105 **Sun Y**, Li Z, Li L, Li J, Liu X, Li W. Effective inhibition of hepatitis B virus replication by small interfering RNAs expressed from human foamy virus vectors. *Int J Mol Med* 2007; **19**: 705-711 [PMID: 17334648]

106 **Rauschhuber C**, Xu H, Salazar FH, Marion PL, Ehrhardt A. Exploring gene-deleted adenoviral vectors for delivery of short hairpin RNAs and reduction of hepatitis B virus infection in mice. *J Gene Med* 2008; **10**: 878-889 [PMID: 18470951 DOI: 10.1002/jgm.1207]

107 **Crowther C**, Mowa MB, Ely A, Arbuthnot PB. Inhibition of HBV replication in vivo using helper-dependent adenovirus vectors to deliver antiviral RNA interference expression cassettes. *Antivir Ther* 2014; **19**: 363-373 [PMID: 24296696 DOI: 10.3851/imp2713]

108 **Deng L**, Li G, Xi L, Yin A, Gao Y, You W, Wang X, Sun B. Hepatitis B virus inhibition in mice by lentiviral vector mediated short hairpin RNA. *BMC Gastroenterol* 2009; **9**: 73 [PMID: 19804649 DOI: 10.1186/1471-230X-9-73]

109 **Xiangji L**, Feng X, Qingbao C, Weifeng T, Xiaoqing J, Baihe Z, Feng S, Hongyang W, Mengchao W. Knockdown of HBV surface antigen gene expression by a lentiviral microRNA-based system inhibits HBV replication and HCC growth. *J Viral Hepat* 2011; **18**: 653-660 [PMID: 20642484 DOI: 10.1111/j.1365-2893.2010.01346.x]

110 **Starkey JL**, Chiari EF, Isom HC. Hepatitis B virus (HBV)-specific short hairpin RNA is capable of reducing the formation of HBV covalently closed circular (CCC) DNA but has no effect on established CCC DNA in vitro. *J Gen Virol* 2009; **90**: 115-126 [PMID: 19088280 DOI: 10.1099/vir.0.004408-0]

111 **Morrissey DV**, Lockridge JA, Shaw L, Blanchard K, Jensen K, Breen W, Hartsough K, Machemer L, Radka S, Jadhav V, Vaish N, Zinnen S, Vargeese C, Bowman K, Shaffer CS, Jeffs LB, Judge A, MacLachlan I, Polisky B. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. *Nat Biotechnol* 2005; **23**: 1002-1007 [PMID: 16041363 DOI: 10.1038/nbt1122]

112 **Peng J**, Zhao Y, Mai J, Pang WK, Wei X, Zhang P, Xu Y. Inhibition of hepatitis B virus replication by various RNAi constructs and their pharmacodynamic properties. *J Gen Virol* 2005; **86**: 3227-3234 [PMID: 16298967 DOI: 10.1099/vir.0.81171-0]

113 **Wu K**, Gong Y, Zhang X, Zhang Y, Mu Y, Liu F, Song D, Zhu Y, Wu J. Inhibition of hepatitis B virus replication by recombinant small interfering RNAs. *Acta Virol* 2005; **49**: 235-241 [PMID: 16402680]

114 **Wu KL**, Zhang X, Zhang J, Yang Y, Mu YX, Liu M, Lu L, Li Y, Zhu Y, Wu J. Inhibition of Hepatitis B virus gene expression by single and dual small interfering RNA treatment. *Virus Res* 2005; **112**: 100-107 [PMID: 16022904 DOI: 10.1016/j.virusres.2005.04.001]

115 **Weinberg MS**, Ely A, Barichievy S, Crowther C, Mufamadi S, Carmona S, Arbuthnot P. Specific inhibition of HBV replication in vitro and in vivo with expressed long hairpin RNA. *Mol Ther* 2007; **15**: 534-541 [PMID: 17213835 DOI: 10.1038/sj.mt.6300077]

116 **Ely A**, Naidoo T, Arbuthnot P. Efficient silencing of gene expression with modular trimeric Pol II expression cassettes comprising microRNA shuttles. *Nucleic Acids Res* 2009; **37**: e91 [PMID: 19474340 DOI: 10.1093/nar/gkp446]

117 **Gish RG**, Satishchandran C, Young M, Pachuk C. RNA interference and its potential applications to chronic HBV treatment: results of a Phase I safety and tolerability study. *Antivir Ther* 2011; **16**: 547-554 [PMID: 21685542 DOI: 10.3851/IMP1798]

118 **Grimm D**, Streetz KL, Jopling CL, Storm TA, Pandey K, Davis CR, Marion P, Salazar F, Kay MA. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature* 2006; **441**: 537-541 [PMID: 16724069 DOI: 10.1038/nature04791]

119 **Ely A**, Naidoo T, Mufamadi S, Crowther C, Arbuthnot P. Expressed anti-HBV primary microRNA shuttles inhibit viral replication efficiently in vitro and in vivo. *Mol Ther* 2008; **16**: 1105-1112 [PMID: 18431360 DOI: 10.1038/mt.2008.82]

120 **Jenke AC**, Wilhelm AD, Orth V, Lipps HJ, Protzer U, Wirth S. Long-term suppression of hepatitis B virus replication by short hairpin RNA expression using the scaffold/matrix attachment region-based replicating vector system pEPI-1. *Antimicrob Agents Chemother* 2008; **52**: 2355-2359 [PMID: 18474581 DOI: 10.1128/aac.00067-08]

121 **Wooddell CI**, Rozema DB, Hossbach M, John M, Hamilton HL, Chu Q, Hegge JO, Klein JJ, Wakefield DH, Oropeza CE, Deckert J, Roehl I, Jahn-Hofmann K, Hadwiger P, Vornlocher HP, McLachlan A, Lewis DL. Hepatocyte-targeted RNAi therapeutics for the treatment of chronic hepatitis B virus infection. *Mol Ther* 2013; **21**: 973-985 [PMID: 23439496 DOI: 10.1038/mt.2013.31]

122 **Li Z.** Arrowhead Completes Enrollment of First Cohort in Phase 2a Trial of Chronic Hepatitis B Candidate ARC-520. 2014. Available from: URL: http://www.arrowheadresearch.com/press-releases/arrowhead-completes-enrollment-first-cohort-phase-2a-trial-chronic-hepatitis-b-0

123 **Chinnappan M**, Singh AK, Kakumani PK, Kumar G, Rooge SB, Kumari A, Varshney A, Rastogi A, Singh AK, Sarin SK, Malhotra P, Mukherjee SK, Bhatnagar RK. Key elements of the RNAi pathway are regulated by hepatitis B virus replication and HBx acts as a viral suppressor of RNA silencing. *Biochem J* 2014; **462**: 347-358 [PMID: 24902849 DOI: 10.1042/BJ20140316]

124 **Bibikova M**, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, Chandrasegaran S. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. *Mol Cell Biol* 2001; **21**: 289-297 [PMID: 11113203 DOI: 10.1128/MCB.21.1.289-297.2001]

125 **Lombardo A**, Genovese P, Beausejour CM, Colleoni S, Lee YL, Kim KA, Ando D, Urnov FD, Galli C, Gregory PD, Holmes MC, Naldini L. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. *Nat Biotechnol* 2007; **25**: 1298-1306 [PMID: 17965707 DOI: 10.1038/nbt1353]

126 **Hockemeyer D**, Soldner F, Beard C, Gao Q, Mitalipova M, DeKelver RC, Katibah GE, Amora R, Boydston EA, Zeitler B, Meng X, Miller JC, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Jaenisch R. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. *Nat Biotechnol* 2009; **27**: 851-857 [PMID: 19680244 DOI: 10.1038/nbt.1562]

127 **Cermak T**, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res* 2011; **39**: e82 [PMID: 21493687 DOI: 10.1093/nar/gkr218]

128 **Li T**, Huang S, Zhao X, Wright DA, Carpenter S, Spalding MH,

Weeks DP, Yang B. Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. *Nucleic Acids Res* 2011; **39**: 6315-6325 [PMID: 21459844 DOI: 10.1093/nar/gkr188]

129 **Jinek M**, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012; **337**: 816-821 [PMID: 22745249 DOI: 10.1126/science.1225829]

130 **Cong L**, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science* 2013; **339**: 819-823 [PMID: 23287718 DOI: 10.1126/science.1231143]

131 **Mali P**, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM. RNA-guided human genome engineering via Cas9. *Science* 2013; **339**: 823-826 [PMID: 23287722 DOI: 10.1126/science.1232033]

132 **Chapman JR**, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell* 2012; **47**: 497-510 [PMID: 22920291 DOI: 10.1016/j.molcel.2012.07.029]

133 **Blancafort P**, Segal DJ, Barbas CF. Designing transcription factor architectures for drug discovery. *Mol Pharmacol* 2004; **66**: 1361-1371 [PMID: 15340042 DOI: 10.1124/mol.104.002758]

134 **Cathomen T**, Joung JK. Zinc-finger nucleases: the next generation emerges. *Mol Ther* 2008; **16**: 1200-1207 [PMID: 18545224 DOI: 10.1038/mt.2008.114]

135 **Carroll D**. Progress and prospects: zinc-finger nucleases as gene therapy agents. *Gene Ther* 2008; **15**: 1463-1468 [PMID: 18784746 DOI: 10.1038/gt.2008.145]

136 **Kim YG**, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. *Proc Natl Acad Sci USA* 1996; **93**: 1156-1160 [PMID: 8577732 DOI: 10.1073/pnas.93.3.1156]

137 **Zimmerman KA**, Fischer KP, Joyce MA, Tyrrell DL. Zinc finger proteins designed to specifically target duck hepatitis B virus covalently closed circular DNA inhibit viral transcription in tissue culture. *J Virol* 2008; **82**: 8013-8021 [PMID: 18524822 DOI: 10.1128/JVI.00366-08]

138 **Cradick TJ**, Keck K, Bradshaw S, Jamieson AC, McCaffrey AP. Zinc-finger nucleases as a novel therapeutic strategy for targeting hepatitis B virus DNAs. *Mol Ther* 2010; **18**: 947-954 [PMID: 20160705 DOI: 10.1038/mt.2010.20]

139 **Weber ND**, Stone D, Sedlak RH, De Silva Feelixge HS, Roychoudhury P, Schiffer JT, Aubert M, Jerome KR. AAV-mediated delivery of zinc finger nucleases targeting hepatitis B virus inhibits active replication. *PLoS One* 2014; **9**: e97579 [PMID: 24827459 DOI: 10.1371/journal.pone.0097579]

140 **McCarty DM**, Monahan PE, Samulski RJ. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Ther* 2001; **8**: 1248-1254 [PMID: 11509958 DOI: 10.1038/sj.gt.3301514]

141 **Boch J**, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, Lahaye T, Nickstadt A, Bonas U. Breaking the code of DNA binding specificity of TAL-type III effectors. *Science* 2009; **326**: 1509-1512 [PMID: 19933107 DOI: 10.1126/science.1178811]

142 **Neff KL**, Argue DP, Ma AC, Lee HB, Clark KJ, Ekker SC. Mojo Hand, a TALEN design tool for genome editing applications. *BMC Bioinformatics* 2013; **14**: 1 [PMID: 23323762 DOI: 10.1186/1471-2105-14-1]

143 **Reyon D**, Tsai SQ, Khayter C, Foden JA, Sander JD, Joung JK. FLASH assembly of TALENs for high-throughput genome editing. *Nat Biotechnol* 2012; **30**: 460-465 [PMID: 22484455 DOI: 10.1038/nbt.2170]

144 **Schmid-Burgk JL**, Schmidt T, Kaiser V, Höning K, Hornung V. A ligation-independent cloning technique for high-throughput assembly of transcription activator–like effector genes. *Nat Biotechnol* 2013; **31**: 76-81 [PMID: 23242165 DOI: 10.1038/nbt.2460]

145 **Uhde-Stone C**, Gor N, Chin T, Huang J, Lu B. A do-it-yourself protocol for simple transcription activator-like effector assembly. *Biol Proced Online* 2013; **15**: 3 [PMID: 23316790 DOI: 10.1186/1480-9222-15-3]

146 **Bloom K**, Ely A, Mussolino C, Cathomen T, Arbuthnot P. Inactivation of hepatitis B virus replication in cultured cells and in vivo with engineered transcription activator-like effector nucleases. *Mol Ther* 2013; **21**: 1889-1897 [PMID: 23883864 DOI: 10.1038/mt.2013.170]

147 **Chen J**, Zhang W, Lin J, Wang F, Wu M, Chen C, Zheng Y, Peng X, Li J, Yuan Z. An efficient antiviral strategy for targeting hepatitis B virus genome using transcription activator-like effector nucleases. *Mol Ther* 2014; **22**: 303-311 [PMID: 24025750 DOI: 10.1038/mt.2013.212]

148 **Lin SR**, Yang HC, Kuo YT, Liu CJ, Yang TY, Sung KC, Lin YY, Wang HY, Wang CC, Shen YC, Wu FY, Kao JH, Chen DS, Chen PJ. The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates In Vivo. *Mol Ther Nucleic Acids* 2014; **3**: e186 [PMID: 25137139 DOI: 10.1038/mtna.2014.38]

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REVIEW

Targeting the tumor stroma in hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common and deadly cancers worldwide. In ninety percent of the cases it develops as a result of chronic liver damage and it is thus a typical inflammation-related cancer characterized by the close relation between the tumor microenvironment and tumor cells. The stromal environment consists out of several cell types, including hepatic stellate cells, macrophages and endothelial cells. They are not just active bystanders in the pathogenesis of HCC, but play an important and active role in tumor initiation, progression and metastasis. Furthermore, the tumor itself influences these cells to create a background that is beneficial for sustaining tumor growth. One of the key players is the hepatic stellate cell, which is activated during liver damage and differentiates towards a myofibroblast-like cell. Activated stellate cells are responsible for the deposition of extracellular matrix, increase the production of angiogenic factors and stimulate the recruitment of macrophages. The increase of angiogenic factors (which are secreted by macrophages, tumor cells and activated stellate cells) will induce the formation of new blood vessels, thereby supplying the tumor with more oxygen and nutrients, thus supporting tumor growth and offering a passageway in the circulatory system. In addition, the secretion of chemokines by the tumor cells leads to the recruitment of tumor associated macrophages. These tumor associated macrophages are key actors of cancer-related inflammation, being the main type of inflammatory cells infiltrating the tumor environment and exerting a tumor promoting effect by secreting growth factors, stimulating angiogenesis and influencing the activation of stellate cells. This complex interplay between the several cell types involved in liver cancer emphasizes the need for targeting the tumor stroma in HCC patients.

Key words: Hepatocellular carcinoma; Stellate cells; Cirrhosis; Angiogenesis; Macrophages; Inflammation

Core tip: Hepatocellular carcinoma is a primary liver tumor that usually develops in a background of chronic liver disease and fibrosis. It is the underlying chronic inflammation that creates an environment that not only causes but also enhances the formation and growth of tumors. The stromal compartment-including hepatic stellate cells, macrophages and endothelial cells-actively contribute to tumorigenesis, while the tumor itself influences these cells to create a background that is beneficial for tumor growth. This review focuses on the interplay between stroma and tumor cells, as well as therapeutic strategies that aim to target these complex interactions.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary liver tumor that usually develops in a background of chronic liver disease. It is the underlying chronic inflammation that creates an environment that not only causes but also enhances the formation and growth of tumors. Firstly, the continuous state of inflammation as a result of sustained liver damage can lead to hepatocyte cell death as well as compensatory proliferation, which can generate an accumulation of genomic lesions in hepatocytes. Secondly, the initiated cells are surrounded by an inflammatory niche that facilitates their progression towards malignant tumors. For instance, the fibrotic liver is characterized by an increased formation of blood vessels[1], which will benefit tumor cells for their blood supply as well as facilitating metastasis[2]. In addition, several factors produced by macrophages and activated stellate cells are known to directly stimulate and enhance tumor growth. Once the cancer has been established, the microenvironment continues to regulate the tumor behavior, influencing the development, progression and even response to therapy. All players within the tumor stroma strongly interact with each other, creating an environment that supports tumor growth (Figure 1). It is therefore not unlikely that future therapies will more and more focus on targeting these complex interactions in the tumor stroma (Figure 2). Ongoing clinical trials are listed in Table 1.

HEPATIC STELLATE CELLS

One major player in the formation of the perfect tumor environment is the activated hepatic stellate cell (HSC)[3,4]. During liver injury, the stellate cells undergo a transformation from quiescent cells that serve as the liver's resident vitamin-A storing cells, towards "activated" myofibroblast-like cells. These activated HSCs are characterized by increased proliferation and contractility, altered matrix protease activity and the secretion of extracellular matrix (ECM) proteins, as well as tumor growth factors and pro-angiogenic factors.

Several studies have shown that co-culturing HSC with different HCC cell lines induces phenotypic changes in the behavior of the tumor cells[5,6]. *In vitro* studies show that HSCs can directly influence the tumor cells (through the secretion of growth factors[7], matrix proteases[8] and/or ECM proteins[9]) and there is also evidence from *in vivo* studies that activated HSCs can create an immunosuppressive environment that promotes HCC growth[10,11]. The interaction between the tumor cells and HSCs is bidirectional, thereby allowing the tumor to alter the stellate cells (and the overall stromal environment) towards a more pro-tumoral phenotype[8]. Consistent with these findings, several *in vivo* studies have shown that inducing stellate cell activation increases liver fibrosis and hepatocarcinogenesis[12-15].

One of the key factors in this HSC-HCC cross talk is transforming growth factor (TGF)-β[14,16,17]. Activated HSC are the main source of TGF-β, however most liver cells (including malignant hepatocytes) have the ability to produce TGF-β as well. The TGF-β signaling pathway consists of three distinct ligands, TGF-β1, TGF-β2, and TGF-β3 which all bind to a specific receptor by first engaging with the TGF-βR1, which then heterodimerizes with the TGF-βR2. This causes the phosphorylation of Smad2 and 3, initiating an activation cascade leading to the induction several nuclear transduction proteins. Alternative pathway activation is possible, including the activation of AKT and other intracellular activation proteins. Interestingly, Smad7 antagonizes TGF-β mediated activation of hepatic stellate cells and protects against liver damage[18]. TGF-β signaling promotes HCC by several distinct mechanisms (reviewed more in detail by Dooley *et al*[17]): firstly, through functioning as a growth factor, by which it can act oncogenic or as tumor suppressor depending on the temporal and spatial availability of TGF-β in tumor and stromal cells[19,20]. And secondly, by transforming HSC to activated myofibroblasts. Interestingly, Inhibitors of TGF-β signaling have been shown to block HCC in different experimental models[21], leading to the clinical investigation of the TGF-β inhibitor LY2157299 (NCT01246986 and NCT02178358). LY2157299 is a small molecule kinase inhibitor that binds to TGF-βR1 and hence inhibits TGF-β signaling.

The connective tissue growth factor (CTGF) is an extracellular matrix-associated heparin binding protein that is overexpressed in fibrotic lesions, and the overexpression correlates with the severity of fibrosis and can be linked to malignant transformation in patients with chronic hepatitis B[22]. CTGF is a downstream mediator of some TGF-β effects and it is induced by TGF-β in a SMAD2/3 and stat3 dependent way. Furthermore, IL-13 is able to induce CTGF expression in HSCs by activating TGF-β-independent Smad signaling *via* the Erk-MAPK pathway instead of the canonical JAK/Stat6 pathway[23]. CTGF expression in HSC leads to increased migration, proliferation, and collagen expression of these cells. In addition, studies have shown that TGF-β can elicit a direct effect on hepatocytes *via* CTGF, thus making it an interesting therapeutic target for multiple cell types involved in the fibrogenesis[18]. CTGF blocking antibodies have been tested in patients with idiopathic pulmonary fibrosis (NCT00074698) and animal studies have shown that CTGF-inhibition prevents liver fibrosis in

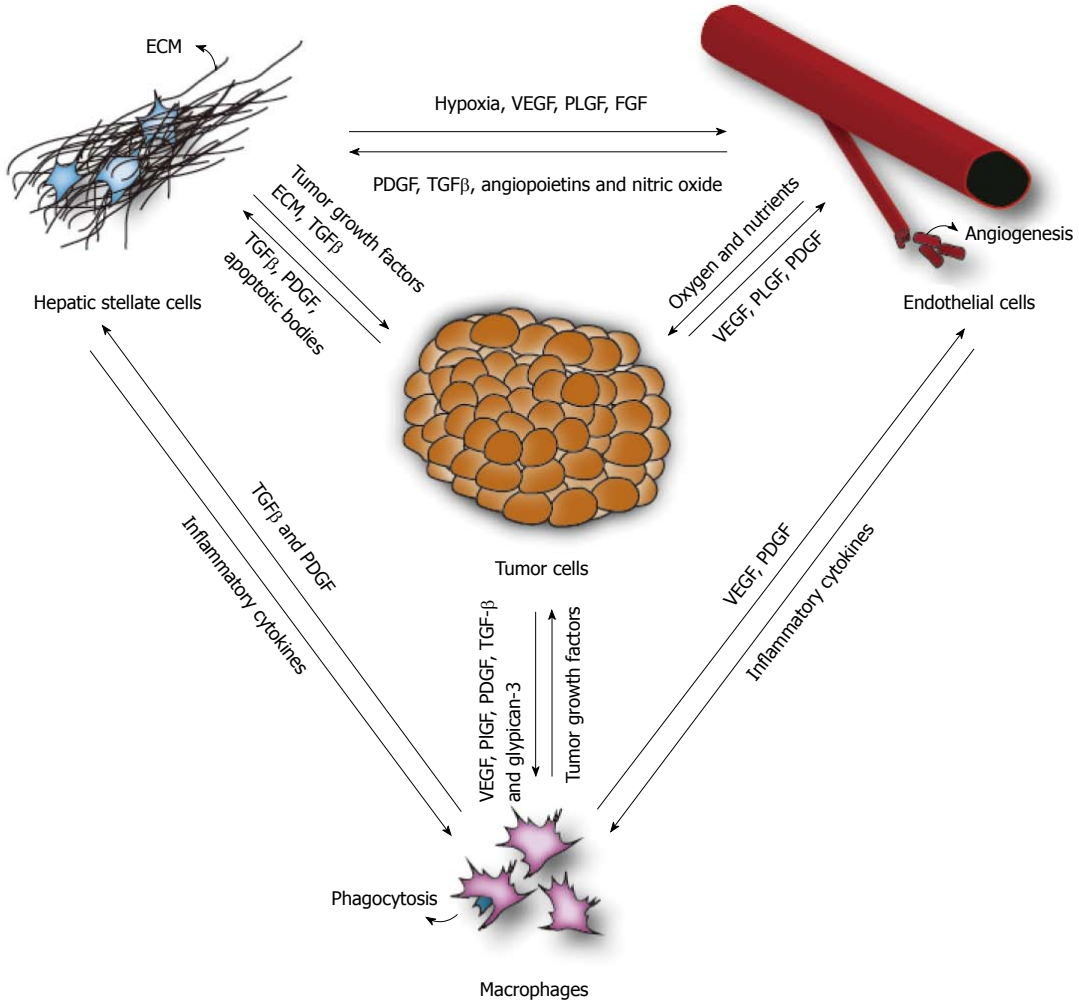

Figure 1 The interaction between tumor stroma and tumor cells in hepatocellular carcinoma. The several actors of the stromal compartment-including hepatic stellate cells, macrophages and endothelial cells-actively contribute to tumorigenesis, while the tumor itself influences these cells to create a background that is beneficial for tumor growth. Tumor cells activate the hepatic stellate cells, leading to the deposition of extracellular matrix (ECM), an increased production of angiogenic factors and the recruitment of macrophages. The increase of angiogenic factors (secreted by macrophages, tumor cells and activated stellate cells) will induce the formation of new blood vessels, thereby supplying the tumor with more oxygen and nutrients, thus supporting tumor growth. The increase of inflammatory cytokines, leads to the recruitment of macrophages, which can exert a pro-tumoral effect by secreting growth factor and influences the activation of stellate cells. This complex interplay between the several cell types involved in liver cancer emphasizes the need for a multi-targeted approach in hepatocellular carcinoma (HCC) patients. VEGF: Vascular endothelial growth factor; PDGF: Platelet-derived growth factor; FGF: Fibroblast growth factor.

rats[24]. However, no clinical trials on the effect on liver fibrosis have been done.

Another driver of HSC activation are members of the platelet derived growth factor (PDGF) family. PDGFs are potent mitogens for mesenchymal cells and work synergistically with TGF to activate stellate cells. Specific hepatic over-expression of PDGF-C leads to an increase in fibrosis and enhances hepatocarcinogenesis[12,15]. PDGF-B is also involved in different stages of liver cancer development and is an essential regulator in the development of liver fibrosis[25]. Hepatic overexpression of PDGF-B accelerates liver cancer, possibly by up regulating TGF-β receptor and by increasing expression of β-catenin as well as VEGF, CD31 and FGF. Several protein tyrosine kinase inhibitors-such as sorafenib, orantinib, sunitinib and SU6668-target PDGFR amongst other targets including VEGFR and FGFR. The protein tyrosine kinase inhibitor imatinib reduces stromal cell proliferation in this mouse model, which successfully inhibits tumor progression[13]. Imatinib is currently used to treat gastrointestinal stromal tumors[26] and could possibly benefit HCC patients.

The deposition of ECM proteins is one of the most characteristic hallmarks of the activated stellate cell. Several of the ECM components such as proteoglycans, laminins, collagens, and fibronectin interact directly and indirectly with HCC cells and the different stroma cell types. This not only changes the tumor phenotype, but also prepares a microenvironment that facilitates tumor growth. Since the ECM acts as a reservoir for growth factors and cytokines, it can rapidly release them to support the tumor's needs.

Heparan sulfate (HS) proteoglycans (PG) are expressed in the ECM and composed of a protein core to which HS is covalently attached as side

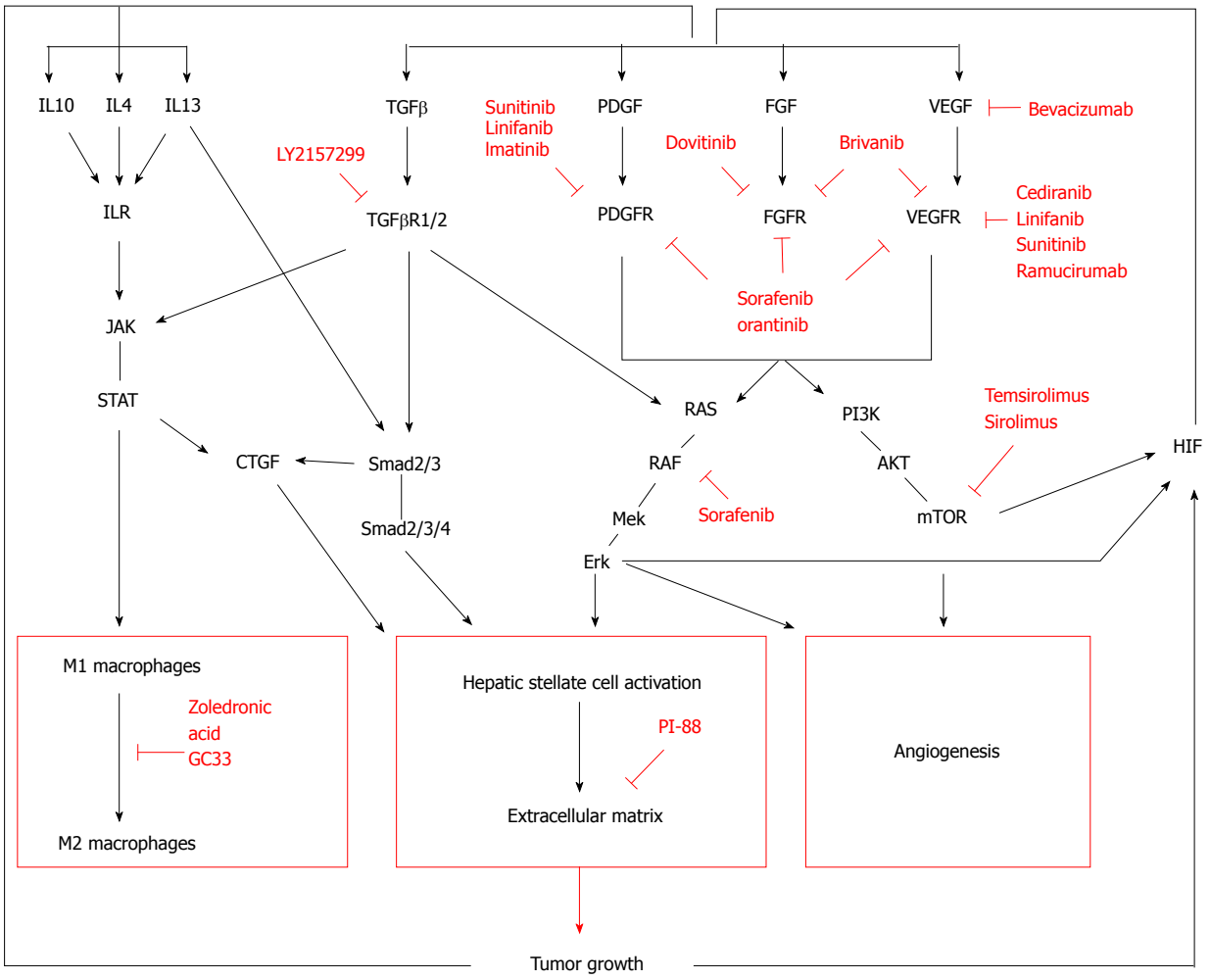

Figure 2 Schematic overview of (simplified) signaling pathways involved in the tumor-stroma interaction, and therapeutic targets that are currently tested in hepatocellular carcinoma. IL: Interleukin; TGF: Transforming growth factor; mTOR: Mammalian target of rapamycin.

chains. They maintain the structural framework of the tissue, store growth factors within the ECM or function as co-receptors. Desulfation of these co-receptor-PG's can abrogate growth factor signaling and inhibit tumor growth[27,28]. Heparanase cleaves the HS side chains of HSPG, leading to the release of HS-bound proteins, such as growth factors. PI-88 is a heparin sulfate mimic that specifically targets heparanase in cancer, thus preventing the release of growth factors that otherwise would contribute to tumor growth, angiogenesis and metastasis[29]. The safety and efficiency of PI-88 as an adjuvant therapy for post-operative HCC has been shown in a phase Ⅱ trial[30] and a recent follow up study revealed significant clinical benefits for patients with HCC[31]. Phase Ⅲ trials are currently ongoing (NCT01402908).

Another important glycoprotein is laminin-5. Laminin-5 is a member of the laminin family, which has been widely reported to be involved in the malignant phenotype of several cancers, including HCC[32]. Laminin-5 is expressed higher in metastatic HCC and has been shown to stimulate HCC cell migration[9,32].

Collagen is the major insoluble fibrous protein in the extracellular matrix. Besides its function as a supportive scaffold, collagens can also provoke a cellular response through the integrin family of transmembrane receptors. Several collagen types have been implicated in tumor growth and angiogenesis in different tumors[33-35] and a recent study has shown that collagen matrix protects malignant hepatocytes from apoptosis[36]. Antibodies targeting cleaved collagen epitopes have been clinically tested and show promising results in patients with solid tumors[37,38].

This deposition of ECM leads to an increase in liver stiffness, an important hallmark of the cirrhotic liver, which is also used as a diagnostic tool for patients with CLD[39]. This change in the mechanical properties of the tumor's surrounding has been associated with a higher risk of developing HCC[40]. In addition, the increase of ECM and the capillarization of hepatic sinusoids cause a vascular resistance that leads to hypoxia, stimulating the production of pro-angiogenic factors and subsequently inducing angiogenesis[1,41-43]. The activated HSCs also produce

Table 1 Overview of clinical trials that focus on the tumor environment of hepatocellular carcinoma

	Drug	Targets	Trial	Phase	Status	Ref.
Tyrosine kinase inhibitors	Sorafenib	PDGFR VEGFR RAF/MEK/ERK	NCT00105443	Ⅲ	Completed[1]	[90]
	Orantinib	PDGFR, FGFR VEGFR	NCT02178358	Ⅰ / Ⅱ	Completed	[91]
	Sunitinib	VEGFR	NCT00699374	Ⅲ	Terminated	[71]
		PDGFR	NCT00514228	Ⅱ	Completed	[70]
		RET	NCT00361309	Ⅱ	Completed	[69]
		CSF	NCT00428220	N/A	Ongoing	
	Linifanib	VEGF, PDGF, PDGFR-β, KDR	NCT01009593	Ⅲ	Terminated	
		CSF	NCT00517920	Ⅱ	Completed	[68]
	Brivanib	VEGFR	NCT00858871	Ⅲ	Completed	[59]
		FGFR	NCT00908752	Ⅲ	Ongoing	
			NCT00825955	Ⅲ	Ongoing	
			NCT01108705	Ⅲ	Terminated	
			NCT00355238	Ⅱ	Completed	[92]
			NCT00437424	Ⅰ	Completed	[93]
	Cediranib	VEGFR	NCT00238394	Ⅱ		
			NCT00427973	Ⅱ	Terminated	[54]
	Dovitinib	VEGFR, PDGFR, FGFR	NCT01232296	Ⅱ	Ongoing	
Antibodies	Bevacizumab	VEGF	NCT00335829	Ⅱ	Completed	[94]
			NCT00162669	Ⅱ	Completed	[50]
			NCT00605722	Ⅱ	Completed	[51]
			NCT00049322	Ⅱ	Completed	[52]
			NCT00280007	Ⅱ	Terminated	
			NCT01180959	Ⅱ	Ongoing	
	Ramucirumab	VEGFR	NCT00627042	Ⅱ	Completed	[53]
			NCT01140347	Ⅲ	Ongoing	
	GC33	Glypican-3	NCT01507168	Ⅱ	Completed	
			NCT00746317	Ⅰ	Completed	[84]
			NCT00976170	Ⅰ	Ongoing	
Other kinase inhibitors	Temsirolimus	mTOR	NCT01008917	Ⅰ	Ongoing	
			NCT01687673	Ⅱ	Recruiting	
	Everolimus	mTOR	NCT01035229	Ⅲ	Completed	[95]
			NCT01488487	Ⅱ	Ongoing	
			NCT00516165	Ⅰ / Ⅱ	Completed	[66]
			NCT00828594	Ⅰ	Terminated	
	LY2157299	TGF-βR1	NCT01246986	Ⅱ	Recruiting	
			NCT02178358	Ⅱ	Recruiting	
	PI-88	Heparanase	NCT00568308	Ⅲ	Terminated	
			NCT01402908	Ⅲ	Ongoing	
			NCT00247728	Ⅱ	Completed	[30]
	Zoledronic acid	Macrophages	NCT01259193	Ⅱ	Ongoing	

[1]Sorafenib is currently used as the standard-of-care for advanced hepatocellular carcinoma. CSF: Colony stimulating facto-1-receptor; mTOR: Mammalian target of rapamycin.

angiogenic growth factors, thus enhancing neo-angiogenesis[8]. This increased vasculature will allow small HCC lesions to progress and eventually metastasize.

ENDOTHELIAL CELLS

The prolonged fibrogenic process leads to an abnormal angioarchitecture distinctive for cirrhosis. Anatomical changes in the cirrhotic liver, such as fibrotic scar tissue compressing portal and central venules are responsible for an increased intrahepatic vascular resistance. In addition, the formation of fibrotic septa, as well as sinusoidal capillarisation, results in an increased resistance to blood flow and oxygen delivery. This causes hypoxia and the transcription of hypoxia-sensitive pro-angiogenic genes, thus stimulating the formation of new vessels. These new vessels can contribute to the inflammatory response by expressing chemokines and adhesion molecules, thus promoting the recruitment of inflammatory cells, such as macrophages. In addition, hepatic stellate cells are recruited to the angiogenic areas (*via* a number of signaling pathways, including PDGF, TGF-β, angiopoetins and nitric oxide) to contribute in vascular remodeling and stabilization[44]. Therefore, angiogenesis may contribute to the progression of liver cirrhosis and stimulate the growth of small dysplastic lesions to advanced solid tumors.

HCC is solid tumor that rapidly outgrows its blood

supply and therefore stimulates the formation of new blood vessels to fulfill its high needs in oxygen and nutrients. The malignant hepatocytes, as well as other actors in the microenvironment such as activated stellate cells and macrophages, secrete a number of angiogenic growth factors[1]. This induces an "angiogenic switch", which activates endothelial cells and basement membranes to remodel existing vessels, and form new vessels. These new vessels allow the tumor to rapidly expand and offer a passage in the circulatory system, thus facilitating metastasis. Therefore, targeting angiogenesis has become a common cancer therapy to treat solid tumors.

The vascular endothelial growth factor A (VEGF) is one of the key factors regulating angiogenesis. It is secreted by tumor cells, macrophages and stellate cells. VEGF binds to its receptors (VEGFR1 and VEGFR2) on the present endothelial cells, simulating endothelial cell proliferation and migration into the tumor, which results in vascular sprouting. Elevated VEGF levels are associated with tumor vascularity, metastasis, chemoresistance and poor prognosis[45-47].

Significant progress on the treatment of advanced HCC has been made possible by sorafenib. Sorafenib is a small molecular inhibitor targeting several tyrosine protein kinases in the Raf/MEK/ERK-pathway (anti-proliferative effect); and PDGF, VEGFR1 and VEGFR2 (anti-angiogenic effect). Sorafenib has become the standard-of-care for patients with advanced HCC and for those progressing after loco-regional therapies[48]. The success of sorafenib has opened the door for several anti-angiogenic agents to enter clinical studies on HCC[49]. At the moment, several multikinase inhibitors are being tested in clinical trials, including sunitinib, brivanib, linifanib, cediranib, pazopanib, lenvatinib and axitinib, as well as blocking-antibodies targeting angiogenic pathways.

Bevacizumab, a humanized monoclonal antibody that targets VEGF, has been approved for the treatment of various solid tumors and is currently being investigated as a treatment for HCC. Several phase Ⅱ trials have been completed and show that bevacizumab is well tolerated in HCC-patients, and could be a promising therapy as a single-agent[50], in combination with erlotinib[51] or after loco-regional therapies[52]. Ramucirumab is a monoclonal antibody targeting VEGFR2 which has been tested as a first line treatment (NCT00627042) for HCC-patients with promising results[53] and is currently being investigated as second line treatment after sorafenib (NCT01140347)[53]. Cediranib is a tyrosine kinase inhibitor that targets all VEGF receptors, which has been tested in two clinical trials (NCT00427973 and NCT00238394). Despite some anti-tumor effects, the high toxicity of cediranib makes it an unsuitable drug for HCC-patients HCC[54,55].

However, targeting VEGF has been shown to induce therapy escape mechanisms and many patients treated with VEGF-inhibitors or with sorafenib obtain a secondary resistance to therapy. Alternative angiogenic factors, such as the placental growth factor (PlGF), PDGF and fibroblast growth factor (FGF) have been implicated in this acquired tumor resistance and combination therapies could open the door for sustained treatment response[56]. Additionally, combining sorafenib with conventional chemotherapy could improve outcome and is currently tested in several phase Ⅲ trials (NCT01015833, NCT01214343)[57].

Brivanib and dovitinib are tyrosine kinase inhibitors of VEGF and fibroblast growth factor (FGF) signaling pathways, hence anticipating FGF-mediated resistance to anti-VEGF therapy[58]. Brivanib has been or is being investigated in several phase Ⅲ trials, including first-line treatment with brivanib *vs* sorafenib (NCT00858871)[59], second-line treatment with brivanib after progression on sorafenib treatment (NCT01108705), second-line treatment with brivanib after sorafenib (NCT00825955) and trans-arterial chemoembolization in combination with brivanib (NCT00908752). However, results from the study testing brivanib and sorafenib as first-line therapy in patients with HCC indicate there are no benefits of using brivanib over sorafenib[59] and study NCT01108705-testing brivanib after sorfanib treatment-has been terminated before completing the trial. Dovitinib trials are still ongoing (NCT01232296).

A drawback of anti-angiogenic therapies is that they aim to deprive the tumor from oxygen, leading to a hypoxic environment that stimulates cancer cells towards a more aggressive phenotype[60]. Therefore, long-term administration of anti-angiogenic treatment could trigger escape mechanisms and lead to increased metastasis[61,62].

An interesting way to indirectly target VEGF signaling and the HIF-pathway, is through inhibitors of the mammalian target of rapamycin (mTOR) pathway. mTOR signaling increases VEGF expression by up-regulating hypoxia inducible factor 1α[63]. Furthermore, mTOR-inhibitors can directly influence tumor growth by inhibiting the expression of anti-apoptotic proteins and by inducing autophagy[64]. Everolimus binds the cyclophilin FKBP-12, which binds the serine-threonine (ST) kinase mTOR when it is associated with raptor and mLST8 to form a complex (mTORC1), and subsequently inhibits downstream signaling, which involves cell cycle regulators and transcription factors such as HI. mTORC1 lies downstream of phosphatidylinositol 3′ kinase (PI3K), which is frequently activated in human cancers. Everolimus has been used in several clinical trials[65,66], but data from the latest phase Ⅲ trial (NCT01035229) show no improval in overall survival[67]. Temsirolimus is a sirolimus ester, which binds the same receptors. Trials using

temsirolimus are currently ongoing (NCT01008917 and NCT01687673).

The activated stellate cells also play a pivotal role in vascular remodeling, by creating a hypoxic environment, by producing angiogenic factors and also by migrating to angiogenic sites to contribute in the stabilization and maturation of (tumor) blood vessels. Current anti-angiogenic strategies for cancer have mostly focused on endothelial cells. However, combining drugs that target endothelial cells and stellate cells (or pericytes) could work synergistically as a therapy.

Several receptor tyrosine kinase inhibitors target VEGF and PDGF. Linifanib is a potent inhibitor of VEGF, PDGF, PDGFR-β, KDR and colony stimulating facto-1-receptor (CSF). A phase Ⅱ trial (NCT00517920) showed initial benefits for linifanib in HCC patients[68], however, the subsequent phase Ⅲ trial (NCT01009593) had to be terminated for unknown reasons. Sunitinib inhibits receptors for PDGF and VEGF, as well as other receptor tyrosine kinases such as CSF. While several phase Ⅱ trials (NCT00514228, NCT00361309) have shown promising results[69,70], it is inferior to sorafenib and the latest phase Ⅲ trial had to be terminated for safety reasons[71].

Orantinib is a receptor tyrosine kinase inhibitor that binds and inhibits the autophosphorylation of VEGFR2, PDGF-receptor and fibroblast growth factor receptor (FGFR), thereby inhibiting angiogenesis and cell proliferation. A phase Ⅰ/Ⅱ trial has shown a trend towards prolonged progression free survival in patients treated with orantinib after transarterial chemoembolization[72], and a phase Ⅲ trial is still ongoing (NCT01465464). Blocking PDGF signaling in mouse models of pancreatic carcinogenesis with orantinib caused regression of blood vessels, as a result of the detachment of pericytes from tumor vessels. The fact that tumor vessels lacking pericytes are more vulnerable suggests that they could be more responsive to other anti-angiogenic drugs[73,74]. Combining receptor tyrosine kinase inhibitors targeting ECs and pericytes successfully diminished tumor angiogenesis and decreased tumor size compared to a monotherapeutic approach in colon cancer[75]. Similar effects were seen when PDGF inhibitors were combined with anti-angiogenic treatments[74]. Thus, targeting stellate cells and endothelial cells may destabilize the existing tumor vasculature more potently than targeting each cell type individually.

MACROPHAGES

After liver damage, the pool of the liver's resident macrophages-Kupffer cells-is rapidly expanded. A harmful incident causes the hepatic macrophages to secrete pro-inflammatory cytokines and chemokines such as IL-1β, TNF, CCL2, and CCL5, resulting in the activation of protective or apoptotic signaling pathways of hepatocytes and the recruitment of immune cells that support hepatic injury. There is increasing evidence suggesting that phagocytosis of apoptotic bodies by HSC and by macrophages may directly stimulate fibrogenesis through upregulation of TGF-β[76]. Furthermore, these repeated cycles of hepatocyte death and compensatory proliferation provide a mitogenic and mutagenic environment that fuels the development of HCC.

The location of Kupffer cells in the sinusoids allows close interactions with other non-parenchymal liver cells. Firstly, Kupffer cells interact with other immune cells by secreting inflammatory cytokines and chemokines. Secondly, they can activate HSC *via* paracrine mechanisms, likely involving TGF-β and PDGF. These profibrotic functions of Kupffer cells during chronic liver injury possibly contribute to a tumor-stimulating environment in the cirrhotic liver. *In vivo* studies have shown that depleting macrophages reduces angiogenesis and slows down tumor progression in mouse models, and enhances the response to sorafenib[77].

Macrophages can be classified into two main classes depending on their phenotypic polarization: the M1-phenotype, triggering a Th1 immune response and exerting cytotoxic activity; and the M2-phenotype, which activates a Th2 immune response and promotes angiogenesis, tissue remodeling and tumor progression[78]. Macrophages can adapt to signals from the microenvironment and change their functional phenotype accordingly[79]. M1 macrophages are activated as a response to microbial stimuli and interferon gamma, while in a tumor environment the tumour-associated macrophages (TAMs) are mainly polarized towards a M2 phenotype. Increased numbers of M2-macrophages have been associated with angiogenesis, metastasis and poor prognosis.

Tumor associated macrophages are key actors of cancer-related inflammation, being the main type of inflammatory cells infiltrating the tumor environment[80]. In HCC, tumor cells have been shown to recruit and activate TAMs by the secretion of VEGF, PlGF, PDGF, TGF-β and glypican-3. Glypican-3 is a member of the glypican family of heparin-sulfate proteoglycans linked to the cell surface. It is highly expressed in the majority of HCC cells and is known for its role in the regulation of cell proliferation and apoptosis[81]. In addition, studies have suggested an involvement in the recruitment of M2-polarized TAM's in human HCC tissues[82]. Possibly glypican-3 present on the cell surface of malignant cells, binds to CCL5 and CCL3, which are chemokines that attract TAMs. Glypican-3 antibodies could therefore block the recruitment of TAMs *via* CCL5 and CCL3. Antibodies targeting glypican-3 have been tested in several phase Ⅰ trials for advanced HCC, with promising results. The antibody was well tolerated and preliminary antitumor activity show a threefold prolongation of the median time to progression in patients receiving glypican-3-antibodies compared to

untreated patients[83,84].

Zoledronic acid (ZA) is a compound widely used to prevent skeletal complications associated with bone metastases. Recent studies have shown a possible direct role as an anti-tumor agent by targeting the TAMs. ZA is taken up by macrophages *via* phagocytosis and leads to apoptosis specifically in TAMs, thus causing a repolarization of the macrophage population[85,86]. *In vivo* studies of ZA in combination with sorafenib have shown that the latter leads to an increase of M2-macrophages infiltrating the tumor stroma, which can be effectively depleted with ZA. This significantly inhibits angiogenesis, metastasis and tumor progression compared to sorafenib alone[77]. A phase Ⅱ study of sorafenib and ZA in advanced HCC has been conducted (NCT01259193), but no results have been published.

DISCUSSION

Several studies have shown that the stroma regulates the malignant transformation, survival, progression and metastasis of hepatocellular carcinoma. Factors derived from the tumor cells in their turn alter the tumor stroma to generate a tumor-permissive microenvironment. This complex interplay between the tumor and the different actors in the stroma establishes a promising axis for therapeutic targets (Figure 2).

VEGF targeting therapies have represented the first success in treating HCC patients in many years, reviving research in this field and leading to an explosion of clinical trials with anti-angiogenic therapies[49]. However, the success of treatments such as sorafenib needs to be followed by better understanding of the mechanisms that underlie the intrinsic and acquired resistance to anti-angiogenic therapies. Perhaps targeting several actors of the stromal environment and the tumor cells at the same time could be the key for optimal treatment in future therapies.

Sorafenib does not only inhibit angiogenesis, but also alters the inflammatory environment. Sorafenib has been shown to suppress natural killer cells and facilitate tumor growth and metastasis[87]. Furthermore, multi-tyrosine kinase inhibitors have been shown to increase infiltration of tumor-associated macrophages in the tumor environment which could contribute to the resistance or escape to anti-angiogenic treatment[77] (although it is important to note that some studies have shown the opposite effect[88]). Hence the solution could be the use of adjuvant immunotherapy along with tyrosine kinase inhibitors for patients with unresectable HCC in order to obtain long-term response. In fact, one of the first trials to confirm the efficacy of sorafenib in advanced, metastasized renal cell carcinoma, was performed in combination with immunotherapy with IL-2 and interferon-alpha[89].

Tumor associated macrophages are important actors of cancer-related inflammation, being the main type of inflammatory cells infiltrating the tumor environment. Targeting macrophages as a therapeutic strategy could be done by depleting the overall population of macrophages, or by altering their phenotype from a M2 towards an M1 orientation. Again, macrophages are known to not only interact with the tumor cells and stimulate their growth, they also influence stellate cell activation and angiogenesis.

The stellate cells are one of the key players in the formation of the perfect tumor environment. Not only do they directly affect tumor growth by secreting growth factors[7], matrix proteases[8] and/or ECM proteins[9], they also alter the mechanical properties of the tumor's surrounding. Activated stellate cells are known to stimulate angiogenesis, which allows the tumor cells to grow rapidly and invade in the circulatory system. The deposition of ECM proteins, such as collagens and proteoglycans, serve as a reservoir for growth factors, but also directly provoke a pro-tumoral cellular response. Indeed, the thick layer of ECM in the cirrhotic liver could impair drug delivery and hence decrease response to therapy. Therefore, preventing or reversing the activation of stellate cells could inhibit HCC growth, decrease angiogenesis and increase response to other therapies, such as classic chemotherapy or sorafenib.

As our understanding of the complex interplay between tumor and stroma evolves, the next-generation cancer drugs could target several actors in the tumor-stroma axis and offer a durable treatment for advanced HCC.

REFERENCES

1 **Coulon S**, Heindryckx F, Geerts A, Van Steenkiste C, Colle I, Van Vlierberghe H. Angiogenesis in chronic liver disease and its complications. *Liver Int* 2011; **31**: 146-162 [PMID: 21073649 DOI: 10.1111/j.1478-3231.2010.02369.x]

2 **Welti J**, Loges S, Dimmeler S, Carmeliet P. Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer. *J Clin Invest* 2013; **123**: 3190-3200 [PMID: 23908119 DOI: 10.1172/JCI70212]

3 **Puche JE**, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. *Compr Physiol* 2013; **3**: 1473-1492 [PMID: 24265236 DOI: 10.1002/cphy.c120035]

4 **Otranto M**, Sarrazy V, Bonté F, Hinz B, Gabbiani G, Desmoulière A. The role of the myofibroblast in tumor stroma remodeling. *Cell Adh Migr* 2012; **6**: 203-219 [PMID: 22568985 DOI: 10.4161/cam.20377]

5 **Geng ZM**, Li QH, Li WZ, Zheng JB, Shah V. Activated human hepatic stellate cells promote growth of human hepatocellular carcinoma in a subcutaneous xenograft nude mouse model. *Cell Biochem Biophys* 2014; **70**: 337-347 [PMID: 24676678 DOI: 10.1007/s12013-014-9918-3]

6 **Carloni V**, Luong TV, Rombouts K. Hepatic stellate cells and extracellular matrix in hepatocellular carcinoma: more complicated than ever. *Liver Int* 2014; **34**: 834-843 [PMID: 24397349 DOI: 10.1111/liv.12465]

7 **Amann T**, Bataille F, Spruss T, Mühlbauer M, Gäbele E, Schölmerich J, Kiefer P, Bosserhoff AK, Hellerbrand C. Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. *Cancer Sci* 2009; **100**: 646-653 [PMID: 19175606 DOI:

10.1111/j.1349-7006.2009.01087.x]
8 **Coulouarn C**, Corlu A, Glaise D, Guénon I, Thorgeirsson SS, Clément B. Hepatocyte-stellate cell cross-talk in the liver engenders a permissive inflammatory microenvironment that drives progression in hepatocellular carcinoma. *Cancer Res* 2012; **72**: 2533-2542 [PMID: 22419664 DOI: 10.1158/0008-5472.CAN-11-3317]
9 **Santamato A**, Fransvea E, Dituri F, Caligiuri A, Quaranta M, Niimi T, Pinzani M, Antonaci S, Giannelli G. Hepatic stellate cells stimulate HCC cell migration via laminin-5 production. *Clin Sci* (Lond) 2011; **121**: 159-168 [PMID: 21413933 DOI: 10.1042/CS20110002]
10 **Zhao W**, Zhang L, Xu Y, Zhang Z, Ren G, Tang K, Kuang P, Zhao B, Yin Z, Wang X. Hepatic stellate cells promote tumor progression by enhancement of immunosuppressive cells in an orthotopic liver tumor mouse model. *Lab Invest* 2014; **94**: 182-191 [PMID: 24296878 DOI: 10.1038/labinvest.2013.139]
11 **Zhao W**, Zhang L, Yin Z, Su W, Ren G, Zhou C, You J, Fan J, Wang X. Activated hepatic stellate cells promote hepatocellular carcinoma development in immunocompetent mice. *Int J Cancer* 2011; **129**: 2651-2661 [PMID: 21213212 DOI: 10.1002/ijc.25920]
12 **Campbell JS**, Hughes SD, Gilbertson DG, Palmer TE, Holdren MS, Haran AC, Odell MM, Bauer RL, Ren HP, Haugen HS, Yeh MM, Fausto N. Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma. *Proc Natl Acad Sci USA* 2005; **102**: 3389-3394 [PMID: 15728360 DOI: 10.1073/pnas.0409722102]
13 **Campbell JS**, Johnson MM, Bauer RL, Hudkins KL, Gilbertson DG, Riehle KJ, Yeh MM, Alpers CE, Fausto N. Targeting stromal cells for the treatment of platelet-derived growth factor C-induced hepatocellular carcinogenesis. *Differentiation* 2007; **75**: 843-852 [PMID: 17999742 DOI: 10.1111/j.1432-0436.2007.00235.x]
14 **Nitta T**, Kim JS, Mohuczy D, Behrns KE. Murine cirrhosis induces hepatocyte epithelial mesenchymal transition and alterations in survival signaling pathways. *Hepatology* 2008; **48**: 909-919 [PMID: 18712785 DOI: 10.1002/hep.22397]
15 **Wright JH**, Johnson MM, Shimizu-Albergine M, Bauer RL, Hayes BJ, Surapisitchat J, Hudkins KL, Riehle KJ, Johnson SC, Yeh MM, Bammler TK, Beyer RP, Gilbertson DG, Alpers CE, Fausto N, Campbell JS. Paracrine activation of hepatic stellate cells in platelet-derived growth factor C transgenic mice: evidence for stromal induction of hepatocellular carcinoma. *Int J Cancer* 2014; **134**: 778-788 [PMID: 23929039 DOI: 10.1002/ijc.28421]
16 **Giannelli G**, Villa E, Lahn M. Transforming growth factor-β as a therapeutic target in hepatocellular carcinoma. *Cancer Res* 2014; **74**: 1890-1894 [PMID: 24638984 DOI: 10.1158/0008-5472.CAN-14-0243]
17 **Dooley S**, Weng H, Mertens PR. Hypotheses on the role of transforming growth factor-beta in the onset and progression of hepatocellular carcinoma. *Dig Dis* 2009; **27**: 93-101 [PMID: 19546546 DOI: 10.1159/000218340]
18 **Dooley S**, Hamzavi J, Ciuclan L, Godoy P, Ilkavets I, Ehnert S, Ueberham E, Gebhardt R, Kanzler S, Geier A, Breitkopf K, Weng H, Mertens PR. Hepatocyte-specific Smad7 expression attenuates TGF-beta-mediated fibrogenesis and protects against liver damage. *Gastroenterology* 2008; **135**: 642-659 [PMID: 18602923 DOI: 10.1053/j.gastro.2008.04.038]
19 **Bogaerts E**, Heindryckx F, Vandewynckel YP, Van Grunsven LA, Van Vlierberghe H. The roles of transforming growth factor-β, Wnt, Notch and hypoxia on liver progenitor cells in primary liver tumours (Review). *Int J Oncol* 2014; **44**: 1015-1022 [PMID: 24504124 DOI: 10.3892/ijo.2014.2286]
20 **Ikushima H**, Miyazono K. TGFbeta signalling: a complex web in cancer progression. *Nat Rev Cancer* 2010; **10**: 415-424 [PMID: 20495575 DOI: 10.1038/nrc2853]
21 **Dituri F,** Mazzocca A, Peidro FJ, Papappicco P, Fabregat I, De Santis F, Paradiso A, Sabba C, Giannelli G. Differential Inhibition of the TGF-β Signaling Pathway in HCC Cells Using the Small Molecule Inhibitor LY2157299 and the D10 Monoclonal Antibody against TGF-β Receptor Type II. *PLoS One* 2013; **8**: e67109 [PMID: 23826206 DOI: 10.1371/journal.pone.0067109]
22 **Gressner OA**, Fang M, Li H, Lu LG, Gressner AM, Gao CF. Connective tissue growth factor (CTGF/CCN2) in serum is an indicator of fibrogenic progression and malignant transformation in patients with chronic hepatitis B infection. *Clin Chim Acta* 2013; **421**: 126-131 [PMID: 23501329 DOI: 10.1016/j.cca.2013.02.029]
23 **Liu Y**, Meyer C, Müller A, Herweck F, Li Q, Müllenbach R, Mertens PR, Dooley S, Weng HL. IL-13 induces connective tissue growth factor in rat hepatic stellate cells via TGF-β-independent Smad signaling. *J Immunol* 2011; **187**: 2814-2823 [PMID: 21804025 DOI: 10.4049/jimmunol.1003260]
24 **Li G**, Xie Q, Shi Y, Li D, Zhang M, Jiang S, Zhou H, Lu H, Jin Y. Inhibition of connective tissue growth factor by siRNA prevents liver fibrosis in rats. *J Gene Med* 2006; **8**: 889-900 [PMID: 16652398 DOI: 10.1002/jgm.894]
25 **Maass T**, Thieringer FR, Mann A, Longerich T, Schirmacher P, Strand D, Hansen T, Galle PR, Teufel A, Kanzler S. Liver specific overexpression of platelet-derived growth factor-B accelerates liver cancer development in chemically induced liver carcinogenesis. *Int J Cancer* 2011; **128**: 1259-1268 [PMID: 20506153 DOI: 10.1002/ijc.25469]
26 **Patel S**. Long-term efficacy of imatinib for treatment of metastatic GIST. *Cancer Chemother Pharmacol* 2013; **72**: 277-286 [PMID: 23503753 DOI: 10.1007/s00280-013-2135-8]
27 **Lai JP**, Sandhu DS, Shire AM, Roberts LR. The tumor suppressor function of human sulfatase 1 (SULF1) in carcinogenesis. *J Gastrointest Cancer* 2008; **39**: 149-158 [PMID: 19373441 DOI: 10.1007/s12029-009-9058-y]
28 **Lai JP**, Thompson JR, Sandhu DS, Roberts LR. Heparin-degrading sulfatases in hepatocellular carcinoma: roles in pathogenesis and therapy targets. *Future Oncol* 2008; **4**: 803-814 [PMID: 19086847 DOI: 10.2217/14796694.4.6.803]
29 **Parish CR**, Freeman C, Brown KJ, Francis DJ, Cowden WB. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. *Cancer Res* 1999; **59**: 3433-3441 [PMID: 10416607]
30 **Liu CJ**, Lee PH, Lin DY, Wu CC, Jeng LB, Lin PW, Mok KT, Lee WC, Yeh HZ, Ho MC, Yang SS, Lee CC, Yu MC, Hu RH, Peng CY, Lai KL, Chang SS, Chen PJ. Heparanase inhibitor PI-88 as adjuvant therapy for hepatocellular carcinoma after curative resection: a randomized phase II trial for safety and optimal dosage. *J Hepatol* 2009; **50**: 958-968 [PMID: 19303160 DOI: 10.1016/j.jhep.2008.12.023]
31 **Liu CJ**, Chang J, Lee PH, Lin DY, Wu CC, Jeng LB, Lin YJ, Mok KT, Lee WC, Yeh HZ, Ho MC, Yang SS, Yang MD, Yu MC, Hu RH, Peng CY, Lai KL, Chang SS, Chen PJ. Adjuvant heparanase inhibitor PI-88 therapy for hepatocellular carcinoma recurrence. *World J Gastroenterol* 2014; **20**: 11384-11393 [PMID: 25170226 DOI: 10.3748/wjg.v20.i32.11384]
32 **Giannelli G**, Fransvea E, Bergamini C, Marinosci F, Antonaci S. Laminin-5 chains are expressed differentially in metastatic and nonmetastatic hepatocellular carcinoma. *Clin Cancer Res* 2003; **9**: 3684-3691 [PMID: 14506159]
33 **Duan W**, Ma J, Ma Q, Xu Q, Lei J, Han L, Li X, Wang Z, Wu Z, Lv S, Ma Z, Liu M, Wang F, Wu E. The Activation of β1-integrin by Type I Collagen Coupling with the Hedgehog Pathway Promotes the Epithelial-Mesenchymal Transition in Pancreatic Cancer. *Curr Cancer Drug Targets* 2014 [PMID: 24720337]
34 **Sok JC**, Lee JA, Dasari S, Joyce S, Contrucci SC, Egloff AM, Trevelline BK, Joshi R, Kumari N, Grandis JR, Thomas SM. Collagen type XI α1 facilitates head and neck squamous cell cancer growth and invasion. *Br J Cancer* 2013; **109**: 3049-3056 [PMID: 24231953 DOI: 10.1038/bjc.2013.624]
35 **Shields MA**, Dangi-Garimella S, Redig AJ, Munshi HG. Biochemical role of the collagen-rich tumour microenvironment in pancreatic cancer progression. *Biochem J* 2012; **441**: 541-552 [PMID: 22187935 DOI: 10.1042/BJ20111240]
36 **Mailloux A,** Epling-Burnette P. Collagen matrix deposition by hepatic stellate cells protects hepatocellular carcinoma from NK-

mediated cytotoxicity. *J Immunol* 2013; **190.** Available from: http: //www.jimmunol.org/cgi/content/meeting_abstract/190/1_MeetingAbstracts/53.7

37 **Robert F,** Gordon MS, Rosen LS, Mendelson DS, Mulay M, Adams BJ, Alvarez D, Theuer CP, Leigh BR. Final results from a phase I study of TRC093 (humanized anti-cleaved collagen antibody) in patients with solid cancer. *J Clin Oncol* 2010; **28:** 15

38 **Gordon MS,** Rosen LS, Robert F, Mendelson DS, Kleinzweig D, Adams BJ, Theuer CP. A phase 1, open-label, dose escalation study of the humanized monoclonal antibody (HuMAb) TRC093, an inhibitor of angiogenesis that binds to cleaved collagen, in patients with locally advanced or metastatic solid tumors. *Ejc Supplements* 2008; **6:** 130-130 [DOI: 10.1016/S1359-6349(08)72348-6]

39 **Wong GL**. Transient elastography: Kill two birds with one stone? *World J Hepatol* 2013; **5**: 264-274 [PMID: 23717737 DOI: 10.4254/wjh.v5.i5.264]

40 **Wang HM**, Hung CH, Lu SN, Chen CH, Lee CM, Hu TH, Wang JH. Liver stiffness measurement as an alternative to fibrotic stage in risk assessment of hepatocellular carcinoma incidence for chronic hepatitis C patients. *Liver Int* 2013; **33**: 756-761 [PMID: 23405889 DOI: 10.1111/liv.12118]

41 **Coulon S**, Legry V, Heindryckx F, Van Steenkiste C, Casteleyn C, Olievier K, Libbrecht L, Carmeliet P, Jonckx B, Stassen JM, Van Vlierberghe H, Leclercq I, Colle I, Geerts A. Role of vascular endothelial growth factor in the pathophysiology of nonalcoholic steatohepatitis in two rodent models. *Hepatology* 2013; **57**: 1793-1805 [PMID: 23299577 DOI: 10.1002/hep.26219]

42 **Heindryckx F**, Coulon S, Terrie E, Casteleyn C, Stassen JM, Geerts A, Libbrecht L, Allemeersch J, Carmeliet P, Colle I, Van Vlierberghe H. The placental growth factor as a target against hepatocellular carcinoma in a diethylnitrosamine-induced mouse model. *J Hepatol* 2013; **58**: 319-328 [PMID: 23046674 DOI: 10.1016/j.jhep.2012.09.032]

43 **Van Steenkiste C**, Ribera J, Geerts A, Pauta M, Tugues S, Casteleyn C, Libbrecht L, Olievier K, Schroyen B, Reynaert H, van Grunsven LA, Blomme B, Coulon S, Heindryckx F, De Vos M, Stassen JM, Vinckier S, Altamirano J, Bataller R, Carmeliet P, Van Vlierberghe H, Colle I, Morales-Ruiz M. Inhibition of placental growth factor activity reduces the severity of fibrosis, inflammation, and portal hypertension in cirrhotic mice. *Hepatology* 2011; **53**: 1629-1640 [PMID: 21520176 DOI: 10.1002/hep.24238]

44 **Hellström M**, Gerhardt H, Kalén M, Li X, Eriksson U, Wolburg H, Betsholtz C. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol* 2001; **153**: 543-553 [PMID: 11331305]

45 **Zhan P**, Qian Q, Yu LK. Serum VEGF level is associated with the outcome of patients with hepatocellular carcinoma: a meta-analysis. *Hepatobiliary Surg Nutr* 2013; **2**: 209-215 [PMID: 24570945 DOI: 10.3978/j.issn.2304-3881.2013.06.07]

46 **Tsuchiya K**, Asahina Y, Matsuda S, Muraoka M, Nakata T, Suzuki Y, Tamaki N, Yasui Y, Suzuki S, Hosokawa T, Nishimura T, Ueda K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Izumi N. Changes in plasma vascular endothelial growth factor at 8 weeks after sorafenib administration as predictors of survival for advanced hepatocellular carcinoma. *Cancer* 2014; **120**: 229-237 [PMID: 24122122 DOI: 10.1002/cncr.28384]

47 **Huang GW**, Yang LY, Lu WQ. Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival. *World J Gastroenterol* 2005; **11**: 1705-1708 [PMID: 15786555]

48 **Germano D**, Daniele B. Systemic therapy of hepatocellular carcinoma: current status and future perspectives. *World J Gastroenterol* 2014; **20**: 3087-3099 [PMID: 24696596 DOI: 10.3748/wjg.v20.i12.3087]

49 **Pircher A**, Medinger M, Drevs J. Liver cancer: Targeted future options. *World J Hepatol* 2011; **3**: 38-44 [PMID: 21423913 DOI: 10.4254/wjh.v3.i2.38]

50 **Boige V**, Malka D, Bourredjem A, Dromain C, Baey C, Jacques N, Pignon JP, Vimond N, Bouvet-Forteau N, De Baere T, Ducreux M, Farace F. Efficacy, safety, and biomarkers of single-agent bevacizumab therapy in patients with advanced hepatocellular carcinoma. *Oncologist* 2012; **17**: 1063-1072 [PMID: 22707516 DOI: 10.1634/theoncologist.2011-0465]

51 **Hsu CH**, Kang YK, Yang TS, Shun CT, Shao YY, Su WC, Sandoval-Tan J, Chiou TJ, Jin K, Hsu C, Cheng AL. Bevacizumab with erlotinib as first-line therapy in Asian patients with advanced hepatocellular carcinoma: a multicenter phase II study. *Oncology* 2013; **85**: 44-52 [PMID: 23838576 DOI: 10.1159/000350841]

52 **Britten CD**, Gomes AS, Wainberg ZA, Elashoff D, Amado R, Xin Y, Busuttil RW, Slamon DJ, Finn RS. Transarterial chemoembolization plus or minus intravenous bevacizumab in the treatment of hepatocellular cancer: a pilot study. *BMC Cancer* 2012; **12**: 16 [PMID: 22244160 DOI: 10.1186/1471-2407-12-16]

53 **Zhu AX**, Finn RS, Mulcahy M, Gurtler J, Sun W, Schwartz JD, Dalal RP, Joshi A, Hozak RR, Xu Y, Ancukiewicz M, Jain RK, Nugent FW, Duda DG, Stuart K. A phase II and biomarker study of ramucirumab, a human monoclonal antibody targeting the VEGF receptor-2, as first-line monotherapy in patients with advanced hepatocellular cancer. *Clin Cancer Res* 2013; **19**: 6614-6623 [PMID: 24088738 DOI: 10.1158/1078-0432.CCR-13-1442]

54 **Zhu AX**, Ancukiewicz M, Supko JG, Sahani DV, Blaszkowsky LS, Meyerhardt JA, Abrams TA, McCleary NJ, Bhargava P, Muzikansky A, Sheehan S, Regan E, Vasudev E, Knowles M, Fuchs CS, Ryan DP, Jain RK, Duda DG. Efficacy, safety, pharmacokinetics, and biomarkers of cediranib monotherapy in advanced hepatocellular carcinoma: a phase II study. *Clin Cancer Res* 2013; **19**: 1557-1566 [PMID: 23362324 DOI: 10.1158/1078-0432.CCR-12-3041]

55 **Alberts SR**, Fitch TR, Kim GP, Morlan BW, Dakhil SR, Gross HM, Nair S. Cediranib (AZD2171) in patients with advanced hepatocellular carcinoma: a phase II North Central Cancer Treatment Group Clinical Trial. *Am J Clin Oncol* 2012; **35**: 329-333 [PMID: 21422991 DOI: 10.1097/COC.0b013e3182118cdf]

56 **Lieu CH**, Tran H, Jiang ZQ, Mao M, Overman MJ, Lin E, Eng C, Morris J, Ellis L, Heymach JV, Kopetz S. The association of alternate VEGF ligands with resistance to anti-VEGF therapy in metastatic colorectal cancer. *PLoS One* 2013; **8**: e77117 [PMID: 24143206 DOI: 10.1371/journal.pone.0077117]

57 **Miyahara K**, Nouso K, Yamamoto K. Chemotherapy for advanced hepatocellular carcinoma in the sorafenib age. *World J Gastroenterol* 2014; **20**: 4151-4159 [PMID: 24764653 DOI: 10.3748/wjg.v20.i15.4151]

58 **Tai WT**, Cheng AL, Shiau CW, Liu CY, Ko CH, Lin MW, Chen PJ, Chen KF. Dovitinib induces apoptosis and overcomes sorafenib resistance in hepatocellular carcinoma through SHP-1-mediated inhibition of STAT3. *Mol Cancer Ther* 2012; **11**: 452-463 [PMID: 22180308 DOI: 10.1158/1535-7163.Mct-11-0412]

59 **Johnson PJ**, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng AL. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013; **31**: 3517-3524 [PMID: 23980084 DOI: 10.1200/JCO.2012.48.4410]

60 **Comito G**, Calvani M, Giannoni E, Bianchini F, Calorini L, Torre E, Migliore C, Giordano S, Chiarugi P. HIF-1α stabilization by mitochondrial ROS promotes Met-dependent invasive growth and vasculogenic mimicry in melanoma cells. *Free Radic Biol Med* 2011; **51**: 893-904 [PMID: 21703345 DOI: 10.1016/j.freeradbiomed.2011.05.042]

61 **Pàez-Ribes M**, Allen E, Hudock J, Takeda T, Okuyama H, Viñals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* 2009; **15**: 220-231 [PMID: 19249680 DOI: 10.1016/j.ccr.2009.01.027]

62 **Loges S**, Mazzone M, Hohensinner P, Carmeliet P. Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited. *Cancer Cell* 2009; **15**: 167-170 [PMID: 19249675 DOI: 10.1016/j.ccr.2009.02.007]

63 **Zhong H**, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 2000; **60**: 1541-1545 [PMID: 10749120]

64 **Thomas HE**, Mercer CA, Carnevalli LS, Park J, Andersen JB, Conner EA, Tanaka K, Matsutani T, Iwanami A, Aronow BJ, Manway L, Maira SM, Thorgeirsson SS, Mischel PS, Thomas G, Kozma SC. mTOR inhibitors synergize on regression, reversal of gene expression, and autophagy in hepatocellular carcinoma. *Sci Transl Med* 2012; **4**: 139ra84 [PMID: 22539746 DOI: 10.1126/scitranslmed.3003923]

65 **Finn RS**, Poon RT, Yau T, Klümpen HJ, Chen LT, Kang YK, Kim TY, Gomez-Martin C, Rodriguez-Lope C, Kunz T, Paquet T, Brandt U, Sellami D, Bruix J. Phase I study investigating everolimus combined with sorafenib in patients with advanced hepatocellular carcinoma. *J Hepatol* 2013; **59**: 1271-1277 [PMID: 23928403 DOI: 10.1016/j.jhep.2013.07.029]

66 **Zhu AX**, Abrams TA, Miksad R, Blaszkowsky LS, Meyerhardt JA, Zheng H, Muzikansky A, Clark JW, Kwak EL, Schrag D, Jors KR, Fuchs CS, Iafrate AJ, Borger DR, Ryan DP. Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. *Cancer* 2011; **117**: 5094-5102 [PMID: 21538343 DOI: 10.1002/cncr.26165]

67 **Zhu AX,** Kudo M, Assenat E, Cattan S, Kang YK, Lim HY, Poon RTP, Blanc JF, Vogel A, Chen CL, Dorval E, Peck-Radosavljevic M, Santoro A, Daniele B, Furuse J, Jappe A, Perraud K, Anak O, Sellami DB, Chen LT. EVOLVE-1: Phase 3 study of everolimus for advanced HCC that progressed during or after sorafenib. *J Clin Oncol* 2014; **32**: 3

68 **Toh HC**, Chen PJ, Carr BI, Knox JJ, Gill S, Ansell P, McKeegan EM, Dowell B, Pedersen M, Qin Q, Qian J, Scappaticci FA, Ricker JL, Carlson DM, Yong WP. Phase 2 trial of linifanib (ABT-869) in patients with unresectable or metastatic hepatocellular carcinoma. *Cancer* 2013; **119**: 380-387 [PMID: 22833179 DOI: 10.1002/cncr.27758]

69 **Sahani DV**, Jiang T, Hayano K, Duda DG, Catalano OA, Ancukiewicz M, Jain RK, Zhu AX. Magnetic resonance imaging biomarkers in hepatocellular carcinoma: association with response and circulating biomarkers after sunitinib therapy. *J Hematol Oncol* 2013; **6**: 51 [PMID: 23842041 DOI: 10.1186/1756-8722-6-51]

70 **Koeberle D**, Montemurro M, Samaras P, Majno P, Simcock M, Limacher A, Lerch S, Kovàcs K, Inauen R, Hess V, Saletti P, Borner M, Roth A, Bodoky G. Continuous Sunitinib treatment in patients with advanced hepatocellular carcinoma: a Swiss Group for Clinical Cancer Research (SAKK) and Swiss Association for the Study of the Liver (SASL) multicenter phase II trial (SAKK 77/06). *Oncologist* 2010; **15**: 285-292 [PMID: 20203173 DOI: 10.1634/theoncologist.2009-0316]

71 **Cheng AL**, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol* 2013; **31**: 4067-4075 [PMID: 24081937 DOI: 10.1200/JCO.2012.45.8372]

72 **Inaba Y**, Kanai F, Aramaki T, Yamamoto T, Tanaka T, Yamakado K, Kaneko S, Kudo M, Imanaka K, Kora S, Nishida N, Kawai N, Seki H, Matsui O, Arioka H, Arai Y. A randomised phase II study of TSU-68 in patients with hepatocellular carcinoma treated by transarterial chemoembolisation. *Eur J Cancer* 2013; **49**: 2832-2840 [PMID: 23764238 DOI: 10.1016/j.ejca.2013.05.011]

73 **Bergers G**, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 2003; **111**: 1287-1295 [PMID: 12727920 DOI: 10.1172/JCI17929]

74 **Pietras K**, Hanahan D. A multitargeted, metronomic, and maximum-tolerated dose "chemo-switch" regimen is antiangiogenic, producing objective responses and survival benefit in a mouse model of cancer. *J Clin Oncol* 2005; **23**: 939-952 [PMID: 15557593 DOI: 10.1200/JCO.2005.07.093]

75 **Shaheen RM**, Tseng WW, Davis DW, Liu W, Reinmuth N, Vellagas R, Wieczorek AA, Ogura Y, McConkey DJ, Drazan KE, Bucana CD, McMahon G, Ellis LM. Tyrosine kinase inhibition of multiple angiogenic growth factor receptors improves survival in mice bearing colon cancer liver metastases by inhibition of endothelial cell survival mechanisms. *Cancer Res* 2001; **61**: 1464-1468 [PMID: 11245452]

76 **Canbay A**, Taimr P, Torok N, Higuchi H, Friedman S, Gores GJ. Apoptotic body engulfment by a human stellate cell line is profibrogenic. *Lab Invest* 2003; **83**: 655-663 [PMID: 12746475]

77 **Zhang W**, Zhu XD, Sun HC, Xiong YQ, Zhuang PY, Xu HX, Kong LQ, Wang L, Wu WZ, Tang ZY. Depletion of tumor-associated macrophages enhances the effect of sorafenib in metastatic liver cancer models by antimetastatic and antiangiogenic effects. *Clin Cancer Res* 2010; **16**: 3420-3430 [PMID: 20570927 DOI: 10.1158/1078-0432.CCR-09-2904]

78 **Roderfeld M**, Rath T, Lammert F, Dierkes C, Graf J, Roeb E. Innovative immunohistochemistry identifies MMP-9 expressing macrophages at the invasive front of murine HCC. *World J Hepatol* 2010; **2**: 175-179 [PMID: 21160992 DOI: 10.4254/wjh.v2.i5.175]

79 **Stout RD**, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol* 2005; **175**: 342-349 [PMID: 15972667]

80 **Capece D**, Fischietti M, Verzella D, Gaggiano A, Cicciarelli G, Tessitore A, Zazzeroni F, Alesse E. The inflammatory microenvironment in hepatocellular carcinoma: a pivotal role for tumor-associated macrophages. *Biomed Res Int* 2013; **2013**: 187204 [PMID: 23533994 DOI: 10.1155/2013/187204]

81 **Yao M**, Wang L, Dong Z, Qian Q, Shi Y, Yu D, Wang S, Zheng W, Yao D. Glypican-3 as an emerging molecular target for hepatocellular carcinoma gene therapy. *Tumour Biol* 2014; **35**: 5857-5868 [PMID: 24633918 DOI: 10.1007/s13277-014-1776-5]

82 **Takai H**, Ashihara M, Ishiguro T, Terashima H, Watanabe T, Kato A, Suzuki M. Involvement of glypican-3 in the recruitment of M2-polarized tumor-associated macrophages in hepatocellular carcinoma. *Cancer Biol Ther* 2009; **8**: 2329-2338 [PMID: 19838081]

83 **Ikeda M**, Ohkawa S, Okusaka T, Mitsunaga S, Kobayashi S, Morizane C, Suzuki I, Yamamoto S, Furuse J. Japanese phase I study of GC33, a humanized antibody against glypican-3 for advanced hepatocellular carcinoma. *Cancer Sci* 2014; **105**: 455-462 [PMID: 24521523 DOI: 10.1111/cas.12368]

84 **Zhu AX**, Gold PJ, El-Khoueiry AB, Abrams TA, Morikawa H, Ohishi N, Ohtomo T, Philip PA. First-in-man phase I study of GC33, a novel recombinant humanized antibody against glypican-3, in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2013; **19**: 920-928 [PMID: 23362325 DOI: 10.1158/1078-0432.CCR-12-2616]

85 **Rogers TL**, Wind N, Hughes R, Nutter F, Brown HK, Vasiliadou I, Ottewell PD, Holen I. Macrophages as potential targets for zoledronic acid outside the skeleton-evidence from in vitro and in vivo models. *Cell Oncol* (Dordr) 2013; **36**: 505-514 [PMID: 24177992 DOI: 10.1007/s13402-013-0156-2]

86 **Coscia M**, Quaglino E, Iezzi M, Curcio C, Pantaleoni F, Riganti C, Holen I, Mönkkönen H, Boccadoro M, Forni G, Musiani P, Bosia A, Cavallo F, Massaia M. Zoledronic acid repolarizes tumour-associated macrophages and inhibits mammary carcinogenesis by targeting the mevalonate pathway. *J Cell Mol Med* 2010; **14**: 2803-2815 [PMID: 19818098 DOI: 10.1111/j.1582-4934.2009.00926.x]

87 **Zhang QB**, Sun HC, Zhang KZ, Jia QA, Bu Y, Wang M, Chai ZT, Zhang QB, Wang WQ, Kong LQ, Zhu XD, Lu L, Wu WZ, Wang L, Tang ZY. Suppression of natural killer cells by sorafenib contributes to prometastatic effects in hepatocellular carcinoma. *PLoS One* 2013; **8**: e55945 [PMID: 23409093 DOI: 10.1371/journal.pone.0055945]

88 **Sprinzl MF**, Reisinger F, Puschnik A, Ringelhan M, Ackermann K, Hartmann D, Schiemann M, Weinmann A, Galle PR, Schuchmann M, Friess H, Otto G, Heikenwalder M, Protzer U. Sorafenib perpetuates cellular anticancer effector functions by modulating the crosstalk between macrophages and natural killer cells. *Hepatology* 2013; **57**: 2358-2368 [PMID: 23424039 DOI: 10.1002/hep.26328]

89 **Escudier B**, Lassau N, Angevin E, Soria JC, Chami L, Lamuraglia M, Zafarana E, Landreau V, Schwartz B, Brendel E, Armand JP, Robert C. Phase I trial of sorafenib in combination with IFN alpha-2a in patients with unresectable and/or metastatic renal cell carcinoma or malignant melanoma. *Clin Cancer Res* 2007; **13**: 1801-1809 [PMID: 17363536 DOI: 10.1158/1078-0432.CCR-06-1432]

90 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

91 **Kanai F**, Yoshida H, Tateishi R, Sato S, Kawabe T, Obi S, Kondo Y, Taniguchi M, Tagawa K, Ikeda M, Morizane C, Okusaka T, Arioka H, Shiina S, Omata M. A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2011; **67**: 315-324 [PMID: 20390419 DOI: 10.1007/s00280-010-1320-2]

92 **Park JW**, Finn RS, Kim JS, Karwal M, Li RK, Ismail F, Thomas M, Harris R, Baudelet C, Walters I, Raoul JL. Phase II, open-label study of brivanib as first-line therapy in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2011; **17**: 1973-1983 [PMID: 21349999 DOI: 10.1158/1078-0432.CCR-10-2011]

93 **El-Khoueiry A**, Posey JA, Castillo Ferrando JR, Krishnamurthi SS, Syed S, Kollia G, Walters I, Fischer BS, Masson E. The effects of liver impairment on the pharmacokinetics of brivanib, a dual inhibitor of fibroblast growth factor receptor and vascular endothelial growth factor receptor tyrosine kinases. *Cancer Chemother Pharmacol* 2013; **72**: 53-64 [PMID: 23719718 DOI: 10.1007/s00280-013-2168-z]

94 **Buijs M**, Reyes DK, Pawlik TM, Blackford AL, Salem R, Messersmith WA, Weekes CD, Mulcahy M, Kamel IR, Geschwind JF. Phase 2 trial of concurrent bevacizumab and transhepatic arterial chemoembolization in patients with unresectable hepatocellular carcinoma. *Cancer* 2013; **119**: 1042-1049 [PMID: 23132335 DOI: 10.1002/cncr.27859]

95 **Zhu AX**, Kudo M, Assenat E, Cattan S, Kang YK, Lim HY, Poon RT, Blanc JF, Vogel A, Chen CL, Dorval E, Peck-Radosavljevic M, Santoro A, Daniele B, Furuse J, Jappe A, Perraud K, Anak O, Sellami DB, Chen LT. Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib: the EVOLVE-1 randomized clinical trial. *JAMA* 2014; **312**: 57-67 [PMID: 25058218 DOI: 10.1001/jama.2014.7189]

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REVIEW

Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver

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Abstract

Elevated iron stores as indicated by hyperferritinemia with normal or mildly elevated transferrin saturation and mostly mild hepatic iron deposition are a characteristic finding in subjects with non-alcoholic fatty liver disease (NAFLD). Excess iron is observed in approximately one third of NAFLD patients and is commonly referred to as the "dysmetabolic iron overload syndrome". Clinical evidence suggests that elevated body iron stores aggravate the clinical course of NAFLD with regard to liver-related and extrahepatic disease complications which relates to the fact that excess iron catalyses the formation of toxic hydroxyl-radicals subsequently resulting in cellular damage. Iron removal improves insulin sensitivity, delays the onset of type 2 diabetes mellitus, improves pathologic liver function tests and likewise ameliorates NAFLD histology. Several mechanisms contribute to pathologic iron accumulation in NAFLD. These include impaired iron export from hepatocytes and mesenchymal Kupffer cells as a consequence of imbalances in the concentrations of iron regulatory factors, such as hepcidin, cytokines, copper or other dietary factors. This review summarizes the knowledge about iron homeostasis in NAFLD and the rationale for its therapeutic implications.

Key words: Dysmetabolic iron overload syndrome; Hepcidin; Iron overload; Metabolic syndrome; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis

Core tip: Hyperferritinemia with normal transferrin saturation and mostly mild hepatic iron deposition is a frequent finding in subjects with non-alcoholic fatty liver disease. Excess iron in non-alcoholic fatty liver disease (NAFLD) patients is referred to as the "dysmetabolic iron overload syndrome". Clinical evidence suggests that elevated body iron stores aggravate the clinical course of NAFLD with regard to liver-related and extrahepatic disease complications. Iron removal improves insulin sensitivity, delays the onset of type 2 diabetes mellitus,

improves pathologic liver function tests and ameliorates NAFLD histology The mechanisms contributing to iron excess in fatty liver include impaired iron export from hepatocytes and mesenchymal Kupffer.

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INTRODUCTION

Physiological regulation of iron homeostasis

Iron is essential for life of mammalian organisms due to its paradigmatic role in oxygen transport and also in being a central component of many enzymes and proteins involved in mitochondrial respiration, DNA biosynthesis and the citric acid cycle, among others. However, excess iron is detrimental and may lead to severe organ damage as it facilitates the formation of reactive oxygen species (ROS) *via* the Fenton reaction. On the other hand, iron deficiency can lead to anemia and fatigue which are among the most common disorders in the world. In order to provide enough iron for biological function and at the same time avoid iron overload and toxicity, iron trafficking and storage are diligently balanced by a mechanisms involving bone marrow, intestine, liver and the reticuloendothelial system (RES)[1,2].

Many aspects of iron metabolism have been unravelled in recent years. Dietary iron is taken up as Fe^{2+} in the duodenum by the cation transporter divalent metal transporter 1[3,4]. After transfer through the duodenal baso-lateral membrane *via* the iron exporter ferroportin (FPN)[5,6], iron is oxidized by the copper containing ferroxidase hephaestin and loaded onto transferrin for systemic distribution[7]. Most cells facilitae iron uptake by transferrin bound Fe^{3+} *via* the transferrin-receptor (TfR1). Most iron is required for erythropoiesis and the biosynthesis of other heme enzymes like cytochromes, and excess iron is stored in hepatocytes[5,8]. Most iron for physiological requirements, mainly erythropoiesis, is obtained from re-utilisation of senescent erythrocytes which are taken up and degraded in splenic macrophages. Only approximately 1-2 mg of daily body iron requirements which are used for compensation of iron losses *via* bleeding, enteric and cutaneous cell desquamation are replenished *via* duodenal iron absorption. Iron export is facilitated by FPN from hepatocytes, macrophages and all other cells[9].

Systemic iron homeostasis is equilibrated by the peptide hepcidin (hepatic bactericidal protein) mainly derived from hepatocytes and regulated by iron status, hypoxia, anemia and inflammation[10-12]. Hepcidin impacts on iron trafficking by attaching to FPN which leads to the degradation of FPN and thereby to down-regulation of iron export inducing a decline in serum iron concentrations[13]. Quantitatively hepatocytes are the most important source for hepcidin, however, expression has also been reported in adipose tissue, pancreatic islets, macrophages, and even cardiac myocytes. Hence, iron homeostasis *via* FPN mediated iron export may be regulated in an autocrine fashion in these cells[14-16].

Perturbations of iron homeostasis are frequently observed in patients suffering from non-alcoholic fatty liver disease (NAFLD)[17,18]. As the prevalence of obesity rises, NAFLD with or without associated metabolic syndrome (MetS), has become the most frequent cause of hyperferritinemia. The first report of non-hemochromatotic iron overload linked to metabolic characteristics such as insulin resistance and overweight in a French study subsequently stimulated extensive research on the potential mechanisms underlying iron accumulation in NAFLD[19]. The dysmetabolic iron overload syndrome (DIOS) commonly refers to the characteristic association of fatty liver with moderate histological iron deposition (hemosiderosis) and increased serum ferritin[17,20].

WHAT IS THE IRON PHENOTYPE OF NAFLD?

An increase in ferritin concentrations is the key feature of iron dysregulation in subjects with NAFLD. It is found in one third to half of patients with NAFLD and ranges from mild elevations to rarely 1000-1500 ng/mL[17]. Serum ferritin concentrations increase with the number of features of the MetS[21]. Transferrin saturation (TfS) is typically in the upper range of normal or mildly elevated (45%-50%) which is distinct from hereditary hemochromatosis, where hyperferritinemia is accompanied by markedly elevated TfS and usually TfS is elevated before the development of hyperferritinemia in early stages of hemochromatosis[22].

Iron deposits in NAFLD are found in Kupffer cells which are the resident liver macrophages as well as in hepatocytes[20]. Mesenchymal iron deposition is more frequent than hepatocellular iron accumulation but mostly both compartments are affected[23]. This is different from tissue iron deposition in primary genetic iron overload, hemochromatosis, where the metal is almost exclusively found in the hepatocellular compartment (with the exception of ferroportin disease) and macrophages are iron deficient as a result of uninhibited iron export from these cells[24,25]. The extent of hyperferritinemia in subjects with NAFLD and/or the MetS overestimates the degree of iron overload compared to hemochromatosis. Phlebotomy studies demonstrated that in DIOS patients the amount of iron need to be removed

for normalisation of circulating iron parameters is usually significantly less than in hemochromatosis, indicating only mild body iron excess[26,27]. Few studies have performed liver iron quantification in NAFLD subjects and these results confirm the mild degree of tissue iron excess compared to genetic iron overload disorders[19,28]. The mild degree of body iron excess compared to markedly raised serum ferritin concentrations suggests that iron overload in NAFLD subjects results from a combination of alimentary and inflammatory driven iron loading and retention[20,29,30]. This is in line with the current evidence that NAFLD is both a metabolic and an inflammatory disease[31].

WHAT IS THE CLINICAL RELEVANCE OF ELEVATED IRON STORES?

IR and associated metabolic conditions

In 1981 Sullivan[32] suggested that the postponed occurrence of cardiovascular diseases in women compared to men and the subsequent postmenopausal increase could be caused by low premenopausal iron stores. This report likely is the first report of an impact of iron stores in non-hemochromatotic metabolic disorders. An association of iron stores with type 2 diabetes mellitus (T2DM) and various manifestations of IR has been repeatedly confirmed and a detailed discussion thereof is beyond the scope of this review[33]. However, glucose metabolism and iron homeostasis appear to be functionally interconnected, due to the fact that gluconeogenetic signals regulate iron homeostasis *via* hepcidin[34] while iron loading or deficiency directly affect circulating glucose concentrations in mammals most likely *via* its effects on citric acid cycle enzyme activities[35,36], thereby also affecting lipid profiles[37]. Ferritin concentrations were associated with an increased rate of diabetes and gestational diabetes[38-43], with BMI[44], visceral fat mass[45], serum glucose levels and insulin sensitivity[46], blood pressure[47], the MetS[21,48], the polycystic ovary syndrome (PCOS)[49] and cholesterol[50]. Higher parameters of iron storage clustered with metabolic risk markers in a study of obese[51] and healthy lean adolescents[52]. These observations are epidemiologically important as patients with IR have a higher risk of developing cerebrovascular or cardiovascular disease[53,54]. However, the most convincing argument for causative involvement of iron in obesity-related conditions is derived from iron removal studies mentioned in detail below. In summary, available studies convincingly suggest a direct impact of body iron on manifestations of IR or the MetS.

NAFLD

NAFLD has been firmly established as the hepatic manifestation of the MetS/IR[55]. The disease spectrum of NAFLD ranges from simple steatosis which is generally considered benign to steatosis with various stages of inflammation, hepatocellular ballooning and fibrosis called non-alcoholic steatohepatitis (NASH). NASH is the potentially progressive manifestation leading to cirrhosis, end-stage liver disease and hepatocellular carcinoma in a minority of patients[56]. To our knowledge, there is no data available suggesting that excess iron is linked to the extent of hepatic steatosis. Although multiple associations between iron homeostasis and lipid metabolism have been reported[57], no characteristic lipid phenotype has been documented to distinguish NAFLD with iron overload from NAFLD without iron. Underlying NAFLD may explain the link between MetS features and ferritin on the population level[58].

Several studies provide evidence that iron may contribute to more advanced fibrosis and thus to progression of NAFLD[18,59-63], however, this association was not confirmed in all studies[64-66]. The to date largest study reported that iron in NAFLD liver biopsies, particularly in Kupffer cells, was linked to more fibrosis and disease severity[67]. Iron deposition particularly in the Kupffer cell compartment was associated with higher markers of hepatocellular apoptosis and oxidative stress[68]. Some studies also suggested that an increased rate of HFE mutations could account for more progressed stages of NAFLD, but this was not reported in all studies[65,69-72]. Additionally the beta-globin trait[73], TMPRSS6[74], and the alpha-1-antitrypsin genotype[75] may modify the iron phenotype of NAFLD. It appears reasonable to conclude that the contribution of the genetic background may vary according to the geographic region. Data evaluating causality of iron in disease progression is limited by the feasibility of a prospective study with serial liver biopsies in enough patients to adjust for known co-factors of disease progression[64,76]. Retrospective studies demonstrated that, hyperferritinemia was linked to mortality of patients on the transplantation waiting list and it also had an impact on post-transplant mortality[77,78]. It is important to note that particularly sinusoidal iron deposition may be linked to the development of HCC in NASH[79].

In summary, the prevailing body of evidence suggests that excess iron is a contributing factor for the progression of steatosis to NASH, liver cirrhosis and also hepatocellular carcinoma. It remains to be established to what extent different patterns of iron deposition affect outcomes such as cirrhosis, HCC or cardiovascular diseases. The data mentioned above suggest that the pattern of iron deposition may have distinct effects.

HOW DOES IRON LEAD TO DISEASE PROGRESSION IN NAFLD?

It has been well recognized that iron overload leads to diabetes in patients with hemochromatosis where

IR increases and insulin secretion decreases with the rise of body iron stores[25,80-82]. Hepatic insulin sensitivity and insulin secretion are re-established in the majority once iron is removed[83,84]. However, the prediabetic stage in hemochromatotic mice and humans displays impaired β-cell function along with increased insulin sensitivity, whereas dietary iron overload similar to the prediabetic state in humans are characterized by peripheral IR[85]. Hence, lessons drawn from hemochromatosis models are likely not fully applicable to the role of iron in human IR and NAFLD.

Iron is well-recognized as a catalyst for the production of reactive oxygen intermediates *via* the Fenton reaction, and it is generally held that an increase of oxidative stress is a central mechanism for IR although direct proof for this hypothesis has not been obtained so far. Oxidative stress is a central pathogenic factor in NAFLD, T2DM and obesity[86-88] and markers of oxidative stress were increased in NAFLD with iron loading as compared to NAFLD without iron excess[68,89,90]. Generation of ROS may induce lipid peroxidation and cellular damage which may contribute to the progression of NAFLD. Importantly, oxidative stress induced molecules such as malonyldialdehyd and 4-hydroxynonenal may induce the formation of *de-novo* antigens with subsequent activation of T-lymphocytes and development of immunoglobulin G reactive against these antigens. This response was further enhanced by previous immunization against these antigens with a stimulated M1 macrophage response[91]. Although no studies have been performed, iron may contribute to this process by further augmentation of oxidative stress.

In cell culture, iron chelation re-established insulin receptor signalling and iron inhibited insulin receptor activity[92]. Desferoxamine increased the phosphorylation of Akt/protein kinase B (Akt/PKB), forkhead transcription factor O1 (FoxO1) and glycogen synthase kinase 3β (GSK3β) reflecting insulin effects on gluconeogenesis and glycogen synthesis. Likewise, genes playing a role in glucose utilization such as GLUT1 or hypoxia-inducible factor 1α (HIF1α) were up-regulated in hepatoma cells resulting in enhanced glucose removal[92]. In summary, these molecular observations indicate that iron affects IR by modulating insulin receptor signalling as has been recently reviewed[93].

Importantly, dietary iron intake may impact on glucose metabolism by affecting circadian rhythm *via* heme mediated effects on RevErb-α. Disruption of circadian rhythms, *e.g.,* through night-shift work is an established risk factor for metabolic and cardiovascular diseases[94,95].

In cultured fat cells, iron favored an IR, characterised by impaired glucose uptake and suppression of lipolysis in response to insulin[96,97]. Ferritin was inversely associated with adiponectin concentrations in insulin resistant and sensitive patients[98,99]. Knockout of FPN1 in adipocytes increased intracellular iron and subsequently reduced adiponectin biosynthesis, thus establishing a molecular link between adipocyte iron concentration and insulin resistance[100]. Furthermore, excess iron the diet may be routed to visceral adipose tissue and change the expression of adipokines, as demonstrated for resistin[101] Adipokines represent a diverse group of hormones which mediate the metabolic effects of diseased adipose tissue to organs and tissues. Associations have been observed between retinol-binding protein 4 (RBP4) and visfatin serum concentrations and parameters of iron metabolism[102,103]. However, these reports may reflect the co-incidence of elevated iron stores with surrogate markers of IR and do not prove causality[93].

Liver macrophages named Kupffer cells, which are an important site of iron storage in NAFLD, are tightly involved in the initiation of the hepatic inflammatory cascade in response to the uptake of oxidized lipoproteins[104] or oxidized phosphatidylcholines[105]. It is well known that macrophage iron status affects their inflammatory response pattern and polarization towards a pro-inflammatory phenotype[106], however, the particular role of these potential interactions have to our knowledge not been investigated in NAFLD.

Thus, the potential mechanisms of iron-induced NAFLD disease progression are complex and involve protean effects of iron in extrahepatic tissues as well direct liver damage.

WHAT ARE THE MECHANISMS UNDERLYING IRON ACCUMULATION IN NAFLD?

Hepcidin is the key regulator of systemic iron homeostasis and plays a role for the hemochromatotic and the inflammatory driven misdistribution of iron. Whereas the lack of hepcidin in hemochromatosis leads to uncoordinated duodenal iron absorption and iron accumulation in parenchymal tissues such as the liver[107]. the inflammation driven iron retention occurs mainly in monocytes/macrophages as a consequence of increased iron accumulation and reduced FPN mediated iron export from these cells, the latter being due to increased circulating hepcidin levels along with negative effects of certain cytokines on FPN expression[108]. The histological hallmarks of hemochromatosis, *i.e.*, hepatocellular iron, and also the inflammatory phenotype iron deposition in macrophages are both observed concurrently, suggesting that iron dysregulation is multifaceted in NAFLD. Several stimuli of hepcidin regulation have been reported which may be of particular relevance in NAFLD and also be related to different iron phenotypes. These stimuli and their relation

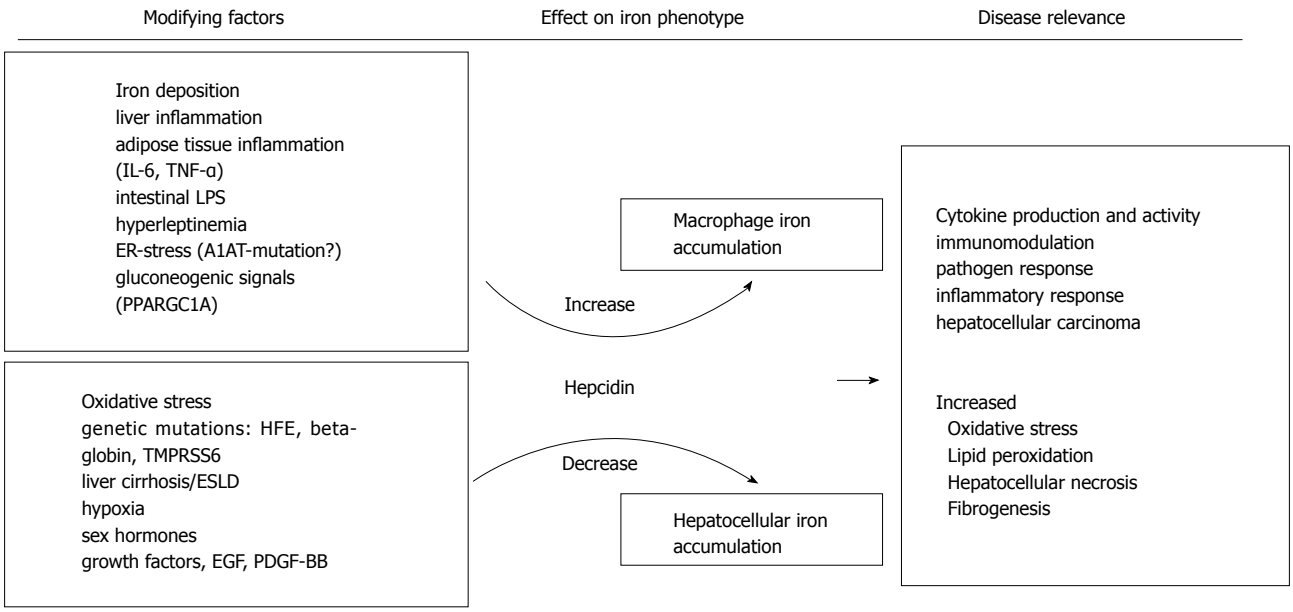

Figure 1 Summary of the potential stimuli that may affect iron homeostasis in non-alcoholic fatty liver disease. Both, increasing and decreasing stimuli have been reported in non-alcoholic fatty liver disease and it appears likely that the net balance of these frequently counteracting forces finally determines the iron phenotype in the individual. Patterns of iron deposition may also be linked to distinct clinical consequences. IL-6: Interleukin 6; TNF-α: Tumor necrosis factor-α; LPS: Lipopolysaccharide; ER: Endoplasmatic reticulum; A1AT: α-1-antitrypsin; PPARGC1A: Peroxisome proliferator-activated receptor gamma coactivator 1-α; TMPRSS6: Transmembrane protease, serine 6; ESLD: End-stage liver disease; EGF: Epidermal growth factor; PDGF-BB: Platelet derived growth factor BB.

to NAFLD iron accumulation are summarized in Figure 1. For several of these, like sex hormones, growth factors and hypoxia-induced circulating factors, the contribution to the dys-regulation of iron homeostasis in NAFLD has not been directly demonstrated but is physiologically plausible and these have therefore been included in the summary figure[109-112]. Additionally, alcohol consumption may decrease hepcidin expression and thus modify iron accumulation in NAFLD subjects[113], and although relevant alcohol consumption should be excluded in NAFLD subjects both conditions frequently co-exist. Thus, in NAFLD multiple, potentially counteracting signals impacting on hepcidin expression may be present at the same time. It is likely that the net balance of these signals finally determines the pattern of iron accumulation in the fatty liver of the individual patient.

Hepcidin levels in urin, serum and liver were elevated in NAFLD patients with iron excess compared to healthy subjects, hemochromatosis patients and NAFLD subjects without excess iron[28,114-116]. Hepcidin expression correlated directly with liver iron indicating an intact physiological response of hepcidin biosynthesis to iron in the liver[28,114]. Additionally, hepcidin is expressed in adipocytes of morbidly obese subjects[15]. Moreover, obesity is characterised by a chronic subclinical inflammation and in humans hepcidin concentrations and TNF-α were directly related, suggesting that both iron and inflammation contribute to hepcidin biosynthesis in NAFLD[28]. Furthermore, hepcidin and cytokines may be derived from both, the inflamed adipose and the liver[117,118]. Activation of gluconeogenesis *via* starvation, namely activation of peroxisome proliferator activated receptor gamma co-activator-1 α (PGC1α) increased hepcidin expression in a mouse model[34]. Likewise, iron fortification decreased gluconeogenesis *via* PGC1α in a murine model[119]. Hence, although PGC1α offers an intriguing cellular link between glucose and iron homeostasis, its relevance to human NAFLD remains to be elucidated. Leptin, was demonstrated to up-regulate hepcidin in hepatocytes in vitro by activation of the JAK2/STAT3 pathway. Hence, hyperleptinemia may directly contribute to higher hepcidin and thereby to iron deposition in NAFLD[120,121].

In NAFLD with iron overload the iron exporter FPN is lower than in controls and hemochromatosis patients in the liver and in the duodenum[28,114,122,123]. In NAFLD without liver iron accumulation, FPN levels were comparable to control subjects, but were significantly lower in NAFLD with hepatic iron on histology[28]. Along the same line of the observations, duodenal iron absorption was decreased in DIOS patients[124]. Obesity also represents a risk factor for an inadequate dietary iron fortification, linked to high hepcidin and low FPN expression[125]. Along this line mice feed a high fat diet presented with significantly reduced iron absorption which could be traced back diminished intestinal iron uptake. Mechanistically, the impaired iron absorption was independent of hepcidin but resulted from reduced metal uptake into the mucosa and transfer of iron across enterocyte membranes as a consequence of dietary induced discordant membrane-bound oxidoreductase expression[126].

An additional mechanism may be the phagocytosis of fragile erythrocytes by liver Kupffer cells. This was documented in rabbits on a high-fat diet and the phagocytosis of fragile erythrocytes was observed *in vitro*. Accumulation of erythrocytes was microscopically detected in inflamed regions in human NAFLD[127] suggesting that uptake of heme-iron *via* erythrophagocytosis may contribute to NAFLD iron accumulation, then promoting oxidative stress and inflammation.

Although cellular iron uptake *via* TfR1 is the most important route of iron uptake under physiological circumstances TfR1 appears not to be involved in excess iron uptake in NAFLD[128,129]. Hepatic TfR1 expression in NAFLD patients with low iron was increased compared to NAFLD and iron accumulation or patients with hemochromatosis suggesting physiologically intact TfR1 expression in response to iron stimuli[28] (Figure 2).

WHAT IS THE ROLE OF COPPER IN NAFLD?

Similar to iron, an adequate supply of copper is essential for proper biological function. Chronic copper deficiency can elicit anemia, leucopenia, myelopathy or skin abnormalities and excess copper may also facilitate the formation of ROS.

Copper affects lipid and glucose metabolism

There are several ways in which inadequate copper supply may be involved in the pathogenesis of NAFLD. Epidemiological studies found that copper deficiency is linked to atherogenic dyslipidemia and dietary copper supplementation improved cardiovascular risk markers in healthy adults[130] Investigations in rodent models demonstrated that dietary copper restriction induces hypertension or cardiac dysfunction, hypertriglyceridemia, hypercholesterolemia and modifies LDL and VLDL composition[131,132]. We recently reported low intrahepatic copper concentrations in human NAFLD compared to other liver diseases and that rats on a copper depleted diet developed IR and liver steatosis[133]. Increased oxidative stress is considered a key trigger in the pathogenesis of human NAFLD and one of the enzymes counteracting oxidative stress, Cu/Zn superoxide dismutase (SOD) depends on adequate copper availability, suggesting a potential link between copper availability and impaired antioxidant defense in NAFLD[134]. Sprague-Dawley rats exhibited an increased activity of the pro-inflammatory protein cyclo-oxygenase-2, when fed a diet with a low copper content[135]. Systemic copper deficiency causes mitochondrial dysfunction in mice and similar morphological and functional alterations have also been described in human NAFLD[136]. Recently, a detailed examination revealed an interaction of a high-fructose diet (which is also a culprit in the rise of obesity-related conditions) with low copper intake in triggering liver steatosis and damage as well as iron overload. Fructose acts as an inhibitor of duodenal copper absorption thereby leading to impaired oxidant defense and augmented lipid peroxidation[137]. As dietary copper content of the Western diet is rather low whereas iron and fructose are consumed in excess, this model offers attractive data to speculate that a dysbalance in micronutrient intake may have a significant role in NAFLD beyond calorie excess. Hence, animal and human data suggest that the therapeutic effect of dietary copper supplementation should be investigated as a subset of patients may potentially benefit.

Copper affects NAFLD iron homeostasis

Copper modulates iron homeostasis and is also linked to the iron perturbations of NAFLD. Hephaestin ferroxidase activity in duodenal enterocytes is critically dependent on copper as it oxidizes ferrous to ferric iron which is subsequently loaded onto Tf[7]. Similarly, copper is necessary for ceruloplasmin function to export iron from the liver or the RES and also for FPN expression[138]. Expression of a membrane-bound form of ceruloplasmin is mandatory for stable FPN expression[139,140]. Accordingly, a lack of ceruloplasmin as found in the heritable disease aceruloplasminemia leads to tissue iron accumulation and damage most notably in the brain[141].

Low liver and serum copper concentrations were reported in iron overloaded NAFLD and were linked to decreased ferroxidase activity of ceruloplasmin[122]. The expression of FPN was found to be decreased in livers of rats on a copper deficient diet. These observations provide evidence that in addition to decreased FPN expression due to low-grade systemic inflammation, low copper bioavailability contributes to iron retention in NAFLD.

WHAT IS THE THERAPEUTIC POTENTIAL OF MODULATING IRON STORES IN NAFLD?

Elimination of iron may confer a beneficial effect on IR-associated conditions. Removal of iron using phlebotomies is usually well tolerated, with the caution that DIOS patients frequently show a fast decline in TfS[142]. These clinical observations are expected due to the underlying molecular mechanisms of impaired iron export. The incidence of diabetes, postprandial serum insulin and pancreatic insulin sensitivity, *i.e.,* beta cell function were al improved in subjects with previous phlebotomy treatment[143]. Iron removal also improved coronary vascular dysfunction in patients with T2DM[144] and endothelial function in patients with known coronary artery disease and in subjects with primary iron

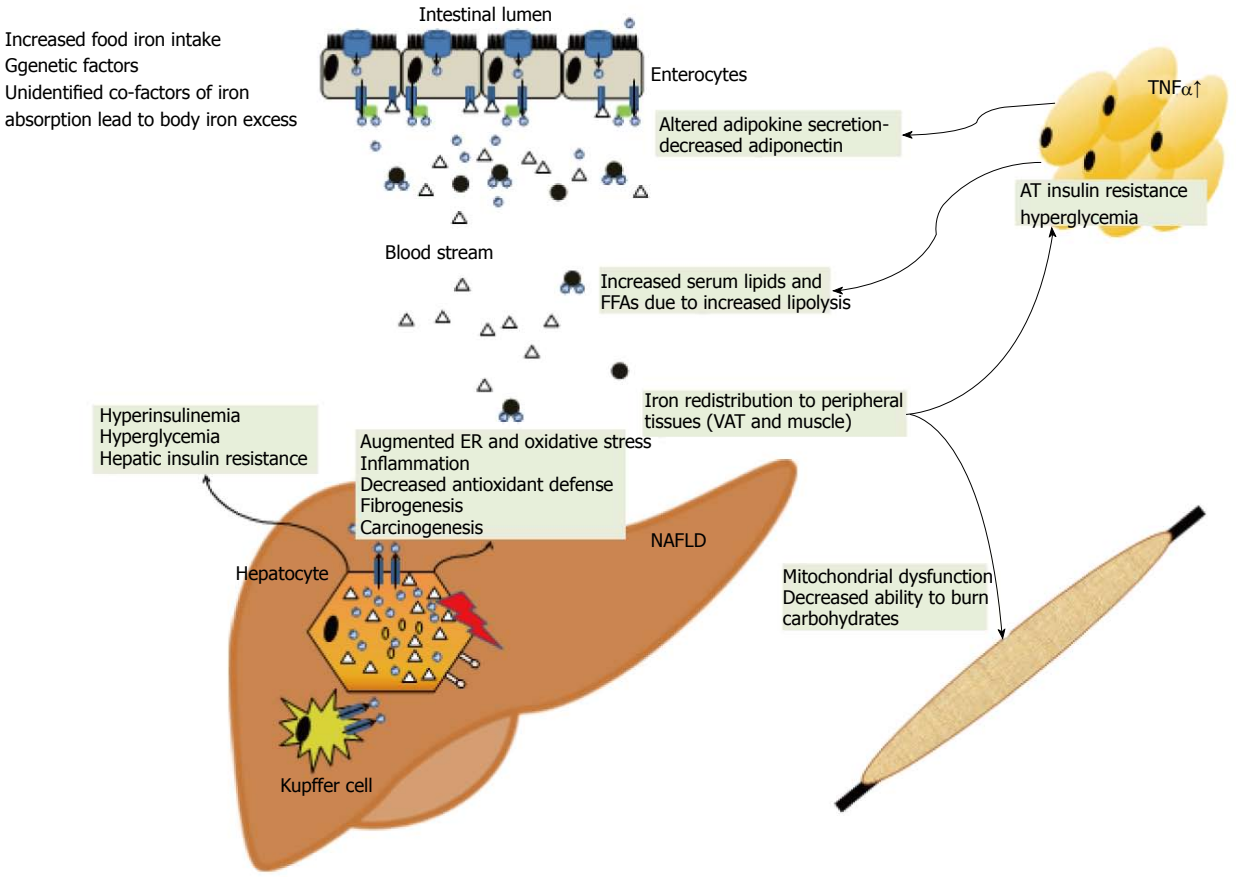

Figure 2 Summary of how iron excess and low copper availability may affect whole body glucose and lipid homeostasis. Iron excess may promote insulin resistance in the liver, muscle and adipose tissue. Iron may increase ER and oxidative stress whereas low copper is potentially associated with an impaired antioxidant defence. These factors may result in the propagation of inflammation, fibrogenesis and hepatocarcinogenesis. TNF-α: Tumor necrosis factor-α; ER: Endoplasmatic reticulum; FFA: Free fatty acid; VAT: Visceral adipose tissue; AT: Adipose tissue.

overload[145,146]. Blood donations were linked to insulin sensitivity even in healthy subjects[46]. Studies on iron depletion in NAFLD in humans have demonstrated benefits regarding systemic or hepatic insulin resistance and pancreatic insulin sensitivity[142,147,148]. A randomized trial demonstrated improved HbA1c, insulin sensitivity and secretion subjects who received phlebotomy treatment[149]. The effects of iron depletion were additive to successful lifestyle modifications[150]. Similar observations were reported the effect of iron depletion on other cardiovascular risk factors[151] and iron removal may prevent development and progression of malignancies[152].

As far as practical treatment of iron excess in NAFLD patients with elevated ferritin is concerned, available data suggest that iron removal may thus be beneficial in addition to weight loss, diet and lifestyle modification or antidiabetic medication as indicated in an individual patient. We have adopted the practice to perform biweekly phlebotomies in these subjects until serum ferritin concentrations are between 50 and 100 ng/L, however, no evidence-based recommendation for this is currently available. In contrast to hemochromatosis patients, NAFLD subjects have impaired iron mobilisation from storage sites and may therefore develop anemia in response to phlebotomy treatment. We therefore recommend close monitoring of serum ferritin, TfS and hemoglobin at each visit for the period of time while these patients are on phlebotomy treatment[26,153].

CONCLUSION

Elevated serum ferritin concentrations are a frequent finding in NAFLD. Excess iron is linked to IR, accelerated disease progression and adverse outcomes. Removing excess iron *via* phlebotomies is safe and has clinical benefits. We suggest that on the basis of available evidence it can be offered to NAFLD patients as it is linked to improvement of IR and inflammation. The mechanisms underlying iron accumulation in NAFLD are tightly linked to impaired iron export from liver cells as a consequence of low expression of the iron export molecule FPN and elevated hepcidin concentrations. Inflammation of adipose tissue as indicated by TNF-α and IL-6 and altered adipokine secretion (leptin, resistin) or hepcidin represent potent signals from diseased

adipose tissue to dysregulate iron as well as glucose or lipid homeostasis.

REFERENCES

1 **Hentze MW**, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. *Cell* 2010; **142**: 24-38 [PMID: 20603012 DOI: 10.1016/j.cell.2010.06.028]

2 **Wang J**, Pantopoulos K. Regulation of cellular iron metabolism. *Biochem J* 2011; **434**: 365-381 [PMID: 21348856 DOI: 10.1042/BJ20101825]

3 **Fleming MD**, Trenor CC, Su MA, Foernzler D, Beier DR, Dietrich WF, Andrews NC. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet* 1997; **16**: 383-386 [PMID: 9241278]

4 **Gunshin H**, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* 1997; **388**: 482-488 [PMID: 9242408 DOI: 10.1038/41343]

5 **McKie AT**, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, Miret S, Bomford A, Peters TJ, Farzaneh F, Hediger MA, Hentze MW, Simpson RJ. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol Cell* 2000; **5**: 299-309 [PMID: 10882071 DOI: 10.1016/S1097-2765(00)80425-6]

6 **Donovan A**, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, Law TC, Brugnara C, Lux SE, Pinkus GS, Pinkus JL, Kingsley PD, Palis J, Fleming MD, Andrews NC, Zon LI. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* 2000; **403**: 776-781 [PMID: 10693807 DOI: 10.1038/35001596]

7 **Vulpe CD**, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, Gitschier J, Anderson GJ. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. *Nat Genet* 1999; **21**: 195-199 [PMID: 9988272 DOI: 10.1038/5979]

8 **Wrighting DM**, Andrews NC. Iron homeostasis and erythropoiesis. *Curr Top Dev Biol* 2008; **82**: 141-167 [PMID: 18282520 DOI: 10.1016/S0070-2153(07)00006-3]

9 **Abboud S**, Haile DJ. A novel mammalian iron-regulated protein involved in intracellular iron metabolism. *J Biol Chem* 2000; **275**: 19906-19912 [PMID: 10747949 DOI: 10.1074/jbc.M000713200]

10 **De Domenico I**, McVey Ward D, Kaplan J. Regulation of iron acquisition and storage: consequences for iron-linked disorders. *Nat Rev Mol Cell Biol* 2008; **9**: 72-81 [PMID: 17987043 DOI: 10.1038/nrm2295]

11 **Ganz T**. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood* 2003; **102**: 783-788 [PMID: 12663437 DOI: 10.1182/blood-2003-03-0672]

12 **Nicolas G**, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, Beaumont C, Kahn A, Vaulont S. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 2002; **110**: 1037-1044 [PMID: 12370282 DOI: 10.1172/JCI0215686]

13 **Nemeth E**, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; **306**: 2090-2093 [PMID: 15514116]

14 **Nguyen NB**, Callaghan KD, Ghio AJ, Haile DJ, Yang F. Hepcidin expression and iron transport in alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2006; **291**: L417-L425 [PMID: 16648237 DOI: 10.1152/ajplung.00484.2005]

15 **Bekri S**, Gual P, Anty R, Luciani N, Dahman M, Ramesh B, Iannelli A, Staccini-Myx A, Casanova D, Ben Amor I, Saint-Paul MC, Huet PM, Sadoul JL, Gugenheim J, Srai SK, Tran A, Le Marchand-Brustel Y. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* 2006; **131**: 788-796 [PMID: 16952548 DOI: 10.1053/j.gastro.2006.07.007]

16 **Theurl I**, Theurl M, Seifert M, Mair S, Nairz M, Rumpold H, Zoller H, Bellmann-Weiler R, Niederegger H, Talasz H, Weiss G. Autocrine formation of hepcidin induces iron retention in human monocytes. *Blood* 2008; **111**: 2392-2399 [PMID: 18073346 DOI: 10.1182/blood-2007-05-090019]

17 **Mendler MH**, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, Le Gall JY, Brissot P, David V, Deugnier Y. Insulin resistance-associated hepatic iron overload. *Gastroenterology* 1999; **117**: 1155-1163 [PMID: 10535879 DOI: 10.1016/S0016-5085(99)70401-4]

18 **Valenti L**, Dongiovanni P, Fracanzani AL, Santorelli G, Fatta E, Bertelli C, Taioli E, Fiorelli G, Fargion S. Increased susceptibility to nonalcoholic fatty liver disease in heterozygotes for the mutation responsible for hereditary hemochromatosis. *Dig Liver Dis* 2003; **35**: 172-178 [PMID: 12779071 DOI: 10.1016/S1590-8658(03)00025-2]

19 **Moirand R**, Mortaji AM, Loréal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. *Lancet* 1997; **349**: 95-97 [PMID: 8996422 DOI: 10.1016/S0140-6736(96)06034-5]

20 **Turlin B**, Mendler MH, Moirand R, Guyader D, Guillygomarc'h A, Deugnier Y. Histologic features of the liver in insulin resistance-associated iron overload. A study of 139 patients. *Am J Clin Pathol* 2001; **116**: 263-270 [PMID: 11488074 DOI: 10.1309/WWNE-KW2C-4KTW-PTJ5]

21 **Wrede CE**, Buettner R, Bollheimer LC, Schölmerich J, Palitzsch KD, Hellerbrand C. Association between serum ferritin and the insulin resistance syndrome in a representative population. *Eur J Endocrinol* 2006; **154**: 333-340 [PMID: 16452549 DOI: 10.1530/eje.1.02083]

22 **Nairz M**, Weiss G. Molecular and clinical aspects of iron homeostasis: From anemia to hemochromatosis. *Wien Klin Wochenschr* 2006; **118**: 442-462 [PMID: 16957974 DOI: 10.1007/s00508-006-0653-7]

23 **Nelson JE**, Wilson L, Brunt EM, Yeh MM, Kleiner DE, Unalp-Arida A, Kowdley KV. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 448-457 [PMID: 21274866 DOI: 10.1002/hep.24038]

24 **Weiss G**. Genetic mechanisms and modifying factors in hereditary hemochromatosis. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 50-58 [PMID: 19918260 DOI: 10.1038/nrgastro.2009.201]

25 **Pietrangelo A**. Hereditary hemochromatosis--a new look at an old disease. *N Engl J Med* 2004; **350**: 2383-2397 [PMID: 15175440 DOI: 10.1056/NEJMra031573]

26 **Valenti L**, Fracanzani AL, Dongiovanni P, Rovida S, Rametta R, Fatta E, Pulixi EA, Maggioni M, Fargion S. A randomized trial of iron depletion in patients with nonalcoholic fatty liver disease and hyperferritinemia. *World J Gastroenterol* 2014; **20**: 3002-3010 [PMID: 24659891 DOI: 10.3748/wjg.v20.i11.3002]

27 **Valenti L**, Moscatiello S, Vanni E, Fracanzani AL, Bugianesi E, Fargion S, Marchesini G. Venesection for non-alcoholic fatty liver disease unresponsive to lifestyle counselling--a propensity score-adjusted observational study. *QJM* 2011; **104**: 141-149 [PMID: 20851820 DOI: 10.1093/qjmed/hcq170]

28 **Aigner E**, Theurl I, Theurl M, Lederer D, Haufe H, Dietze O, Strasser M, Datz C, Weiss G. Pathways underlying iron accumulation in human nonalcoholic fatty liver disease. *Am J Clin Nutr* 2008; **87**: 1374-1383 [PMID: 18469261]

29 **Diehl AM**, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2005; **54**: 303-306 [PMID: 15647199 DOI: 10.1136/gut.2003.024935]

30 **Chen J**, Wildman RP, Hamm LL, Muntner P, Reynolds K, Whelton PK, He J. Association between inflammation and insulin resistance in U.S. nondiabetic adults: results from the Third National Health and Nutrition Examination Survey. *Diabetes Care* 2004; **27**: 2960-2965 [PMID: 15562214 DOI: 10.2337/diacare.27.12.2960]

31 **Tilg H**, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010; **52**: 1836-1846 [PMID: 21038418 DOI: 10.1002/hep.24001]

32 **Sullivan JL**. Iron and the sex difference in heart disease risk. *Lancet* 1981; **1**: 1293-1294 [PMID: 6112609 DOI: 10.1016/S0140-6736(81)92463-6]

33 **Wood RJ**. The iron-heart disease connection: is it dead or just hiding? *Ageing Res Rev* 2004; **3**: 355-367 [PMID: 15231242 DOI: 10.1016/j.arr.2004.04.002]

34 **Vecchi C**, Montosi G, Garuti C, Corradini E, Sabelli M, Canali S, Pietrangelo A. Gluconeogenic signals regulate iron homeostasis via hepcidin in mice. *Gastroenterology* 2014; **146**: 1060-1069 [PMID: 24361124 DOI: 10.1053/j.gastro.2013.12.016]

35 **Choi JS**, Koh IU, Lee HJ, Kim WH, Song J. Effects of excess dietary iron and fat on glucose and lipid metabolism. *J Nutr Biochem* 2013; **24**: 1634-1644 [PMID: 23643521 DOI: 10.1016/j.jnutbio.2013.02.004]

36 **Davis MR**, Rendina E, Peterson SK, Lucas EA, Smith BJ, Clarke SL. Enhanced expression of lipogenic genes may contribute to hyperglycemia and alterations in plasma lipids in response to dietary iron deficiency. *Genes Nutr* 2012; **7**: 415-425 [PMID: 22228222 DOI: 10.1007/s12263-011-0278-y]

37 **Oexle H**, Gnaiger E, Weiss G. Iron-dependent changes in cellular energy metabolism: influence on citric acid cycle and oxidative phosphorylation. *Biochim Biophys Acta* 1999; **1413**: 99-107 [PMID: 10556622 DOI: 10.1016/S0005-2728(99)00088-2]

38 **Ford ES**, Cogswell ME. Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care* 1999; **22**: 1978-1983 [PMID: 10587829 DOI: 10.2337/diacare.22.12.1978]

39 **Forouhi NG**, Harding AH, Allison M, Sandhu MS, Welch A, Luben R, Bingham S, Khaw KT, Wareham NJ. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. *Diabetologia* 2007; **50**: 949-956 [PMID: 17333112 DOI: 10.1007/s00125-007-0604-5]

40 **Lao TT**, Tam KF. Maternal serum ferritin and gestational impaired glucose tolerance. *Diabetes Care* 1997; **20**: 1368-1369 [PMID: 9283781 DOI: 10.2337/diacare.20.9.1368]

41 **Lao TT**, Chan PL, Tam KF. Gestational diabetes mellitus in the last trimester - a feature of maternal iron excess? *Diabet Med* 2001; **18**: 218-223 [PMID: 11318843 DOI: 10.1046/j.1464-5491.2001.00453.x]

42 **Chen X**, Scholl TO, Stein TP. Association of elevated serum ferritin levels and the risk of gestational diabetes mellitus in pregnant women: The Camden study. *Diabetes Care* 2006; **29**: 1077-1082 [PMID: 16644640 DOI: 10.2337/dc06-0164]

43 **Jiang R**, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA* 2004; **291**: 711-717 [PMID: 14871914 DOI: 10.1001/jama.291.6.711]

44 **Gillum RF**. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men--the Third National Health and Nutrition Examination Survey. *Int J Obes Relat Metab Disord* 2001; **25**: 639-645 [PMID: 11360145 DOI: 10.1038/sj.ijo.0801561]

45 **Iwasaki T**, Nakajima A, Yoneda M, Yamada Y, Mukasa K, Fujita K, Fujisawa N, Wada K, Terauchi Y. Serum ferritin is associated with visceral fat area and subcutaneous fat area. *Diabetes Care* 2005; **28**: 2486-2491 [PMID: 16186284 DOI: 10.2337/diacare.28.10.2486]

46 **Fernández-Real JM**, López-Bermejo A, Ricart W. Iron stores, blood donation, and insulin sensitivity and secretion. *Clin Chem* 2005; **51**: 1201-1205 [PMID: 15976100 DOI: 10.1373/clinchem.2004.046847]

47 **Piperno A**, Trombini P, Gelosa M, Mauri V, Pecci V, Vergani A, Salvioni A, Mariani R, Mancia G. Increased serum ferritin is common in men with essential hypertension. *J Hypertens* 2002; **20**: 1513-1518 [PMID: 12172312 DOI: 10.1097/00004872-200208000-00013]

48 **Jehn M**, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care* 2004; **27**: 2422-2428 [PMID: 15451911 DOI: 10.2337/diacare.27.10.2422]

49 **Martínez-García MA**, Luque-Ramírez M, San-Millán JL, Escobar-Morreale HF. Body iron stores and glucose intolerance in premenopausal women: role of hyperandrogenism, insulin resistance, and genomic variants related to inflammation, oxidative stress, and iron metabolism. *Diabetes Care* 2009; **32**: 1525-1530 [PMID: 19401444 DOI: 10.2337/dc09-0420]

50 **Galan P**, Noisette N, Estaquio C, Czernichow S, Mennen L, Renversez JC, Briançon S, Favier A, Hercberg S. Serum ferritin, cardiovascular risk factors and ischaemic heart diseases: a prospective analysis in the SU.VI.MAX (SUpplementation en VItamines et Minéraux AntioXydants) cohort. *Public Health Nutr* 2006; **9**: 70-74 [PMID: 16480536 DOI: 10.1079/PHN2005826]

51 **Dubern B**, Girardet JP, Tounian P. Insulin resistance and ferritin as major determinants of abnormal serum aminotransferase in severely obese children. *Int J Pediatr Obes* 2006; **1**: 77-82 [PMID: 17907318 DOI: 10.1080/17477160600569594]

52 **Aigner E**, Hinz C, Steiner K, Rossmann B, Pfleger J, Hohla F, Steger B, Stadlmayr A, Patsch W, Datz C. Iron stores, liver transaminase levels and metabolic risk in healthy teenagers. *Eur J Clin Invest* 2010; **40**: 155-163 [PMID: 20050877 DOI: 10.1111/j.1365-2362.2009.02238.x]

53 **van der A DL**, Grobbee DE, Roest M, Marx JJ, Voorbij HA, van der Schouw YT. Serum ferritin is a risk factor for stroke in postmenopausal women. *Stroke* 2005; **36**: 1637-1641 [PMID: 16002760 DOI: 10.1161/01.STR.0000173172.82880.72]

54 **Ramakrishnan U**, Kuklina E, Stein AD. Iron stores and cardiovascular disease risk factors in women of reproductive age in the United States. *Am J Clin Nutr* 2002; **76**: 1256-1260 [PMID: 12450891]

55 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047 DOI: 10.2337/diabetes.50.8.1844]

56 **Dyson JK,** Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. *Frontline Gastroenterol* 2014; **5**: 211-218 [PMID: 25018867 DOI: 10.1136/flgastro-2013-100403]

57 **Ahmed U**, Latham PS, Oates PS. Interactions between hepatic iron and lipid metabolism with possible relevance to steatohepatitis. *World J Gastroenterol* 2012; **18**: 4651-4658 [PMID: 23002334 DOI: 10.3748/wjg.v18.i34.4651]

58 **Zelber-Sagi S**, Nitzan-Kaluski D, Halpern Z, Oren R. NAFLD and hyperinsulinemia are major determinants of serum ferritin levels. *J Hepatol* 2007; **46**: 700-707 [PMID: 17150278]

59 **Fargion S**, Mattioli M, Fracanzani AL, Sampietro M, Tavazzi D, Fociani P, Taioli E, Valenti L, Fiorelli G. Hyperferritinemia, iron overload, and multiple metabolic alterations identify patients at risk for nonalcoholic steatohepatitis. *Am J Gastroenterol* 2001; **96**: 2448-2455 [PMID: 11513189 DOI: 10.1111/j.1572-0241.2001.04052.x]

60 **Fargion S**, Dongiovanni P, Guzzo A, Colombo S, Valenti L, Fracanzani AL. Iron and insulin resistance. *Aliment Pharmacol Ther* 2005; **22** Suppl 2: 61-63 [PMID: 16225476 DOI: 10.1111/j.1365-2036.2005.02599.x]

61 **George DK**, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, Jazwinska EC, Powell LW. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1998; **114**: 311-318 [PMID: 9453491 DOI: 10.1016/S0016-5085(98)70482-2]

62 **Fierbinţeanu-Braticevici C**, Bengus A, Neamţu M, Usvat R. The risk factors of fibrosis in nonalcoholic steatohepatitis. *Rom J Intern Med* 2002; **40**: 81-88 [PMID: 15526543]

63 **Bhattacharya R**, Kowdley KV. Iron and HFE mutations in nonalcoholic steatohepatitis: innocent bystanders or accessories to the crime? *Gastroenterology* 2003; **125**: 615-666; discussion 617 [PMID: 12891567 DOI: 10.1016/S0016-5085(03)00963-6]

64 **Chitturi S**, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, Samarasinghe D, Lin R, Abeygunasekera S, George J. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. *Hepatology* 2002; **36**: 142-149 [PMID: 12085358]

65 **Bugianesi E**, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179-187 [PMID: 14752836]

66 **Younossi ZM**, Gramlich T, Bacon BR, Matteoni CA, Boparai N, O'

Neill R, McCullough AJ. Hepatic iron and nonalcoholic fatty liver disease. *Hepatology* 1999; **30**: 847-850 [PMID: 10498632]
67 **Kowdley KV**, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, Sanyal AJ, Nelson JE. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 77-85 [PMID: 21953442 DOI: 10.1002/hep.24706]
68 **Maliken BD**, Nelson JE, Klintworth HM, Beauchamp M, Yeh MM, Kowdley KV. Hepatic reticuloendothelial system cell iron deposition is associated with increased apoptosis in nonalcoholic fatty liver disease. *Hepatology* 2013; **57**: 1806-1813 [PMID: 23325576 DOI: 10.1002/hep.26238]
69 **Nelson JE**, Brunt EM, Kowdley KV. Lower serum hepcidin and greater parenchymal iron in nonalcoholic fatty liver disease patients with C282Y HFE mutations. *Hepatology* 2012; **56**: 1730-1740 [PMID: 22611049 DOI: 10.1002/hep.25856]
70 **Raszeja-Wyszomirska J**, Kurzawski G, Lawniczak M, Miezynska-Kurtycz J, Lubinski J. Nonalcoholic fatty liver disease and HFE gene mutations: a Polish study. *World J Gastroenterol* 2010; **16**: 2531-2536 [PMID: 20503453 DOI: 10.3748/wjg.v16.i20.2531]
71 **Valenti L**, Dongiovanni P, Fracanzani AL, Fargion S. HFE mutations in nonalcoholic fatty liver disease. *Hepatology* 2008; **47**: 1794-1795; author reply 1795-1796 [PMID: 18438784]
72 **Valenti L**, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, Canavesi E, Lattuada E, Roviaro G, Marchesini G, Fargion S. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010; **138**: 905-912 [PMID: 19931264 DOI: 10.1053/j.gastro.2009.11.013]
73 **Valenti L**, Canavesi E, Galmozzi E, Dongiovanni P, Rametta R, Maggioni P, Maggioni M, Fracanzani AL, Fargion S. Beta-globin mutations are associated with parenchymal siderosis and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2010; **53**: 927-933 [PMID: 20739079 DOI: 10.1016/j.jhep.2010.05.023]
74 **Valenti L**, Rametta R, Dongiovanni P, Motta BM, Canavesi E, Pelusi S, Pulixi EA, Fracanzani AL, Fargion S. The A736V TMPRSS6 polymorphism influences hepatic iron overload in nonalcoholic fatty liver disease. *PLoS One* 2012; **7**: e48804 [PMID: 23144979 DOI: 10.1371/journal.pone.0048804]
75 **Valenti L**, Dongiovanni P, Piperno A, Fracanzani AL, Maggioni M, Rametta R, Loria P, Casiraghi MA, Suigo E, Ceriani R, Remondini E, Trombini P, Fargion S. Alpha 1-antitrypsin mutations in NAFLD: high prevalence and association with altered iron metabolism but not with liver damage. *Hepatology* 2006; **44**: 857-864 [PMID: 17006922 DOI: 10.1002/hep.21329]
76 **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. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]
77 **Weismüller TJ**, Kirchner GI, Scherer MN, Negm AA, Schnitzbauer AA, Lehner F, Klempnauer J, Schlitt HJ, Manns MP, Strassburg CP. Serum ferritin concentration and transferrin saturation before liver transplantation predict decreased long-term recipient survival. *Hepatology* 2011; **54**: 2114-2124 [PMID: 21898488 DOI: 10.1002/hep.24635]
78 **Walker NM**, Stuart KA, Ryan RJ, Desai S, Saab S, Nicol JA, Fletcher LM, Crawford DH. Serum ferritin concentration predicts mortality in patients awaiting liver transplantation. *Hepatology* 2010; **51**: 1683-1691 [PMID: 20225256 DOI: 10.1002/hep.23537]
79 **Sorrentino P**, D'Angelo S, Ferbo U, Micheli P, Bracigliano A, Vecchione R. Liver iron excess in patients with hepatocellular carcinoma developed on non-alcoholic steato-hepatitis. *J Hepatol* 2009; **50**: 351-357 [PMID: 19070395 DOI: 10.1016/j.jhep.2008.09.011]
80 **Dmochowski K**, Finegood DT, Francombe W, Tyler B, Zinman B. Factors determining glucose tolerance in patients with thalassemia major. *J Clin Endocrinol Metab* 1993; **77**: 478-483 [PMID: 8345055]
81 **Frayling T**, Ellard S, Grove J, Walker M, Hattersley AT. C282Y mutation in HFE (haemochromatosis) gene and type 2 diabetes. *Lancet* 1998; **351**: 1933-1934 [PMID: 9654270 DOI: 10.1016/S0140-6736(05)78618-9]
82 **Merkel PA**, Simonson DC, Amiel SA, Plewe G, Sherwin RS, Pearson HA, Tamborlane WV. Insulin resistance and hyperinsulinemia in patients with thalassemia major treated by hypertransfusion. *N Engl J Med* 1988; **318**: 809-814 [PMID: 3281000 DOI: 10.1056/NEJM198803313181303]
83 **Abraham D**, Rogers J, Gault P, Kushner JP, McClain DA. Increased insulin secretory capacity but decreased insulin sensitivity after correction of iron overload by phlebotomy in hereditary haemochromatosis. *Diabetologia* 2006; **49**: 2546-2551 [PMID: 17019598 DOI: 10.1007/s00125-006-0445-7]
84 **McClain DA**, Abraham D, Rogers J, Brady R, Gault P, Ajioka R, Kushner JP. High prevalence of abnormal glucose homeostasis secondary to decreased insulin secretion in individuals with hereditary haemochromatosis. *Diabetologia* 2006; **49**: 1661-1669 [PMID: 16538487 DOI: 10.1007/s00125-006-0200-0]
85 **Huang J**, Gabrielsen JS, Cooksey RC, Luo B, Boros LG, Jones DL, Jouihan HA, Soesanto Y, Knecht L, Hazel MW, Kushner JP, McClain DA. Increased glucose disposal and AMP-dependent kinase signaling in a mouse model of hemochromatosis. *J Biol Chem* 2007; **282**: 37501-37507 [PMID: 17971451 DOI: 10.1074/jbc.M703625200]
86 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400 DOI: 10.1172/JCI21625]
87 **Urakawa H**, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka K, Maruyama N, Kitagawa N, Tanaka T, Hori Y, Nakatani K, Yano Y, Adachi Y. Oxidative stress is associated with adiposity and insulin resistance in men. *J Clin Endocrinol Metab* 2003; **88**: 4673-4676 [PMID: 14557439 DOI: 10.1210/jc.2003-030202]
88 **Houstis N**, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006; **440**: 944-948 [PMID: 16612386 DOI: 10.1038/nature04634]
89 **Tan TC**, Crawford DH, Jaskowski LA, Subramaniam VN, Clouston AD, Crane DI, Bridle KR, Anderson GJ, Fletcher LM. Excess iron modulates endoplasmic reticulum stress-associated pathways in a mouse model of alcohol and high-fat diet-induced liver injury. *Lab Invest* 2013; **93**: 1295-1312 [PMID: 24126888 DOI: 10.1038/labinvest.2013.121]
90 **van de Wier B**, Balk JM, Haenen GR, Giamouridis D, Bakker JA, Bast BC, den Hartog GJ, Koek GH, Bast A. Elevated citrate levels in non-alcoholic fatty liver disease: the potential of citrate to promote radical production. *FEBS Lett* 2013; **587**: 2461-2466 [PMID: 23792160 DOI: 10.1016/j.febslet.2013.06.019]
91 **Sutti S**, Jindal A, Locatelli I, Vacchiano M, Gigliotti L, Bozzola C, Albano E. Adaptive immune responses triggered by oxidative stress contribute to hepatic inflammation in NASH. *Hepatology* 2014; **59**: 886-897 [PMID: 24115128 DOI: 10.1002/hep.26749]
92 **Dongiovanni P**, Valenti L, Ludovica Fracanzani A, Gatti S, Cairo G, Fargion S. Iron depletion by deferoxamine up-regulates glucose uptake and insulin signaling in hepatoma cells and in rat liver. *Am J Pathol* 2008; **172**: 738-747 [PMID: 18245813 DOI: 10.2353/ajpath.2008.070097]
93 **Datz C**, Felder TK, Niederseer D, Aigner E. Iron homeostasis in the metabolic syndrome. *Eur J Clin Invest* 2013; **43**: 215-224 [PMID: 23289518 DOI: 10.1111/eci.12032]
94 **Carter R**, Mouralidarane A, Soeda J, Ray S, Pombo J, Saraswati R, Novelli M, Fusai G, Rappa F, Saracino C, Pazienza V, Poston L, Taylor PD, Vinciguerra M, Oben JA. Non-alcoholic fatty pancreas disease pathogenesis: a role for developmental programming and altered circadian rhythms. *PLoS One* 2014; **9**: e89505 [PMID: 24657938 DOI: 10.1371/journal.pone.0089505]
95 **Cho H**, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, Chong LW, DiTacchio L, Atkins AR, Glass CK, Liddle C, Auwerx J, Downes M, Panda S, Evans RM. Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. *Nature* 2012; **485**: 123-127 [PMID: 22460952 DOI: 10.1038/nature11048]
96 **Green A**, Basile R, Rumberger JM. Transferrin and iron induce insulin resistance of glucose transport in adipocytes. *Metabolism* 2006; **55**: 1042-1045 [PMID: 16839839 DOI: 10.1016/j.metabol.2006.03.015]

97 **Rumberger JM**, Peters T, Burrington C, Green A. Transferrin and iron contribute to the lipolytic effect of serum in isolated adipocytes. *Diabetes* 2004; **53**: 2535-2541 [PMID: 15448081 DOI: 10.2337/diabetes.53.10.2535]

98 **Aso Y**, Takebayashi K, Wakabayashi S, Momobayashi A, Sugawara N, Terasawa T, Naruse R, Hara K, Suetsugu M, Morita K, Inukai T. Relation between serum high molecular weight adiponectin and serum ferritin or prohepcidin in patients with type 2 diabetes. *Diabetes Res Clin Pract* 2010; **90**: 250-255 [PMID: 20888657 DOI: 10.1016/j.diabres.2010.09.008]

99 **Ku BJ**, Kim SY, Lee TY, Park KS. Serum ferritin is inversely correlated with serum adiponectin level: population-based cross-sectional study. *Dis Markers* 2009; **27**: 303-310 [PMID: 20075513 DOI: 10.3233/DMA-2009-0676]

100 **Gabrielsen JS**, Gao Y, Simcox JA, Huang J, Thorup D, Jones D, Cooksey RC, Gabrielsen D, Adams TD, Hunt SC, Hopkins PN, Cefalu WT, McClain DA. Adipocyte iron regulates adiponectin and insulin sensitivity. *J Clin Invest* 2012; **122**: 3529-3540 [PMID: 22996660 DOI: 10.1172/JCI44421]

101 **Dongiovanni P**, Fracanzani AL, Fargion S, Valenti L. Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target. *J Hepatol* 2011; **55**: 920-932 [PMID: 21718726 DOI: 10.1016/j.jhep.2011.05.008]

102 **Fernández-Real JM**, Moreno JM, Chico B, López-Bermejo A, Ricart W. Circulating visfatin is associated with parameters of iron metabolism in subjects with altered glucose tolerance. *Diabetes Care* 2007; **30**: 616-621 [PMID: 17327330 DOI: 10.2337/dc06-1581]

103 **Fernández-Real JM**, Moreno JM, Ricart W. Circulating retinol-binding protein-4 concentration might reflect insulin resistance-associated iron overload. *Diabetes* 2008; **57**: 1918-1925 [PMID: 18426863 DOI: 10.2337/db08-0041]

104 **Wouters K**, van Gorp PJ, Bieghs V, Gijbels MJ, Duimel H, Lütjohann D, Kerksiek A, van Kruchten R, Maeda N, Staels B, van Bilsen M, Shiri-Sverdlov R, Hofker MH. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 474-486 [PMID: 18666236 DOI: 10.1002/hep.22363]

105 **Ikura Y**, Ohsawa M, Suekane T, Fukushima H, Itabe H, Jomura H, Nishiguchi S, Inoue T, Naruko T, Ehara S, Kawada N, Arakawa T, Ueda M. Localization of oxidized phosphatidylcholine in nonalcoholic fatty liver disease: impact on disease progression. *Hepatology* 2006; **43**: 506-514 [PMID: 16496325 DOI: 10.1002/hep.21070]

106 **Nairz M,** Schroll A, Demetz E, Tancevski I, Theurl I, Weiss G. 'Ride on the ferrous wheel' - The cycle of iron in macrophages in health and disease. *Immunobiology* 2015; **220**: 280-294 [PMID: 25240631 DOI: 10.1016/j.imbio.2014.09.010]

107 **Pietrangelo A**. Hemochromatosis: an endocrine liver disease. *Hepatology* 2007; **46**: 1291-1301 [PMID: 17886335]

108 **Weiss G**, Schett G. Anaemia in inflammatory rheumatic diseases. *Nat Rev Rheumatol* 2013; **9**: 205-215 [PMID: 23147894 DOI: 10.1038/nrrheum.2012.183]

109 **Sonnweber T**, Nachbaur D, Schroll A, Nairz M, Seifert M, Demetz E, Haschka D, Mitterstiller AM, Kleinsasser A, Burtscher M, Trübsbach S, Murphy AT, Wroblewski V, Witcher DR, Mleczko-Sanecka K, Vecchi C, Muckenthaler MU, Pietrangelo A, Theurl I, Weiss G. Hypoxia induced downregulation of hepcidin is mediated by platelet derived growth factor BB. *Gut* 2014; **63**: 1951-1959 [PMID: 24598129 DOI: 10.1136/gutjnl-2013-305317]

110 **Hou Y**, Zhang S, Wang L, Li J, Qu G, He J, Rong H, Ji H, Liu S. Estrogen regulates iron homeostasis through governing hepatic hepcidin expression via an estrogen response element. *Gene* 2012; **511**: 398-403 [PMID: 23041085 DOI: 10.1016/j.gene.2012.09.060]

111 **Goodnough JB**, Ramos E, Nemeth E, Ganz T. Inhibition of hepcidin transcription by growth factors. *Hepatology* 2012; **56**: 291-299 [PMID: 22278715 DOI: 10.1002/hep.25615]

112 **Latour C**, Kautz L, Besson-Fournier C, Island ML, Canonne-Hergaux F, Loréal O, Ganz T, Coppin H, Roth MP. Testosterone perturbs systemic iron balance through activation of epidermal growth factor receptor signaling in the liver and repression of hepcidin. *Hepatology* 2014; **59**: 683-694 [PMID: 23907767 DOI: 10.1002/hep.26648]

113 **Harrison-Findik DD**, Schafer D, Klein E, Timchenko NA, Kulaksiz H, Clemens D, Fein E, Andriopoulos B, Pantopoulos K, Gollan J. Alcohol metabolism-mediated oxidative stress down-regulates hepcidin transcription and leads to increased duodenal iron transporter expression. *J Biol Chem* 2006; **281**: 22974-22982 [PMID: 16737972 DOI: 10.1074/jbc.M602098200]

114 **Barisani D**, Pelucchi S, Mariani R, Galimberti S, Trombini P, Fumagalli D, Meneveri R, Nemeth E, Ganz T, Piperno A. Hepcidin and iron-related gene expression in subjects with Dysmetabolic Hepatic Iron Overload. *J Hepatol* 2008; **49**: 123-133 [PMID: 18462824 DOI: 10.1016/j.jhep.2008.03.011]

115 **Détivaud L**, Nemeth E, Boudjema K, Turlin B, Troadec MB, Leroyer P, Ropert M, Jacquelinet S, Courselaud B, Ganz T, Brissot P, Loréal O. Hepcidin levels in humans are correlated with hepatic iron stores, hemoglobin levels, and hepatic function. *Blood* 2005; **106**: 746-748 [PMID: 15797999 DOI: 10.1182/blood-2004-12-4855]

116 **Lainé F**, Deugnier Y. Increased expression of hepcidin in obese patients: impact on phenotypic expression of hemochromatosis and pathophysiology of dysmetabolic iron overload syndrome. *Gastroenterology* 2006; **131**: 2028 [PMID: 17188970 DOI: 10.1053/j.gastro.2006.10.046]

117 **Dallalio G**, Law E, Means RT. Hepcidin inhibits in vitro erythroid colony formation at reduced erythropoietin concentrations. *Blood* 2006; **107**: 2702-2704 [PMID: 16332970 DOI: 10.1182/blood-2005-07-2854]

118 **Weiss G**, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; **352**: 1011-1023 [PMID: 15758012 DOI: 10.1056/NEJMra041809]

119 **Huang J**, Simcox J, Mitchell TC, Jones D, Cox J, Luo B, Cooksey RC, Boros LG, McClain DA. Iron regulates glucose homeostasis in liver and muscle via AMP-activated protein kinase in mice. *FASEB J* 2013; **27**: 2845-2854 [PMID: 23515442 DOI: 10.1096/fj.12-216929]

120 **Chung B**, Matak P, McKie AT, Sharp P. Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J Nutr* 2007; **137**: 2366-2370 [PMID: 17951471]

121 **Amato A**, Santoro N, Calabrò P, Grandone A, Swinkels DW, Perrone L, del Giudice EM. Effect of body mass index reduction on serum hepcidin levels and iron status in obese children. *Int J Obes* (Lond) 2010; **34**: 1772-1774 [PMID: 20877286 DOI: 10.1038/ijo.2010.204]

122 **Aigner E**, Theurl I, Haufe H, Seifert M, Hohla F, Scharinger L, Stickel F, Mourlane F, Weiss G, Datz C. Copper availability contributes to iron perturbations in human nonalcoholic fatty liver disease. *Gastroenterology* 2008; **135**: 680-688 [PMID: 18505688 DOI: 10.1053/j.gastro.2008.04.007]

123 **Zoller H**, Koch RO, Theurl I, Obrist P, Pietrangelo A, Montosi G, Haile DJ, Vogel W, Weiss G. Expression of the duodenal iron transporters divalent-metal transporter 1 and ferroportin 1 in iron deficiency and iron overload. *Gastroenterology* 2001; **120**: 1412-1419 [PMID: 11313311 DOI: 10.1053/gast.2001.24033]

124 **Ruivard M**, Lainé F, Ganz T, Olbina G, Westerman M, Nemeth E, Rambeau M, Mazur A, Gerbaud L, Tournilhac V, Abergel A, Philippe P, Deugnier Y, Coudray C. Iron absorption in dysmetabolic iron overload syndrome is decreased and correlates with increased plasma hepcidin. *J Hepatol* 2009; **50**: 1219-1225 [PMID: 19398238 DOI: 10.1016/j.jhep.2009.01.029]

125 **Zimmermann MB**, Zeder C, Muthayya S, Winichagoon P, Chaouki N, Aeberli I, Hurrell RF. Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. *Int J Obes* (Lond) 2008; **32**: 1098-1104 [PMID: 18427564]

126 **Sonnweber T**, Ress C, Nairz M, Theurl I, Schroll A, Murphy AT, Wroblewski V, Witcher DR, Moser P, Ebenbichler CF, Kaser S, Weiss G. High-fat diet causes iron deficiency via hepcidin-independent reduction of duodenal iron absorption. *J Nutr Biochem* 2012; **23**: 1600-1608 [PMID: 22444869 DOI: 10.1016/j.jnutbio.2011.10.013]

127 **Otogawa K**, Kinoshita K, Fujii H, Sakabe M, Shiga R, Nakatani K, Ikeda K, Nakajima Y, Ikura Y, Ueda M, Arakawa T, Hato F, Kawada N. Erythrophagocytosis by liver macrophages (Kupffer cells) promotes oxidative stress, inflammation, and fibrosis in a rabbit model of steatohepatitis: implications for the pathogenesis

of human nonalcoholic steatohepatitis. *Am J Pathol* 2007; **170**: 967-980 [PMID: 17322381 DOI: 10.2353/ajpath.2007.060441]
128 **Davis RJ**, Corvera S, Czech MP. Insulin stimulates cellular iron uptake and causes the redistribution of intracellular transferrin receptors to the plasma membrane. *J Biol Chem* 1986; **261**: 8708-8711 [PMID: 3013860]
129 **Yokomori N**, Iwasa Y, Aida K, Inoue M, Tawata M, Onaya T. Transcriptional regulation of ferritin messenger ribonucleic acid levels by insulin in cultured rat glioma cells. *Endocrinology* 1991; **128**: 1474-1480 [PMID: 1999166 DOI: 10.1210/endo-128-3-1474]
130 **Klevay LM**. Is the Western diet adequate in copper? *J Trace Elem Med Biol* 2011; **25**: 204-212 [PMID: 21982501 DOI: 10.1016/j.jtemb.2011.08.146]
131 **al-Othman AA**, Rosenstein F, Lei KY. Copper deficiency alters plasma pool size, percent composition and concentration of lipoprotein components in rats. *J Nutr* 1992; **122**: 1199-1204 [PMID: 1588437]
132 **al-Othman AA**, Rosenstein F, Lei KY. Copper deficiency increases in vivo hepatic synthesis of fatty acids, triacylglycerols, and phospholipids in rats. *Proc Soc Exp Biol Med* 1993; **204**: 97-103 [PMID: 8372103 DOI: 10.3181/00379727-204-43640]
133 **Aigner E**, Strasser M, Haufe H, Sonnweber T, Hohla F, Stadlmayr A, Solioz M, Tilg H, Patsch W, Weiss G, Stickel F, Datz C. A role for low hepatic copper concentrations in nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2010; **105**: 1978-1985 [PMID: 20407430 DOI: 10.1038/ajg.2010.170]
134 **Prohaska JR**, Geissler J, Brokate B, Broderius M. Copper, zinc-superoxide dismutase protein but not mRNA is lower in copper-deficient mice and mice lacking the copper chaperone for superoxide dismutase. *Exp Biol Med* (Maywood) 2003; **228**: 959-966 [PMID: 12968068]
135 **Schuschke DA**, Adeagbo AS, Patibandla PK, Egbuhuzo U, Fernandez-Botran R, Johnson WT. Cyclooxygenase-2 is upregulated in copper-deficient rats. *Inflammation* 2009; **32**: 333-339 [PMID: 19609832 DOI: 10.1007/s10753-009-9140-4]
136 **Wei YZ,** Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial dysfunction. *World J Gastroenterol* 2008; **14**: 193-199 [PMID: 18186554 DOI: 10.3748/wjg.14.193]
137 **Song M**, Schuschke DA, Zhou Z, Chen T, Pierce WM, Wang R, Johnson WT, McClain CJ. High fructose feeding induces copper deficiency in Sprague-Dawley rats: a novel mechanism for obesity related fatty liver. *J Hepatol* 2012; **56**: 433-440 [PMID: 21781943 DOI: 10.1016/j.jhep.2011.05.030]
138 **Chung J**, Haile DJ, Wessling-Resnick M. Copper-induced ferroportin-1 expression in J774 macrophages is associated with increased iron efflux. *Proc Natl Acad Sci USA* 2004; **101**: 2700-2705 [PMID: 14973193 DOI: 10.1073/pnas.0306622101]
139 **De Domenico I**, Ward DM, di Patti MC, Jeong SY, David S, Musci G, Kaplan J. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. *EMBO J* 2007; **26**: 2823-2831 [PMID: 17541408 DOI: 10.1038/sj.emboj.7601735]
140 **Musci G**, Polticelli F, Bonaccorsi di Patti MC. Ceruloplasmin-ferroportin system of iron traffic in vertebrates. *World J Biol Chem* 2014; **5**: 204-215 [PMID: 24921009 DOI: 10.4331/wjbc.v5.i2.204]
141 **Levi S**, Finazzi D. Neurodegeneration with brain iron accumulation: update on pathogenic mechanisms. *Front Pharmacol* 2014; **5**: 99 [PMID: 24847269 DOI: 10.3389/fphar.2014.00099]
142 **Piperno A**, Vergani A, Salvioni A, Trombini P, Viganò M, Riva A, Zoppo A, Boari G, Mancia G. Effects of venesections and restricted diet in patients with the insulin-resistance hepatic iron overload syndrome. *Liver Int* 2004; **24**: 471-476 [PMID: 15482345 DOI: 10.1111/j.1478-3231.2004.0988.x]
143 **Facchini FS**. Effect of phlebotomy on plasma glucose and insulin concentrations. *Diabetes Care* 1998; **21**: 2190 [PMID: 9839115 DOI: 10.2337/diacare.21.12.2190a]
144 **Nitenberg A**, Ledoux S, Valensi P, Sachs R, Antony I. Coronary microvascular adaptation to myocardial metabolic demand can be restored by inhibition of iron-catalyzed formation of oxygen free radicals in type 2 diabetic patients. *Diabetes* 2002; **51**: 813-818 [PMID: 11872685 DOI: 10.2337/diabetes.51.3.813]
145 **Duffy SJ**, Biegelsen ES, Holbrook M, Russell JD, Gokce N, Keaney JF, Vita JA. Iron chelation improves endothelial function in patients with coronary artery disease. *Circulation* 2001; **103**: 2799-2804 [PMID: 11401935 DOI: 10.1161/01.CIR.103.23.2799]
146 **Gaenzer H**, Marschang P, Sturm W, Neumayr G, Vogel W, Patsch J, Weiss G. Association between increased iron stores and impaired endothelial function in patients with hereditary hemochromatosis. *J Am Coll Cardiol* 2002; **40**: 2189-2194 [PMID: 12505233 DOI: 10.1016/S0735-1097(02)02611-6]
147 **Guillygomarc'h A**, Mendler MH, Moirand R, Lainé F, Quentin V, David V, Brissot P, Deugnier Y. Venesection therapy of insulin resistance-associated hepatic iron overload. *J Hepatol* 2001; **35**: 344-349 [PMID: 11592595 DOI: 10.1016/S0168-8278(01)00147-7]
148 **Valenti L**, Fracanzani AL, Fargion S. Effect of iron depletion in patients with nonalcoholic fatty liver disease without carbohydrate intolerance. *Gastroenterology* 2003; **124**: 866; author reply 866-867 [PMID: 12612935 DOI: 10.1053/gast.2003.50130]
149 **Fernández-Real JM**, Peñarroja G, Castro A, García-Bragado F, Hernández-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes* 2002; **51**: 1000-1004 [PMID: 11916918 DOI: 10.2337/diabetes.51.4.1000]
150 **Valenti L**, Fracanzani AL, Dongiovanni P, Bugianesi E, Marchesini G, Manzini P, Vanni E, Fargion S. Iron depletion by phlebotomy improves insulin resistance in patients with nonalcoholic fatty liver disease and hyperferritinemia: evidence from a case-control study. *Am J Gastroenterol* 2007; **102**: 1251-1258 [PMID: 17391316 DOI: 10.1111/j.1572-0241.2007.01192.x]
151 **Facchini FS**, Saylor KL. Effect of iron depletion on cardiovascular risk factors: studies in carbohydrate-intolerant patients. *Ann N Y Acad Sci* 2002; **967**: 342-351 [PMID: 12079862 DOI: 10.1111/j.1749-6632.2002.tb04290.x]
152 **Zacharski LR**, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, Malenka DJ, Ozaki CK, Lavori PW. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. *J Natl Cancer Inst* 2008; **100**: 996-1002 [PMID: 18612130 DOI: 10.1093/jnci/djn209]
153 **Beaton MD**, Chakrabarti S, Levstik M, Speechley M, Marotta P, Adams P. Phase II clinical trial of phlebotomy for non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2013; **37**: 720-729 [PMID: 23441892 DOI: 10.1111/apt.12255]

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REVIEW

Antiviral treatment for chronic hepatitis B in renal transplant patients

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Abstract

Chronic hepatitis B infection is frequent in renal transplant patients. It negatively impacts long term outcomes reducing graft and patient survival. Current guidelines clearly define who needs treatment, when to start, what is the first line therapy, how to monitor treatment response, when to stop, and how patients must be controlled for its safety. There is some data showing a favorable safety and efficacy profile of nucleos(t)ide analogue (NUC) treatment in the renal transplant setting. Entecavir, a drug without major signs of nephrotoxicity, appears to be the first option for NUC naïve patients and tenofovir remains the preferred choice for patients with previous resistance to lamivudine or any other NUC. Renal transplant recipients under antiHBV therapy should be monitored for its efficacy against HBV but also for its safety with a close renal monitoring. Studies including a large number of patients with long term treatment and follow up are still needed to better demonstrate the safety and efficacy of newer NUCs in this population.

Key words: Tenofovir; Long term outcome; Hepatitis B; Renal transplantation; Entecavir

Core tip: Nucleos(t)ide analogue treatment is safe and effective in renal transplant patients. It improves long term patients and graft survival.

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INTRODUCTION

Renal transplantation (RT) is the preferred treatment for patients with end-stage renal disease (ESRD) undergoing renal replacement therapy. Moreover, RT improves quality of life and survival when compared with remaining on the waiting list[1]. In the last twenty years, improvement in immunosuppressive therapy resulted in a decline in acute rejection

prevalence and in 1-year improvement in graft survival. In spite of short-term survival, both long-term patients and graft survival has not improved as expected[2,3]. Cardiovascular diseases, malignancy and infections are the most frequent determinants of death in RT recipients. Liver failure appears as the fourth cause of death in long-term survivors after RT[3-6]. In these patients liver failure is mostly related to chronic viral hepatitis B (HBV) and C (HCV). Both HBV and HCV negatively impact on renal transplantation outcomes by reducing long term graft and patient survival; the magnitude of this impact may vary between these viruses and may differ from different series. Treatment strategies of these viruses are clearly different in patients with ESRD. Nowadays HCV must be treated with Peg-interferon combined with low dose ribavirin before transplantation; in the near future new antivirals will allow HCV treatment after renal transplantation. Conversely, HBV can be treated with the same drugs across all stages of chronic renal disease: before and in dialysis, and after RT.

In the last decades HBV prevalence has decreased in dialysis units due to the implementation vaccination programs and infection control procedures. Today it varies between 0% to 20% according to different sources[7,8]. But prevalence in RT patients tends to vary and can be higher since some of these patients were transplanted before these programs were widely available. The present review focuses on the current management of patients with HBV after renal transplantation.

PRE-TRANSPLANT EVALUATION

Chronic HBV infected patients with ESRD must be adequately evaluated before being transplanted. Two key aspects must be taken into account: evaluation of HBV status and the severity of liver disease. Regarding HBV status, all HBsAg (hepatitis B surface antigen) positive patients and all patients with previous known history of acute or chronic hepatitis B or the presence of antiHBc (hepatitis B core antibody) with/without antiHBs (hepatitis B surface antibody) require a full virological evaluation including HBeAg (hepatitis B envelope antigen) and antiHBe (hepatitis B envelope antibody) determination and HBV DNA levels measurement. This evaluation will allow classifying these patients into different clinical situations[9,10].

Chronic hepatitis B: (1) HBsAg positive for more than 6 mo; (2) Serum HBV DNA $\geqslant$ 2000 (EASL)-20000 (AASLD) IU/mL (10^4-10^5 copies/mL), lower values 2000-20000 IU/mL (10^4-10^5 copies/mL) are often seen in HBeAg-negative chronic hepatitis B; (3) Persistent or intermittent elevation in ALT/AST levels; (4) Liver biopsy showing chronic hepatitis with moderate or severe necroinflammation; and (5) Chronic hepatitis B can be subdivided into HBeAg positive and HBeAg negative chronic hepatitis B.

Inactive HBsAg carrier state: (1) HBsAg positive for more than 6 mo; (2) HBeAg negative, antiHBe positive; (3) Serum HBV DNA < 2000 IU/mL; (4) Persistently normal ALT/AST levels; and (5) Liver biopsy confirms absence of significant hepatitis.

Resolved hepatitis B: (1) Previous known history of acute or chronic hepatitis B or the presence of antiHBc with/without antiHBs; (2) HBsAg negative; (3) Undetectable serum HBV DNA; and (4) Normal ALT levels.

Concomitantly, the severity of liver disease must be evaluated before RT usually by obtaining a liver biopsy. There is some debate about the better route to perform the liver biopsy given that patients with ESRD undergoing hemodialysis have an increased risk of bleeding associated with clotting diseases, uremia-associated platelet dysfunction and intradialysis antiaggregants and anticoagulant treatments[11]. Once transplanted this risk disappears with the restoration of normal renal function. In some centres the transyugular route is the preferred one since is associated with less a reduced risk of bleeding and pain, and may allow measuring the hepatic venous pressure gradient (HVPG) for confirming and grading portal hypertension[12,13]. However in many centres the percutaneous trans-thoracic route is still widely and safely used[14].

There are some noninvasive tests to evaluate the severity of liver fibrosis but they have not been widely evaluated in dialysis and RT patients[11,15]. FibroTest (FT) and liver stiffness measurement (LSM) for noninvasive assessment of liver fibrosis had been evaluated in RT patients with chronic HBV or HCV infection[16]. It had been reported that FT and LSM are sufficiently accurate for diagnosing mild liver fibrosis ($\leqslant$ F2), but differed by 38.4% from the histological data in patients with more severe fibrosis ($\geqslant$ F3); their predictive value for diagnosing severe liver disease needs to be confirmed. More information is needed in HBV infection to recommend its use.

Once the HBV clinical situation and the severity of liver diseases have been established, treatment indication and possibility of RT has to be defined. HBsAg positive patients receiving immunosuppressive therapy after renal transplantation must antiHBV prophylaxis or treatment (based on HBV DNA levels) with a NUC.

In the general population HBV inactive carriers do not need to be treated[9,10]. But RT candidates who are inactive carriers have a higher risk of reactivation after transplantation. In HBsAg positive inactive carriers, treatment can be used as prophylactic (HBV DNA undetectable, no hepatocellular injury), preemptive (HBV DNA < 2000 IU/mL, no hepatocellular injury), and salvage

Table 1 Dosage adjustment of nucleos(t)ide analogue for patients with reduced creatinine clearance

	Recommended dosage		Dosage forms
Creatinine clearance (mL/min)	Lamivudine[25]		Lamivudine[25]
> 50	100 mg once daily		Tablets: 100 mg
30-49	100 mg first dose, then 50 mg once daily		Oral solution: 10 mg/mL
15-29	100 mg first dose, then 25 mg once daily		
5-14	35 mg first dose, then 15 mg once daily		
< 5	35 mg first dose, then 10 mg once daily		
	Adefovir[26]		Adefovir[26]
> 50	10 mg every 24 h		Tablets: 10 mg
30-49	10 mg every 48 h		Oral solution: not available
10-29	10 mg every 72 h		
Hemodialysis	10 mg every 7 d following dialysis		
	Telbivudine[27]		Telbivudine[27]
> 50	600 mg every 24 h		Tablets: 600 mg
30-49	600 mg every 48 h		Oral solution: 100 mg/5 mL
10-29	600 mg every 72 h		
Hemodialysis	600 mg every 96 h following dialysis		
	Entecavir[28]	Entecavir in Lamivudine-Refractory[28]	Entecavir[28]
> 50	0.5 mg once daily	1 mg once daily	Tablets: 0.5 mg and 1 mg
30-49	0.25 mg once daily OR	0.5 mg once daily OR	Oral solution: 0.05 mg/mL
	0.5 mg every 48 h	1 mg every 48 h	
10-29	0.15 mg once daily OR	0.3 mg once daily OR	
	0.5 mg every 72 h	1 mg every 72 h	
Hemodialysis	0.05 mg once daily OR	0.1 mg once daily OR	
	0.5 mg every 7 d following dialysis	1 mg every 7 d following dialysis	
	Tenofovir[29]		Tenofovir[29]
> 50	300 mg every 24 h		Tablets: 300 mg
30-49	300 mg every 48 h		Oral powder: 40 mg per 1 g of oral powder
10-29	300 mg every 72 to 96 h		
Hemodialysis	300 mg every 7 d or after approximately 12 h of dialysis		

therapy after reactivation (HBV DNA > 2000 IU/mL, with hepatocellular injury). Even if the prophylactic/preemptive initiation is the generally accepted treatment, the data comparing these treatments are few[17]. All RT candidates with *chronic HBV* need to be treated before transplantation with NUCs[9,10]. Patients with *resolved HBV* have a low reactivation risk in the RT setting varying between 0.6% to 6%[18-20]. Since there is a low reactivation risk in HBsAg negative patients, universal prophylaxis is not recommended in them. Among antiHBc positive patients, those having low antiHBs titers (< 100 IU/mL) have the higher risk of reactivation. Even though there is limited evidence, repeat vaccination may be considered for this group. Current HBV DNA tests allows to diagnose true occult infection in patients with isolated antiHBc positive serology. There is not enough information about the absolute risk of reactivation in this sub-population, so it is unclear whether prophylaxis is beneficial[21].

The severity of liver disease will determine if the patient is a good candidate for RT or not. Evidence of decompensated liver disease (ascites, encephalopathy, variceal bleeding, *etc.*) precludes RT and is a clear indication of combined liver-renal transplantation (LRT). The presence of compensated cirrhosis with signs of portal hypertension is also an indication for LRT. Cirrhotic patients without portal hypertension must be carefully evaluated for RT since cirrhosis is correlated with an higher mortality risk[22]. Non cirrhotic patients are adequate candidates for RT.

TREATMENT

There are many guidelines regarding HBV treatment. Patients with ESRD and RT can be considered a special population and there are particular recommendations for them that may vary from those implemented in the general population[9,10,15,23]. Patients with chronic hepatitis B are candidates for treatment and those inactive hepatitis B carriers are candidates for prophylactic or preemptive therapy.

There are two main treatment options in hepatitis B: interferon and NUCs. Interferon therapy has many disadvantages when compared with NUCs: poorly tolerated due to side effects, limited efficacy in this populations, subcutaneous administration and there is certain risk of graft rejection[24]. So, there is agreement that in RT patients with chronic HBV infection interferon based therapy should be avoided[15,21,23]. On the contrary, NUCs have a high antiviral potency, have a good safety and tolerability profile and can be orally administrated. These drugs can be easily used in RT and doses can be adjusted according to creatinine clearance[25-29] (Table 1). The main limitations of NUCs include the need for long-

term therapy, which may be for indefinite time in HBeAg negative patients; the risk of development of NUC's resistant viral strains; and the unknown safety profile with long-term treatment[11].

Regarding NUCs, there are five drugs currently approved for HBV treatment: lamivudine (LAM), adefovir (ADV), telbivudine (LdT), entecavir (ETV) and tenofovir disoproxil fumarate (TDF). Treatment with TDF or ETV is preferable to LAM in NUC naïve patients, since they are more effective due to a high antiviral potency and have a high barrier to resistance reducing the risk of drug resistance and treatment failure[9,10,15,21,23].

Since LAM was the first NUC approved for clinical use, it has yielded the majority of data on the management of HBsAg-positive renal transplant recipients. Several observational studies have shown that LAM can improve liver function[15]. A meta-analysis including 14 prospective cohort studies (184 patients) showed that LAM normalized ALT levels in 81% (95%CI: 70%-92%), cleared HBV-DNA in 91% (95%CI: 86%-96%) cleared HBeAg in 27% (95%CI: 16%-39%) of the patients. In most studies (11 of 14) LAM was administered for 6 to 12 mo[30]. Even though LAM was associated with significantly improved patient survival[31], prolonged treatment is associated with progressive increase in drug resistance and the cumulative probability of developing LAM resistance (LAM-R) was approximately 60% after 69 mo[30,32,33]. LAM-R leads to treatment failure and can be associated with progressive liver disease and a negative impact in patient and graft survival. Fortunately today there are good treatment options for LAM-R. Given that there are better options for HBV treatment, LAM cannot be consider within the first treatment choices for these patients[9,10,15,21,23].

Adefovir was the second available oral drug for HBV treatment infection. It has similar antiviral activity against both LAM-R and wild-type HBV, but it may be nephrotoxic (especially in high doses). Currently its major clinical application is as add on therapy for the management of lamivudine-resistance since it has lower antiviral activity than ETV and TDF for naïve patients[9,10,15].

There have been reports on ADV short-term efficacy either as mono- or add-on therapy in LAM-R RT patients[34-39]. One year ADV monotherapy showed a significant viral response in 11 patients with a median HBV DNA decline of 5.5 $\log_{10}$. Only one patient cleared HBV DNA, one of the six HBeAg positive patients cleared HBeAg but without antiHBe seroconversion; none cleared HBsAg. Importantly, there were no significant clinical and laboratory adverse events[34]. ADV as add-on therapy to LAM resulted in significant HBV suppression LAM-R RT recipients[38,39]. In 11 ADV add on treated patients, HBV DNA was undetectable in 80%-83% after 36 to 42 mo[38]. However, six patients (54%) had to lower ADV dose due to a decline in glomerular filtration rate after a median of 11 mo (range: 9-42)[38]. After 12, 24 and 36 mo of ADV treatment treatment 35.7%, 42.8% and 88.0% of treated patients cleared HBV DNA; there was no virological breakthrough and 92.8% of patients achieved normal ALT levels after 12 mo of treatment[39]. Patients treated with add-on ADV therapy tended to normalize ALT levels and to reduce HBV DNA levels more effectively than those treated with ADV monotherapy[39]. In this study 29% of the participants developed moderate to severe renal failure[39].

However, when compared with treatment-naïve the virological response could be fluctuating and relatively slow in LAM-R patients[40]. Nevertheless, rescue therapy with ADV resulted in significantly better viral suppression and liver biochemistry compared with continuation of LAM (75% *vs* 14.3% had persistent normalization of ALT), and the clinical response was sustained for at least 24 mo[31]. Evidence of nephrotoxicity in the absence of proximal tubulopathy, despite dosage adjustment, was frequently observed, and could necessitate treatment discontinuation[38,39]. ADV has a low antiviral potency at the currently approved dose and its efficacy could be further reduced with dose adjustment according to renal dysfunction. For these reasons ADV is not a first line option for naïve patients and its benefits for LAM-R may be less when compared with TDF.

There are currently no results about telbivudine treatment in RT recipients but it would be worthwhile to explore the use of this agent in treatment-naïve kidney allograft recipients given its relatively low resistance rate, lack of nephrotoxicity, and the relatively lower cost compared with other nucleoside/tide analogues[40].

Entecavir is one of the first line treatment options for HBV[9,10]. This drug has a high antiviral potency, a high genetic barrier for resistance and a good safety profile. It is very effective for treatment naïve patients but has a lower efficacy for LAM-R patients, and it is not the first option for this latter population[9,10,15]. A recent 2-year prospective study included 27 RT patients, 18 (67%) were treatment naïve and 9 (33%) had been previously treated with LAM but had no resistant mutations. ETV cleared HBV DNA in 70%, 74%, 96% and 100% of patients after 12, 24, 52 and 104 wk respectively[41]. There was no change of creatinine clearance, and no episodes of lactic acidosis or muscle damage during treatment. There were higher rates of undetectable HBV DNA levels in ETV treated than LAM treated patients (32%, 37%, 63% and 63% at 12, 24, 52 and 104 wk, respectively; $P < 0.005$)[41]. In an analysis excluding 9 patients from the ETV group who were also LAM experienced, the remaining 18 ETV naïve subjects exhibited a better virological response at 52 and 104 wk than 19 previously treated with LAM ($P < 0.05$)[41].

Other studies reported results with ETV in cohorts including both naïve and LAM-R patients, unfortunately with limited number of patient[42-45]. Experience regarding the use of ETV in RT recipients who had developed LAM- or ADV-resistance had been examined in a small study with 10 solid organ transplant recipients (8 kidney allograft recipients)[42]. Treatment with ETV resulted in an appreciable drop in HBV DNA levels and a 50% HBV undetectability in both HBeAg positive and HBeAg negative patients after 16.5 mo of treatment without significant changes in glomerular filtration rate[42]. In our small experience we reported ETV use in 11 patients with several chronic renal diseases: 1 with stage 4 CKD, 7 in dialysis, and 3 RT recipients[43,44]. HBV DNA was cleared in 54.5% (n = 6); 77.7% of HBeAg-positive patients (7/9) seroconverted to antiHBe positive; and only one patient (9.1%) showed antiHBs seroconversion. There were no significant changes in renal or hematological biochemical parameters[43,44]. In the most recent report, twenty-one RT patients (10 treatment naïve, 11 with LAM resistance) were treated with ETV for 34.7 ± 22.9 mo (range 6-75 mo)[45]. The cumulative rate of HBV DNA undetectability at 12, 24, and 36 mo was 60%, 100%, and 100% for treatment naïve group, and 27%, 45%, and 45% for LAM-R group, respectively. Genotypic resistance to ETV emerged after 20.0 ± 3.5 mo with increase in ALT and HBV DNA in two patients with LAM-R, but was not observed in the treatment-naïve group. There were no significant changes in glomerular filtration rate[45]. Also, ETV was used in RT patients who developed hepatic flares duo to the appearance of LAM-R[46]. Four patients were treated with ADV and two with ETV. After 18 mo, HBV DNA was < 10^5 copies/mL in 4 subjects and < 10^2 copies/mL in 1 subject. There were no remarkable adverse events and no changes in renal function[46]. ETV appears as one of the best options for NUC naïve RT patients; it is less effective in LAM-R and better options are available.

Tenofovir was the last NUC to be approved for HBV monoinfection and is the other first line option together with ETV[9,10,15]. It has a high antiviral potency, a high genetic barrier for resistance and a good safety profile[9,10,15], but there is some concern about its potential nephrotoxicity[47]. There is little data in the renal transplant setting: only one study reports the results of three RT treated patients together with 3 liver, and 1 heart transplant recipients[48]. HBV DNA viral became significantly decreased and 3 patients cleared HBV DNA at the end of the study period. There were no adverse events related to tenofovir treatment. No episodes of acute rejection were reported under therapy. There were no statistically significant changes in renal function represented by stable creatinine levels, estimated creatinine clearance, serum phosphorus level, or daily microalbuminuria level[48]. TDF appears as one of the best options for both NUC naïve and LAM-R RT patients; treatment results have to be extrapolated from the general population since there is little experience in RT.

TIMING OF INITIATION OF TREATMENT: PREEMPTIVE OR PROPHYLACTIC THERAPY

Patients with chronic renal disease go through different phases: varying stages of renal failure, ESRD, hemodialysis (HD)/peritoneal dialysis (PD), and transplantation. Once transplanted could suffer various kidney disease and finally lose the graft and return to dialysis. HBV infection will go with the patient along the road. The timing of HBV treatment initiation may vary depending on the stage of renal disease.

Patients undergoing HD or PD who are not RT candidates can start NUC therapy if HBV DNA levels are ⩾ 2000 IU/mL regardless of ALT levels, especially if they have moderate fibrosis in the liver biopsy (METAVR score F ⩾ 2) or estimated by a non-invasive methods[11].

All HBsAg-positive RT recipients are considered candidates for NUC treatment. RT candidates with HBV DNA levels > 2000 IU/mL must initiate treatment at HBV diagnosis, those with HBV DNA ⩽ 2000 IU/mL should start therapy at least 2 wk before RT. NUC therapy has to be continued indefinitely as long as the patients are under any immunosuppressive treatment[9,10,11,15]. It should be remembered that compensated cirrhotic patients are not candidates for RT, and cirrhotic patients with decompensated disease should be evaluated for combined liver-kidney transplantation[11,49].

As previously mentioned, RT candidates who are inactive carriers have an increased reactivation risk after transplantation. In this subgroup of HBsAg positive patients treatment can be used as prophylactic (HBV DNA undetectable, no hepatocellular injury), preemptive (HBV DNA ⩽ 2000 IU/mL, no hepatocellular injury), and salvage therapy after reactivation (HBV DNA > 2000 IU/mL, with hepatocellular injury). Even if the prophylactic/preemptive initiation is the generally accepted treatment, the data comparing these treatments are few. The disappearance of viral load is a prerequisite for a HBV positive patient on hemodialysis to be enrolled in the RT list. Therapy with ETV, TDF or LAM on adjusted doses for renal function is included in the current guidelines for prophylaxis of HBV positive RT candidates. The optimal NUC regimen has not been proposed yet, so prophylaxis may start before or at the time of RT and continue thereafter[9,10,15,50]. ETV should be the first line option for avoidance of short term resistance and ADV nephrotoxicity, while TDF had better be applied in case of LAM-R[9,10,50].

Lamivudine is the most extensively drug used in prophylactic/preemptive therapy in RT patients. In a small study, LAM given as either prophylactic or pre-emptive treatment was proven superior to salvage therapy when liver dysfunction is evident[51]. None of the HBsAg positive patients receiving prophylactic or pre-emptive therapy developed reactivation, while 50% of the patients not been treated suffered reactivation[51,52]. These results were confirmed by others, but there is some controversy about the clinical impact of prophylactic/preemptive therapy *vs* salvage therapy[51-55]. One study showed that there was no differences in survival between HBsAg positive RT patients treated preemptively with LAM and HBsAg negative controls. HBsAg positive patients transplanted without preemptive therapy had in increased mortality rate [relative risk of death, 9.7 (P < 0.001); relative risk of liver-related mortality, 68.0 (P < 0.0001)[53]. Twenty five RT candidates received pre-transplantation prophylactic/preemptive NUC therapy, 22 (88%) were treated with LAM and 3 (12%) with ETV[54]. When compared with a historical control group NUC treated patients has a significant improvement in 10 year graft (82% *vs* 34%) and patient (91% *vs* 57%) survivals. There was no liver-related death in NUC treated patients. In contrast, in untreated controls patient death (68%) was the most frequent cause of graft failure, which was mostly caused by liver diseases. Prophylactic and preemptive therapy resulted in the same graft and patient survival, but patients receiving preemptive therapy had a higher HBV reactivation incidence. NUC treatment was independently associated with better patient survival (P = 0.005)[54]. On the contrary, a retrospective analysis using LAM in the majority of patients found no benefit of prophylactic/preemptive treatment[55]. Ninety four RT candidates were evaluated, 56 received antiviral prophylaxis (Group 1), 51 with LAM and 5 with ETV, and 38 did not (Group 2). In group 2 20 patients experienced HBV reactivation: 16 received LAM, 2 received ETV and 2 received no antiviral treatment. Using the Cox-regression model, prophylactic treatment did not improve patient survival (OR = 1.29, 95%CI: 0.37-4.49, P = 0.693), graft survival (OR = 1.25, 95%CI: 0.45-3.46, P = 0.666) or reduce the risk of hepatic decompensation (OR = 2.01, 0.35-11.57, P = 0.434)[55]. LAM-R occurred in 21 LAM-treated Group 1 and 4 LAM-treated Group 2 patients (P = 0.243), with mean times of resistance after RT of 82 and 132 mo, respectively (P = 0.001)[55].

A recent retrospective study compared both treatment strategies[17]. It included 58 HBsAg positive RT recipients: 24 in the prophylactic group (all patients used LAM) and the 34 in the preemptive group (32 patients used LAM and 2 patients used ETV). The graft/patient survival rates for HBsAg positive were the same as those of hepatitis-free recipients (P = 0.18). In the prophylactic group, there were fewer hepatic dysfunctions (12.5% *vs* 30%, P = 0.12), viral breakthroughs (16% *vs* 32%, P = 0.17) and elevated alanine aminotransferase concentrations (37% *vs* 52%, P = 0.24), however these differences were not statistically significant. In the prophylactic group, one patient was switched to ETV and then to TDF due to partial response finally achieving complete virological response. In the preemptive group, LAM was withdrawn and changed to TDF in 3 patients and to ADV in another one achieving an adequate virologic/biochemical response. These NUCs were almost as safe as LAM, as there were no significant differences among proteinuria and estimated glomerular filtration rate[17].

Results from these studies support the clinical guidelines recommendations: prophylactic or preemptive therapy with NUCs provides comparable graft/patient survival with hepatitis-free RT recipients and may be better in preventing hepatic dysfunction than salvage therapy. Given its high risk for developing resistant mutations, LAM is no longer a first option, and ETV should be the first one. TDF can be an effective and safe treatment for LAM-R in RT recipients (Figure 1)[9,11,15,21,56].

TREATMENT IMPACT ON LONG TERM EVOLUTION

In the last years, several cohort studies had demonstrated that HBV infection is associated with higher patient mortality and risk of graft failure in RT patients[15,40,50,57]. These results had been validated in two meta-analysis[58,59]. The first meta-analysis was published in 2005 and included 6050 patients from six observational cohort retrospective studies. Pooled results showed that HBsAg positive status was a significant predictor for death (RR = 2.49, 95%CI: 1.64-3.78) and for graft loss after RT, when compared to seronegative patients (RR = 1.44, 95%CI: 1.02-2.04) (homogeneity test, P < 0.0001)[58]. These results have been updated in 2014: ten observational studies involving 82690 unique RT recipients were included. In this study, HBsAg positive status was associated with an increase risk for all-cause mortality (adjusted RR = 2.214, 95%CI: 1.56-3.137, P < 0.0001) and for all-cause graft failure (aRR = 1.44, 95%CI: 1.26-1.63, P < 0.0001)[59]. Both meta-analyses of observational studies concluded that untreated RT HBsAg positive patients have an reduced patient and graft survival.

As previously mentioned oral NUC therapy safely and effectively can suppress HBV replication in RT recipients. Several studies had shown that this antiviral effect may impact on long term graft and patients outcome[31,54,60-62]. In 63 LAM treated HBsAg positive RT recipients 10-year survival rate was 81% and such results were nearly comparable to HBsAg

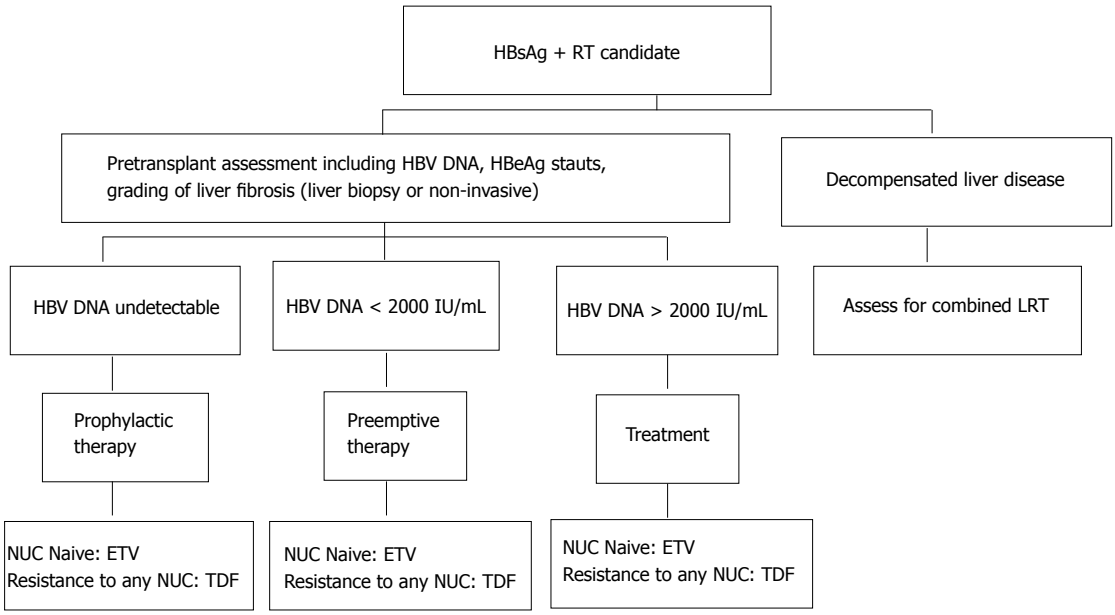

Figure 1 Treatment algorithm for management of renal transplant candidates with chronic hepatitis B virus infection. RT: Renal transplantation; LRT: Liver renal transplantation; NUC: Nucleos(t)ide analogue; ETV: Entecavir; TDF: Tenofovir disoproxil fumarate.

negative patients[31]. Initiating treatment with LAM is associated with a 62% chance of developing drug resistance after 4 years of continuous therapy. Six months after beginning rescue therapy with ADV or ETV, HBV DNA decrease three-log in 75% of patients without significant adverse effects. When compared with untreated patients, those treated with NUCs showed a significant improvement in survival after 20 years of follow up (34% *vs* 83% respectively, $P < 0.006$). Even though NUC therapy reduced overall mortality by reducing liver related disease ($P < 0.036$), 40% of death in chronic HBV infected patients are still related to liver complications and 22.2% of them developed in patients being treated[31].

Forty two RT patients were treated for long term with different NUCs regimens: at the end of follow up 18 patients were receiving monotherapy (9 LAM, 2 ADV, and ETV) and 24 combination therapy (11 LAM + ADV, 2 LAM + TDF, 4 ETV + ADV, 6 ETV + TDF and 1 TDF + emtricitabine, FTC)[60]. At the end of the study 100% (18/18) of patients under monotherapy and 87.5% (21/24) of patients under combination therapy cleared HBV DNA. The 3 patients (12.5%) in the combination therapy group with detectable HBV DNA, had HBV DNA levels below300 IU/mL. At the end of follow up, 92.8% of the entire cohort had cleared HBV DNA. Patient survival was 97.6% at 10 years, 95.2% at 15 years, and 90.4% at 20 years after renal transplantation, while graft survival was 100% at 5 years, 97.6% at 10 years, 95.2% at 15 years, and 88.1% at 20 years after renal transplantation. At the end of the study, 8 patients (19.04%) died and 1 received a liver transplantation due to end stage liver disease. Four deaths were liver related: 4 patients (3 cirrhotics and 1 with only mild fibrosis at baseline) developed an hepatocellular carcinoma (HCC) despite complete virological response; three of the other patients died of non liver cancer and the remaining from stroke[60]. During the study, 11.9% (6/42) of patients returned to dialysis due to chronic rejection leading to graft failure. The NUC dose was modified according to creatinine clearance in 45.2% (19/42) of the patients[60].

Thirty RT patients underwent long term NUC therapy; at the end of follow up 25 were still alive and being treated and 24 were HBV DNA non detectable. Eight patients were receiving LAM monotherapy, 1 on ETV, 1 on TDF, 6 on LAM + ADV combination and 9 LAM + TDF[61]. Five patients died from no liver related disease and 9 returned to dialysis after graft failure due to chronic allograft nephropathy. In this cohort, 10 year patient survival was 92% and 10 year graft survival was 86%. There were no renal adverse effects related to ADV/TDF therapy[61].

Twenty five patients received pre-RT treatment with LAM (22 patients) and ETV (3 patients): 18 were HBV DNA undetectable (prophylactic group) and 8 were HBV DNA positive (preemptive group), and were compared to a historical control group[54]. Unadjusted 10-year graft survival rates in the treatment cohort *vs* historical control cohort were 81.8% and 34.3%, respectively ($P = 0.003$). Graft lost occurred more frequently in the historical control than in the treated cohort (70.3% *vs* 4.3%, respectively); this was mainly related to patient death. Treated patients showed a better 10-year patient survival than the control group: 90.0% *vs* 57.4%; $P = 0.013$. Pre-transplantation NUC

Table 2 Definition of response to nucleos(t)ide analogue antiviral therapy of chronic hepatitis B

Category of response	
Biochemical (BR)	Decrease in serum ALT to within the normal range
Virologic (VR)	Decrease in serum HBV DNA to undetectable levels by PCR assays, and loss of HBeAg in patients who were initially HBeAg positive
Primary non-response	Decrease in serum HBV DNA by 2 log10 IU/mL after at least 24 wk of therapy
Virologic relapse	Increase in serum HBV DNA of 1 log10 IU/mL after discontinuation of treatment in at least two determinations more than 4 wk apart
Histologic (HR)	Decrease in histology activity index by at least 2 points and no worsening of fibrosis score compared to pre-treatment liver biopsy
Complete (CR)	Fulfill criteria of biochemical and virological response and loss of HBsAg
Time of assessment	
On-therapy	During therapy
Maintained	Persist throughout the course of treatment
End-of-treatment	At the end of a defined course of therapy
Off-therapy	After discontinuation of therapy
Sustained (SR-6)	6 mo after discontinuation of therapy
Sustained (SR-12)	12 mo after discontinuation of therapy

HBV: Hepatitis B; HBsAg: Hepatitis B surface antigen.

treatment was an independent factor for the improved patient survival [odds ratio (OR) = 0.052; *P* = 0.005]. Liver-related disease was the main cause of death in the historical control cohort (84.6% of the cases); sepsis was the second most frequent cause (15.4% of the cases)[54]. Overall, graft (100% *vs* 71.4%) and patient survivals (100% *vs* 85.7%) were similar in the 2 treated cohorts (*P* = 0.601)[54].

Only one study showed some conflicting results on NUC therapy impact in patients' survival[62]. The study included 94 HBV-positive and 282 age/sex-matched HBV negative RT patients: 56 patients received an antiviral agent for prophylaxis (LAM 51, ETV 5), and other 18 for HBV reactivation. Although the patient survival rate was lower for HBV positive than HBV negative RTRs (89% *vs* 94% at 5 years, 78% *vs* 88% at 10 years, *P* = 0.031), there was no difference in graft survival between the two groups (86% *vs* 92% at 5 years, 73% *vs* 81% at 10 years, *P* = 0.113). In multivariate analysis, HBsAg positive status was a significant risk factor for death (OR = 2.19, 95%CI: 1.14-4.20, *P* = 0.019), but not significant for graft loss (OR = 1.64, 95%CI: 0.94-2.86, *P* = 0.079)[62]. HBeAg and HBV DNA Pretransplant status was not available for all the patients. Of the 26 HBeAg-positive patients, 14 were receiving antiviral prophylaxis at transplantation: 8 showed reactivation while 6/12 of the untreated developed reactivation. All survived with stable liver chemistry, except for one dying form an HCC. Of 57 HBeAg-negative patients, 35 were started on antiviral prophylaxis at transplantation: 14 showed reactivation while 14/22 of the untreated developed reactivation. Among them, 12 died, whereas the remaining 45 survived without hepatic dysfunction[62]. Even though treated patients showed a reduced survival, it appears to be better than the survival reported in untreated patients.

NUC therapy in HBsAg positive RT patients is associated with a higher long term patient and graft survival rate. Studies have some limitations since most of the used LAM, which is not the best treatment option. More potent NUCs may add some benefit over LAM, but this still has to be demonstrated. Salvage therapy with TDF or ADV is safe and effective in patients developing LAM-R. Despite this clear benefit, all HBV infected patients must be closely follow up and HCC screening must be performed every six months, since the risk of HCC development may not entirely disappears even in the presence of virological response[60,63].

DURATION OF THERAPY AND EVALUATION OF RESPONSE

In RT patients it is unclear what is the optimal treatment extent that assures long term viral suppression, preserving adequate liver function with the minimal risk of viral resistance development[15]. Current guidelines clearly define how to monitor on treatment response, what are the therapeutic endpoints and when it is possible to stop treatment (Table 2)[9,10]. In the case of NUC therapy, there are some terms regarding resistance that have also been defined. These is particularly important in this population, since some patients had initiated treatment long time ago with old NUCs such as LAM (Table 3)[9,10].

Table 3 Definition of terms relating to antiviral resistance to nucleos(t)ide analogue treatment

Term	Definition
Virologic breakthrough	Increase in serum HBV DNA by > 1 log10 (10-fold) above nadir after achieving virologic response, during continued treatment
Viral rebound	Increase in serum HBV DNA to > 20000 IU/mL or above pretreatment level after achieving virologic response, during continued treatment
Biochemical breakthrough	Increase in ALT above upper limit of normal after achieving normalization, during continued treatment
Genotypic resistance	Detection of mutations that have been shown in "*in vitro*" studies to confer resistance to the NA that is being administered
Phenotypic resistance	*In vitro* confirmation that the mutation detected decreases susceptibility (as demonstrated by increase in inhibitory concentrations) to the NUC administered

NUC: Nucleos(t)ide analogue.

The duration of treatment depends on HBeAg status. HBeAg positive patients should be treated until HBV DNA and HBeAg are cleared and antiHBe seroconversion develops. Additional treatment, also known as "consolidation therapy", is needed for at least 6 to 12 mo after antiHBe seroconversion to prevent virological relapse. It is recommended to closely monitoring for relapse after treatment withdrawal. Relapse, even in patients achieving adequate virological response is a possibility, but their rates tend to be low[64]. HBeAg negative patients should treated until HBsAg clearance is achieve[9,10]. These recommendations might be applied to treatment in RT recipients to ensure treatment success, but outcomes after NUCs withdrawal in RT immunosuppressed patients is unknown.

A small recent study evaluated the long term results in HBV positive RT patients after NUC treatment discontinuation[65]. Fourteen patients treated with LAM (11 patients), ADV (1 patient), ETV (1 patient), and LdT (1 patient) were included in this study. Patients were allowed to discontinue treatment if they have all of the following: (1) no clinical and histologic evidence of cirrhosis; (2) normal liver biochemistry; (3) negative for both HBV DNA and HBeAg; (4) no viral resistance; (5) antiviral therapy > 9 mo; (6) maintenance dosage of immunosuppressant for > 3 mo; and (7) no history of acute rejection during recent 6 mo[65]. All patients were followed at 3 to 6 mo interval for liver biochemistry, viral serology, and HBV DNA level after treatment discontinuation. In 6 (42.9%) of 14 patients who meet the pre-specified criteria treatment was discontinued. In 4 of them (66.7%) it was successfully discontinued and HBV DNA was still undetectable for a median 60.5 mo (range, 47-82 mo). In the other 2 patients HBV reactivated, but HBV DNA was again cleared after immediately resuming NUC therapy[65]. On the contrary, in LAM treatment discontinuation in 19 RT recipients after 2 years of treatment without adequate virological response, relapse rate was high (75%)[66]. Even though evidence is scarce, it seems that in certain RT patients, after complete viral suppression and sufficient duration, antiviral therapy can be successfully and safely withdrawn.

IMPACT OF NUCLEOS(T)IDE ANALOGUES ON RENAL FUNCTION

Nucleos(t)ide analogues are primarily eliminated without changes in the urine following ingestion, and appropriate dose modifications are proposed for patients with impaired renal function (eGFR < 50 mL/min) (Table 1). Treatment guidelines recommend that all patients initiating NUC treatment should be tested for serum creatinine levels and estimated creatinine clearance before therapy; and baseline renal risk should be assessed for all of them[9,10]. High baseline renal risk includes one or more of the following clinical situations: decompensated cirrhosis, creatinine clearance < 60 mL/min, poorly controlled hypertension, proteinuria, uncontrolled diabetes, active glomerulonephritis, concomitant nephrotoxic medications and solid organ transplantation. In consequence, RT recipients may have many of these basal renal risk factors.

In clinical trials outside renal transplant setting, minimal decline in renal function have been showed with all NUCs, except for LdT which appears to improve renal function[67,68]. Impact of LdT on renal function was analyzed from a database including all patients treated in the GLOBE Study (2 years), in the long term extension study CN04E1 (4 to 6 years) and in patients with decompensated cirrhosis (2 years)[69]. Renal function improved in LdT treated patients in GLOBE trial (+8.5% increase in mean eGFR,) and it was sustained for 4 to 6 years. Improvement in renal function in LdT treated patients was also observed in those at increased risk for renal impairment: patients with baseline eGFRs of 60-89 mL/min per 1.73 m^2 (+17.2%), > 50 years (+11.4%), and with advanced liver fibrosis or cirrhosis (+7.2% for patients with Ishak fibrosis score 5-6). In patients with the highest renal risk such as decompensated cirrhotics, eGFR was also improved with LdT (+2.0%). In patients who received 2 years of LAM in GLOBE/015 studies and

rolled over to extension study to receive LdT for 2 additional years, eGFR also improved after treatment switch (+8.9%)[69]. Although this data may suggest that LdT may be renal protective, it is not clear whether this protective effect is specific to this NUC. This potential benefit, particularly relevant in the RT population, does not overcome the high risk of treatment resistance and neuromuscular adverse events. As previously mentioned, this beneficial safety profile does not support the use of LdT as a first-line NUC in hepatitis B treatment[69].

Nucleotide, specially ADV, appear to be more nephrotoxic than nucleoside analogues[70-74]. In a real-life setting study, 145 patients ADV treated patients were compared with 145 untreated patients regarding its impact on renal function[71]. During follow-up, 30% of ADV treated patients show a mild decrease in renal function (10%-20% reduction in eGFR from baseline) compared with 16% in the untreated group, 15% *vs* 6% showed a moderate decrease (20%-30%), and 7% *vs* 1% showed a severe decrease (> 30%) respectively (P > 0.0001). In the ADV group 6.9% of the patients discontinued treatment (P > 0.004). In a multivariate analysis ADV treatment significantly predicts renal dysfunction [hazard ratio (HR) = 3.94, P = 0.03]. In the same analysis, age > 50 years (HR = 3.49, p = 0.087), baseline mild renal dysfunction (HR = 4.49, P = 0.073), and hypertension and/or diabetes mellitus (HR = 2.36, P = 0.074) were not significant predictors[71]. In a retrospective study, 687 patients receiving ADV monotherapy (18.2%) or in combination with LAM (81.8%) for 1 year or more were enrolled to evaluate the incidence and risk factors of renal impairment[72]. Renal dysfunction was defined as mild (20%-30% reduction in eGFR), moderate (30%-50%), or severe (more than 50%). Patients were treated for a median of 27 mo, 10.5% (n = 72) developed renal dysfunction being mild in 77.8% of patients, moderate in 20.8% of patients, and severe in only 1 patient. The cumulative incidence of renal dysfunction at 1, 3, and 5 years was 2.6%, 14.8%, and 34.7%, respectively. ADV dose was modified in 7 patients and it was discontinued in 3 patients; after these changes, eGFR remained stable[72]. In 271 ADV treated patients, after 6 years of treatment GFR $\leqslant$ 60 mL/min incidence was 38.3% and after 5 years, serum creatinine increased $\geqslant$ 0.5 mg/dL in 21.48%. Switching ADV to other NUC or reducing its dose was associated with reversal of renal dysfunction in almost all patients; there were no differences between the two approaches (P = 0.737)[73]. On the contrary, a study including 46 HBeAg negative LAM-R patients treated with ADV add on for up to 90 mo found no impact on renal function when compared with a matched control group of untreated inactive HBV carriers[74].

The number of patients treated with ADV in the RT setting is smaller than in the general population. In this subgroup, ADV treatment may also impact on renal function. A significant decrease of estimated GFR and an increase in serum creatinine from 1.42 (± 0.39) to 1.6 (± 0.36) mg/dL, (P = 0.02) was found in 11 patients treated for 2 or more years[37]. It was also associated with an increase in proteinuria, changes in renal tubular parameters and changes in phospho-calcic metabolism[37]. Another study including also 11 LAM-R patients did not show significant changes in median creatinine clearance (CLcr), in serum phosphorus or in urinary protein level from baseline to the last available visit. However, after a median treatment time of 11 mo (range: 9-42), 54% (n = 6) of patients reduce ADV dose due to renal dysfunction. Renal function remained stable (n = 5) or improved (n = 1) 22 mo (range: 6-34) after dose modification[38].

Fourteen patients were treated with long term ADV (5 monotherapy, and 9 ADV + LAM combination therapy). Eight patients (57.2%) developed impaired renal function; it was mild (5%-20% reduction in the eGFR compared to baseline values) in 4, moderate (20%-30%) in 2, and severe (> 30%) in the 2 remaining patients. Acute graft rejection was diagnosed by kidney biopsy in 2 of these patients. Calcineurin inhibitors nephrotoxicity was presumed in 2 of these patients and their doses were accordingly adjusted. ADV dose was reduced in 3 patients due to severe renal dysfunction (eGFR 30-50 mL/min) and it was discontinued in 1 patient (eGFR < 20 mL/min) without impact on virological response[39]. Renal dysfunction in long-term ADV treated patients appears relatively frequent, but serious nephrotoxicity is unusual. Renal dysfunction can be safely managed by dose reduction or switching to another NUC without impact on virological response.

In TDF treated patients, also a nucleotide analogue, renal dysfunction is less frequently seen than with ADV. The majority of previously nephrotoxic events reported, which were similar to those reported under ADV treatment, were in HIV infected patients[68]. There is recently presented data about TDF impact on renal function in HBV mono-infected patients[75-80]. A study evaluated the pooled results from three global randomized clinical trials including 426 TDF treated patients for 144 wk. In this study 0.5% (2/426) of patients developed a creatinine increase $\geqslant$ 0.5 mg/dL from pre-treatment values and none showed an eGFR decrease < 50 mL/min, showing a minimal impact of TDF on renal function even in high risk patients such as cirrhotics or diabetics[75]. Moreover, when 74 patients with mild renal dysfunction (CrCl 50-80 mL/min) were compared with 206 with normal renal function (CrCl $\geqslant$ 80 mL/min), none of them showed signs of renal impairment defined as a creatinine increase $\geqslant$ 0.5 mg/dL after 96 wk of therapy[76]. Among 441 patients from the Vireal cohort, 114 with baseline impaired

renal function were classified as stage 2 (GFR 60-89 mL/min), stage 3 (GFR 30-59 mL/min), stage 4 (GFR 15-29 mL/min) and stage 5 (GFR < 15 mL/min or dialysis) and included in the study. When compared from baseline, after 48 wk of treatment, TDF did not significantly modified GFR in patients with stage 2 (76 mL/min *vs* 77 mL/min), 3 (50 mL/min *vs* 49 mL/min), or 4 (23 mL/min *vs* 23 mL/min) renal failure[77]. Two RT recipients were included and had a stable GFR under therapy. Nine patients needed to adjust TDF dose. At the end of the study, 67% had a stable renal failure stage, 22% had an improvement and 11% had a decreased in it[77]. In a retrospective study, 195 refractory patients were treated with TDF monotherapy for 30 ± 16 (6-90) mo were compared with 89 asymptomatic HBsAg carriers[78]. After 48 mo, TDF treated patients in showed a significantly greater reduction in eGFR when compared to untreated patients [-16 ± 36 (-48 - +23) and -9.6 ± 36 (-21 - +22) mL/min, respectively, P = 0.03]. TDF dose was reduced in only 1 patient after 15 mo of treatment due to a 0.38 mg/dL increase in creatinine levels[78]. In 26 LAM-R patients treated with TDF, there were no significant variations in phosphatemia and GFR from baseline after one year of treatment[79]. Even if there is no impairment on renal function, TDF may have some potential effects on the proximal tubule. In 61 TDF treated patients for a mean time of 29 mo, there were no significant change in mean GFR in the overall population but 58% of patients showed an impairment in GFR (median 8.1%, range 0.01% to 20.5%) and two patients developed an GFR to < 60 mL/min[80]. At least one sign of proximal tubular damage appeared in 26 (42%) individuals: glucosuria without diabetes mellitus, increased alpha1-microglobulinuria/creatinine ratio, hypophosphatemia, reduced tubular resorption of phosphate rate and reduced tubular maximum reabsorption rate[80]. The effects of TDF on renal function were evaluated in 321 naïve patients treated for 4 years in clinical practice[81]. In this large European cohort, there were no modifications in creatinine and phosphorus serum levels and eGFR was reduced from 84 to 80 mL/min. At year 4, patients with eGFR < 50 increased from 2% to 3% and those with eGFR < 60 mL/min increased from7% to 11%. At the same time point, hypophosphatemia (serum phosphate < 2.3 mg/dL) increased from 2% to 5.1 %, while 1% of the patients had phosphate levels < 2.0 along the study period. TDF dose was reduced in 17 patients due to reduction in eGFR and in 2 due to hypophosphatemia. Seven patients had to withdraw treatment and were switched to ETV. Overall, some renal adverse effect was reported in 26 patients (7%)[81].

In comparison to nucleotide analogues, nucleoside analogues, such as ETV and LdT, show not significant renal toxicity[67,68]. Studies have been performed comparing ETV and TDF nephrotoxicity[82-86]. After 2 years of treatment, there was no significant modifications in eGFR in 74 ETV and 50 TDF ± LAM treated patients[82]. In the ETV group 2.7% showed a reduction ⩾ 40% in eGFR *vs* 3.92% in the TDF ± LMV group (P = NS). When compared with an untreated control group, in ETV treated patients eGFR was reduced by -7.6 mL/min (95%CI: -15.8-+0.6, P = 0.07) and by -8.7 mL/min (95%CI: -18.3-+1.0, P = 0.08) in TDF ± LMV treated patients. In untreated controls, eGFR remained stable or even improved by +7.4 mL/min (95%CI: 0.78-14.1, P = 0.03)[82]. In another real-life cohort of 212 patients were treated with TDF and 79 with ETV and its impact on renal function was evaluated[83]. No significant differences were found in urea, creatinine and phosphorus levels and in eGFR after 12 mo of TDF treatment. Also in the same group, there was no difference in the proportion of patients with eGFR < 60 mL/min when compared with baseline levels. In ETV treated patients, there was a significantly reduction in serum phosphorus (0.96 *vs* 1.06, P = 0.016), increased in creatinine levels (1.0 *vs* 0.89, P < 0.05) and reduction in eGFR (80 *vs* 89, P < 0.05) after 12 mo of treatment. In ETV treated patients, 3.8% of patients had a 25% increase in creatinine levels while 0.47% of TDF treated patients had a 25% decrease in eGFR after 12 mo of treatment[83]. In a community-based retrospective cohort study, 80 patients treated with TDF monotherapy or in combination with other NUCs were matched with 80 ETV treated patients and incidences of serum creatinine increments and eGFR decrease were evaluated[84,85]. More patients in the ETV group had creatinine increments ⩾ 0.5 mg/dL (3 *vs* 11; P = 0.025), whereas more patients treated with TDF had eGFR reductions of < 60 mL/min (15 *vs* 6; P = 0.022) and at least 1 dose modification (13 *vs* 4; P = 0.021). In a multivariate analysis, previous organ transplantation (aOR, 6.740; 95%CI: 1.799-28.250; P = 0.005) and pre-treatment renal failure (aOR, 10.960; 95%CI: 2.419-48.850; P = 0.002) were significantly associated with increases in serum creatinine levels[84]. Renal function was evaluated in 197 HBV mono-infected patients from two outpatient clinics and who were classified according to the received treatment: LAM (n = 36), ADV (n = 32), ETV (n = 32), TDF (n = 37), and untreated HBsAg-positive patients (n = 60)[86]. The CKD-EPI equation was used to calculate eGFR and the individual change in eGFR over time was modeled with linear mixed effects models. Patients with previous renal dysfunction, diabetes mellitus, or arterial hypertension were excluded from the analysis. The yearly predicted median individual changes in eGFR according to this model were: -2.05 mL/min in untreated patients, and -0.92 mL/min, -1.02 mL/min, -1.00 mL/min, and -0.92 mL/min in LAM, ADV, ETV and TDF treated patients,

respectively. A decrease of eGFR > 20 mL/min from baseline developed in 3.3% of untreated patients, and in 5.5%, 0%, 6.25%, and 2.7% in LAM, ADV, ETV and TDF treated patients, respectively. Renal insufficiency stage 3 (eGFR of < 60 mL/min) was uncommon and not different between all patient groups[86].

First line NUCs, ETV and TDF, appears to have little impact on renal function in the general population when compared with untreated controls and with the others NUCs. Markers of renal function indicated that TDF treated patients, suspected to be more nephrotoxic, have similar risks of developing changes in renal function than ETV treated patients. Although there is some evidence showing some degree of renal dysfunction in ETV treated patients, its clinical significance remains unclear and it may represent a physiological decrease in renal function in this group and/or reflect the potential limitations of standard biochemical tests of renal function in patients with liver disease[83]. Baseline renal risk factors may play a role in the nephrotoxic effects of NUCs. Data on RT patients is limited but these results can be extrapolated to this population, taking into account that these RT recipients can be considered within the high renal risk group. Therefore, it is recommended in all HBV treated patients to measure serum creatinine levels and estimated creatinine clearance, and in ADV or TDF treated patients it is also recommended to measure serum phosphate levels, especially in patients at high renal risk. In patients at low renal risk these tests can be performed every 3 mo during the first year and every 6 mo thereafter, in case of no renal adverse events. In patients at high renal risk these tests can be performed every month for the first 3 mo, every 3 mo until the end of the first year and every 6 mo thereafter, in case of no renal adverse events. Closer renal monitoring is required in patients who develop reductions in creatinine clearance < 60 mL/min or reductions in serum phosphate levels < 2 mg/dL[9,10,15,87].

CONCLUSION

Current guidelines clearly define who needs treatment, when to start, what is the first line therapy, how to monitor treatment response, when to stop, and how patients must be controlled for its safety. There is some data showing a favorable safety and efficacy profile of NUC treatment in the renal transplant setting. ETV, an agent without signs of major nephrotoxicity, appears to be the best option for NUC naïve patients and TDF is still the preferred agent in patients with resistance to LAM or any other NUC. Renal transplant recipients under antiHBV treatment should be closely monitored for its efficacy against HBV and for its safety, especially regarding its impact on renal function. Studies including a large number of patients with long term treatment and follow up are still needed to better demonstrate the safety and efficacy of newer NUCs in this population.

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REFERENCES

1 **McDonald SP**, Russ GR. Survival of recipients of cadaveric kidney transplants compared with those receiving dialysis treatment in Australia and New Zealand, 1991-2001. *Nephrol Dial Transplant* 2002; **17**: 2212-2219 [PMID: 12454235 DOI: 10.1093/ndt/17.12.2212]

2 **Pascual M**, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 2002; **346**: 580-590 [PMID: 11856798 DOI: 10.1056/NEJMra011295]

3 **Morales JM**, Marcén R, del Castillo D, Andres A, Gonzalez-Molina M, Oppenheimer F, Serón D, Gil-Vernet S, Lampreave I, Gainza FJ, Valdés F, Cabello M, Anaya F, Escuin F, Arias M, Pallardó L, Bustamante J. Risk factors for graft loss and mortality after renal transplantation according to recipient age: a prospective multicentre study. *Nephrol Dial Transplant* 2012; **27** Suppl 4: iv39-iv46 [PMID: 23258810 DOI: 10.1093/ndt/gfs544]

4 **Howard RJ**, Patton PR, Reed AI, Hemming AW, Van der Werf WJ, Pfaff WW, Srinivas TR, Scornik JC. The changing causes of graft loss and death after kidney transplantation. *Transplantation* 2002; **73**: 1923-1928 [PMID: 12131689]

5 **Shu KH**, Ho HC, Wen MC, Wu MJ, Chen CH, Cheng CH, Yu TM, Chuang YW, Huang ST, Tsai SF, Lo YC, Weng SC. Changing pattern of mortality in renal transplant recipients: a single-center, 30-year experience. *Transplant Proc* 2014; **46**: 442-444 [PMID: 24655983 DOI: 10.1016/j.transproceed.2013.11.032]

6 **Fabrizi F**, Martin P. Management of hepatitis B and C virus infection before and after renal transplantation. *Curr Opin Organ Transplant* 2006; **11**: 583-588 [DOI: 10.1097/MOT.0b013e3280105c5c]

7 **Burdick RA**, Bragg-Gresham JL, Woods JD, Hedderwick SA, Kurokawa K, Combe C, Saito A, LaBrecque J, Port FK, Young EW. Patterns of hepatitis B prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney Int* 2003; **63**: 2222-2229 [PMID: 12753311 DOI: 10.1046/j.1523-1755.2003.00017.x]

8 **Urbánek P**. Viral hepatitis infections in chronic kidney disease patients and renal transplant recipients. *Kidney Blood Press Res* 2012; **35**: 454-467 [PMID: 22677941 DOI: 10.1159/000338309]

9 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]

10 **European Association for the Study of the Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]

11 **Pipili C**, Cholongitas E, Papatheodoridis G. Review article: nucleos(t)ide analogues in patients with chronic hepatitis B virus infection and chronic kidney disease. *Aliment Pharmacol Ther* 2014; **39**: 35-46 [PMID: 24299322 DOI: 10.1111/apt.12538]

12 **Ahmad A**, Hasan F, Abdeen S, Sheikh M, Kodaj J, Nampoory MR, Johny KV, Asker H, Siddique I, Thalib L, Al-Nakib B. Transjugular liver biopsy in patients with end-stage renal disease. *J Vasc Interv Radiol* 2004; **15**: 257-260 [PMID: 15028810 DOI: 10.1097/01.RVI.0000109403.52762.C4]

13 **García Agudo R**, Aoufi Rabih S, Pérez Roldán F, Guzmán Ames

F, González Carro P, Ruiz Carrillo F, Cuesta Domínguez R. Hepatic venous pressure gradient and transjugular liver biopsy to assess patients with kidney failure and chronic liver disease. *Nefrologia* 2011; **31**: 490-492 [PMID: 21738254 DOI: 10.3265/Nefrologia.pre2011.May.10878]

14 **Kidney Disease: Improving Global Outcomes (KDIGO)**. KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. *Kidney Int Suppl* 2008; **73** (Suppl 109): S1-99 [PMID: 18382440 DOI: 10.1038/ki.2008.81]

15 **Kidney Disease: Improving Global Outcomes (KDIGO)**. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9** Suppl 3: S1-155 [PMID: 19845597 DOI: 10.1111/j.1600-6143.2009.02834.x]

16 **Alric L**, Kamar N, Bonnet D, Danjoux M, Abravanel F, Lauwers-Cances V, Rostaing L. Comparison of liver stiffness, fibrotest and liver biopsy for assessment of liver fibrosis in kidney-transplant patients with chronic viral hepatitis. *Transpl Int* 2009; **22**: 568-573 [PMID: 19196449 DOI: 10.1111/j.1432-2277.2009.00834.x]

17 **Ruhi C**, Süleymanlar I, Koçak H, Dinçkan A, Ersoy F, Süleymanlar G. Effect of Prophylactic Versus Preemptive Lamivudine Treatment and Tenofovir on HBsAg (+) Kidney Transplant Recipients. *Exp Clin Transplant* 2014 Jun 25; Epub ahead of print [PMID: 25019317 DOI: 10.6002/ect.2013.0280]

18 **Berger A**, Preiser W, Kachel HG, Stürmer M, Doerr HW. HBV reactivation after kidney transplantation. *J Clin Virol* 2005; **32**: 162-165 [PMID: 15653420 DOI: 10.1016/j.jcv.2004.10.006]

19 **Kanaan N**, Kabamba B, Maréchal C, Pirson Y, Beguin C, Goffin E, Hassoun Z. Significant rate of hepatitis B reactivation following kidney transplantation in patients with resolved infection. *J Clin Virol* 2012; **55**: 233-238 [PMID: 22921412 DOI: 10.1016/j.jcv.2012.07.015]

20 **Duhart BT**, Honaker MR, Shokouh-Amiri MH, Riely CA, Vera SR, Taylor SL, Al-jedai AH, Gaber AO. Retrospective evaluation of the risk of hepatitis B virus reactivation after transplantation. *Transpl Infect Dis* 2003; **5**: 126-131 [PMID: 14617300 DOI: 10.1034/j.1399-3062.2003.00021.x]

21 **Barclay S**, Pol S, Mutimer D, Benhamou Y, Mills PR, Hayes PC, Cameron S, Carman W. The management of chronic hepatitis B in the immunocompromised patient: recommendations from a single topic meeting. *J Clin Virol* 2008; **41**: 243-254 [PMID: 18203658 DOI: 10.1016/j.jcv.2007.11.017]

22 **Gane E**, Pilmore H. Management of chronic viral hepatitis before and after renal transplantation. *Transplantation* 2002; **74**: 427-437 [PMID: 12352899]

23 **Kasiske BL**, Zeier MG, Chapman JR, Craig JC, Ekberg H, Garvey CA, Green MD, Jha V, Josephson MA, Kiberd BA, Kreis HA, McDonald RA, Newmann JM, Obrador GT, Vincenti FG, Cheung M, Earley A, Raman G, Abariga S, Wagner M, Balk EM. KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary. *Kidney Int* 2010; **77**: 299-311 [PMID: 19847156]

24 **Durlik M**, Gaciong Z, Rowińska D, Rancewicz Z, Lewandowska D, Kozłowska B, Wyzgał J, Soluch L, Walewska-Zielecka B, Rowiński W, Lao M. Long-term results of treatment of chronic hepatitis B, C and D with interferon-alpha in renal allograft recipients. *Transpl Int* 1998; **11** Suppl 1: S135-S139 [PMID: 9664963]

25 Lamivudine (Epivir®) prescribing information. NC 27709, USA: GlaxoSmithKline Research Triangle Park, 2013

26 Adefovir (Hepsera®) prescribing information. Foster City, CA 94404, USA: Gilead Sciences, Inc., 2012

27 Telbivudine (Tyzeka®) Novartis Pharmaceuticals Corporation. New Jersey 07936, USA: East Hanover, 2013

28 Entecavir (Baraclude®) Bristol-Myers Squibb Company. NJ 08543, USA: Princeton, 2014

29 Tenofovir (Viread®) prescribing information. Foster City, CA 94404, USA: Gilead Sciences, Inc., 2013

30 **Fabrizi F**, Dulai G, Dixit V, Bunnapradist S, Martin P. Lamivudine for the treatment of hepatitis B virus-related liver disease after renal transplantation: meta-analysis of clinical trials. *Transplantation* 2004; **77**: 859-864 [PMID: 15077027]

31 **Yap DY**, Tang CS, Yung S, Choy BY, Yuen MF, Chan TM. Long-term outcome of renal transplant recipients with chronic hepatitis B infection-impact of antiviral treatments. *Transplantation* 2010; **90**: 325-330 [PMID: 20562676 DOI: 10.1097/TP.0b013e3181e5b811]

32 **Thabut D**, Thibault V, Bernard-Chabert B, Mouquet C, Di Martino V, Le Calvez S, Opolon P, Benhamou Y, Bitker MO, Poynard T. Long-term therapy with lamivudine in renal transplant recipients with chronic hepatitis B. *Eur J Gastroenterol Hepatol* 2004; **16**: 1367-1373 [PMID: 15618847]

33 **Chan TM**, Tse KC, Tang CS, Lai KN, Ho SK. Prospective study on lamivudine-resistant hepatitis B in renal allograft recipients. *Am J Transplant* 2004; **4**: 1103-1109 [PMID: 15196068 DOI: 10.1111/j.1600-6143.2004.00467.x]

34 **Fontaine H**, Vallet-Pichard A, Chaix ML, Currie G, Serpaggi J, Verkarre V, Varaut A, Morales E, Nalpas B, Brosgart C, Pol S. Efficacy and safety of adefovir dipivoxil in kidney recipients, hemodialysis patients, and patients with renal insufficiency. *Transplantation* 2005; **80**: 1086-1092 [PMID: 16278590 DOI: 10.1097/01.tp.0000178305.39231.a2]

35 **Garcia A**, Mazuecos A, González P, Diaz F, Garcia T, Ceballos M, Rivero M. Treatment with adefovir dipivoxil in a renal transplant patient with renal insufficiency and lamivudine-resistant hepatitis B infection. *Transplant Proc* 2005; **37**: 1462-1463 [PMID: 15866639 DOI: 10.1016/j.transproceed.2005.02.010]

36 **de Silva HJ**, Dassanayake AS, Manamperi A, de Silva AP. Treatment of lamivudine-resistant hepatitis B infection in post-renal transplant patients with adefovir dipivoxil: preliminary results. *Transplant Proc* 2006; **38**: 3118-3120 [PMID: 17112914 DOI: 10.1016/j.transproceed.2006.08.186]

37 **Kamar N**, Huart A, Tack I, Alric L, Izopet J, Rostaing L. Renal side effects of adefovir in hepatitis B virus-(HBV) positive kidney allograft recipients. *Clin Nephrol* 2009; **71**: 36-42 [PMID: 19203548]

38 **Lampertico P**, Viganò M, Facchetti F, Invernizzi F, Aroldi A, Lunghi G, Messa PG, Colombo M. Long-term add-on therapy with adefovir in lamivudine-resistant kidney graft recipients with chronic hepatitis B. *Nephrol Dial Transplant* 2011; **26**: 2037-2041 [PMID: 21486869 DOI: 10.1093/ndt/gfr174]

39 **Lai HW**, Chang CC, Chen TH, Tsai MC, Chen TY, Lin CC. Safety and efficacy of adefovir therapy for lamivudine-resistant hepatitis B virus infection in renal transplant recipients. *J Formos Med Assoc* 2012; **111**: 439-444 [PMID: 22939662 DOI: 10.1016/j.jfma.2011.05.010]

40 **Yap DY**, Chan TM. Evolution of hepatitis B management in kidney transplantation. *World J Gastroenterol* 2014; **20**: 468-474 [PMID: 24574715 DOI: 10.3748/wjg.v20.i2.468]

41 **Hu TH**, Tsai MC, Chien YS, Chen YT, Chen TC, Lin MT, Chang KC, Chiu KW. A novel experience of antiviral therapy for chronic hepatitis B in renal transplant recipients. *Antivir Ther* 2012; **17**: 745-753 [PMID: 22522918 DOI: 10.3851/IMP2097]

42 **Kamar N**, Milioto O, Alric L, El Kahwaji L, Cointault O, Lavayssière L, Sauné K, Izopet J, Rostaing L. Entecavir therapy for adefovir-resistant hepatitis B virus infection in kidney and liver allograft recipients. *Transplantation* 2008; **86**: 611-614 [PMID: 18724232 DOI: 10.1097/TP.0b013e3181806c8c]

43 **Ridruejo E**, Adrover R, Alonso C, Mando OG, Silva MO. Entecavir in the treatment of chronic hepatitis B in end stage renal disease and kidney transplantation. *Dial Transplant* 2010; **39**: 397-400 [DOI: 10.1002/dat.20485]

44 **Ridruejo E**, Adrover R, Mandó OG, Silva MO. Entecavir in the treatment of chronic hepatitis B in kidney transplantation. *J Hepatol* 2012; **56**: 997-998; author reply 999 [PMID: 22075262 DOI: 10.1016/j.jhep.2011.09.019]

45 **Yap DY**, Yung S, Tang CS, Seto WK, Ma MK, Mok MM, Kwan LP, Chan GC, Choy BY, Yuen MF, Chan TM. Entecavir treatment in kidney transplant recipients infected with hepatitis B. *Clin Transplant* 2014; **28**: 1010-1015 [PMID: 24974788 DOI: 10.1111/ctr.12410]

46 **Tse KC**, Yap DY, Tang CS, Yung S, Chan TM. Response to adefovir

or entecavir in renal allograft recipients with hepatitic flare due to lamivudine-resistant hepatitis B. *Clin Transplant* 2010; **24**: 207-212 [PMID: 19758269 DOI: 10.1111/j.1399-0012.2009.01090.x]
47 **Tourret J**, Deray G, Isnard-Bagnis C. Tenofovir effect on the kidneys of HIV-infected patients: a double-edged sword? *J Am Soc Nephrol* 2013; **24**: 1519-1527 [PMID: 24052632 DOI: 10.1681/ASN.2012080857]
48 **Daudé M**, Rostaing L, Sauné K, Lavayssière L, Basse G, Esposito L, Guitard J, Izopet J, Alric L, Kamar N. Tenofovir therapy in hepatitis B virus-positive solid-organ transplant recipients. *Transplantation* 2011; **91**: 916-920 [PMID: 21325995 DOI: 10.1097/TP.0b013e3182100f59]
49 **Olsen SK**, Brown RS. Hepatitis B treatment: Lessons for the nephrologist. *Kidney Int* 2006; **70**: 1897-1904 [PMID: 17021602 DOI: 10.1038/sj.ki.5001908]
50 **Pipili C**, Cholongitas E. Management of patients with hepatitis B and C before and after liver and kidney transplantation. *World J Hepatol* 2014; **6**: 315-325 [PMID: 24868325 DOI: 10.4254/wjh.v6.i5.315]
51 **Han DJ**, Kim TH, Park SK, Lee SK, Kim SB, Yang WS, Park JS, Jung JG, Yu ES, Kim SC. Results on preemptive or prophylactic treatment of lamivudine in HBsAg (+) renal allograft recipients: comparison with salvage treatment after hepatic dysfunction with HBV recurrence. *Transplantation* 2001; **71**: 387-394 [PMID: 11233898]
52 **Filik L**, Karakayali H, Moray G, Dalgiç A, Emiroğlu R, Ozdemir N, Colak T, Gür G, Yilmaz U, Haberal M. Lamivudine therapy in kidney allograft recipients who are seropositive for hepatitis B surface antigen. *Transplant Proc* 2006; **38**: 496-498 [PMID: 16549158 DOI: 10.1016/j.transproceed.2005.12.047]
53 **Chan TM**, Fang GX, Tang CS, Cheng IK, Lai KN, Ho SK. Preemptive lamivudine therapy based on HBV DNA level in HBsAg-positive kidney allograft recipients. *Hepatology* 2002; **36**: 1246-1252 [PMID: 12395336 DOI: 10.1053/jhep.2002.36156]
54 **Kim HG**, Kim EY, Yu YJ, Kim GH, Jeong JW, Byeon JH, Chung BH, Yang CW. Comparison of clinical outcomes in hepatitis B virus-positive kidney transplant recipients with or without pretransplantation antiviral therapy. *Transplant Proc* 2013; **45**: 1374-1378 [PMID: 23726576 DOI: 10.1016/j.transproceed.2013.01.088]
55 **Park KS**, Yang WS, Han DJ, Park JB, Park JS, Park SK. Long-term impact of prophylactic antiviral treatment in Hepatitis B surface antigen positive renal allograft recipients. *Clin Nephrol* 2012; **78**: 303-311 [PMID: 22541690 DOI: 10.5414/CN107617]
56 **Hoofnagle JH**. Reactivation of hepatitis B. *Hepatology* 2009; **49**: S156-S165 [PMID: 19399803 DOI: 10.1002/hep.22945]
57 **Ridruejo E**, Díaz C, Michel MD, Soler Pujol G, Martínez A, Marciano S, Mandó OG, Vilches A. Short and long term outcome of kidney transplanted patients with chronic viral hepatitis B and C. *Ann Hepatol* 2010; **9**: 271-277 [PMID: 20720267]
58 **Fabrizi F**, Martin P, Dixit V, Kanwal F, Dulai G. HBsAg seropositive status and survival after renal transplantation: meta-analysis of observational studies. *Am J Transplant* 2005; **5**: 2913-2921 [PMID: 16303005 DOI: 10.1111/j.1600-6143.2005.01113.x]
59 **Fabrizi F,** Dixit V, Martin P, Messa P. Hepatitis B and survival after renal transplant: meta-analysis of observational studies. *J Viral Hepat* 2014; **21**: 542-550 [DOI: 10.1111/jvh.12184]
60 **Cosconea S**, Fontaine H, Méritet JF, Corouge M, Sogni P, Vallet-Pichard A, Mallet V, Legendre C, Pol S. Benefits associated with antiviral treatment in kidney allograft recipients with chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 55-60 [PMID: 22414762 DOI: 10.1016/j.jhep.2012.02.020]
61 **Sperl J**, Frankova S, Spicak J, Viklicky O. Further evidence of the benefits associated with antiviral treatment in kidney allograft recipients with chronic hepatitis B virus infection. *J Hepatol* 2013; **58**: 833-835 [PMID: 23207145 DOI: 10.1016/j.jhep.2012.11.032]
62 **Park KS**, Han DJ, Park JB, Park JS, Park S. Long-term outcome of Hepatitis B-positive renal allograft recipients after development of antiviral treatment. *Clin Nephrol* 2012; **78**: 391-398 [PMID: 22541689 DOI: 10.5414/CN107565]
63 **Ridruejo E**, Mandó OG, Dávalos M, Díaz C, Vilches A. Hepatocellular carcinoma in renal transplant patients. *Transplant Proc* 2005; **37**: 2086-2088 [PMID: 15964346 DOI: 10.1016/j.transproceed.2005.03.010]
64 **Ridruejo E**, Marciano S, Galdame O, Reggiardo MV, Muñoz AE, Adrover R, Cocozzella D, Fernandez N, Estepo C, Mendizábal M, Romero GA, Levi D, Schroder T, Paz S, Fainboim H, Mandó OG, Gadano AC, Silva MO. Relapse rates in chronic hepatitis B naïve patients after discontinuation of antiviral therapy with entecavir. *J Viral Hepat* 2014; **21**: 590-596 [PMID: 24188363 DOI: 10.1111/jvh.12200]
65 **Cho JH**, Lim JH, Park GY, Kim JS, Kang YJ, Kwon O, Choi JY, Park SH, Kim YL, Kim HK, Huh S, Kim CD. Successful withdrawal of antiviral treatment in kidney transplant recipients with chronic hepatitis B viral infection. *Transpl Infect Dis* 2014; **16**: 295-303 [PMID: 24628837 DOI: 10.1111/tid.12202]
66 **Hu TH**, Tsai MC, Chen YT, Chien YS, Hung CH, Chen TC, Tseng PL, Chang KC, Yen YH. The therapeutic response of antiviral therapy in HBsAg-positive renal transplant recipients and a long-term follow-up. *Hepatol Int* 2011; **16**: [PMID: 21744310 DOI: 10.1007/s12072-011-9295-6]
67 **Fontana RJ**. Side effects of long-term oral antiviral therapy for hepatitis B. *Hepatology* 2009; **49**: S185-S195 [PMID: 19399802 DOI: 10.1002/hep.22885]
68 **Fung J**, Seto WK, Lai CL, Yuen MF. Extrahepatic effects of nucleoside and nucleotide analogues in chronic hepatitis B treatment. *J Gastroenterol Hepatol* 2014; **29**: 428-434 [PMID: 24372662 DOI: 10.1111/jgh.12499]
69 **Gane EJ**, Deray G, Liaw YF, Lim SG, Lai CL, Rasenack J, Wang Y, Papatheodoridis G, Di Bisceglie A, Buti M, Samuel D, Uddin A, Bosset S, Trylesinski A. Telbivudine improves renal function in patients with chronic hepatitis B. *Gastroenterology* 2014; **146**: 138-146.e5 [PMID: 24067879 DOI: 10.1053/j.gastro.2013.09.031]
70 **Yapali S**, Lok AS. Potential benefit of telbivudine on renal function does not outweigh its high rate of antiviral drug resistance and other adverse effects. *Gastroenterology* 2014; **146**: 15-19 [PMID: 24275236 DOI: 10.1053/j.gastro.2013.11.028]
71 **Ha NB**, Ha NB, Garcia RT, Trinh HN, Vu AA, Nguyen HA, Nguyen KK, Levitt BS, Nguyen MH. Renal dysfunction in chronic hepatitis B patients treated with adefovir dipivoxil. *Hepatology* 2009; **50**: 727-734 [PMID: 19517525 DOI: 10.1002/hep.23044]
72 **Kim YJ**, Cho HC, Sinn DH, Gwak GY, Choi MS, Koh KC, Paik SW, Yoo BC, Lee JH. Frequency and risk factors of renal impairment during long-term adefovir dipivoxil treatment in chronic hepatitis B patients. *J Gastroenterol Hepatol* 2012; **27**: 306-312 [PMID: 21777281 DOI: 10.1111/j.1440-1746.2011.06852.x]
73 **Hartono JL**, Aung MO, Dan YY, Gowans M, Lim K, Lee YM, Lee GH, Low HC, Tan PS, Thwin MA, Soon C, Chiu LL, Khoo MJ, Koay E, Lim SG. Resolution of adefovir-related nephrotoxicity by adefovir dose-reduction in patients with chronic hepatitis B. *Aliment Pharmacol Ther* 2013; **37**: 710-719 [PMID: 23432107 DOI: 10.1111/apt.12251]
74 **Manolakopoulos S**, Striki A, Deutsch M, Mela M, Ketikoglou I, Tzourmakliotis D, Manesis EK, Papatheodoridis GV. Long-term adefovir plus lamivudine therapy does not decrease creatinine clearance in HBeAg-negative chronic hepatitis B patients. *Liver Int* 2011; **31**: 1525-1532 [PMID: 22093327 DOI: 10.1111/j.1478-3231.2011.02616.x]
75 **Marcellin P,** Heathcote EJ, Berg T, Anderson J, Mondou E, Coombs D, Ebrahimi R, Reddy S, Lopatin U, McNally J, Ng C. Effects of tenofovir disoproxil fumarate on renal function in chronic HBV patients in three global randomized studies. *J Hepatol* 2011; **54** (Suppl 1): S296-297 [DOI: 10.1016/S0168-8278(11)60741-1]
76 **Fung S,** Kwan P, Horban A, Pelemis M, Husa P, Hann HW, Flaherty J, Massetto B, Dinh P, Custodio J, Subramanian GM, Fabri M, Gane E. Tenofovir DF (TDF) is safe and well tolerated in chronic hepatitis B (CHB) patients with pre-existing mild renal impairment. *J Hepatol* 2013; **58** (Suppl 1): S301-302. [DOI: 10.1016/S0168-8278(13)60746-1]
77 **Marcellin P**, Zoulim F, Causse X, Hézode C, Larrey D, Pageaux

G, Roche B, Truchi R, Ouzan D, Pauwels A, Dumortier J, Stern C, Libert O, Cadranel JF, Zarski JP, Vireal Group. Tenofovir DF treatment is safe and well tolerated in chronic hepatitis B (CHB) patients with baseline decreased glomerular filtration rate. *J Hepatol* 2012; **56** (Suppl 1): S210 [DOI: 10.1016/S0168-8278(12)60544-3]

78 **van Bommel F**, De Man RA, Ferenci P, Reijnders JG, Bronowicki JP, Wedemeyer H, Brodzinski A, Fülöp B, Deterding K, Erhardt A, Hüppe D, Bourlière M, Sarrazin C, Trojan J, Buggisch PB, Petersen J, Spengler U, Pariente A, Schuchmann M, Wasmuth H, Stein K, Rutter K, Feucht HH, Wiedenmann B, Berg T. Renal safety and antiviral efficacy of tenofovir disoproxil fumarate (TDF) monotherapy in nucleos(t)ide analogue refractory patients with hepatitis B virus (HBV) mono-infection. *J Hepatol* 2010; **52** (Suppl 1): S398 [DOI: 10.1016/S0168-8278(10)61029-X]

79 **Jorquera F**, Pascasio JM, Fraga E, Fuentes J, Prieto M, Sánchez-Antolín G, Calleja JL, Molina E, Tomé S, Bonet L, Blanco MA, García-Buey ML, Salmerón J, Pons JA, González JM, Rodríguez M. Tenofovir monotherapy versus lamivudine plus adefovir in lamivudine-failure patients rescued with lamivudine plus adefovir combination: interim analysis of the TENOSIMP-B clinical trial. *J Hepatol* 2014; **60** (Suppl 1): S422 [DOI: 10.1016/S0168-8278(14)61201-0]

80 **Brodzinski A**, Fueloep B, Schlosser B, Biermer M, Wiegand J, Berg T, van Bommel F. Prevalence of renal alterations indicative for proximal tubular damage (PTD) in patients with chronic hepatitis B virus (HBV) infection during long-term treatment with tenofovir disoproxil fumarate (TDF). *Hepatology* 2010; **52** (Suppl 1): 515-6A

81 **Lampertico P,** Soffredini R, Yurdaydin C, Idilman R, Papatheodoridis GV, Margariti E, Buti M, Esteban R, Zaltron S, Vavassori A, Castelli C, Viganò M, Rumi MG, Vinci M, Belli LS, Cologni G, Rizzi M, Milanese M, Strazzabosco M, Minola E, Giorgini AM, Zuin M, Salmi A, Colombo S, Fracassetti O, Del Poggio P, Bruno S, Fagiuoli S, Andreoletti M, Colli A, Colombo AE, Bellati GA, Magni CF, Angeli E, Gubertini GA, Fasano M, Santantonio TA, Terreni NM, Mangia G, Colombo M. Four years of tenofovir monotherapy for NUC naïve field practice European patients suppresses HBV replication in most patients with a favorable renal safety profile but does not prevent HCC in patients with or without cirrhosis. *Hepatology* 2013; **58** (Suppl 1): 653A

82 **Le ST**, Sahhar L, Lim J, He T, Ha P, Rusli F, Ratnam D, Moore GT, Pianko S, Dev A, Polkinghorne K, Sievert W. Do tenofovir and entecavir affect renal function in patients with chronic hepatitis B (CHB)? A two-year observational study from a single Australian centre. *Hepatology* 2012; **56** (Suppl 1): 410A

83 **Nguyen HL**, Al-Freah MA, Joe D, Carey I, Boyer T, Suddle A, Harrison PM, Agarwal K. A single centre, large "real-life" cohort treated with tenofovir versus entecavir: no deterioration in renal function in tenofovir cohort over 12 months of therapy. *Hepatology* 2011; **54** (Suppl 1): 604A

84 **Gish RG**, Clark MD, Kane SD, Shaw RE, Mangahas MF, Baqai S. Similar risk of renal events among patients treated with tenofovir or entecavir for chronic hepatitis B. *Clin Gastroenterol Hepatol* 2012; **10**: 941-946; quiz e68 [PMID: 22507876 DOI: 10.1016/j.cgh.2012.04.008]

85 **Gish R**, Mangahas M, Baqai S, Shaw R. Risk of renal toxicity with tenofovir DF (TDF) for chronic hepatitis B (CHB). *J Hepatol* 2010; **52** (Suppl 1): S388-389 [DOI: 10.1016/S0168-8278(10)61008-2]

86 **Mauss S**, Berger F, Filmann N, Hueppe D, Henke J, Hegener P, Athmann C, Schmutz G, Herrmann E. Effect of HBV polymerase inhibitors on renal function in patients with chronic hepatitis B. *J Hepatol* 2011; **55**: 1235-1240 [PMID: 21703180 DOI: 10.1016/j.jhep.2011.03.030]

87 **Ridruejo E**, Silva MO. Safety of long-term nucleos(t)ide treatment in chronic hepatitis B. *Expert Opin Drug Saf* 2012; **11**: 357-360 [PMID: 22417072 DOI: 10.1517/14740338.2012.672972]

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REVIEW

Recent advances in dietary supplementation, in treating non-alcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is currently known as the most common liver problem, characterized by excessive lipid accumulation in hepatocytes, which may progress to other liver diseases such as nonalcoholic steatohepatitis, hepatic tissue fibrosis, liver cirrhosis, and failure or hepatocellular carcinoma. Since NAFLD is positively associated with the development of obesity, insulin resistance, and ultimately type 2 diabetes mellitus, it is often regarded as the hepatic manifestation of the metabolic syndrome. No pharmacologic treatment has yet been proven for this disease. For most patients with presumed or confirmed NAFLD, the only proven strategy is to offer lifestyle advice that can lead to sustained weight loss. Since insulin resistance, oxidative stress, inflammation, and necro-apoptosis are involved in NAFLD pathogenesis, it seems that every potential therapeutic agent should target one or some of these pathologic events. There are many well known anti-oxidants, anti-inflammatory, and insulin sensitizer dietary supplements which have shown beneficial effects on NAFLD improvement in animal and human studies. The purpose of this review is to explore the existing evidences on dietary supplements considered to have hepatoprotective properties, and to present some proposed mechanisms by which they may protect against NAFLD.

Key words: Nonalcoholic fatty liver disease; Dietary supplementation; Treatment

Core tip: This review explores the existing evidences on dietary supplements considered to have anti-oxidant, anti-inflammatory, and/or insulin sensitizer properties, and their role in management of nonalcoholic fatty liver disease while addressing some of their proposed mechanism of action.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a range of conditions caused by fatty infiltration of the hepatocytes without significant amounts of alcohol use, that can be originated from multiple factors[1]. NAFLD begins with simple hepatocyte steatosis, and progresses to nonalcoholic steatohepatitis (NASH), fibrosis of the hepatocytes, and liver cirrhosis, which can further progress to hepatocellular carcinoma (HCC)[2]. Patients with NAFLD are usually asymptomatic and are diagnosed accidentally through routine checkup exams. Currently liver biopsies are considered gold standard for the diagnosis and staging of NASH, since there are no specific symptoms to differentiate between this disease and other liver disorders. Magnetic resonance spectroscopy (H^1-MRS) and Fibroscan are noninvasive modalities for diagnosis and staging, assessing a larger section of the liver in comparison to liver biopsy[3,4]. Other clinical diagnostic indices such as increased serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as evidence of liver steatosis in ultrasonography are also routinely used[5]. Developing effective therapies with minimal side-effects against NAFLD is critical for controlling the progression of this disease to end-stage liver disorders[6].

EPIDEMIOLOGY

NAFLD is the most common diagnosis in subjects with altered aminotransferases in the Western world[7], where one third of the population is affected[8]. In Asia, recent reports showed a similar prevalence of NAFLD[9,10]. About 20%-25% of adults with NASH have been reported to develop liver cirrhosis[11]. About 30% to 40% of patients who develop cirrhosis secondary to NAFLD, will die of liver-related problems[12]. The prevalence of NAFLD is different among men and women, and it increases with age, occurring in less than 20% of individuals younger than 20 years of age, and in more than 40% of those over the age of 60[13]. NAFLD has also been identified in the pediatric population, prevailing at 2.6%, although it is estimated that its prevalence will increase to 22.5%-58.5% in obese children[14]. Parallel to the rising prevalence of conditions such as obesity and type 2 diabetes mellitus (T2DM), the rate and prevalence of NAFLD is also increasing[15]. NAFLD affects 40%-75% of patients with T2DM, 33%-76% of obese and 90% of morbidly obese people[16].

PATHOGENESIS

The pathogenesis of NAFLD is complicated and while its exact mechanism remains largely unknown, different genetic factors and/or environmental elements seem to influence it[12,17]. The "two-hit hypothesis" of NASH, originally explained by Day and James suggests that lipid deposition in the liver (first hit) is followed by a series of other, oxidative and hepatotoxic processes (second hit), caused by a mechanism currently not known[18]. Several factors such as genetics, epigenetic mechanisms, as well as environmental elements, appear to promote hepatocyte fat deposition and insulin resistance, both of which further lead to the secondary pathologic events[19], such as oxidative stress, lipid peroxidation, increased inflammatory responses, hepatic fibrosis and apoptosis[20]. Other triggers such as lipotoxicity, endotoxemia, and adipocytokines or other inflammatory signals released from fat-infiltrated hepatocytes and adipose tissue, may promote oxidative stress in the liver, inducing the progression of NAFLD to NASH[21,22].

MANAGEMENT OF NAFLD

Currently, the only proven strategy for NAFLD management is lifestyle modification techniques such as weight loss through diet and exercise. Since obesity strongly influences the development of NAFLD, weight loss is again the main objective in NAFLD management, and the first-line therapy. All NAFLD patients are encouraged to follow a low caloric diet, increase their physical activity, and stop smoking (if applicable)[11,23,24]. Moreover, a wide range of drugs and supplements, including antioxidants, anti-inflammations, insulin sensitizers, and lipid lowering agents, have been evaluated in patients and experimental models of NAFLD, however none of them have shown long term efficacy[25,26].

In the recent years, however, the beneficial effects of dietary supplements on NAFLD progression have received increasing attention since these substances have several advantages such as being widely available, while having low or minimal side effects[27]. In the present review, we mainly focus on the recent advances of dietary supplements in NAFLD amelioration.

RECENT FINDINGS FROM DIETARY SUPPLEMENTATION IN THE TREATMENT OF NAFLD

Antioxidants agents

Vitamin E and vitamin C: Since oxidative stress is one of the factors involved in the pathogenesis of NAFLD, it was thought that antioxidant agents could be beneficial in its treatment. Thus, many clinical trials have evaluated the effects of vitamin E and/or vitamin C, as the main dietary sources of antioxidants to treat NAFLD. Nobili *et al*[28] have shown that vitamin E supplementation does not provide a greater benefit for NAFLD treatment, than diet and physical exercise[28]. Akcam *et al*[29] have reported that

metformin is more efficacious in reducing metabolic parameters such as insulin resistance, fasting insulin and lipid levels, than dietary advice and vitamin E use in obese patients with NAFLD. A clinical trial using atorvastatin and vitamins E + C *vs* placebo, showed improved hepatic steatosis on computed tomography scans. It was not however detected whether this improvement was due to the combination treatment or a single compound alone[30]. The TONIC randomized controlled trial showed that neither vitamin E nor metformin are superior to placebo in sustaining a reduction in ALT levels of pediatric NAFLD patients[31]. A recent review article concluded that vitamin E is only recommended in adults with NASH who do not have diabetes or cirrhosis, or an aggressive histology[32]. In a meta-analysis, adjuvant vitamin E was not shown to have a significant effect on normalizing serum ALT levels. Using higher doses of vitamin E, a longer duration of therapy or adding vitamin C did not alter the effect of these antioxidants on the measured outcomes either[33]. There seems to be lacking evidence on the long-term effects of vitamin E use on histological improvements of NAFLD patients, which calls for larger, well-designed randomized controlled trials (RCTs) with histological endpoints, to really determine the efficacy of its use.

Resveratrol: Resveratrol (3,5,4-trihydroxystilbene) is a natural phenol produced by certain plants and found in the skin of red grapes. Resveratrol has been widely accepted as a chemopreventive agent that exerts other positive health effects as well because of its ability to take part in many biological activities. Resveratrol is thought to have antioxidative, anti-inflammatory, anti-cancer, anti-obesity, anti-diabetic, and anti-aging properties. Its positive effects on animal NAFLD models have been shown in several studies. In different studies, Resveratrol decreased NAFLD severity in animal models in the following ways: through TNF-alpha inhibition and antioxidant activities[34], through the activation of AMPK[35,36], by induction of skeletal muscle SIRT1 and SIRT4 expression[37], by increasing the number of mitochondria, and specially, by increasing hepatic uncoupling protein 2 expression[38], decreasing hepatic LDL receptor and SR-BI mRNA and protein expressions[39], and the reduction of nuclear factor-kappaB (NF-kappaB) activity[40].

Clinical trials evaluating the effects of Resveratrol supplementation on NAFLD characteristics are scarce. A recent study, administering Resveratrol *vs* placebo for eight weeks, not only failed to show any significantly improvements in any NAFLD features in the Resveratrol group, it also showed an increase in hepatic stress, based on increased liver enzyme levels[41]. A different trial however, did find a significant improvement in NAFLD characteristics after 12 wk of supplementation with 500 mg Resveratrol[42]. It appears that the dose and duration of Resveratrol administration is important in its efficacy. Future clinical studies with different dosages and durations are needed to clarify the true impact of Resveratrol treatment in NAFLD/NASH patients[43].

Anthocyanin: Anthocyanins (ACNs) are water-soluble bioactive compounds of the polyphenol class that are present in many plant based products. It has been reported that ACNs decrease hepatic lipid accumulation and may counteract oxidative stress and hepatic inflammation in animal studies, but their benefits in patients with NAFLD has not yet been well elucidated[44]. There is only one study evaluating the effects of ACN on NAFLD patients; Suda *et al*[45] have reported that supplementation with 400 mg of acylated ACNs could reduce levels of liver enzymes, in particular gamma-glutamyl transferases in patients with NAFLD. This clinical trial had many limitations; liver damage was not directly assessed, fatty liver was not confirmed by direct imaging, and the effect of acylated ACNs was not compared to that of a control food or to the lack of intervention[45]. More research studies are therefore required to evaluate the effects of ACNs supplementation on NAFLD features.

Green tea extract: It has been shown that the main important green tea polyphenol, epigallocatechin-3-gallate (EGCG), has a positive therapeutic effect on obesity, features of metabolic syndrome, and liver steatosis in mice[46]. In experimental models of NAFLD, EGCG supplementation significantly decreased weight gain, total and visceral body fat, insulin resistance, liver steatosis, serum cholesterol, and monocyte chemoattractant protein concentrations[46].

Both *in vitro* and *in vivo* experiments have revealed that green tea and EGCG could prevent steatosis by reducing dietary absorption of lipids and carbohydrates, and by the inhibition of adipose tissue breakdown, and *de novo* lipogenesis in both hepatic and adipose tissues, through the stimulation of β-oxidation and thermogenesis in the liver, and by improving insulin sensitivity. Furthermore, EGCG may inhibit the development of steatohepatitis from fatty liver disease, through its antioxidant and anti-inflammatory characteristics[47]. Currently, there are no randomized, controlled trials in humans, evaluating the effects of green tea on NAFLD. These studies are needed to provide enough evidence that green tea can effectively prevent the development and/or progression of NAFLD[48].

Coffee: Both epidemiological and animal studies have shown that drinking coffee on a regular basis can decrease the risk of T2DM development[49-51]. A recent case-control study comparing coffee *vs* non-coffee drinkers showed that fatty liver occurred less frequently in coffee drinkers, and that

drinking coffee was inversely associated with the degree of liver brightness, as well as obesity and insulin resistance[52]. Among NASH patients, coffee consumption has been shown to be significantly associated with a reduced risk of fibrosis[53].

More research is needed to determine the protective properties of caffeine against NAFLD. Coffee contains certain phytochemicals with potential antioxidant properties, which may be protective against cardiovascular and liver diseases, and malignancies. The anti-oxidative, anti-inflammatory, and anti-fibrotic properties of coffee might explain its hepatoprotective effects in NAFLD[54,55].

Garlic: Garlic-derived S-allylmercaptocysteine (SAMC) has a therapeutic role in diabetes and nonalcoholic fatty liver disease due to its properties in the regulation of lipogenesis and glucose metabolism[56]. Results of two studies show that SAMC decreases the liver injury caused by NAFLD, while decreasing fat build-up, and collagen formation. This may occur because SAMC takes part in different activities at the molecular level that affect NAFLD, by for example decreasing lipogenesis and restoring lipolysis markers. The expression of pro-fibrogenic factors is also reduced by SAMC, as well as oxidative stress in the liver, by means of cytochrome P450 2E1-dependent pathway inhibition. SAMC may partially prevent NAFLD-induced inflammation as well, by reducing pro-inflammatory mediators, chemokines and suppressor of cytokine signaling. The protective effects of SAMC are also partly shown through its ability to restore the altered phosphorylation status of FFAs-dependent MAP kinase pathways, and to diminish the activity of nuclear transcription factors such as NF-kappaB and AP-1, while reducing apoptosis and enhancing autophagy during NAFLD development[57,58].

In addition, garlic essential oil (GEO) and its major organosulfur component diallyl disulfide (DADS), also have therapeutic effects on the development of NAFLD. They exert anti-obesity and anti-hyperlipidemic effects by reducing weight gain, adipose tissue weight, and serum lipid parameters. They significantly decrease the release of pro-inflammatory cytokines in the serum, while at the same time elevating in the hepatic antioxidant capacity by inhibiting cytochrome P450 2E1 expression during NAFLD development. The anti-NAFLD effects of GEO and DADS are mediated through the down-regulation of sterol regulatory element binding protein-1c, acetyl-CoA carboxylase, fatty acid synthase, and 3-hydroxy-3-methylglutaryl-coenzyme[59]. Clinical trials are needed to confirm these experimental studies.

Ginger: Several mechanisms have been proposed by which ginger may prevent NAFLD or slow its progression to other liver diseases, such as increasing insulin sensitivity, inducing the activation of peroxisome proliferator-activated receptor gamma, which in turn induces adiponectin and down-regulates pro-inflammatory cytokines, changing the balance between adiponectin and tumor necrosis factor-alpha in favor of adiponectin, promoting considerable antioxidant effects and antidyslipidemic properties, and reducing hepatic triglyceride content which can prevent steatosis. These mechanisms indicate that ginger possesses interesting potentials for serving as a natural supplement for the prevention and treatment of NAFLD[60]. It might suppress fructose-stimulated overexpression of carbohydrate response element-binding protein (ChREBP) at the mRNA and protein levels in hepatocytes, which results in down regulation of the ChREBP-targeted lipogenic genes responsible for fatty acid biosynthesis, while expression of neither peroxisome proliferator-activated receptor- (PPAR-) alpha and its downstream genes, nor PPAR-gamma and sterol regulatory element-binding protein 1c is altered[61]. Randomized clinical trials are needed to confirm these effects in patients with NAFLD.

Anti-inflammatory agents

Polyunsaturated fatty acids and monounsaturated fatty acids supplementation: Polyunsaturated fatty acids (PUFAs), especially n-3 PUFAs, are used to promote weight loss, and to reduce hepatic triglyceride accumulation, while improving insulin sensitivity and reducing steatosis, and hepatic damage in patients with NAFLD[62-64]. They are also thought to exert anti-inflammatory effects[65]. N-3 fatty acids affect lipid metabolism by mediating genomic pathways and regulating the transcription of genes involved in lipid metabolism[66]. They improve insulin sensitivity by decreasing hepatic TNFα expression, repress fatty acid synthesis by negatively controlling sterol regulatory element binding protein-1c (SREBP-1c), and enhance fatty acid oxidation by positively controlling peroxisome proliferator-activated receptor-α (PPARα)[67,68]. Several studies support the protective effects of n-3 PUFAs in NAFLD. Among these, Capanni *et al*[69] investigated the effects of n-3 PUFA supplementation (1 g/d for 12 mo) in 56 NAFLD patients. Their results indicated that n-3 PUFAs improved NAFLD characteristics such as ALT, AST, GGT, triglyceride and fasting glucose concentrations. Another clinical trial conducted in 23 patients with NASH found the same results[70]. A recent systematic review reported a beneficial effect of omega-3 supplementation on hepatic fat content and AST, although the effect size was relatively small[71]. The optimal dose and duration of this therapy need to be addressed in larger clinical trials in the future. Data on omega-6 fatty acids are very limited and mainly restricted to animal models.

Moreover, dietary monounsaturated fatty

acids (MUFAs) may prevent the development of NAFLD by reducing the oxidization of low-density lipoprotein (LDL), serum concentrations of LDL and total cholesterol (TC) and triacylglycerols, while decreasing body fat accumulation and postprandial adiponectin expression. It is shown that the replacing dietary carbohydrate and saturated fat consumption with MUFAs, reduces the blood pressure and glucose concentrations, and increases serum high-density lipoprotein (HDL) levels[72]. The probable mechanisms for the beneficial effects of MUFA on liver fat content may be related to their roles in the regulation of insulin sensitizing gene expression[7], and in the reduction of inflammation[73], as well as to their inhibitory effects on nuclear factor-κB (NF-κB)[74]. In a study, MUFA decreased the expression of hepatic lipogenesis and gluconeogenesis genes and SREBP in fatty rats[75]. Further investigations are warranted to ascertain the role of MUFA on NAFLD.

Vitamin D: Evidence supporting the immunoregulatory roles of vitamin D continues to increase. Recent studies have indicated that deficiencies in vitamin D can result in insulin resistance, metabolic syndrome, and NAFLD[76]. In one study, rats who were fed a western diet along with vitamin D depletion had significantly more steatosis, lobular inflammation, and NAFLD activity scores in comparison to animals with sufficient vitamin D intakes[77]. In humans, vitamin D deficiency has been correlated with a more severe NAFLD activity score and hepatic fibrosis[78], perhaps owing to the greater oxidative stress resulting from vitamin D deficiency[79]. Hepatic expression of vitamin D receptors, CYP2R1 and CYP 27A1, negatively correlates with the severity of steatosis, inflammation, and NAFLD scores in patients with this disease[80]. A recent study found a significant association between NAFLD and low serum vitamin D levels[81]; this relationship remained significant even after adjustments were made for the presence of other metabolic syndrome features. Evidence from liver biopsies have shown that serum vitamin D levels are significantly related with the stage of hepatic fibrosis[82]. Clinical trials have not yet been published to evaluate the effect of vitamin D supplementation on NAFLD characteristics.

Probiotics, prebiotics and symbiotic: It is known that the liver is susceptible to the exposure of intestine-derived bacterial products because of a close anatomic and functional connection between the intestinal lumen and the liver through the portal system[83,84]. The gut-liver axis is an important pathway in NAFLD development, which is associated with small intestinal bacterial overgrowth and increased intestinal permeability[85,86]. The contribution of microflora in NAFLD progression is mainly based on increased oxidative stress in the liver, which is caused by the increased ethanol and lipopolysaccharide production in the intestine, further causing the release of inflammatory cytokines[87,88].

Probiotics are live microorganisms that are beneficial to human health when ingested[88]. The therapeutic effects of probiotics have been demonstrated in several animal models of NAFLD[86,89-91]; however clinical trials are scarce[92-95]. In a recent double blind, placebo controlled, clinical trial, we found that 28 wk of synbiotic supplementation can significantly decrease liver enzymes, inflammatory cytokines, NF-kB activity, and fibrosis scores so that this supplementation in addition to lifestyle modification was significantly superior to lifestyle modification alone; whether these effects will sustain with longer treatment durations remains to be determined[89].

Insulin sensitizers and lipid lowering agents

Cinnamon: Cinnamon might play a potential role in the reduction of post-prandial intestinal glucose absorption through the inhibition of pancreatic enzymes such as α-amylase and α-glucosidase, and by stimulating cellular glucose uptake by membrane translocation of glucose transporter-4, which stimulates insulin release, glucose metabolism, glycogen synthesis, and inhibits gluconeogenesis. These actions may ameliorate fasting blood glucose, LDL, and hemoglobin A1c, and might increase HDL cholesterol and insulin concentrations[96].

Since one of the most important therapeutic strategies for NAFLD is modulating insulin resistance and oxidative stress, we thought that cinnamon could have beneficial effects on NAFLD features too. Thus, we investigated this hypothesis in a double-blind, placebo-controlled trial, and found that 12 wk of cinnamon supplementation significantly decreases HOMA (Homeostatic Model Assessment) index, FBS (fasting blood glucose), total cholesterol, triglyceride, liver enzymes, and high-sensitivity C-reactive protein in patients with NAFLD, however we did not find any significant changes in serum HDL levels[97]. Further clinical trials of longer durations are recommended to elucidate the exact effects of cinnamon on NAFLD characteristics.

Curcumin: It has shown that curcumin can reduce serum lipid levels, and liver steatosis. Furthermore, it may prevent fatty liver progression to steatohepatitis due to its potent antioxidant and anti-inflammatory activities[98,99]. Curcumin can reduce the expression of lipogenic genes in the liver and inflammatory responses of adipose tissue[100], while enhancing the antioxidant defense system, attenuating mitochondrial dysfunction and inhibiting apoptosis[101,102]. We did not find any clinical trial evaluating the effect of curcumin in patients with NAFLD.

Quercetine: Quercetin, a plant-derived bioflavonoid, has been reported to provide an improved health

status to its consumers, particularly with regard to obesity and diabetes[103]. Studies have demonstrated that quercetin can modestly reduce weight and regulate the expression of genes related to *in vitro* adipogenesis[103,104]. Quercetin reduces inflammatory cytokine levels and improves lipid peroxidation and insulin resistance in animal models of NAFLD, and its beneficial effects are dose dependent[103,105]. There is no clinical trial evaluating its effects on patients with NAFLD.

Carnitin: Carnitine is an essential component of mitochondrial beta oxidation. It takes part in the transportation of long-chain fatty acids into the mitochondria. Abnormalities in the mitochondria have been found to play an important role in NAFLD and NASH development. There are two published clinical trials evaluating the effects of carnitin supplementation on NAFLD characteristics. Lim *et al*[106] showed that 3 mo of carnitine supplementation improved NAFLD features by improving serum liver function tests and mitochondrial DNA copies[106]. Malaguarnera *et al*[107] showed that the addition of an L-carnitine supplement to an individual's diet for 24 wk, reduced TNF-α and CRP, and improved liver function, plasma glucose levels, lipid profile, HOMA-IR, and histological manifestations of NASH[107]; how long these effects will sustain was not evaluated.

CONCLUSION

Since there is no proven pharmacologic treatment for NAFLD, it is critically important to find dietary approaches to the prevention, attenuation, or reversal of hepatic steatosis, and its progression to steatohepatitis. As insulin resistance, oxidative stress, and inflammation are involved in pathogenesis of NAFLD, it seems that dietary supplements that can modulate these pathologies could be useful in the treatment of NAFLD. These supplements have shown beneficial effects in animal models of NAFLD, however clinical trials are scarce. Further clinical trials are needed to support the use of supplements, either as preventative or therapeutic agents that effectively prevent the development and/or worsening of liver steatosis in patients with NAFLD.

REFERENCES

1 **McCullough AJ**. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; **8**: 521-533, viii [PMID: 15331061 DOI: 10.1016/j.cld.2004.04.004]

2 **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. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]

3 **Malekzadeh R**, Poustchi H. Fibroscan for assessing liver fibrosis: An acceptable alternative for liver biopsy: Fibroscan: an acceptable alternative for liver biopsy. *Hepat Mon* 2011; **11**: 157-158 [PMID: 22087136]

4 **Fierbinteanu-Braticevici C**, Dina I, Petrisor A, Tribus L, Negreanu L, Carstoiu C. Noninvasive investigations for non alcoholic fatty liver disease and liver fibrosis. *World J Gastroenterol* 2010; **16**: 4784-4791 [PMID: 20939106 DOI: 10.3748/wjg.v16.i38.4784]

5 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]

6 **Stickel F**, Hellerbrand C. Non-alcoholic fatty liver disease as a risk factor for hepatocellular carcinoma: mechanisms and implications. *Gut* 2010; **59**: 1303-1307 [PMID: 20650925 DOI: 10.1136/gut.2009.199661]

7 **Clark SJ**, Shojaee-Moradie F, Croos P, Seed PT, Umpleby AM, Wendon JA, Miell J. Temporal changes in insulin sensitivity following the development of acute liver failure secondary to acetaminophen. *Hepatology* 2001; **34**: 109-115 [PMID: 11431740 DOI: 10.1053/jhep.2001.25514]

8 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

9 **Eguchi Y**, Hyogo H, Ono M, Mizuta T, Ono N, Fujimoto K, Chayama K, Saibara T. Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol* 2012; **47**: 586-595 [PMID: 22328022 DOI: 10.1007/s00535-012-0533-z]

10 **Farrell GC**, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 307-318 [PMID: 23458891 DOI: 10.1038/nrgastro.2013.34]

11 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470 DOI: 10.1016/j.jhep.2010.04.008]

12 **McCullough AJ**. Pathophysiology of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2006; **40** Suppl 1: S17-S29 [PMID: 16540762]

13 **Dowman JK**, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2011; **33**: 525-540 [PMID: 21198708 DOI: 10.1111/j.1365-2036.2010.04556.x]

14 **Tarantino G**, Saldalamacchia G, Conca P, Arena A. Non-alcoholic fatty liver disease: further expression of the metabolic syndrome. *J Gastroenterol Hepatol* 2007; **22**: 293-303 [PMID: 17295757 DOI: 10.1111/j.1440-1746.2007.04824.x]

15 **Gastaldelli A**, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, Buzzigoli E, Sironi AM, Cersosimo E, Ferrannini E, Defronzo RA. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007; **133**: 496-506 [PMID: 17681171 DOI: 10.1053/j.gastro.2007.04.068]

16 **Lazo M**, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 2008; **28**: 339-350 [PMID: 18956290]

17 **Cohen JC**, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011; **332**: 1519-1523 [PMID: 21700865 DOI: 10.1126/science.1204265]

18 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102 DOI: 10.1016/S0016-5085(98)70599-2]

19 **Day CP**. Genetic and environmental susceptibility to non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 255-260 [PMID: 20460920 DOI: 10.1159/000282098]

20 **Polyzos SA**, Kountouras J, Zavos Ch. The multi-hit process and the antagonistic roles of tumor necrosis factor-alpha and adiponectin in non alcoholic fatty liver disease. *Hippokratia* 2009; **13**: 127; author reply 128 [PMID: 19561788]

21 **Polyzos SA**, Kountouras J, Zavos C, Deretzi G. Nonalcoholic fatty liver disease: multimodal treatment options for a pathogenetically

multiple-hit disease. *J Clin Gastroenterol* 2012; **46**: 272-284 [PMID: 22395062 DOI: 10.1097/MCG.0b013e31824587e0]
22 **Giby VG**, Ajith TA. Role of adipokines and peroxisome proliferator-activated receptors in nonalcoholic fatty liver disease. *World J Hepatol* 2014; **6**: 570-579 [PMID: 25232450 DOI: 10.4254/wjh.v6.i8.570]
23 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]
24 **Nascimbeni F**, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, Lonardo A. From NAFLD in clinical practice to answers from guidelines. *J Hepatol* 2013; **59**: 859-871 [PMID: 23751754 DOI: 10.1016/j.jhep.2013.05.044]
25 **Gossard AA**, Lindor KD. Current therapies for nonalcoholic fatty liver disease. *Drugs Today* (Barc) 2011; **47**: 915-922 [PMID: 22348916 DOI: 10.1358/dot.2011.47.12.1688530]
26 **Keating SE**, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012; **57**: 157-166 [PMID: 22414768 DOI: 10.1016/j.jhep.2012.02.023]
27 **Firenzuoli F**, Gori L. Herbal medicine today: clinical and research issues. *Evid Based Complement Alternat Med* 2007; **4**: 37-40 [PMID: 18227931 DOI: 10.1093/ecam/nem096]
28 **Nobili V**, Manco M, Devito R, Ciampalini P, Piemonte F, Marcellini M. Effect of vitamin E on aminotransferase levels and insulin resistance in children with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2006; **24**: 1553-1561 [PMID: 17206944 DOI: 10.1111/j.1365-2036.2006.03161.x]
29 **Akcam M**, Boyaci A, Pirgon O, Kaya S, Uysal S, Dundar BN. Therapeutic effect of metformin and vitamin E versus prescriptive diet in obese adolescents with fatty liver. *Int J Vitam Nutr Res* 2011; **81**: 398-406 [PMID: 22673924 DOI: 10.1024/0300-9831/a000086]
30 **Arendt BM**, Allard JP. Effect of atorvastatin, vitamin E and C on nonalcoholic fatty liver disease: is the combination required? *Am J Gastroenterol* 2011; **106**: 78-80 [PMID: 21212755 DOI: 10.1038/ajg.2010.310]
31 **Lavine JE**, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, Abrams SH, Scheimann AO, Sanyal AJ, Chalasani N, Tonascia J, Ünalp A, Clark JM, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA* 2011; **305**: 1659-1668 [PMID: 21521847 DOI: 10.1001/jama.2011.520]
32 **Pacana T**, Sanyal AJ. Vitamin E and nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care* 2012; **15**: 641-648 [PMID: 23075940 DOI: 10.1097/MCO.0b013e328357f747]
33 **Sarkhy AA**, Al-Hussaini AA, Nobili V. Does vitamin E improve the outcomes of pediatric nonalcoholic fatty liver disease? A systematic review and meta-analysis. *Saudi J Gastroenterol* 2014; **20**: 143-153 [PMID: 24976277 DOI: 10.4103/1319-3767.132983]
34 **Bujanda L**, Hijona E, Larzabal M, Beraza M, Aldazabal P, García-Urkia N, Sarasqueta C, Cosme A, Irastorza B, González A, Arenas JI. Resveratrol inhibits nonalcoholic fatty liver disease in rats. *BMC Gastroenterol* 2008; **8**: 40 [PMID: 18782455 DOI: 10.1186/1471-230X-8-40]
35 **Shang J**, Chen LL, Xiao FX, Sun H, Ding HC, Xiao H. Resveratrol improves non-alcoholic fatty liver disease by activating AMP-activated protein kinase. *Acta Pharmacol Sin* 2008; **29**: 698-706 [PMID: 18501116 DOI: 10.1111/j.1745-7254.2008.00807.x]
36 **Choi YJ**, Suh HR, Yoon Y, Lee KJ, Kim DG, Kim S, Lee BH. Protective effect of resveratrol derivatives on high-fat diet induced fatty liver by activating AMP-activated protein kinase. *Arch Pharm Res* 2014; **37**: 1169-1176 [PMID: 24633463 DOI: 10.1007/s12272-014-0347-z]
37 **Tauriainen E**, Luostarinen M, Martonen E, Finckenberg P, Kovalainen M, Huotari A, Herzig KH, Lecklin A, Mervaala E. Distinct effects of calorie restriction and resveratrol on diet-induced obesity and Fatty liver formation. *J Nutr Metab* 2011; **2011**: 525094 [PMID: 21977315 DOI: 10.1155/2011/525094]
38 **Poulsen MM**, Larsen JØ, Hamilton-Dutoit S, Clasen BF, Jessen N, Paulsen SK, Kjær TN, Richelsen B, Pedersen SB. Resveratrol up-regulates hepatic uncoupling protein 2 and prevents development of nonalcoholic fatty liver disease in rats fed a high-fat diet. *Nutr Res* 2012; **32**: 701-708 [PMID: 23084643 DOI: 10.1016/j.nutres.2012.08.004]
39 **Xin P**, Han H, Gao D, Cui W, Yang X, Ying C, Sun X, Hao L. Alleviative effects of resveratrol on nonalcoholic fatty liver disease are associated with up regulation of hepatic low density lipoprotein receptor and scavenger receptor class B type I gene expressions in rats. *Food Chem Toxicol* 2013; **52**: 12-18 [PMID: 23127599 DOI: 10.1016/j.fct.2012.10.026]
40 **Li L**, Hai J, Li Z, Zhang Y, Peng H, Li K, Weng X. Resveratrol modulates autophagy and NF-κB activity in a murine model for treating non-alcoholic fatty liver disease. *Food Chem Toxicol* 2014; **63**: 166-173 [PMID: 23978414 DOI: 10.1016/j.fct.2013.08.036]
41 **Chachay VS**, Macdonald GA, Martin JH, Whitehead JP, O'Moore-Sullivan TM, Lee P, Franklin M, Klein K, Taylor PJ, Ferguson M, Coombes JS, Thomas GP, Cowin GJ, Kirkpatrick CM, Prins JB, Hickman IJ. Resveratrol Does Not Benefit Patients With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2014; **12**: 2092-2103.e1-e6 [PMID: 24582567 DOI: 10.1016/j.cgh.2014.02.024]
42 **Faghihzadeh F,** Adibi P, Rafiei R, Hekmatdoost A. Resveratrol supplementation improves inflammatory biomarkers in patients with nonalcoholic fatty liver disease. *Nutr Res* 2014; In press [PMID: 25311610 DOI: 10.1016/j.nutres.2014.09.005]
43 **Heebøll S**, Thomsen KL, Pedersen SB, Vilstrup H, George J, Grønbæk H. Effects of resveratrol in experimental and clinical non-alcoholic fatty liver disease. *World J Hepatol* 2014; **6**: 188-198 [PMID: 24799987 DOI: 10.4254/wjh.v6.i4.188]
44 **Valenti L**, Riso P, Mazzocchi A, Porrini M, Fargion S, Agostoni C. Dietary anthocyanins as nutritional therapy for nonalcoholic fatty liver disease. *Oxid Med Cell Longev* 2013; **2013**: 145421 [PMID: 24282628 DOI: 10.1155/2013/145421]
45 **Suda I**, Ishikawa F, Hatakeyama M, Miyawaki M, Kudo T, Hirano K, Ito A, Yamakawa O, Horiuchi S. Intake of purple sweet potato beverage affects on serum hepatic biomarker levels of healthy adult men with borderline hepatitis. *Eur J Clin Nutr* 2008; **62**: 60-67 [PMID: 17299464 DOI: 10.1038/sj.ejcn.1602674]
46 **Bose M**, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS. The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. *J Nutr* 2008; **138**: 1677-1683 [PMID: 18716169]
47 **Xiao J**, Ho CT, Liong EC, Nanji AA, Leung TM, Lau TY, Fung ML, Tipoe GL. Epigallocatechin gallate attenuates fibrosis, oxidative stress, and inflammation in non-alcoholic fatty liver disease rat model through TGF/SMAD, PI3 K/Akt/FoxO1, and NF-kappa B pathways. *Eur J Nutr* 2014; **53**: 187-199 [PMID: 23515587 DOI: 10.1007/s00394-013-0516-8]
48 **Masterjohn C**, Bruno RS. Therapeutic potential of green tea in nonalcoholic fatty liver disease. *Nutr Rev* 2012; **70**: 41-56 [PMID: 22221215 DOI: 10.1111/j.1753-4887.2011.00440.x]
49 **Birerdinc A**, Stepanova M, Pawloski L, Younossi ZM. Caffeine is protective in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2012; **35**: 76-82 [PMID: 22059453 DOI: 10.1111/j.1365-2036.2011.04916.x]
50 **Yamauchi R**, Kobayashi M, Matsuda Y, Ojika M, Shigeoka S, Yamamoto Y, Tou Y, Inoue T, Katagiri T, Murai A, Horio F. Coffee and caffeine ameliorate hyperglycemia, fatty liver, and inflammatory adipocytokine expression in spontaneously diabetic KK-Ay mice. *J Agric Food Chem* 2010; **58**: 5597-5603 [PMID: 20405946 DOI: 10.1021/jf904062c]
51 **Yesil A**, Yilmaz Y. Review article: coffee consumption, the metabolic syndrome and non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2013; **38**: 1038-1044 [PMID: 24024834 DOI: 10.1111/apt.12489]

52 **Catalano D**, Martines GF, Tonzuso A, Pirri C, Trovato FM, Trovato GM. Protective role of coffee in non-alcoholic fatty liver disease (NAFLD). *Dig Dis Sci* 2010; **55**: 3200-3206 [PMID: 20165979 DOI: 10.1007/s10620-010-1143-3]

53 **Molloy JW**, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA. Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. *Hepatology* 2012; **55**: 429-436 [PMID: 21987293 DOI: 10.1002/hep.24731]

54 **Chen S**, Teoh NC, Chitturi S, Farrell GC. Coffee and non-alcoholic fatty liver disease: brewing evidence for hepatoprotection? *J Gastroenterol Hepatol* 2014; **29**: 435-441 [PMID: 24199670 DOI: 10.1111/jgh.12422]

55 **Salomone F**, Li Volti G, Vitaglione P, Morisco F, Fogliano V, Zappalà A, Palmigiano A, Garozzo D, Caporaso N, D'Argenio G, Galvano F. Coffee enhances the expression of chaperones and antioxidant proteins in rats with nonalcoholic fatty liver disease. *Transl Res* 2014; **163**: 593-602 [PMID: 24365744 DOI: 10.1016/j.trsl.2013.12.001]

56 **Takemura S**, Minamiyama Y, Kodai S, Shinkawa H, Tsukioka T, Okada S, Azuma H, Kubo S. S-Allyl cysteine improves nonalcoholic fatty liver disease in type 2 diabetes Otsuka Long-Evans Tokushima Fatty rats via regulation of hepatic lipogenesis and glucose metabolism. *J Clin Biochem Nutr* 2013; **53**: 94-101 [PMID: 24062606 DOI: 10.3164/jcbn.13-1]

57 **Xiao J**, Guo R, Fung ML, Liong EC, Chang RC, Ching YP, Tipoe GL. Garlic-Derived S-Allylmercaptocysteine Ameliorates Nonalcoholic Fatty Liver Disease in a Rat Model through Inhibition of Apoptosis and Enhancing Autophagy. *Evid Based Complement Alternat Med* 2013; **2013**: 642920 [PMID: 23861709 DOI: 10.1155/2013/642920]

58 **Xiao J**, Ching YP, Liong EC, Nanji AA, Fung ML, Tipoe GL. Garlic-derived S-allylmercaptocysteine is a hepato-protective agent in non-alcoholic fatty liver disease in vivo animal model. *Eur J Nutr* 2013; **52**: 179-191 [PMID: 22278044 DOI: 10.1007/s00394-012-0301-0]

59 **Lai YS**, Chen WC, Ho CT, Lu KH, Lin SH, Tseng HC, Lin SY, Sheen LY. Garlic essential oil protects against obesity-triggered nonalcoholic fatty liver disease through modulation of lipid metabolism and oxidative stress. *J Agric Food Chem* 2014; **62**: 5897-5906 [PMID: 24857364 DOI: 10.1021/jf500803c]

60 **Sahebkar A**. Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. *World J Gastroenterol* 2011; **17**: 271-272 [PMID: 21246004 DOI: 10.3748/wjg.v17.i2.271]

61 **Gao H**, Guan T, Li C, Zuo G, Yamahara J, Wang J, Li Y. Treatment with ginger ameliorates fructose-induced Fatty liver and hypertriglyceridemia in rats: modulation of the hepatic carbohydrate response element-binding protein-mediated pathway. *Evid Based Complement Alternat Med* 2012; **2012**: 570948 [PMID: 23193424 DOI: 10.1155/2012/570948]

62 **Masterton GS**, Plevris JN, Hayes PC. Review article: omega-3 fatty acids - a promising novel therapy for non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2010; **31**: 679-692 [PMID: 20415840 DOI: 10.1111/j.1365-2036.2009.04230.x]

63 **Storlien LH**, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* 1987; **237**: 885-888 [PMID: 3303333 DOI: 10.1126/science.3303333]

64 **Levy JR**, Clore JN, Stevens W. Dietary n-3 polyunsaturated fatty acids decrease hepatic triglycerides in Fischer 344 rats. *Hepatology* 2004; **39**: 608-616 [PMID: 14999679 DOI: 10.1002/hep.20093]

65 **Mouzaki M**, Allard JP. The role of nutrients in the development, progression, and treatment of nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2012; **46**: 457-467 [PMID: 22469640 DOI: 10.1097/MCG.0b013e31824cf51e]

66 **Jump DB**. N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. *Curr Opin Lipidol* 2008; **19**: 242-247 [PMID: 18460914 DOI: 10.1097/MOL.0b013e3282fffaf6a]

67 **Ghafoorunissa A**, Rajkumar L, Acharya V. Dietary (n-3) long chain polyunsaturated fatty acids prevent sucrose-induced insulin resistance in rats. *J Nutr* 2005; **135**: 2634-2638 [PMID: 16253960]

68 **Teran-Garcia M**, Adamson AW, Yu G, Rufo C, Suchankova G, Dreesen TD, Tekle M, Clarke SD, Gettys TW. Polyunsaturated fatty acid suppression of fatty acid synthase (FASN): evidence for dietary modulation of NF-Y binding to the Fasn promoter by SREBP-1c. *Biochem J* 2007; **402**: 591-600 [PMID: 17313375 DOI: 10.1042/BJ20061722]

69 **Capanni M**, Calella F, Biagini MR, Genise S, Raimondi L, Bedogni G, Svegliati-Baroni G, Sofi F, Milani S, Abbate R, Surrenti C, Casini A. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. *Aliment Pharmacol Ther* 2006; **23**: 1143-1151 [PMID: 16611275 DOI: 10.1111/j.1365-2036.2006.02885.x]

70 **Tanaka N**, Sano K, Horiuchi A, Tanaka E, Kiyosawa K, Aoyama T. Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2008; **42**: 413-418 [PMID: 18277895 DOI: 10.1097/MCG.0b013e31815591aa]

71 **Parker HM**, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2012; **56**: 944-951 [PMID: 22023985 DOI: 10.1016/j.jhep.2011.08.018]

72 **Hâncu N**, Roman G, Nită C, Negrean M. Metabolic syndrome--practical approach. *Rom J Intern Med* 2004; **42**: 237-245 [PMID: 15529614]

73 **Serrano-Martinez M**, Palacios M, Martinez-Losa E, Lezaun R, Maravi C, Prado M, Martínez JA, Martinez-Gonzalez MA. A Mediterranean dietary style influences TNF-alpha and VCAM-1 coronary blood levels in unstable angina patients. *Eur J Nutr* 2005; **44**: 348-354 [PMID: 16151968 DOI: 10.1007/s00394-004-0532-9]

74 **Madigan C**, Ryan M, Owens D, Collins P, Tomkin GH. Dietary unsaturated fatty acids in type 2 diabetes: higher levels of postprandial lipoprotein on a linoleic acid-rich sunflower oil diet compared with an oleic acid-rich olive oil diet. *Diabetes Care* 2000; **23**: 1472-1477 [PMID: 11023139 DOI: 10.2337/diacare.23.10.1472]

75 **Sato K**, Arai H, Mizuno A, Fukaya M, Sato T, Koganei M, Sasaki H, Yamamoto H, Taketani Y, Doi T, Takeda E. Dietary palatinose and oleic acid ameliorate disorders of glucose and lipid metabolism in Zucker fatty rats. *J Nutr* 2007; **137**: 1908-1915 [PMID: 17634263]

76 **Alvarez JA**, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol* 2010; **2010**: 351385 [PMID: 20011094]

77 **Roth CL**, Elfers CT, Figlewicz DP, Melhorn SJ, Morton GJ, Hoofnagle A, Yeh MM, Nelson JE, Kowdley KV. Vitamin D deficiency in obese rats exacerbates nonalcoholic fatty liver disease and increases hepatic resistin and Toll-like receptor activation. *Hepatology* 2012; **55**: 1103-1111 [PMID: 21994008 DOI: 10.1002/hep.24737]

78 **Manco M**, Ciampalini P, Nobili V. Low levels of 25-hydroxyvitamin D(3) in children with biopsy-proven nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 2229; author reply 2230 [PMID: 20513013 DOI: 10.1002/hep.23724]

79 **Wu CC**, Chang JH, Chen CC, Su SB, Yang LK, Ma WY, Zheng CM, Diang LK, Lu KC. Calcitriol treatment attenuates inflammation and oxidative stress in hemodialysis patients with secondary hyperparathyroidism. *Tohoku J Exp Med* 2011; **223**: 153-159 [PMID: 21350317 DOI: 10.1620/tjem.223.153]

80 **Barchetta I**, Carotti S, Labbadia G, Gentilucci UV, Muda AO, Angelico F, Silecchia G, Leonetti F, Fraioli A, Picardi A, Morini S, Cavallo MG. Liver vitamin D receptor, CYP2R1, and CYP27A1 expression: relationship with liver histology and vitamin D3 levels in patients with nonalcoholic steatohepatitis or hepatitis C virus. *Hepatology* 2012; **56**: 2180-2187 [PMID: 22753133 DOI: 10.1002/hep.25930]

81 **Rhee EJ**, Kim MK, Park SE, Park CY, Baek KH, Lee WY, Kang MI, Park SW, Kim SW, Oh KW. High serum vitamin D levels reduce the risk for nonalcoholic fatty liver disease in healthy men independent of metabolic syndrome. *Endocr J* 2013; **60**: 743-752 [PMID: 23411507 DOI: 10.1507/endocrj.EJ12-0387]

82 **Targher G**, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza

G, Arcaro G. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2007; **17**: 517-524 [PMID: 16928437 DOI: 10.1507/endocrj.EJ12-0387]

83 **Yang L**, Seki E. Toll-like receptors in liver fibrosis: cellular crosstalk and mechanisms. *Front Physiol* 2012; **3**: 138 [PMID: 22661952]

84 **Gratz SW**, Mykkanen H, El-Nezami HS. Probiotics and gut health: a special focus on liver diseases. *World J Gastroenterol* 2010; **16**: 403-410 [PMID: 20101763 DOI: 10.3748/wjg.v16.i4.403]

85 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]

86 **Compare D**, Coccoli P, Rocco A, Nardone OM, De Maria S, Cartenì M, Nardone G. Gut--liver axis: the impact of gut microbiota on non alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2012; **22**: 471-476 [PMID: 22546554]

87 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887 [PMID: 19291785 DOI: 10.1002/hep.22848]

88 **Iacono A**, Raso GM, Canani RB, Calignano A, Meli R. Probiotics as an emerging therapeutic strategy to treat NAFLD: focus on molecular and biochemical mechanisms. *J Nutr Biochem* 2011; **22**: 699-711 [PMID: 21292470 DOI: 10.1016/j.jnutbio.2010.10.002]

89 **Li Z**, Yang S, Lin H, Huang J, Watkins PA, Moser AB, Desimone C, Song XY, Diehl AM. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003; **37**: 343-350 [PMID: 12540784 DOI: 10.1053/jhep.2003.50048]

90 **Velayudham A**, Dolganiuc A, Ellis M, Petrasek J, Kodys K, Mandrekar P, Szabo G. VSL#3 probiotic treatment attenuates fibrosis without changes in steatohepatitis in a diet-induced nonalcoholic steatohepatitis model in mice. *Hepatology* 2009; **49**: 989-997 [PMID: 19115316 DOI: 10.1002/hep.22711]

91 **Ma X**, Hua J, Li Z. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. *J Hepatol* 2008; **49**: 821-830 [PMID: 18674841 DOI: 10.1016/j.jhep.2008.05.025]

92 **Shavakhi A**, Minakari M, Firouzian H, Assali R, Hekmatdoost A, Ferns G. Effect of a Probiotic and Metformin on Liver Aminotransferases in Non-alcoholic Steatohepatitis: A Double Blind Randomized Clinical Trial. *Int J Prev Med* 2013; **4**: 531-537 [PMID: 23930163]

93 **Eslamparast T**, Eghtesad S, Hekmatdoost A, Poustchi H. Probiotics and Nonalcoholic Fatty liver Disease. *Middle East J Dig Dis* 2013; **5**: 129-136 [PMID: 24829682]

94 **Eslamparast T**, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Am J Clin Nutr* 2014; **99**: 535-542 [PMID: 24401715 DOI: 10.3945/ajcn.113.068890]

95 **Eslamparast T**, Zamani F, Hekmatdoost A, Sharafkhah M, Eghtesad S, Malekzadeh R, Poustchi H. Effects of synbiotic supplementation on insulin resistance in subjects with the metabolic syndrome: a randomised, double-blind, placebo-controlled pilot study. *Br J Nutr* 2014; **112**: 438-445 [PMID: 24848793 DOI: 10.1017/S0007114514000919]

96 **Ranasinghe P**, Jayawardana R, Galappaththy P, Constantine GR, de Vas Gunawardana N, Katulanda P. Efficacy and safety of 'true' cinnamon (Cinnamomum zeylanicum) as a pharmaceutical agent in diabetes: a systematic review and meta-analysis. *Diabet Med* 2012; **29**: 1480-1492 [PMID: 22671971 DOI: 10.1111/j.1464-5491.2012.03718.x]

97 **Askari F**, Rashidkhani B, Hekmatdoost A. Cinnamon may have therapeutic benefits on lipid profile, liver enzymes, insulin resistance, and high-sensitivity C-reactive protein in nonalcoholic fatty liver disease patients. *Nutr Res* 2014; **34**: 143-148 [PMID: 24461315 DOI: 10.1016/j.nutres.2013.11.005]

98 **Vera-Ramirez L**, Pérez-Lopez P, Varela-Lopez A, Ramirez-Tortosa M, Battino M, Quiles JL. Curcumin and liver disease. *Biofactors* 2013; **39**: 88-100 [PMID: 23303639 DOI: 10.1002/biof.1057]

99 **Girish C**, Pradhan SC. Hepatoprotective activities of picroliv, curcumin, and ellagic acid compared to silymarin on carbon-tetrachloride-induced liver toxicity in mice. *J Pharmacol Pharmacother* 2012; **3**: 149-155 [PMID: 22629090 DOI: 10.4103/0976-500X.95515]

100 **Shao W**, Yu Z, Chiang Y, Yang Y, Chai T, Foltz W, Lu H, Fantus IG, Jin T. Curcumin prevents high fat diet induced insulin resistance and obesity via attenuating lipogenesis in liver and inflammatory pathway in adipocytes. *PLoS One* 2012; **7**: e28784 [PMID: 22253696 DOI: 10.1371/journal.pone.0028784]

101 **Zhang J**, Xu L, Zhang L, Ying Z, Su W, Wang T. Curcumin attenuates D-galactosamine/lipopolysaccharide-induced liver injury and mitochondrial dysfunction in mice. *J Nutr* 2014; **144**: 1211-1218 [PMID: 24899159 DOI: 10.3945/jn.114.193573]

102 **Kuo JJ**, Chang HH, Tsai TH, Lee TY. Positive effect of curcumin on inflammation and mitochondrial dysfunction in obese mice with liver steatosis. *Int J Mol Med* 2012; **30**: 673-679 [PMID: 22751848 DOI: 10.3892/ijmm.2012.1049]

103 **Weidmann AE**. Dihydroquercetin: More than just an impurity? *Eur J Pharmacol* 2012; **684**: 19-26 [PMID: 22513183 DOI: 10.1016/j.ejphar.2012.03.035]

104 **Aguirre L**, Portillo MP, Hijona E, Bujanda L. Effects of resveratrol and other polyphenols in hepatic steatosis. *World J Gastroenterol* 2014; **20**: 7366-7380 [PMID: 24966607 DOI: 10.3748/wjg.v20.i23.7366]

105 **Zhang MH**, Liang ZQ, Qin Q, Li SL, Zhou DS, Tang L. [Effects of quercetin on serum levels of resistin and IL-18 and on insulin resistance in nonalcoholic fatty liver disease rats]. *Zhonghua Ganzangbing Zazhi* 2013; **21**: 66-70 [PMID: 23663767]

106 **Lim CY**, Jun DW, Jang SS, Cho WK, Chae JD, Jun JH. Effects of carnitine on peripheral blood mitochondrial DNA copy number and liver function in non-alcoholic fatty liver disease. *Korean J Gastroenterol* 2010; **55**: 384-389 [PMID: 20571306 DOI: 10.4166/kjg.2010.55.6.384]

107 **Malaguarnera M**, Gargante MP, Russo C, Antic T, Vacante M, Malaguarnera M, Avitabile T, Li Volti G, Galvano F. L-carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis--a randomized and controlled clinical trial. *Am J Gastroenterol* 2010; **105**: 1338-1345 [PMID: 20068559 DOI: 10.1038/ajg.2009.719]

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REVIEW

Management of patients with hepatitis C infection and renal disease

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Abstract

Hepatitis C virus (HCV) infection in patients with end-stage renal disease (ESRD) is associated with more rapid liver disease progression and reduced renal graft and patients' survival following kidney transplantation. Evaluations and management of HCV in patients with renal disease are challenging. The pharmacokinetics of interferons (IFN), ribavirin (RBV) and some direct acting antiviral (DAA), such as sofosbuvir, are altered in patients with ESRD. With dose adjustment and careful monitoring, treatment of HCV in patients with ESRD can be associated with sustained virological response (SVR) rates nearly comparable to that of patients with normal renal function. DAA-based regimens, especially the IFN-free and RBV-free regimens, are theoretically preferred for patients with ESRD and KT in order to increase SVR rates and to reduce treatment side effects. However, based on the data for pharmacokinetics, dosing safety and efficacy of DAA for patients with severe renal impairment are lacking. This review will be focused on the evaluations, available pharmacologic data, and management of HCV in patients with severe renal impairment, patients who underwent KT, and those who suffered from HCV-related renal disease, according to the available treatment options, including DAA.

Key words: Hepatitis C; Renal disease; Chronic kidney disease; Dialysis; Interferon; Direct acting antivirals; Cryoglobulinemia

Core tip: Hepatitis C virus (HCV) infection in patients with end-stage renal disease (ESRD) is associated with more rapid liver disease progression and reduced graft and patients' survival following kidney transplantation. The pharmacokinetics of interferons (IFN), ribavirin (RBV) and sofosbuvir are altered in patients with ESRD. With dose adjustment and careful monitoring, treatment of HCV can be safely utilized and successful in most patients with ESRD. direct acting antiviral (DAA)-based regimens, especially IFN-/RBV-free regimens, are preferred for patients with ESRD and kidney

transplantation (KT). However, due to inadequate data on clinical safety and efficacy, DAA-based therapies are not currently recommended in patients with severe renal disease. This review will be focused on evaluations and management of HCV in ESRD, KT recipients and HCV-related renal disease, according to the available treatment options including DAA.

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INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a leading cause of chronic liver disease and hepatocellular carcinoma worldwide which over is a worldwide health problem in that it has a global prevalence rate of approximately 3% and affects over 170 million individuals[1]. In clinical practice, it is common to see HCV patients with pre-existing renal disease. Thus, the prevalence of HCV infection is apparently increased in patients with end-stage renal disease (ESRD) on chronic replacement therapy, especially hemodialysis. Importantly, the liver-related morbidity and mortality of HCV appear to be higher in patients with ESRD than in the general population[2-4]. For patients undergoing kidney transplantation (KT), HCV infection is associated with an increased rate of liver fibrosis, and the possibility of negatively affecting the renal graft and patients' survival[2-4].

Management of HCV in patients with renal disease presents unique challenges. The pharmacokinetics of interferons (IFN) and ribavirin (RBV) are altered in patients with renal disease, particularly with ESRD[2-4]. With dose adjustment and careful monitoring, treatment with pegylated interferons (PEG-IFN) with or without RBV can eradicate HCV infection in 40%-50% of ESRD patients infected with genotype 1/4 and about 80% of ERSD patients infected with genotype 2 or 3, with an incidence of discontinuation of up to 33%[2-4]. All HCV-positive KT candidates should be assessed to receive antiviral treatment prior to transplantation due to the increased risk of progression of liver disease with immunosuppressive therapy and the inability to receive IFN therapy after KT. Theoretically, the use of direct acting antiviral (DAA)-based regimens, especially the IFN-free and RBV-free regimens, is preferred for patients with ESRD and KT in order to increase sustained virological response (SVR) rates and to reduce treatment side effects. However, the data on pharmacokinetics, dosing safety and efficacy of DAA for patients with severe renal impairment are lacking. In addition, the availability of DAA is currently limited in many countries, especially in the developing world, mainly due to socio-economic reasons. Therefore, this review will be focused on the evaluation and management of HCV in patients with severe renal impairment, patients who underwent KT, and those who suffered from HCV-related renal disease, according to the available treatment options.

NATURAL HISTORY OF HCV IN PATIENTS WITH RENAL DISEASE

End-stage renal disease

The natural history of HCV in patients with ESRD is relatively uncertain[3]. Nevertheless, several studies have demonstrated that ESRD on dialysis is associated with an increased risk for all-cause and liver-related mortality[3,5,6]. Cardiovascular disease remains, however, the major cause of death in dialysis patients irrespective of HCV status[1]. Death from cirrhosis and hepatocellular carcinoma was notably higher among HCV-positive ESRD patients[5]. A meta-analysis on survival in dialysis patients (7 studies; n = 11589) showed an estimated relative risk for death in anti-HCV positive patients of 1.34 (95%CI: 1.13-1.59) with liver-related complications contributing to poorer outcomes[5]. Moreover, HCV infection can adversely affect the quality of life in this population[7].

KT

The impact of immunosuppression on HCV disease progression following renal transplantation is unclear. Serum HCV-RNA levels typically increase. Most data suggested that HCV-infected patients have worsening of hepatic necroinflammation and accelerated hepatic fibrosis following KT[8-10], though some studies reported that liver histology may remain stable or even improve[11,12]. Not only affecting the liver, several studies demonstrated that patients with HCV infection have a poorer patient and graft survival after KT compared to those without[3,13,14]. The exact reason for reduced renal graft survival in HCV patients is unknown, but it may partly explain by de novo immune complex chronic glomerulonephritis in the allograft induced by chronic HCV infection[15,16]. Nevertheless, undergoing KT evidently conferred a long-term survival advantages, particularly on the cardiovascular death, over HCV patients with ESRD on maintenance dialysis on the waitlist, although there was a higher risk for infection-related death during the first 6 mo after KT[11]. As cirrhosis is an key predictor of poor survival after KT, assessment of the stage of liver fibrosis in all HCV-positive KT candidates is recommended[17]. For patients with established cirrhosis and portal hypertension who failed (or are not suitable for) HCV treatment, isolated KT may be inappropriate in this settings and a combined liver and KT should be considered[18].

HCV infection acquired during or after RT showed a severe and rapidly progressive course, which is significantly different from HCV patients without transplantation[19]. In addition, fibrosing cholestatic hepatitis (FCH) can occur in HCV-infected patients following KT[20]. It typically develops during the period of maximal immunosuppression (1-4 mo after KT) and is associated with progressive cholestatic, mild elevation of serum alanine aminotransferase (ALT), and high HCV viremia level[20-22]. FCH is associated with very high morbidity and mortality rates. IFN-based treatment is often ineffective and is associated with a risk of graft rejection[20-22].

EVALUATIONS OF HCV IN PATIENTS WITH RENAL DISEASE

Serum aminotransferases

It is known that serum ALT levels in patients with ESRD are lower than in the general population, so it should not be used to screen for liver diseases[23-25]. This is possibly due to suppression of ALT synthesis in hepatocytes, defective release of ALT into the blood stream, or accelerated clearance in patients with chronic renal insufficiency[24,26]. The lower cut-off ALT level (⩾ 27 U/L) was proposed for patients with ESRD to increase sensitivity (to 50%) and specificity (to 100%) for detecting HCV viremia[23]. In addition, there is a weak correlation between ALT levels and liver disease activity in patients with ESRD, especially those on dialysis[25].

Viral markers

Anti-HCV assay by enzyme immunoassay (EIA) technique is the most commonly used screening tool for HCV infection due to its simplicity, availability and low cost. The second generation EIA (EIA-2) assay was frequently associated with false negative results in patients with ESRD on dialysis, with a reported rate of 2.6%-7%[27,28]. The third generation EIA (EIA-3) testing provided excellent accuracy, with 0.26% false-negative rate, and is the preferred screening tool in this setting[3,25,29]. PCR-based molecular diagnostics are required to confirm viremia, viral load, and genotype to guide management decisions. Notably, HCV-RNA level is transiently decreased during hemodialysis and gradually returns to baseline level within 48 h[3]. This may be explained by several mechanisms, such as interference with PCR technique by heparin used during dialysis, adsorption of HCV onto the dialysis membrane, destruction of HCV particles by the hydraulic pressure, escape of HCV into the dialysate, or increased plasma IFN levels during the dialysis[3,25]. Therefore, it is recommended to determine HCV-RNA level before hemodialysis to avoid the possibility of underestimation[3].

Assessment for liver fibrosis

Liver biopsy is the gold standard for assessing the degree of fibrosis in HCV patients. However, its use is limited by invasive nature, poor patient acceptance, and bleeding risk, especially in uremic patients. The use of non-invasive fibrotic markers has been evaluated in HCV patients with ESRD. The aspartate transaminase: platelet ratio index (APRI) can reliably predict liver fibrosis in HCV patients with ESRD, especially to exclude patients with significant fibrosis[30-32]. In Schiavon *et al*[31] study (203 ESRD HCV-infected subjects), APRI < 0.40 accurately identified patients with fibrosis stage 0 or 1 with negative predictive value of 93%; APRI ⩾ 0.95 can confirm significant fibrosis (⩾ fibrosis stage 2) with positive predictive value of 66%[31]. If biopsy indication was restricted to APRI scores in the intermediate range, about 50% of liver biopsies could be avoided[31]. Transient elastography shows superior diagnostic accuracy to APRI in HCV patients with ESRD[3,32]. The suggested optimized cut-off values were 5.3 kPa, 8.3 kPa, and 9.2 kPa, for fibrosis stage of ⩾ F2, ⩾ F3, and F4, respectively (sensitivity 93%-100% and specificity 88%-99%)[32]. Further, a small study (n = 22) revealed a good correlation between transient elastography and fibrosis stage on histology in HCV-positive KT recipients[33].

EPIDEMIOLOGY AND SCREENING OF HCV IN PATIENTS WITH ESRD ON DIALYSIS

The prevalence of HCV infection in patients with ESRD patients varies among geographical areas and dialysis centers, but it is obviously higher than that of the general population[2]. Risk factors for acquiring HCV infection during dialysis include: the number of transfusions, duration of dialysis, number of procedures for dialysis access, type of dialysis; hemodialysis (HD) > peritoneal dialysis (PD), prevalence of HCV infection and lack of compliance with universal precautions in the dialysis unit[34,35].

In developed countries, the prevalence and incidence of HCV has been declining in the past decades[2,36-38]. In the United States national surveillance (n = 164845), the prevalence of anti-HCV positivity has dropped from 10.4% in 1985 to 7.8% in 2002 (ranged from 5.5%-9.8%)[37]. Similarly, the European multicenter survey reported that the prevalence of anti-HCV positivity has dropped steadily from 13.5% in 1991 to 6.8% in 2000 in the Belgian cohort (n = 1710); prevalence also decreased (P < 0.05) in France (42% to 30%), Sweden (16% to 9%) and Italy (28% to 16%), tended to decrease in United Kingdom (7% to 3%, P = 0.058) and Hungary (26% to 15%, P = 0.057), but

did not change (NS) in Germany (7% to 6%), Spain (5% to 12%) and Poland (42% to 44%)[36]. Despite the elimination of post transfusion HCV infection, the incidence of HCV infection among patients on chronic dialysis treatment, with seroconversion rates ranging between 0.2%-15% per year of dialysis, continues to be a cause of concern[2,36-38]. The data on epidemiology of HCV among patients with ESRD on dialysis in developing countries are less abundant and more heterogeneous, but the overall prevalence and incidence rates seem to be higher than developed countries[2]. The prevalence of anti-HCV positivity in single center surveys from Brazil (2005), Turkey (2005), Tunisia (2006), Iran (2005), Saudi Arabia (2004), Morocco (2005) and Egypt (2000) were 8%, 19%, 20%, 25%, 43%, 76%, and 80%, respectively[2]. Whereas in the Asia-Pacific dialysis registry (173788 HD; 27802 PD), HCV seroprevalences range between 0.7%-18.1% across different countries and were generally higher in HD *vs* PD populations (7.9% ± 5.5% *vs* 3.0% ± 2.0%, $P = 0.01$)[38]. Thus, the annual incidence of HCV infections range from 0% in Thai PD patients to 18.1% in Indian HD patients with the rates were generally higher in HD patients than in PD patients (RR 0.33, 95%CI: 0.13-0.75)[38].

Although prospective trials have shown a reduction in HCV transmission within dialysis units by complete isolation of HCV patients, but this practice has not been universally accepted[2,39]. The Centers for Disease Control and Prevention of the US (CDC) and the Kidney Disease: Improving Global Outcomes (KDIGO) practice guidelines[40] do not recommend dedicated machines, patients isolation, or a ban on reuse in HCV patients on hemodialysis[40,41]. However, strict adherence to "universal precautions", careful attention to hygiene, and sterilization of dialysis machines is emphasized[40,41]. Further, the CDC recommends that all HD patients should be screened for anti-HCV at baseline, and then subsequently tested semiannually[41].

PHARMACOLOGIC ISSUES OF ANTIVIRAL AGENTS IN PATIENTS WITH RENAL DISEASE

Interferons and pegylated interferons

Interferon-alfa is a glycoprotein, produced by immune cells in response to foreign antigens, such as viruses, bacteria, parasites or tumor cells. The elimination half-life of IFN following subcutaneous injections is approximately 2-4 h, then it is filtered through the glomeruli and during proximal tubular reabsorption undergo lysosomal proteolytic degradation[25,42]. Kidney is the main site of degradation of IFN molecule, while liver plays only a minor role. Due to a short elimination half-life of IFN following subcutaneous injections (2-4 h), sustained plasma levels are not maintained when dosed 3 times weekly, which is believed in part to explain the suboptimal response rates[25,42]. Accumulation of IFN occurs in patients with renal dysfunction, especially in ESRD[25,42,43]. Although this may result in higher and more sustained plasma levels of IFN, which is preferable for the anti-viral activity against HCV, it may lead to serious adverse events in such patients as well[25,42,43].

Combing a polyethylene glycol (PEG) polymer to IFN successfully created a molecule with a longer half-life, improved pharmacokinetic profile, and more importantly, a superior clinical response when dosed once weekly[44,45]. PEG-IFN alfa-2a, a branched-PEG (40 kD) attached to an IFN alfa-2a molecule, is absorbed slowly (absorption half-life approximately 50 h), has a restricted volume of distribution (2-12 L), and a long elimination half-life (half-life approximately 77 h; peak through ratio 1.5-2)[44,45]. PEG-IFN alfa-2b, a linear PEG molecule (12 kD) attached to IFN alfa-2b, is absorbed rapidly (absorption half-life approximately 4.6 h), has a large volume of distribution (0.9 L/kg) and a shorter elimination half-life (half-life approximately 40 h; peak through ratio > 10)[44,45]. PEG-IFN alfa-2a is metabolized in the liver and kidneys while PEG-IFN alfa-2b is metabolized exclusively by the kidneys[25,44,45]. The pharmacokinetics of PEG-IFN alfa-2a is less affected by renal failure, and less dose modifications are necessary in the setting of renal impairment as compared to PEG-IFN alfa-2b (Table 1)[44,45]. In patients with severe renal impairment [creatinine clearance (CrCl) < 30 mL/min], the maximum plasma concentration and the area under the curve (AUC) of PEG-IFN alfa-2b are increased by approximately 90% and half-life is increased by approximately 40%[46]. Similar pharmacokinetic profiles have been also observed for PEG-IFN alfa-2a[25,45,46]. In addition, hemodialysis has only a small effect on IFN and PEG-IFN clearance[25,45,46].

Ribavirin

After oral absorption, RBV is rapidly absorbed and distributed with a bioavailability of approximately 50%. It has extensive volume of distribution and the steady state is reached in 7-11 wk after multiple dosing and with a terminal half-life of 12 d[47-49]. The route of RBV elimination is mainly by the kidney. Thus, body weight is also highly correlated with RBV clearance[47-49]. Notably, the optimal dosing strategy of RBV must be calculated according to the renal function, as measured by CrCl[47-49]. The AUC for RBV is increased by 2 folds in patients with CrCl 30-60 mL/min and by 3 folds in patients with CrCl 10-30 mL/min respectively, when compared to those with CrCl > 90 mL/min[50]. Notably, the most pronounced side effect of RBV in patients with renal disease is hemolytic anemia. The mechanism of RBV-induced anemia is unclear, but evidently involves an extensive accumulation of active RBV metabolites

Table 1 Dosage modification for patients with renal impairment

Creatinine clearance	Pegylated-interferon alfa-2a	Pegylated-interferon alfa-2b	Ribavirin
30-50 mL/min	180 μg/wk	1.125 μg/kg per week (25% reduction)	Alternating doses; 200 mg and 400 mg every other day
< 30 mL/min	135 μg/wk (25%-45% reduction)	0.75 μg/kg per week (50% reduction)	200 mg/d
Hemodialysis	135 μg/wk (25%-45% reduction)	0.75 μg/kg per week (50% reduction)	200 mg/d

Table 2 Pharmacokinetic and metabolic parameters of selected direct acting antivirals

Drugs	Metabolism/excretion route	Interaction with CYP3A	Comments
NS3/4A protease inhibitors			
Boceprevir	Hepatic (CYP3A, aldoketoreductase)	Moderate CYP3A inhibitor	Significant DDI with other CYP3A substrate drugs
Telaprevir	Hepatic (CYP3A)	Strong CYP3A inhibitor	Significant DDI with other CYP3A and P-gp substrate drugs
Simeprevir	Hepatic (CYP3A)	Mild CYP1A2 and CYP3A inhibitor	Unconjugated hyperbilirubinemia commonly seen
Faldaprevir	Hepatic (CYP3A)	Moderate CYP3A inhibitor; weak CYP2C9 inhibitor	Inhibition of UGTA1 results in unconjugated hyperbilirubinemia
ABT-450/ ritonavir	Hepatic (CYP3A)	Strong CYP3A inhibitor (by ritonavir)	Unconjugated hyperbilirubinemia
NS5A replication complex inhibitors			
Daclatasvir	Hepatic (CYP3A)	No/minimal	
Ledipasvir	Feces (major); hepatic and renal (minor)	No/minimal	
NS5B nucleos(t)ide polymerase inhibitors			
Sofosbuvir	Renal	No/minimal	Dose reduction if moderate to severe renal impairment
NS5B non-nucleoside polymerase inhibitors			
ABT-333	Hepatic (CYP2C8 60%, CYP3A4 30% and CYP2D6 10%)	No/minimal	

Adapted from Tischer *et al*[55]. CYP3A4: Cytochrome P450 3A4.

and subsequent oxidative stress within red blood cells (RBC)[51]. Ribavirin is actively transported into RBC and accumulates within RBC with the concentration greatly exceeding what is observed in plasma (up to 60-fold)[47,51,52]. Once inside RBC, RBV is phosphorylated to RBV-triphosphate, then it is eliminated slowly from RBC[47,51,52]. An increase in RBV-triphosphate concentrations in RBC enhances oxidative stress with subsequent RBC membrane damage, leading to premature extravascular destruction of RBC by reticuloendothelial system[51,52].

With careful monitoring, reduced dose PEG-IFN plus markedly reduced dose RBV (170-400 mg/d) has been safety utilized in patients with renal impairment, as well as those patients on dialysis[47,49]. However, the use of RBV in HCV patients on dialysis has not been well studied and it should be used with extreme caution and close follow. Further, minimal amount of RBV is removed by dialysis so that there is a potential risk of drug accumulation[25]. Despite this, there are some data to support the use of RBV in dialysis patients by starting empirically with a low dose of 200 mg/d, then adjusting doses according to changes in hemoglobin[47,49,53]. Notably, erythropoiesis-stimulating agents may be used to counteract anemia and help maintain optimal RBV dose in patients with renal disease[47,49]. Until recently, the use of RBV in persons with CrCl < 50 mL/min was contraindicated due to a markedly reduced ribavirin elimination rate, leading to severe hemolytic anemia. However, the US Food and Drug Administration (US-FDA) has approved a labeling change of RBV for patients with severe renal impairment with recommended dosage modifications since 2011[54] (Table 1). It should be noted that breaking RBV tablet into half is not advised due to the potential environmental contamination of this serious teratogen. Given its extensive Vd and long half-life, RBV can be finely adjusted by alternating daily dosage[47,49].

Direct acting antivirals

Most Direct acting antiviral (DAA) are metabolized primarily *via* the liver and dose adjustment is not necessary for patients with renal impairment[55,56] (Table 2). However, it should be noted that these agents have not been adequately evaluated for the treatment of HCV in patients with renal impairment in clinical trials in terms of safety and efficacy. *In vitro* studies indicate that boceprevir (BOC) extensively

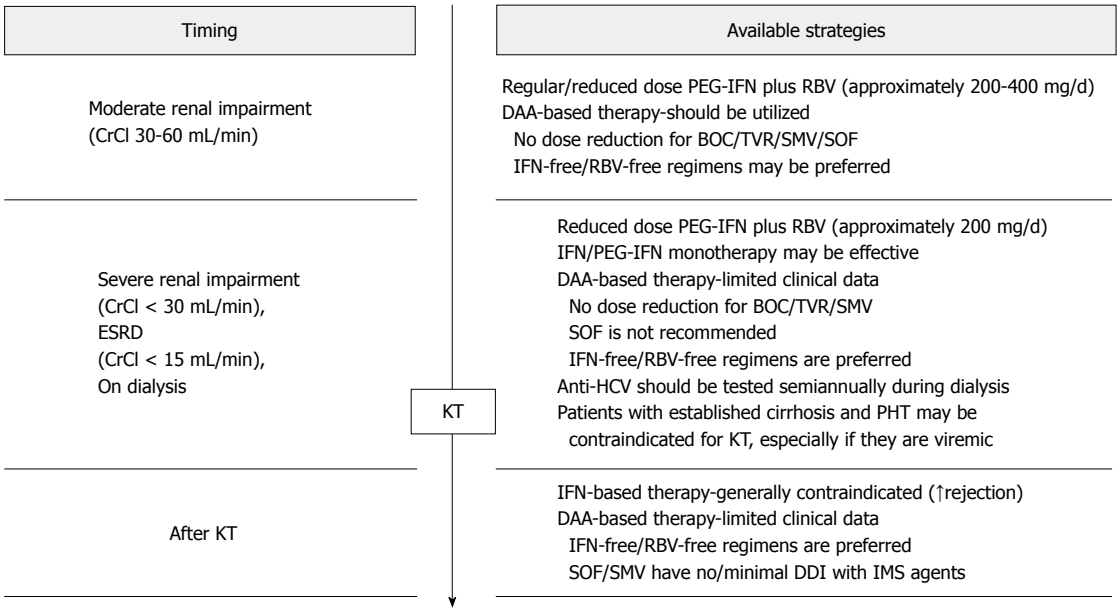

Figure 1 Management of hepatitis C virus in patients with renal disease and kidney transplantation. Empiric dose changes should be done in conjunction with therapeutic drug monitoring. BOC: Boceprevir; TVR: Telaprevir; SMV: Simeprevir; SFV: Sofosbuvir; CYP3A4: Cytochrome P450 3A4; mTOR: Mammalian target of rapamycin; MMF: Mycophenolate mofetil.

undergoes metabolism through the aldo-keto reductase-mediated pathway and, to a lesser extent, oxidative metabolism mediated by CYP3A4 in the liver[54]. Telaprevir (TVR) is primarily metabolized in the liver involving hydrolysis, oxidation, and reduction[54]. No dose adjustment is required for BOC and TVR in patients with any degree of renal impairment[54]. Simeprevir (SMV) is metabolized by liver CYP3A4 and kidney clearance plays an insignificant role (< 1%)[57]. A pharmacokinetic study in volunteers demonstrated that SMV exposure after 7 d of 150 mg/d dosing was 62% higher in patients with severe renal impairment compared with matched healthy volunteers, but safety and tolerability were considered generally favorable[57]. Daclastavir is also metabolized primarily by the liver, and the need for dose adjustment in patients with impaired renal function is unknown[55,56]. Unlike the others, sofosbuvir (SOF) is excreted *via* the kidneys. A single 400-mg dose of SOF resulted in 56%, 90%, and 456% higher levels of the major systemic metabolite, GS-331007, among persons with mild, moderate, and severe renal dysfunction, respectively, compared with individuals with normal renal function[58].

MANAGEMENT OF HCV IN PATIENTS WITH ESRD

Interferon or pegylated interferon monotherapy

There have been at least 4 meta-analyses demonstrating that IFN monotherapy is effective for HCV patients with ESRD, with the overall SVR rates of 33%-41% and the withdrawal rates of 17%-30%[59-62]. The discontinuation of treatment were mainly due to flu-like symptoms, gastrointestinal and hematological adverse events, which is more frequent than in patients with normal renal function[59-62]. Notably, the use of PEG-IFN does not seem to provide an additional benefit in terms of SVR compared to conventional IFN monotherapy in patients on dialysis[61,62]. This finding may largely explain by changes in the pharmacokinetics of IFN toward increasing half-life and AUC (more similar to that of PEG-IFN) in the setting of ESRD. In a recent meta-analysis of 25 studies (included 459 patients treated with IFN38 patients treated with PEG-IFNand 49 patients treated with PEG-IFN/RBV), the overall SVR rate was 41% (95%CI: 33-49) for IFN and 37% (95%CI: 9-77) for PEG-IFN[61]. Treatment discontinuation rates were 26% (95%CI: 20%-34%) for IFN and 28% (95%CI: 12%-53%) for PEG-IFN. The SVR rate tended to be higher when 3 million units or higher of IFN 3 times weekly were used[61] (Figure 1).

Interferon or pegylated interferon plus ribavirin

In patients with normal renal function, the addition of RBV to IFN or PEG-IFN is generally required in order to achieve an optimal SVR, primarily by decreasing relapse rates[47]. Several small studies have demonstrated that the use low-dose RBV (from 200 mg/wk to 400 mg/d) in combination with IFN-based therapy was feasible for treating HCV patients with ESRD[3,50]. Despite the widespread use of high-dose erythropoietin, falling hemoglobin levels were commonly observed[3,50]. Overall, SVR and treatment-related withdrawal rates after 24-48 wk of combination therapy ranged between 17%-90% and 0%-70%, respectively[3,50]. Rendina *et al*[63] reported a case series evaluating PEG-IFN 135 μg/

wk plus RBV 200 mg/d for 24 (non-G1) or 48 wk (G1) in HCV patients on dialysis[63]. In this study, 35 patients received treatment (35 served as untreated controls), and 30 patients completed treatment (drop-out rate 14%)[63]. Overall, 34 of 35 (97%) treated patients, including those with treatment discontinuation due to side effects, achieved SVR[63]. Recently, an open-label, randomized trial in Taiwan randomized 205 HCV patients on hemodialysis to PEG-IFN 135 μg/wk plus RBV 200 mg/d (n = 103) or PEG-IFN 135 μg/wk alone (n = 102) for 48 wk[53]. Compared with monotherapy, combination therapy had a greater SVR rate (64% *vs* 33%, $P < 0.001$)[53]. More patients receiving combination therapy required a higher dosage and longer duration of epoetin-beta and had more hemoglobin levels < 8.5 g/dL than those receiving monotherapy (72% *vs* 6%, $P < 0.001$)[53]. The adverse event-related withdrawal rates were similar between the two groups[53]. The recent AASLD/IDSA guidance recommended patients with renal impairment/ESRD/HD, dosing of PEG-IFN and RBV to follow updated FDA recommendations or package insert recommendations based on calculated glomerular filtration rate[64].

Boceprevir- or telaprevir-based triple therapy

The first wave DAA, boceprevir (BOC) and telaprevir (TVR), in combination with PEG-IFN/RBV has been the standard of care of HCV genotype 1 in many countries since 2011, with an improvement in SVR up to 65%-75% in treatment naïve patients and 60%-65% in previous relapsers/non-responders[1]. Notably, patients with CrCl < 50 mL/min or those with ESRD were excluded from their registration trials[1]. Despite the lack of required dose adjustment, there have been very few small case series evaluating the safety and efficacy of these protease inhibitors in patients with ESRD on dialysis[65-67]. Therefore, the routine use of BOC/TVR-based triple therapy routine use in patients with severe renal impairment cannot be recommended[54,64]. It is possible that treatment-related side effects, particularly anemia, will be increased in patients with renal disease. Thus, the potential drug-drug interactions between the BOC/TVR and concomitant medications that commonly used in patients with ESRD (*e.g.,* antihypertensive and lipid lowering agents) may further complicate the therapy.

Sofosbuvir- and simeprevir-based therapy

SOF and SMV have recently been approved for the treatment of HCV in United States and many countries in Europe. In settings where available, SOF- and SMV-containing regimens are preferred for over BOC/TVR-based triple therapy due to superior efficacy, more convenience dosing, and less drug-drug interactions[64]. Ideally, patients with ESRD should receive an IFN-free, and if possible RBV-free treatment regimen. Based on the available data, the recent AASLD/IDSA guidance advised that no dose reduction is needed when using SOF in patients with HCV infection with mild to moderate renal impairment (CrCl ⩾ 30 mL/min). However, SOF is not recommended in patients with severe renal impairment and ESRD (CrCl < 30 mL/min) or those who require HD until more data available[64]. For SMV, no dosage adjustment is required for patients with any degree of renal impairment. SMV has not been adequately studied in patients with ESRD, including those requiring HD[64].

MANAGEMENT OF HCV IN KIDNEY TRANSPLANT RECIPIENTS

Interferon- or pegylated interferon-based therapy

Outcomes of IFN-based treatment after KT are somewhat disappointing. In a meta-analysis of 12 clinical trials (102 RT recipients with HCV), treatment with IFN with or without RBV is associated with the overall SVR and treatment-related withdrawal rates of 18% and 35%, respectively[68]. More recent meta-analysis (n = 140) reported that the overall SVR rate, drop-out rate and graft rejection rate was 26.6%, 21.1% and 4%, respectively[69]. Thus, PEG-IFN may be a more effective approach for treating HCV post-KT than standard IFN-based treatment (SVR: 40.6% *vs* 20.9%)[69]. These suboptimal SVR rates may largely explain by the interruption of treatment by side effects and the limited efficacy of IFN in immunosuppressed patients. In addition, the immunostimulatory effects of IFN, including increased expression of cytokines and HLA antigens, and enhanced function of cytotoxic T cells and natural killer cells, can lead to an increased risk of acute allograft rejection in KT recipients[4]. The early studies reported that graft dysfunction occurred in 15%-100% of HCV-positive KT recipients treated with IFN, with up to 20% resultant permanent allograft failure[4,70-73]. Although more recent studies revealed that PEG-IFN/RBV treatment may be feasible for KT recipients and the development of graft rejection was in fact relatively rare (0%-5%), but the SVR rates are still relatively low (38%-50%)[74-76]. Taken together, IFN-based therapy should only be initiated in KT recipients under specific clinical situations, such as FCH or severe de novo glomerulonephritis, where DAA are unavailable and when the risk of not treating HCV infection outweighs the risk of graft loss (Figure 1).

Boceprevir- or telaprevir-based triple therapy

Both BOC and TVR are substrates for and inhibitors of the CYP3A4 and the drug transporter P-glycoprotein, so that they are prone to interact with other medications involving this enzyme[55,56,77]. Several studies have demonstrated that co-administration

of BOC and TVR significantly increased the dose exposure of cyclosporine, tacrolimus and mammalian target of rapamycin (mTOR) inhibitors by several folds[55,56,77]. Lessons from LT suggested that the drug-drug interaction issues with BOC- and TVR-based triple therapy may be manageable with preemptive dose reduction and close monitoring of immunosuppressive drug levels[55,56,77]. However, the use of BOC/TVR-based triple therapy may not be suitable for KT setting due to the possible rejection issue with IFN.

Sofosbuvir- and simeprevir-based therapy

KT recipients with an indication for HCV treatment should receive IFN-free regimen. SOF does not undergo metabolism *via* hepatic CYP3A, limiting the likelihood of drug-drug interactions with immunosuppressive agents that are inducers or inhibitors of this enzyme[55,56]. However, SOF is a substrate for P-gp and should not be coadministered with potent P-gp inducers such as rifampin or St John's wort. SMV is a mild CYP3A inhibitor and weak drug-drug interaction may be observed when co-administration with immunosuppressive agents. For example, the AUC of tacrolimus decreased by 17% and that of cyclosporine increased by 19% with SMV co-administration[78]. However, the package insert for SMV advises that no dose adjustments are needed during co-administration with cyclosporine or tacrolimus. Monitoring of sirolimus concentrations may be advisable when given with SMV.

HEPATITIS C VIRUS-RELATED RENAL DISEASE

Epidemiology

Considering HCV is both a hepatotrophic and lymphotrophic virus, in addition to causing a liver disease, it can be associated with a number of lymphoproliferative and immunological disorders of various organ systems, including the kidney[79] (Figure 2). HCV-related renal disease can affect both glomerular and tubular component, which can be presented with a wide array of clinical manifestations, ranging from asymptomatic proteinuria, overt glomerular disease, to ESRD. Notably, the most common and well-established renal involvement of HCV is mixed cryoglobulinemia (MC)-associated glomerulonephritis[16,79-81]. Whereas other conditions such as membranoproliferative glomerulonephritis (MPGN) and membranous nephropathy, focal segmental glomerulosclerosis, fibrillary glomerulonephritis, immunotactoid glomerulopathy, IgA nephropathy, mesangial proliferative glomerular nephritis, renal thrombotic microangiopathy, vasculitic renal involvement and interstitial nephritis are less commonly described[16,80]. These pathologic findings are not specific for HCV and the diagnosis of HCV-related renal disease has to be made cautiously with exclusion of the other secondary causes. In most circumstances, renal involvement itself does not affect overall survival in HCV infected patients but it could modify natural course of disease significantly[82].

The prevalence of HCV-related renal disease varies among reports and also seems to be geographical heterogeneity. For example, the prevalence of MC is clearly more prevalent in Southern Europe (more than 50% of HCV-infected individuals) than in Northern Europe, North America, and Asia[79,81]. One large epidemiologic study among US veterans showed that cryoglobulinemia and MPGN, not membranous glomerulopathy, are more prevalent in HCV-infected patients (n = 34204) compared to non-HCV controls (n = 136816): 0.57% *vs* 0.05%; $P < 0.001$, 0.36% *vs* 0.05%; $P < 0.001$ and 0.33% *vs* 0.19%; $P = 0.86$, respectively[83]. In addition, HCV infection is independently associated with proteinuria[84,85]. Besides, many epidemiological studies have shown an association between HCV and diabetes mellitus, especially type 2[86]. The processes seem to involve direct viral effects, insulin resistance, proinflammatory cytokines, chemokines, and other immune-mediated mechanisms[86]. Therefore, proteinuria and renal disease in such patients may be secondary from diabetic nephropathy, not directly related to HCV itself.

Diagnosis of mixed cryoglobulinemia

Cryoglobulinemia is a chronic systemic disease characterized by the presence of serum immunoglobulins that reversibly precipitate at low temperature[87]. Classically, cryoglobulemia is divided into 3 types. Type I composed of a monoclonal immunoglobulin associated mainly with overt lymphoproliferative disorders and is classically presented with vascular occlusion or hyperviscosity syndrome[80,88]. Type Ⅱ and Ⅲ, so-called MC, consisted of polyclonal IgG and monoclonal/polyclonal IgM with rheumatoid factor. These two types are associated with infectious, immunological and neoplastic disorders (also can be idiopathic), and often present with vasculitis syndrome, *e.g.*, peripheral neuropathy, skin ulcers as well as glomerulonephritis[80,88]. The strong association between HCV and MC type Ⅱ and Ⅲ has been supported by several epidemiological studies[16,79-81,87]. HCV appears to have an important etiologic role in MC, as the evidence of HCV infection can be found in 76%-95% of patients with MC[89,90]. On the other hand, low level of cryoglobulinemia is commonly found in unselected HCV patients with prevalence 19%-54%[91,92]. Most of these patients are asymptomatic and about 5%-20% have an overt clinical of MC[91,92].

Diagnosis of MC is based on clinicopathological and laboratory work-up including cryoglobulin testing, quantitative serum protein and globulins, complement levels, virologic markers, and urine analysis. The

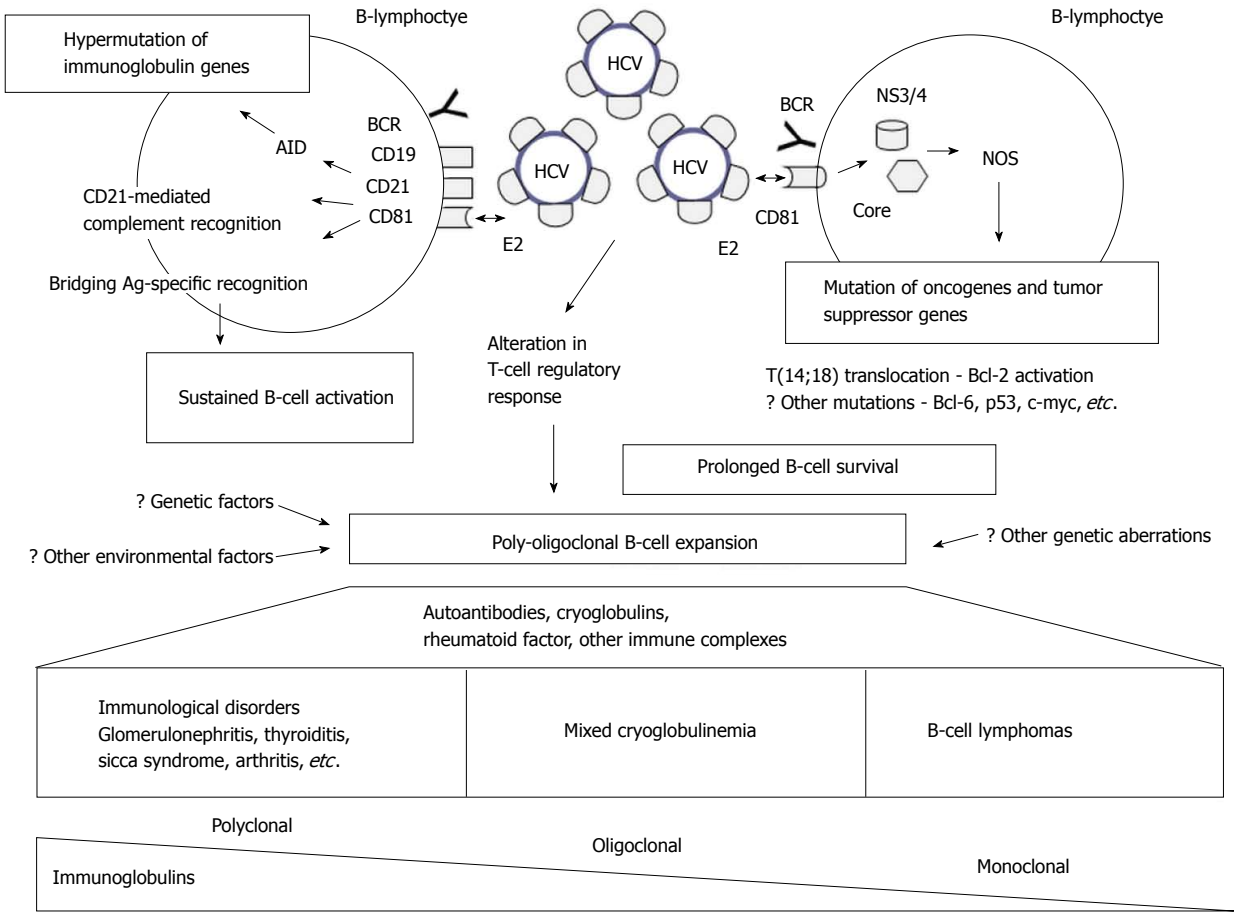

Figure 2 Proposed pathogenesis of hepatitis C virus-related immunogenic and lymphoproliferative disorders. HCV: Hepatitis C virus; NOS: Nitric oxide synthase; ROS: Reactive oxygen species; AID: Activation-induced deaminase.

clinical syndrome of MC caused by deposition of circulating immune complexes in small to medium-sized blood vessels in multiple organs, inducing systemic vasculitis. The main clinical manifestations of MC are palpable purpura, arthralgia, myalgia, peripheral neuropathy and hypocomplementemia[88,90]. Cryoglobulins can precipitate in vitro at temperatures of less than 37 ℃ (typically at 4 ℃[80]) and re-dissolve after rewarming[93].

Renal involvement is presented in up to one third of patients and represents a strong negative prognostic factor, even if their course may vary[90,94]. Nephropathy is observed in 20% at the diagnosis of MC, and in 35%-60% during follow up, in which the majority occurs within a few years[90,95]. Clinically, MC-associated glomerulonephritis may range from asymptomatic abnormal urinalysis (microscopic hematuria, or sub-nephrotic range proteinuria with normal, or mildly impaired, renal function), overt nephritis (20%-25%) and nephrotic syndrome (20%), with variable progression to end-stage renal disease in 10%-33% of patients[90,95]. The typical renal histopathologic pattern is type Ⅰ MPGN, which can be differentiated from idiopathic MPGN by the presence of capillary thrombi, composed of precipitated cryoglobulins, and diffuse IgM deposition in the capillary loops[80,90,96,97].

Treatment of mixed cryoglobulinemia-related glomerulonephritis

Several small studies reported a beneficial effect of PEG-IFN/RBV in patients with HCV-related MC with 62%-78% SVR rates[87,98]. PEG-IFN/RBV is generally well-tolerated (treatment-related side effects 22%-54%) in cryoglobulinemic patients and the dosage should be adjusted according to renal function[86,97]. Notably, the chance to achieve SVR is not affected by the presence of cryoglobulinemia[87,98]. Importantly, significant improvement of clinical MC syndrome and immunologic parameters is typically observed in patients who attained SVR. Cryoglobulin level often declines or even disappears after successful treatment. Though, MC-related vasculitis has been reported to persist or to relapse after achieving in a small proportion of patients[94,99]. A meta-analysis of controlled clinical trials suggested that IFN-based therapies were more effective than immunosuppressive agents in lowering proteinuria of patients with HCV-related cryoglobulinemic glomerulonephritis (OR = 3.86; 95%CI: 1.44-10.33)[100].

Recent studies in HCV-related MC with or without renal involvement demonstrated that triple therapy with TVR, BOC or SOF plus PEG-IFN/RBV are safe and effective in cryoglobulinemic patients[100-102]. However, such therapeutic regimens should be administered cautiously considering the high rate of side effects (up to 35% discontinuation rates)[101,103]. More clinical trials, especially with IFN-free regimens, are eagerly awaited.

Corticosteroids and cyclophosphamides have shown to effectively induce clinical remission in patients with severe MC. However, their effects are not sustainable and they can be associated with significant side effects, liver toxicity, and subsequent increase in HCV viremia[86,99]. Rituximab, a chimeric monoclonal antibody specifically directed against CD20 antigen, has been proven to be safe and effective in the treatment of MC with or without HCV[87,98,104]. A randomized controlled trial comparing between PEG-IFN/RBV with or without rituximab in treatment-naïve MC patients demonstrated that a combination of PEG-IFN/RBV plus rituximab is well tolerated and more effective than PEG-IFN/RBV alone[105]. Thus, its effect may last for more than 3 years[105]. Therefore, a combination of rituximab and antiviral therapy is recommended to treat in HCV-related MC with progressive kidney disease[40,87,106]. In addition, the removal of circulating cryoglobulins by therapeutic plasmapheresis combined with immunosuppressive agents, such as pulse corticosteroids, may be considered as an adjunctive therapy for severe exacerbation of vasculitis, especially rapidly progressive glomerulonephritis[40,87,106,107].

REFERENCES

1 EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2014; **60**: 392-420 [PMID: 24331294 DOI: 10.1016/j.jhep.2013.11.003]

2 **Fabrizi F**, Lunghi G, Ganeshan SV, Martin P, Messa P. Hepatitis C virus infection and the dialysis patient. *Semin Dial* 2007; **20**: 416-422 [PMID: 17897248 DOI: 10.1111/j.1525-139X.2007.00311.x]

3 **Liu CH**, Kao JH. Treatment of hepatitis C virus infection in patients with end-stage renal disease. *J Gastroenterol Hepatol* 2011; **26**: 228-239 [PMID: 21261711 DOI: 10.1111/j.1440-1746.2010.06488.x]

4 **Okoh EJ**, Bucci JR, Simon JF, Harrison SA. HCV in patients with end-stage renal disease. *Am J Gastroenterol* 2008; **103**: 2123-2134 [PMID: 18796105 DOI: 10.1111/j.1572-0241.2008.01981.x]

5 **Fabrizi F**, Takkouche B, Lunghi G, Dixit V, Messa P, Martin P. The impact of hepatitis C virus infection on survival in dialysis patients: meta-analysis of observational studies. *J Viral Hepat* 2007; **14**: 697-703 [PMID: 17875004 DOI: 10.1111/j.1365-2893.2007.00868.x]

6 **Kalantar-Zadeh K**, Kilpatrick RD, McAllister CJ, Miller LG, Daar ES, Gjertson DW, Kopple JD, Greenland S. Hepatitis C virus and death risk in hemodialysis patients. *J Am Soc Nephrol* 2007; **18**: 1584-1593 [PMID: 17429053 DOI: 10.1681/asn.2006070736]

7 **Fabrizi F**, Messa P, Martin P. Health-related quality of life in dialysis patients with HCV infection. *Int J Artif Organs* 2009; **32**: 473-481 [PMID: 19844890]

8 **Izopet J**, Rostaing L, Sandres K, Cisterne JM, Pasquier C, Rumeau JL, Duffaut M, Durand D, Puel J. Longitudinal analysis of hepatitis C virus replication and liver fibrosis progression in renal transplant recipients. *J Infect Dis* 2000; **181**: 852-858 [PMID: 10720504 DOI: 10.1086/315355]

9 **Zylberberg H**, Nalpas B, Carnot F, Skhiri H, Fontaine H, Legendre C, Kreis H, Bréchot C, Pol S. Severe evolution of chronic hepatitis C in renal transplantation: a case control study. *Nephrol Dial Transplant* 2002; **17**: 129-133 [PMID: 11773476]

10 **Töz H**, Ok E, Yilmaz F, Akarca US, Erensoy S, Zeytinoğlu A, Ozkahya M, Karasu Z, Yüce G, Başçi A. Clinicopathological features of hepatitis C virus infection in dialysis and renal transplantation. *J Nephrol* 2002; **15**: 308-312 [PMID: 12116989]

11 **Roth D**, Gaynor JJ, Reddy KR, Ciancio G, Sageshima J, Kupin W, Guerra G, Chen L, Burke GW. Effect of kidney transplantation on outcomes among patients with hepatitis C. *J Am Soc Nephrol* 2011; **22**: 1152-1160 [PMID: 21546575 DOI: 10.1681/asn.2010060668]

12 **Alric L**, Di-Martino V, Selves J, Cacoub P, Charlotte F, Reynaud D, Piette JC, Péron JM, Vinel JP, Durand D, Izopet J, Poynard T, Duffaut M, Rostaing L. Long-term impact of renal transplantation on liver fibrosis during hepatitis C virus infection. *Gastroenterology* 2002; **123**: 1494-1499 [PMID: 12404224]

13 **Mahmoud IM**, Elhabashi AF, Elsawy E, El-Husseini AA, Sheha GE, Sobh MA. The impact of hepatitis C virus viremia on renal graft and patient survival: a 9-year prospective study. *Am J Kidney Dis* 2004; **43**: 131-139 [PMID: 14712436]

14 **Mathurin P**, Mouquet C, Poynard T, Sylla C, Benalia H, Fretz C, Thibault V, Cadranel JF, Bernard B, Opolon P, Coriat P, Bitker MO. Impact of hepatitis B and C virus on kidney transplantation outcome. *Hepatology* 1999; **29**: 257-263 [PMID: 9862875 DOI: 10.1002/hep.510290123]

15 **Filippone EJ**, Chmielewski C, Gulati R, Newman E, Farber JL. De Novo Fibrillary Glomerulonephritis (FGN) in a Renal Transplant with Chronic Hepatitis C. *Case Rep Transplant* 2013; **2013**: 978481 [PMID: 23844313 DOI: 10.1155/2013/978481]

16 **Ozkok A**, Yildiz A. Hepatitis C virus associated glomerulopathies. *World J Gastroenterol* 2014; **20**: 7544-7554 [PMID: 24976695 DOI: 10.3748/wjg.v20.i24.7544]

17 **Gane E**, Pilmore H. Management of chronic viral hepatitis before and after renal transplantation. *Transplantation* 2002; **74**: 427-437 [PMID: 12352899 DOI: 10.1097/01.tp.0000020756.34168.e0]

18 **Van Wagner LB**, Baker T, Ahya SN, Norvell JP, Wang E, Levitsky J. Outcomes of patients with hepatitis C undergoing simultaneous liver-kidney transplantation. *J Hepatol* 2009; **51**: 874-880 [PMID: 19643508 DOI: 10.1016/j.jhep.2009.05.025]

19 **Töz H**, Nart D, Turan I, Ersöz G, Seziş M, Aşçi G, Ozkahya M, Zeytinoğlu A, Erensoy S, Ok E. The acquisition time of infection: a determinant of the severity of hepatitis C virus-related liver disease in renal transplant patients. *Clin Transplant* 2009; **23**: 723-731 [PMID: 19573091 DOI: 10.1111/j.1399-0012.2009.01017.x]

20 **Delladetsima JK**, Boletis JN, Makris F, Psichogiou M, Kostakis A, Hatzakis A. Fibrosing cholestatic hepatitis in renal transplant recipients with hepatitis C virus infection. *Liver Transpl Surg* 1999; **5**: 294-300 [PMID: 10388502 DOI: 10.1002/lt.500050417]

21 **Cimsit B**, Assis D, Caldwell C, Arvelakis A, Taddei T, Kulkarni S, Schilsky M, Emre S. Successful treatment of fibrosing cholestatic hepatitis after liver transplantation. *Transplant Proc* 2011; **43**: 905-908 [PMID: 21486625 DOI: 10.1016/j.transproceed.2011.02.034]

22 **Siddiqui AR**, Abbas Z, Luck NH, Hassan SM, Aziz T, Mubarak M, Naqvi SA, Rizvi SA. Experience of fibrosing cholestatic hepatitis with hepatitis C virus in kidney transplant recipients. *Transplant Proc* 2012; **44**: 721-724 [PMID: 22483477 DOI: 10.1016/j.transproceed.2011.12.019]

23 **Espinosa M**, Martin-Malo A, Alvarez de Lara MA, Soriano S, Aljama P. High ALT levels predict viremia in anti-HCV-positive HD patients if a modified normal range of ALT is applied. *Clin Nephrol* 2000; **54**: 151-156 [PMID: 10968693]

24 **Fabrizi F**, Lunghi G, Andrulli S, Pagliari B, Mangano S, Faranna P, Pagano A, Locatelli F. Influence of hepatitis C virus (HCV) viraemia upon serum aminotransferase activity in chronic dialysis

patients. *Nephrol Dial Transplant* 1997; **12**: 1394-1398 [PMID: 9249775]
25 **Al-Freah MA**, Zeino Z, Heneghan MA. Management of hepatitis C in patients with chronic kidney disease. *Curr Gastroenterol Rep* 2012; **14**: 78-86 [PMID: 22161023 DOI: 10.1007/s11894-011-0238-0]
26 **Wolf PL**, Williams D, Coplon N, Coulson AS. Low aspartate transaminase activity in serum of patients undergoing chronic hemodialysis. *Clin Chem* 1972; **18**: 567-568 [PMID: 5026769]
27 **Bukh J**, Wantzin P, Krogsgaard K, Knudsen F, Purcell RH, Miller RH. High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: failure of commercially available antibody tests to identify a significant number of patients with HCV infection. Copenhagen Dialysis HCV Study Group. *J Infect Dis* 1993; **168**: 1343-1348 [PMID: 7504031]
28 **Kalantar-Zadeh K**, Miller LG, Daar ES. Diagnostic discordance for hepatitis C virus infection in hemodialysis patients. *Am J Kidney Dis* 2005; **46**: 290-300 [PMID: 16112048 DOI: 10.1053/j.ajkd.2005.05.006]
29 **Schneeberger PM**, Keur I, van der Vliet W, van Hoek K, Boswijk H, van Loon AM, van Dijk WC, Kauffmann RH, Quint W, van Doorn LJ. Hepatitis C virus infections in dialysis centers in The Netherlands: a national survey by serological and molecular methods. *J Clin Microbiol* 1998; **36**: 1711-1715 [PMID: 9620405]
30 **Liu CH**, Liang CC, Liu CJ, Hsu SJ, Lin JW, Chen SI, Hung PH, Tsai HB, Lai MY, Chen PJ, Chen JH, Chen DS, Kao JH. The ratio of aminotransferase to platelets is a useful index for predicting hepatic fibrosis in hemodialysis patients with chronic hepatitis C. *Kidney Int* 2010; **78**: 103-109 [PMID: 20357753 DOI: 10.1038/ki.2010.74]
31 **Schiavon LL**, Schiavon JL, Filho RJ, Sampaio JP, Lanzoni VP, Silva AE, Ferraz ML. Simple blood tests as noninvasive markers of liver fibrosis in hemodialysis patients with chronic hepatitis C virus infection. *Hepatology* 2007; **46**: 307-314 [PMID: 17634962 DOI: 10.1002/hep.21681]
32 **Liu CH**, Liang CC, Huang KW, Liu CJ, Chen SI, Lin JW, Hung PH, Tsai HB, Lai MY, Chen PJ, Chen JH, Chen DS, Kao JH. Transient elastography to assess hepatic fibrosis in hemodialysis chronic hepatitis C patients. *Clin J Am Soc Nephrol* 2011; **6**: 1057-1065 [PMID: 21393486 DOI: 10.2215/cjn.04320510]
33 **Muñoz R**, Ramírez E, Fernandez I, Martin A, Romero M, Romero E, Dominguez-Gil B, Hernandez A, Morales E, Andres A, Castellano G, Morales JM. Correlation between fibroscan, liver biopsy, and clinical liver function in patients with hepatitis C virus infection after renal transplantation. *Transplant Proc* 2009; **41**: 2425-2426 [PMID: 19715940 DOI: 10.1016/j.transproceed.2009.06.103]
34 **Bergman S**, Accortt N, Turner A, Glaze J. Hepatitis C infection is acquired pre-ESRD. *Am J Kidney Dis* 2005; **45**: 684-689 [PMID: 15806471]
35 **Gallego E**, López A, Pérez J, Llamas F, Lorenzo I, López E, Illescas ML, Andrés E, Olivas E, Gómez-Roldan C. Effect of isolation measures on the incidence and prevalence of hepatitis C virus infection in hemodialysis. *Nephron Clin Pract* 2006; **104**: c1-c6 [PMID: 16685138 DOI: 10.1159/000093252]
36 **Jadoul M**, Poignet JL, Geddes C, Locatelli F, Medin C, Krajewska M, Barril G, Scheuermann E, Sonkodi S, Goubau P. The changing epidemiology of hepatitis C virus (HCV) infection in haemodialysis: European multicentre study. *Nephrol Dial Transplant* 2004; **19**: 904-909 [PMID: 15031348 DOI: 10.1093/ndt/gfh012]
37 **Finelli L**, Miller JT, Tokars JI, Alter MJ, Arduino MJ. National surveillance of dialysis-associated diseases in the United States, 2002. *Semin Dial* 2005; **18**: 52-61 [PMID: 15663766 DOI: 10.1111/j.1525-139X.2005.18108.x]
38 **Johnson DW**, Dent H, Yao Q, Tranaeus A, Huang CC, Han DS, Jha V, Wang T, Kawaguchi Y, Qian J. Frequencies of hepatitis B and C infections among haemodialysis and peritoneal dialysis patients in Asia-Pacific countries: analysis of registry data. *Nephrol Dial Transplant* 2009; **24**: 1598-1603 [PMID: 19096083 DOI: 10.1093/ndt/gfn684]
39 **Djordjević V**, Stojanović K, Stojanović M, Stefanović V. Prevention of nosocomial transmission of hepatitis C infection in a hemodialysis unit. A prospective study. *Int J Artif Organs* 2000; **23**: 181-188 [PMID: 10795663]
40 KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. *Kidney Int Suppl* 2008; **109**: S1-99 [PMID: 18382440 DOI: 10.1038/ki.2008.81]
41 Recommendations for preventing transmission of infections among chronic hemodialysis patients. *MMWR Recomm Rep* 2001; **50**: 1-43 [PMID: 11349873]
42 **Wills RJ**. Clinical pharmacokinetics of interferons. *Clin Pharmacokinet* 1990; **19**: 390-399 [PMID: 1702693 DOI: 10.2165/00003088-199019050-00003]
43 **Uchihara M**, Izumi N, Sakai Y, Yauchi T, Miyake S, Sakai T, Akiba T, Marumo F, Sato C. Interferon therapy for chronic hepatitis C in hemodialysis patients: increased serum levels of interferon. *Nephron* 1998; **80**: 51-56 [PMID: 9730703]
44 **Noureddin M**, Ghany MG. Pharmacokinetics and pharmacodynamics of peginterferon and ribavirin: implications for clinical efficacy in the treatment of chronic hepatitis C. *Gastroenterol Clin North Am* 2010; **39**: 649-658 [PMID: 20951922 DOI: 10.1016/j.gtc.2010.08.008]
45 **Zeuzem S**, Welsch C, Herrmann E. Pharmacokinetics of peginterferons. *Semin Liver Dis* 2003; **23** Suppl 1: 23-28 [PMID: 12934165 DOI: 10.1055/s-2003-41631]
46 **Gupta SK**, Pittenger AL, Swan SK, Marbury TC, Tobillo E, Batra V, Sack M, Glue P, Jacobs S, Affrime M. Single-dose pharmacokinetics and safety of pegylated interferon-alpha2b in patients with chronic renal dysfunction. *J Clin Pharmacol* 2002; **42**: 1109-1115 [PMID: 12362925]
47 **Bunchorntavakul C,** Reddy KR. Ribavirin: How does it work and dose it still needed? *Curr Hepatitis Rep* 2011; **10**: 168-178 [DOI: 10.1007/s11901-011-0102-6]
48 **Wade JR**, Snoeck E, Duff F, Lamb M, Jorga K. Pharmacokinetics of ribavirin in patients with hepatitis C virus. *Br J Clin Pharmacol* 2006; **62**: 710-714 [PMID: 17118126 DOI: 10.1111/j.1365-2125.2006.02704.x]
49 **Reddy KR**, Nelson DR, Zeuzem S. Ribavirin: current role in the optimal clinical management of chronic hepatitis C. *J Hepatol* 2009; **50**: 402-411 [PMID: 19091439 DOI: 10.1016/j.jhep.2008.11.006]
50 **Berenguer M**. Treatment of chronic hepatitis C in hemodialysis patients. *Hepatology* 2008; **48**: 1690-1699 [PMID: 18972442 DOI: 10.1002/hep.22545]
51 **Russmann S**, Grattagliano I, Portincasa P, Palmieri VO, Palasciano G. Ribavirin-induced anemia: mechanisms, risk factors and related targets for future research. *Curr Med Chem* 2006; **13**: 3351-3357 [PMID: 17168855]
52 **De Franceschi L**, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, Noventa F, Stanzial AM, Solero P, Corrocher R. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. *Hepatology* 2000; **31**: 997-1004 [PMID: 10733558 DOI: 10.1053/he.2000.5789]
53 **Liu CH**, Huang CF, Liu CJ, Dai CY, Liang CC, Huang JF, Hung PH, Tsai HB, Tsai MK, Chen SI, Lin JW, Yang SS, Su TH, Yang HC, Chen PJ, Chen DS, Chuang WL, Yu ML, Kao JH. Pegylated interferon-α2a with or without low-dose ribavirin for treatment-naive patients with hepatitis C virus genotype 1 receiving hemodialysis: a randomized trial. *Ann Intern Med* 2013; **159**: 729-738 [PMID: 24297189 DOI: 10.7326/0003-4819-159-11-201312030-00005]
54 **Sulkowski MS.** Hepatitis C management in special populations. Available from: URL: http: //inpractice.com/Textbooks/Hepatology/ch9_Hep_C_Management_in_Special_Populations
55 **Tischer S**, Fontana RJ. Drug-drug interactions with oral anti-HCV agents and idiosyncratic hepatotoxicity in the liver transplant setting. *J Hepatol* 2014; **60**: 872-884 [PMID: 24280292 DOI: 10.1016/j.jhep.2013.11.013]
56 **Kiser JJ**, Burton JR, Everson GT. Drug-drug interactions during antiviral therapy for chronic hepatitis C. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 596-606 [PMID: 23817323 DOI: 10.1038/nrgastro.2013.106]

57 **Simion A,** Mortier S, Peeters M, Beumont-Mauviel M, Ouwerkerk-Mahadevan S. Pharmacokinetics of simeprevir (TMC435) in volunteers with severe renal impairment. The 8th International Workshop on Clinical Pharmacology of Hepatitis Therapy. Cambridge, Massachusetts: Abstract O-03-PK, June 26-27, 2013. Available from: URL: http://www.mdlinx.com/infectious-disease/conference-details.cfm/30713/

58 **Cornpropst MT**, Denning J, Clemons D, Marbury TC, Alcorn H, Smith WB, Sale M, Fang L, Berrey MM, Symonds WT. The effect of renal impairment and end stage renal disease on the single-dose pharmacokinetics of PSI-7977. *J Hepatol* 2012; **56** (Suppl 2): S433 [DOI: 10.1016/S0168-8278(12)61113-1]

59 **Fabrizi F**, Dulai G, Dixit V, Bunnapradist S, Martin P. Meta-analysis: interferon for the treatment of chronic hepatitis C in dialysis patients. *Aliment Pharmacol Ther* 2003; **18**: 1071-1081 [PMID: 14653826]

60 **Russo MW**, Goldsweig CD, Jacobson IM, Brown RS. Interferon monotherapy for dialysis patients with chronic hepatitis C: an analysis of the literature on efficacy and safety. *Am J Gastroenterol* 2003; **98**: 1610-1615 [PMID: 12873587 DOI: 10.1111/j.1572-0241.2003.07526.x]

61 **Gordon CE**, Uhlig K, Lau J, Schmid CH, Levey AS, Wong JB. Interferon treatment in hemodialysis patients with chronic hepatitis C virus infection: a systematic review of the literature and meta-analysis of treatment efficacy and harms. *Am J Kidney Dis* 2008; **51**: 263-277 [PMID: 18215704 DOI: 10.1053/j.ajkd.2007.11.003]

62 **Fabrizi F**, Dixit V, Messa P, Martin P. Interferon monotherapy of chronic hepatitis C in dialysis patients: meta-analysis of clinical trials. *J Viral Hepat* 2008; **15**: 79-88 [PMID: 18184190 DOI: 10.1111/j.1365-2893.2007.00907.x]

63 **Rendina M**, Schena A, Castellaneta NM, Losito F, Amoruso AC, Stallone G, Schena FP, Di Leo A, Francavilla A. The treatment of chronic hepatitis C with peginterferon alfa-2a (40 kDa) plus ribavirin in haemodialysed patients awaiting renal transplant. *J Hepatol* 2007; **46**: 768-774 [PMID: 17383045 DOI: 10.1016/j.jhep.2006.12.016]

64 **American Association for the Study of Liver Diseases and Infectious Diseases Society of America.** Recommendations for testing, managing, and treating hepatitis C. Available from: URL: http: //www.hcvguidelines.org/full-report-view

65 **Sclair S,** Patel A, Junaidi O, Copado V, Martin P, Rudraraju M, Bhamidimarri KR. Telaprevir or boceprevir therapy in chronic hepatitis C patients with end stage renal disease on hemodialysis is safe, tolerable and feasible. *J Hepatol* 2013; **58**: S370-S371 [DOI: 10.1016/S0168-8278(13)60902-2]

66 **Basu PP,** Siriki R, Shah NJ, Farhat S, Mittimani K, Atluri S, Rahaman M, Brown RS. Telaprevir with adjusted dose of ribavirin in naive CHC-G1: efficacy and treatment in CHC in hemodialysis population. *J Hepatol* 2013; **58**: S30-S31 [DOI: 10.1016/S0168-8278(13)60069-0]

67 **Dumortier J**, Guillaud O, Gagnieu MC, Janbon B, Juillard L, Morelon E, Leroy V. Anti-viral triple therapy with telaprevir in haemodialysed HCV patients: is it feasible? *J Clin Virol* 2013; **56**: 146-149 [PMID: 23149155 DOI: 10.1016/j.jcv.2012.10.009]

68 **Fabrizi F**, Lunghi G, Dixit V, Martin P. Meta-analysis: anti-viral therapy of hepatitis C virus-related liver disease in renal transplant patients. *Aliment Pharmacol Ther* 2006; **24**: 1413-1422 [PMID: 17081162 DOI: 10.1111/j.1365-2036.2006.03151.x]

69 **Wei F**, Liu J, Liu F, Hu H, Ren H, Hu P. Interferon-based anti-viral therapy for hepatitis C virus infection after renal transplantation: an updated meta-analysis. *PLoS One* 2014; **9**: e90611 [PMID: 24699257 DOI: 10.1371/journal.pone.0090611]

70 **Harihara Y**, Kurooka Y, Yanagisawa T, Kuzuhara K, Otsubo O, Kumada H. Interferon therapy in renal allograft recipients with chronic hepatitis C. *Transplant Proc* 1994; **26**: 2075 [PMID: 8066675]

71 **Therret E**, Pol S, Legendre C, Gagnadoux MF, Cavalcanti R, Kreis H. Low-dose recombinant leukocyte interferon-alpha treatment of hepatitis C viral infection in renal transplant recipients. A pilot study. *Transplantation* 1994; **58**: 625-628 [PMID: 7916505]

72 **Magnone M**, Holley JL, Shapiro R, Scantlebury V, McCauley J, Jordan M, Vivas C, Starzl T, Johnson JP. Interferon-alpha-induced acute renal allograft rejection. *Transplantation* 1995; **59**: 1068-1070 [PMID: 7709447]

73 **Rostaing L**, Modesto A, Baron E, Cisterne JM, Chabannier MH, Durand D. Acute renal failure in kidney transplant patients treated with interferon alpha 2b for chronic hepatitis C. *Nephron* 1996; **74**: 512-516 [PMID: 8938673]

74 **Aljumah AA**, Saeed MA, Al Flaiw AI, Al Traif IH, Al Alwan AM, Al Qurashi SH, Al Ghamdi GA, Al Hejaili FF, Al Balwi MA, Al Sayyari AA. Efficacy and safety of treatment of hepatitis C virus infection in renal transplant recipients. *World J Gastroenterol* 2012; **18**: 55-63 [PMID: 22228971 DOI: 10.3748/wjg.v18.i1.55]

75 **Pageaux GP**, Hilleret MN, Garrigues V, Bismuth M, Audin-Mamlouk H, Zarski JP, Mourad G. Pegylated interferon-alpha-based treatment for chronic hepatitis C in renal transplant recipients: an open pilot study. *Transpl Int* 2009; **22**: 562-567 [PMID: 19175562 DOI: 10.1111/j.1432-2277.2008.00831.x]

76 **Sanai FM**, Mousa D, Al-Mdani A, Al-Shoail G, Al-Ashgar H, Al Meshari K, Al-Qahtani A, Saadeh M, Bzeizi KI, Aleid H. Safety and efficacy of peginterferon-α2a plus ribavirin treatment in renal transplant recipients with chronic hepatitis C. *J Hepatol* 2013; **58**: 1096-1103 [PMID: 23428875 DOI: 10.1016/j.jhep.2013.02.004]

77 **Bunchorntavakul C**, Reddy, KR. Management of hepatitis C before and after liver transplantation in the era of rapidly evolving therapeutic advances. *J Clin Translational Hepatol* 2014; **2:** 124-133 [DOI: 10.14218/JCTH.2014.00002]

78 **Ouwerkerk-Mahadevan S,** Simion A, Mortier S, Peeters M, Beumont M. No clinically significant interaction between the investigational HCV protease inhibitor, TMC435 and the immunosuppressives cyclosporine and tacrolimus. *Hepatology* 2012; **58**: 213A

79 **Ferri C**, Antonelli A, Mascia MT, Sebastiani M, Fallahi P, Ferrari D, Pileri SA, Zignego AL. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Dig Liver Dis* 2007; **39** Suppl 1: S13-S21 [PMID: 17936215]

80 **Fabrizi F**, Plaisier E, Saadoun D, Martin P, Messa P, Cacoub P. Hepatitis C virus infection, mixed cryoglobulinemia, and kidney disease. *Am J Kidney Dis* 2013; **61**: 623-637 [PMID: 23102733 DOI: 10.1053/j.ajkd.2012.08.040]

81 **Charles ED**, Dustin LB. Hepatitis C virus-induced cryoglobulinemia. *Kidney Int* 2009; **76**: 818-824 [PMID: 19606079 DOI: 10.1038/ki.2009.247]

82 **Lauletta G**, Russi S, Conteduca V, Sansonno L, Dammacco F, Sansonno D. Impact of Cryoglobulinemic Syndrome on the Outcome of Chronic Hepatitis C Virus Infection: A 15-Year Prospective Study. *Medicine* (Baltimore) 2013 Aug 26; Epub ahead of print [PMID: 23982056 DOI: 10.1097/MD.0b013e31829d2abc]

83 **El-Serag HB**, Hampel H, Yeh C, Rabeneck L. Extrahepatic manifestations of hepatitis C among United States male veterans. *Hepatology* 2002; **36**: 1439-1445 [PMID: 12447870 DOI: 10.1053/jhep.2002.37191]

84 **Huang JF**, Chuang WL, Dai CY, Ho CK, Hwang SJ, Chen SC, Lin ZY, Wang LY, Chang WY, Yu ML. Viral hepatitis and proteinuria in an area endemic for hepatitis B and C infections: another chain of link? *J Intern Med* 2006; **260**: 255-262 [PMID: 16918823 DOI: 10.1111/j.1365-2796.2006.01686.x]

85 **Tsui JI**, Vittinghoff E, Shlipak MG, O'Hare AM. Relationship between hepatitis C and chronic kidney disease: results from the Third National Health and Nutrition Examination Survey. *J Am Soc Nephrol* 2006; **17**: 1168-1174 [PMID: 16524948 DOI: 10.1681/asn.2005091006]

86 **Antonelli A**, Ferrari SM, Giuggioli D, Di Domenicantonio A, Ruffilli I, Corrado A, Fabiani S, Marchi S, Ferri C, Ferrannini E, Fallahi P. Hepatitis C virus infection and type 1 and type 2 diabetes mellitus. *World J Diabetes* 2014; **5**: 586-600 [PMID: 25317237 DOI: 10.4239/wjd.v5.i5.586]

87 **Dammacco F**, Sansonno D. Therapy for hepatitis C virus-related cryoglobulinemic vasculitis. *N Engl J Med* 2013; **369**: 1035-1045 [PMID: 24024840 DOI: 10.1056/NEJMra1208642]

88 **Monti G**, Galli M, Invernizzi F, Pioltelli P, Saccardo F, Monteverde

A, Pietrogrande M, Renoldi P, Bombardieri S, Bordin G. Cryoglobulinaemias: a multi-centre study of the early clinical and laboratory manifestations of primary and secondary disease. GISC. Italian Group for the Study of Cryoglobulinaemias. *QJM* 1995; **88**: 115-126 [PMID: 7704562]

89 **Agnello V**, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; **327**: 1490-1495 [PMID: 1383822 DOI: 10.1056/nejm199211193272104]

90 **Ferri C**, Sebastiani M, Giuggioli D, Cazzato M, Longombardo G, Antonelli A, Puccini R, Michelassi C, Zignego AL. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum* 2004; **33**: 355-374 [PMID: 15190522]

91 **Antonelli A**, Ferri C, Ferrari SM, Colaci M, Fallahi P. Immunopathogenesis of HCV-related endocrine manifestations in chronic hepatitis and mixed cryoglobulinemia. *Autoimmun Rev* 2008; **8**: 18-23 [PMID: 18708169 DOI: 10.1016/j.autrev.2008.07.017]

92 **Wong VS**, Egner W, Elsey T, Brown D, Alexander GJ. Incidence, character and clinical relevance of mixed cryoglobulinaemia in patients with chronic hepatitis C virus infection. *Clin Exp Immunol* 1996; **104**: 25-31 [PMID: 8603528]

93 **Ramos-Casals M**, Stone JH, Cid MC, Bosch X. The cryoglobulinaemias. *Lancet* 2012; **379**: 348-360 [PMID: 21868085 DOI: 10.1016/s0140-6736(11)60242-0]

94 **Landau DA**, Scerra S, Sene D, Resche-Rigon M, Saadoun D, Cacoub P. Causes and predictive factors of mortality in a cohort of patients with hepatitis C virus-related cryoglobulinemic vasculitis treated with antiviral therapy. *J Rheumatol* 2010; **37**: 615-621 [PMID: 20110523 DOI: 10.3899/jrheum.090790]

95 **Roccatello D**, Fornasieri A, Giachino O, Rossi D, Beltrame A, Banfi G, Confalonieri R, Tarantino A, Pasquali S, Amoroso A, Savoldi S, Colombo V, Manno C, Ponzetto A, Moriconi L, Pani A, Rustichelli R, Di Belgiojoso GB, Comotti C, Quarenghi MI. Multicenter study on hepatitis C virus-related cryoglobulinemic glomerulonephritis. *Am J Kidney Dis* 2007; **49**: 69-82 [PMID: 17185147 DOI: 10.1053/j.ajkd.2006.09.015]

96 **Arase Y**, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, Suzuki Y, Saitoh S, Kobayashi M, Kobayashi M, Kobayashi M, Kumada H. Glomerulonephritis in autopsy cases with hepatitis C virus infection. *Intern Med* 1998; **37**: 836-840 [PMID: 9840704]

97 **Latt N**, Alachkar N, Gurakar A. Hepatitis C virus and its renal manifestations: a review and update. *Gastroenterol Hepatol* (N Y) 2012; **8**: 434-445 [PMID: 23293553]

98 **Pietrogrande M**, De Vita S, Zignego AL, Pioltelli P, Sansonno D, Sollima S, Atzeni F, Saccardo F, Quartuccio L, Bruno S, Bruno R, Campanini M, Candela M, Castelnovo L, Gabrielli A, Gaeta GB, Marson P, Mascia MT, Mazzaro C, Mazzotta F, Meroni P, Montecucco C, Ossi E, Piccinino F, Prati D, Puoti M, Riboldi P, Riva A, Roccatello D, Sagnelli E, Scaini P, Scarpato S, Sinico R, Taliani G, Tavoni A, Bonacci E, Renoldi P, Filippini D, Sarzi-Puttini P, Ferri C, Monti G, Galli M. Recommendations for the management of mixed cryoglobulinemia syndrome in hepatitis C virus-infected patients. *Autoimmun Rev* 2011; **10**: 444-454 [PMID: 21303705 DOI: 10.1016/j.autrev.2011.01.008]

99 **Levine JW**, Gota C, Fessler BJ, Calabrese LH, Cooper SM. Persistent cryoglobulinemic vasculitis following successful treatment of hepatitis C virus. *J Rheumatol* 2005; **32**: 1164-1167 [PMID: 15940780]

100 **Fabrizi F**, Lunghi G, Messa P, Martin P. Therapy of hepatitis C virus-associated glomerulonephritis: current approaches. *J Nephrol* 2008; **21**: 813-825 [PMID: 19034865]

101 **Gragnani L**, Fabbrizzi A, Triboli E, Urraro T, Boldrini B, Fognani E, Piluso A, Caini P, Ranieri J, Monti M, Laffi G, Zignego AL. Triple antiviral therapy in hepatitis C virus infection with or without mixed cryoglobulinaemia: a prospective, controlled pilot study. *Dig Liver Dis* 2014; **46**: 833-837 [PMID: 24953206 DOI: 10.1016/j.dld.2014.05.017]

102 **Saadoun D**, Resche Rigon M, Thibault V, Longuet M, Pol S, Blanc F, Pialoux G, Karras A, Bazin-Karra D, Cazorla C, Vittecoq D, Musset L, Decaux O, Ziza JM, Lambotte O, Cacoub P. Peg-IFNα/ribavirin/protease inhibitor combination in hepatitis C virus associated mixed cryoglobulinemia vasculitis: results at week 24. *Ann Rheum Dis* 2014; **73**: 831-837 [PMID: 23606708 DOI: 10.1136/annrheumdis-2012-202770]

103 **Stine JG**, Cornella S, Shah NL. Treatment of chronic hepatitis C complicated by mixed cryoglobulinemia with new protease inhibitor, sofosbuvir. *Ann Rheum Dis* 2014; **73**: e64 [PMID: 25038237 DOI: 10.1136/annrheumdis-2014-206180]

104 **Ferri C**, Cacoub P, Mazzaro C, Roccatello D, Scaini P, Sebastiani M, Tavoni A, Zignego AL, De Vita S. Treatment with rituximab in patients with mixed cryoglobulinemia syndrome: results of multicenter cohort study and review of the literature. *Autoimmun Rev* 2011; **11**: 48-55 [PMID: 21821153 DOI: 10.1016/j.autrev.2011.07.005]

105 **Dammacco F**, Tucci FA, Lauletta G, Gatti P, De Re V, Conteduca V, Sansonno S, Russi S, Mariggiò MA, Chironna M, Sansonno D. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. *Blood* 2010; **116**: 343-353 [PMID: 20308602 DOI: 10.1182/blood-2009-10-245878]

106 **Morales JM**, Kamar N, Rostaing L. Hepatitis C and renal disease: epidemiology, diagnosis, pathogenesis and therapy. *Contrib Nephrol* 2012; **176**: 10-23 [PMID: 22310777 DOI: 10.1159/000333772]

107 **Stefanutti C**, Vivenzio A, Di Giacomo S, Labbadia G, Mazza F, D'Alessandri G, Ferraro PM, Masala C. Immunoadsorption apheresis and immunosuppressive drug therapy in the treatment of complicated HCV-related cryoglobulinemia. *J Clin Apher* 2009; **24**: 241-246 [PMID: 19927363 DOI: 10.1002/jca.20222]

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Micro RNAs in the development of non-alcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease or nonalcoholic fatty liver disease (NAFLD) refers to a group of disorders that arise from the accrual of fat in hepatocytes. Although various factors have been associated with the development of NAFLD, including genetic predisposition and environmental exposures, little is known about the underlying pathogenesis of the disease. Research efforts are ongoing to identify biological targets and signaling pathways that mediate NAFLD. Emerging evidence has implicated a role for micro RNAs (miRNAs), short single-stranded molecules that regulate gene expression either transcriptionally, through targeting of promoter regions, or post-transcriptionally, by blocking translation or promoting cleavage of specific target mRNAs. Several miRNAs have been associated with NAFLD, although our understanding of the biology underlying their role is still emerging. The goal of this review is to present an overview of the current state of knowledge of miRNAs involved in the development of NAFLD across a range of *in vitro* and *in vivo* models, including miRNAs that contribute to pathological mechanisms related to fatty liver in humans. Much less is known about the specific targets of miRNAs in cells, nor the molecular mechanisms involved in the development and progression NAFLD and related outcomes. More recently, the identification and validation of miRNA signatures in serum may facilitate the development of improved methods for diagnosis and clinical monitoring of disease progression.

Key words: MiRNA; Nonalcoholic fatty liver disease; Cell culture; Mouse; Human

Core tip: Available data on miRNAs in nonalcoholic fatty liver disease (NAFLD) are largely derived from various cell culture and animal models. Reflecting an emerging field, little cross-model concordance is present and few human data are available for comparison with cell culture and animal model results. Although the generation of human data may be limited by the availability of tissue samples, recent reports of circulating miRNAs from NAFLD patients hold promise for significant progress for diagnosis and clinical

monitoring of disease progression.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of conditions resulting from excessive accumulation of fat in hepatocytes, a condition known as steatosis. NAFLD can be categorized into nonalcoholic fatty liver representing simple steatosis and non-alcoholic steatohepatitis (NASH) with coincident hepatocyte injury, liver inflammation, and fibrosis. NAFLD is the major cause of chronic liver disease (CLD), which is associated with substantial morbidity and mortality in developed countries[1]. In tandem with the rising rates of obesity and type 2 diabetes mellitus (T2D), the prevalence of NAFLD is also increasing, and is expected to double by 2030 in the United States. NAFLD/NASH has thus become a leading cause of cryptogenic cirrhosis[2] and is currently the 3rd leading clinical indication for liver transplantation in the United States and expected to soon become the primary indication[3]. At present liver biopsy is the only method to accurately assess the severity of liver fibrosis[1] or predict progression of NAFLD to clinically severe forms[4-6], which limits early diagnosis of NAFLD patients who are at high risk for development of liver-related morbidity and mortality. Given the substantial public health burden of NAFLD, novel therapeutic targets are also urgently needed to facilitate the development of improved pharmacological therapies for the treatment and prevention of the disease.

Although genetic predisposition, environmental exposures, and lifestyle factors contribute to the development of NAFLD, little is known about the underlying pathogenesis of the disease. Ongoing research efforts to identify biological targets and signaling pathways that mediate NAFLD are expected to provide the insight necessary to begin to distinguish among different clinical forms of the disease.

Emerging evidence has implicated a role for epigenetic factors, including micro RNAs (miRNAs) in the development of NAFLD. MiRNAs are endogenous, single-stranded RNAs (21-25 nucleotides in length) that regulate gene expression either post-transcriptionally, by blocking translation or promoting cleavage of specific target mRNAs, or transcriptionally, through targeting of promoter regions[7]. Thus, they do not code for proteins, but instead serve to regulate the expression of certain genes. More than 2500 miRNAs may be encoded in the human genome, residing in intergenic regions, introns, and within exons[8]. MiRNAs go through a complex processing pathway following transcription by RNA polymerase Ⅱ (RNA Pol Ⅱ), which has been described in detail elsewhere[9]. MiRNAs can be found both in cells and circulating in the blood, and have the potential to be taken up at sites distant from the cell of origin; as such they may serve as biomarkers of disease processes. They may also act in an endocrine or paracrine fashion to regulate expression at multiple sites. It is not yet known whether miRNAs have specific cell surface receptors or whether they target cells that express their target mRNAs.

MiRNAs have been found to regulate processes relevant to the development and progression of NAFLD; however, our understanding of the biology underlying these processes is presently in its infancy. The goal of this review is to present an overview of the current state of knowledge of miRNAs involved in the development of NAFLD across a range of appropriate *in vitro* and *in vivo* models, with a special focus on miRNAs that contribute to pathological mechanisms related to fatty liver in humans.

In vitro studies

A number of studies have been conducted on miRNAs using cell culture models of NAFLD, primarily those derived from hepatocyte cell lines (Table 1). In one study, immortalized human liver-derived L02 cells, cultured with high levels of free fatty acids (HFFA-treated) to serve as a model for hepatic steatosis, were analyzed using miRNA micro-array[10]. A total of 17 and 15 miRNAs were up- or downregulated, respectively, in these HFFA-treated L02 cells. Of these, miR-10b was the most up-regulated miRNA, and HFFA-cultured L02 cells transfected with anti-miR-10b showed significantly decreased lipid content and the triglyceride level (*i.e.,* steatosis). Peroxisome proliferator-activated receptor-a (*PPARA*) was identified as a potential target for miR-10b, and expression of both transcript and protein levels of *PPARA* were reduced in steatotic L02 cells. Overexpression of miR-10b in HFFA-cultured L02 cells, led to decreased PPARA protein levels, while miR-10b knockdown increased PPARA, indicating that this miRNA may regulate the development of hepatic steatosis through mechanisms involving the *PPARA* pathway. Further investigation involving both animal and human studies will be necessary to confirm this relationship.

In an independent study to identify miRNAs involved in the formation of lipid droplets, the human hepatocellular carcinoma-derived cell line Huh7 was transiently transfected with a library of 327 miRNAs[11]. The Huh7 cell line spontaneously accumulates lipid droplets in culture and is lipogenic, making it an

Table 1 Summary of *in vitro* studies of miRNAs relevant to fatty liver

Model	miRNAs	Biological effect	Validated targets	Ref.
L02	miRNA-10b	Inc expr, decrease steatosis	PPARA	[10]
Huh7	miR-181d	Inc expr, decrease steatosis	Not determined	[11]
Huh7 and Hep3B	miR-122	Dec exp, decrease steatosis	SOCS3	[14]
HepG2	miR-613	Incr exp, increase steatosis	LXRα	[15]
HepG2 and primary human hepatocytes	miR-107	Incr expression, increase steatosis	FASN	[16]

appropriate *in vitro* model for steatosis[12]. Following primary and secondary screening, eleven miRNAs were identified that either increased or decreased intracellular lipid content. Of these, miR-181d showed the strongest influence on steatosis, decreasing lipid droplet formation by approximately 60%. Huh7 cells were also used, along with Hep3B human hepatocellular carcinoma cells, to link expression of miR-122 to decreased fatty acid and cholesterol levels[13]. Expression of the suppressor of cytokine signaling 3 (*SOCS3*) gene is also regulated by miR-122[14]. SOCS3 protein increases expression of sterol regulatory element-binding protein 1 (SREBP1), a transcription factor that regulates cholesterol and lipid metabolism. Silencing of miR-122 in Huh7 cells corresponded with reduced SOCS3 expression, which in turn decreased SREBP1 levels, while restoration of SREBP1 expression when miR-122 levels were depleted through RNA silencing could be achieved by over-expression of SOCS3.

In addition to L02 and Huh7 cells, HepG2 cells have also been used as a model in which to study the role of miRNAs in hepatic lipid metabolism. In these cells, over-expression of miR-613 reduced expression of the nuclear receptor liver X receptor α (LXRA) and several of its target genes including acetyl-CoA carboxylase, sterol-regulatory element binding protein 1c, and fatty acid synthase, and led to the formation of lipid droplets[15]. miR-613 was shown to bind to the 3′-untranslated region of the *LXRA* mRNA. Similarly, miR-107 was shown to bind to the 3′ UTR of the fatty acid synthase gene (*FASN*), reducing its expression and causing malonyl CoA and lipid accumulation in both HepG2 cells and primary hepatocytes[16].

As evident from the studies described, the inherent advantage of cultured cell systems for the study of miRNA physiology in NAFLD is in their ease and economy of manipulating levels of specific molecules. However, NAFLD occurs in the context of multiple cell types that constitute the liver and with molecular interactions with distant cell types and organs; therefore studies involving *in vivo* models have been important in enhancing our understanding of the role miRNAs play in the development of the disease.

In vivo studies

A number of studies of miRNAs have been conducted using animal models of fatty liver, primarily in mice and rats[17], in whom NAFLD is typically induced with a high fat diet. A summary of the main findings from these studies is shown in Table 2. In one study, microarray analysis of 350 miRNAs in liver samples of sprague-dawley rats with diet-induced NASH showed downregulation of miR-122, miR-451, and miR-27a and upregulation of miR-429, miR-200a, and miR-200b compared to animals fed a standard diet[18]. In a similar microarray-based study of diet-induced NASH in sprague-dawley rats, the authors observed that upregulation of miR-146a, miR-210, miR-29c, miR-103, miR-20b-5p, miR-106b, miR-212, miR-31, miR-10a, miR-203, miR-27b, miR-199a, miR-107, let-7b, and downregulation of miR-33, miR-145, miR-196b, miR-93, let-7d, miR-19 could differentiate between steatohepatitis and steatosis[7]. No common mRNA targets were found for the 14 upregulated miRNAs, but 12 common targets were found for the six downregulated miRNAs including stearoyl-coenzyme A desaturase 1 (Scd1). Hepatic expression of miR-15b was also shown to be upregulated in liver RNA of Sprague Dawley rats fed a high fat diet for 16 wk[19]. Surprisingly, no miRNAs were replicated across these rat studies, possibly due to differences in dietary composition and regimen, as well as phenotypic endpoint.

MiRNA microarray analysis of liver RNA from both C57BL/6J and DBA/2J inbred strains of mice fed a lipogenic methyl-deficient diet to induce a form of fatty liver injury similar to human NASH identified significant upregulation in the expression of miR-34a, miR-155, and miR-200b, and downregulation of miR-29c[17]. A strain-specific effect was seen, with more significant changes occurring in DBA/2J mice.

In livers of C57BL/6J mice fed a high fat diet for eight weeks, miR-467b expression was significantly decreased, corresponding to an increase in hepatic lipoprotein lipase (LPL) expression[20]. The authors utilized bioinformatics sequence analysis to identify LPL as a direct target of miR-467b and confirmed the miRNA-mRNA interaction *in vitro*. Interestingly, the interaction between miR-467b and its target gene was associated with insulin resistance, which strongly increases the risk of NAFLD. In a separate study, apoE(-/-) mice treated with intra-peritoneal injection of an miR-467b mimic or agomirna (synthetic chemically modified RNA duplexes) led to reduced lipid accumulation and inflammatory cytokine secretion by macrophages *via* downregulation of LPL expression,

Table 2 MiRNAs associated with nonalcoholic fatty liver disease

miRNA	Species	Model	Ref.
Downregulation of miR-122, miR-451, and miR-27a and upregulation of miR-429, miR- 200a, and miR-200b	Sprague-dawley rats	High fat diet induced NASH *vs* standard diet	[18]
Upregulation of miR-146a, miR-210, miR-29c, miR-103, miR-20b.5p, miR-106b, miR-212, miR-31, miR-10a, miR-203, miR-27b, miR-199a, miR-107, let-7b, and downregulation of miR-33, miR-145, miR-196b, miR-93, let-7d, miR-19	Sprague-dawley rats	High fat diet induced NASH *vs* steatosis	[7]
miR-15b	Sprague-dawley rats	High fat diet induced NASH *vs* standard diet	[19]
Decreased expression miR-29c, and increased expression miR-34a, miR-155, and miR-200b	C57BL/6J and DBA/2J mice	methyl-deficient diet induced NASH	[17]
Decreased miR-467b	C57BL/6J mice	High fat diet *vs* standard diet	[20]
Increased miR-103 and miR-107	C57BL/6J mice	High fat diet *vs* standard diet	[22]
Decreased miR-21	C57BL/6J mice	High fat diet *vs* standard diet	[23]
miR-122 decreased in all strains, while expression of miR-34a, miR-200b, and miR-181a	A/J, C57BL/6J, C3H/HeJ, 129S/SvImJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ strains	choline- and folate-deficient diet	[24]
miR-122 (-/-)	129SvJ	Steatosis on a Normal Diet	[25]
miR-155 (-/-)	C57BL/6	High fat diet *vs* standard diet	[26]
Up regulation of miR-122, miR-24, miR-195a, miR-106b, miR-15b, miR-802, miR-185, miR-214, miR-378, and let-7c; downregulation of miR-224, miR-126, miR-7a, miR-128, miR-455, miR-452, miR-135b, miR-145, miR-18a, and miR-196a	*ob/ob*	Standard diet	[27]
Increased miR-16, miR-122, miR-126 decreased miR-27b	Gankyrin transgenic zebrafish	Standard diet	[29]

NASH: Non-alcoholic steatohepatitis.

leading to protection from atherosclerosis in these animals[21]. Together, these findings suggest that the miR-467b-LPL interaction may play an important role in lipid accumulation, which may exert diverse effects on the development of both hepatic steatosis and atherosclerotic vascular disease.

In LDL receptor knockout ($LDLR^{-/-}$) mice derived from a C57BL/6J background and fed a high-fat diet for 10 wk, increased expression of miR-103 and miR-107 was abolished by daily dosing of a mixture of concentrated plant-derived polyphenol compounds[22], although weight gain and liver steatosis were ameliorated. The expression of miR-122 was not altered by the high fat diet, but was decreased by dietary polyphenols. Further studies on polyphenol administration for 8 wk to C57BL/6J mice fed a high-fat diet demonstrated that lycopene also ameliorated hepatic steatosis and prevented down-regulation of miR-21[23]. Expression of fatty acid-binding protein 7 (FABP7) was downregulated *via* interaction of miR-21 with the FABP7 3′ UTR.

Administration of a choline- and folate-deficient diet for 12 wk to induce NAFLD-like liver injury in inbred male mice of the A/J, C57BL/6J, C3H/HeJ, 129S/SvImJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ strains induced strain-related differences in levels of hepatic and plasma miRNAs[24]. Hepatic expression of miR-122 decreased in all strains, while expression of miR-34a, miR-200b, and miR-181a increased and was correlated with histological severity of liver injury. Serum levels of miR-34a, miR-122, miR-181a, miR-192, miR-200b, and miR-221 were correlated with histological severity across all strains, providing evidence that release of miRNAs may serve as biomarkers of liver injury.

Knockout studies have also been conducted targeting specific miRNAs, although few have been reported thus far. A recent study by Hsu *et al*[25], in which mice with either germline or liver-specific knockdown of miR-122 were generated, showed that deficient animals developed steatosis, steatohepatitis, fibrosis, and spontaneous tumors that were histologically similar to hepatocellular carcinoma. These findings support a critical role for miR-122 in mediating the progression of steatosis to more clinically severe phenotypes and the subsequent development of cancer.

An independent study also using knockout mice investigated the role of a second miRNA, miR-155, in the development of hepatic steatosis[26]. In that study, $miR\text{-}155^{-/-}$ mice fed a high fat diet for six months developed significantly more hepatic steatosis, which was associated with increased liver weight and lipid levels, than C57BL/6 wild-type controls. Hepatic expression of genes involved in glucose regulation, fatty acid uptake, and lipid metabolism were also elevated in the $miR\text{-}155^{-/-}$ mice. Among the differentially expressed genes, the authors identified and validated only one, Nr1h3 (LXRα) as a direct target of miR-155. Together these data indicate that miR-155 plays a protective role in liver lipid metabolism and that downregulation of miR-155 expression may contribute to the development of hepatic steatosis.

In addition to microarray and knockdown studies of specific miRNAs, approaches using next generation sequence analysis have also been used to quantify miRNA expression in NAFLD. Sequencing of liver

non-coding RNA in *ob/ob* and control mice identified 37 differentially expressed hepatic miRNAs[27]. Although miR-122 showed the greatest alteration in expression between the two groups, miR-24, miR-195a, miR-106b, miR-15b, miR-802, miR-185, miR-214, miR-378, and let-7c were also significantly upregulated. In contrast, levels of miR-224, miR-126, miR-7a, miR-128, miR-455, miR-452, miR-135b, miR-145, miR-18a, and miR-196a were significantly downregulated. To determine whether overexpression of miR-126 or inhibition of miR-24 played a mechanistic role, AML-12 liver cells were treated with free fatty acids. Up-regulation of hepatic miR-126 using a miR-126 mimic or down-regulation of hepatic miR-24 using antagomiR-24 was correlated with decreased fat accumulation, suggesting that both may potentially mediate liver steatosis. Additional studies will be necessary to address this possibility.

In addition to mice and rats, zebrafish are presently becoming recognized as suitable models for lipid-related diseases, including hepatic steatosis[28]. In this model, transgenic over-expression of gankyrin, a small ankyrin-repeat protein that plays a role in cellular proliferation, led to increased lipid content in > 90% of viable adult fish. Overexpression of gankyrin led to the development of hepatic steatosis and was associated with increased levels of miR-16, miR-122, and miR-126, and decreased miR-27b[29]. This study provides evidence supporting a link between gankyrin and miRNAs in modulating the development of hepatic steatosis in zebrafish; however, the role of this network in humans is not yet known.

HUMAN STUDIES

A large number of studies on the role of miRNAs in viral hepatitis and hepatocellular carcinoma in humans have been reported[30], which is in contrast to the relatively limited investigations of these molecules in modulating the pathogenesis of NAFLD and liver-related outcomes. Of the studies that have been published, most have been performed using very small sample sizes, thereby limiting the impact of any conclusions drawn from the results. For example, one study recently profiled liver miRNA in 15 individuals with NASH and 15 individuals with normal liver histology[31]. Out of the 474 miRNAs represented on the array, six showed differential expression between the two groups. The authors confirmed overexpression of miR-34a and miR-146 b and underexpression of miR-122 using RT-PCR, although miRNA levels were not associated with NASH severity. Examination of miR-122 target genes, including SREBP-1c, FAS, and HMG-CoA reductase, showed significant increases in mRNA and protein levels in individuals with NASH, which is consistent with *in vitro* findings in HepG2 cells following miR-122 silencing. Similarly, an inverse correlation between miR-122 and levels of SOCS3 and SREBP1 was observed in human liver samples[13]. In obese individuals, levels of miR-34a were approximately 2-fold higher in mild NASH, increasing to more than 3-fold higher in severe NASH relative to steatosis[32]. In comparisons of steatotic and severe NASH liver samples, levels of miR-122, miR-143, and miR-451 were decreased.

In addition to studies using liver tissue, miRNA levels in adipose tissue have also been investigated. A total of 664 miRNAs were profiled in visceral adipose tissue obtained from 12 extremely obese bariatric surgery patients with biopsy-proven NASH and 12 with without NASH[33]. Expression of miR-132, miR-150, miR-433, miR-28-3p, miR-511, miR-517a, and miR-671-3p were all significantly decreased in individuals with NASH. In addition, expression of miR-197 and miR-99 were also decreased in NASH peri-sinusoidal fibrosis compared to non-fibrosis. *IL6* was identified as a target gene for all seven miRNAs, and the authors found that serum IL6 levels were inversely correlated with levels of these candidates. A study with a similar sample size was also conducted using visceral adipose tissue obtained from patients undergoing bariatric surgery[34]. In that study, Drosha, DGCR8, and Dicer1, all of which represent key components of miRNA processing, and seven pri-miRNAs including pri-miR-125b-2, pri-miR-16-2, pri-miR-26a-1, pri-miR-26a-2, pri-miR-7-1, pri-miR-7-2, and pri-miR-7-3 were assayed. Of these, levels of Dicer1, Drosha, DGCR8, and pri-miR-7-1 were significantly increased in NASH patients compared to normal controls. These results indicate that even in the context of severe obesity, specific miRNAs may serve to differentiate liver function, although the small sample sizes of these studies limit the generalizability of the results.

In addition to expression in tissue, miRNAs can also circulate freely in blood or be packaged within microvesicles that provide a high level of protection from degradation. Some studies report a correlation of miRNA levels in tissue and biofluids. A summary of findings from studies of circulating miRNAs in NAFLD is shown in Table 3. For example, levels of miR-122 in serum and liver were significantly correlated (R = 0.461; P = 0.005) in patients with NAFLD[35], suggesting that miR-122 released from hepatic cells enters the bloodstream. Serum levels of miR-122 were lower in individuals with mild steatosis, compared to those with severe steatosis, but higher in patients with mild fibrosis compared to those with severe fibrosis. This result is in agreement with those of previous studies, reporting decreased levels of hepatic miR-122 at advanced stages of fibrosis in patients with liver disease[36]. The reason for the

Table 3 Changes in circulating miRNAs associated with nonalcoholic fatty liver disease in humans

Population	Study design	Source of miRNA	miRNAs	Effect	Ref.
NAFLD	Mild *vs* Severe steaosis; Severe *vs* Mild fibrosis	Serum	miR-122	Decrease	[35]
NAFLD	NAFLD *vs* normal	Serum	miR-122, miR-34a, miR-16, and miR-21	Increased	[36]
NAFLD	NAFLD *vs* normal	Serum	miR-15b	Increased	[19]
NAFLD	NAFLD *vs* normal	Serum	miR-122, miR-192, miR-19a, miR-19b, miR-125b, and miR-375	Increased	[37]

NAFLD: Nonalcoholic fatty liver disease.

discrepancy in miR-122 levels in NAFLD stage may represent the loss of hepatocytes in worsening liver injury. Because hepatocytes are the primary source of miR-122 and since worsening of liver fibrosis results in the replacement of hepatocytes with extracellular matrix, hepatic miR-122 levels may be expected to decrease with severe fibrosis. These results indicate that levels of miR-122 may have significant prognostic value for patients with NAFLD.

Similarly, Cermelli *et al*[36] investigated serum levels of four miRNAs commonly dysregulated in liver fibrosis: miR-122, miR-34a, miR-16, and miR-21. In a study sample comprised of 34 individuals with NAFLD and 19 healthy controls, serum levels of miR-122, miR-34a, and miR-16 were significantly higher in NAFLD patients. Levels of miR-21 showed no difference between the two groups. Interestingly, levels of miR-122 and miR-34a were positively correlated with disease severity from simple steatosis to steatohepatitis, supporting the potential value of these two miRNAs to serve as noninvasive biomarkers for progressive NAFLD.

Zhang *et al*[19] also recently examined miR-15b as a potential biomarker for NAFLD in 69 individuals with fatty liver and 42 healthy controls. Levels of miR-15b were higher in the NAFLD patients compared to the control group. However, because there were significant differences in BMI, blood glucose, triglyceride levels, total cholesterol, and ALT between the two groups, these findings must be interpreted with caution. Additional studies will be necessary to demonstrate the appropriateness of miR-15b as a biomarker for fatty liver disease.

A case hyphen control, multi-phased study that analyzed 84 miRNAs in serum of patients with biopsy-proven NAFLD and healthy controls identified a greater than 2-fold up-regulation of miR-122, miR-192, miR-19a, miR-19b, miR-125b, and miR-375 with steatosis or more advanced NAFLD[37]. Only miR-122 was associated with severe *vs* no or mild fibrosis. Interestingly, miR-122 was 10-fold and miR-192 2-fold down-regulated in the liver. In situ hybridization of miR-122 in liver showed that the miRNA staining was concentrated at the hepatocyte membrane, not more broadly distributed throughout the cytoplasm, consistent with preparation for export to the circulation.

CROSS STUDY COMPARISONS

About a dozen miRNAs have been analyzed in two or more model systems (Table 4). miR-122, the most studied, was also the most discrepant across systems. miR-122, the most abundantly expressed miRNA in hepatocytes has been associated with a variety of liver diseases[38], suggesting complex regulation. Indeed, each miRNA may play a role in regulating the expression of tens to hundreds of genes, which may be regulated by multiple miRNAs. This complex regulatory landscape may thus have species specificity, accounting for differences within or across model systems. Mechanistic differences in the protocols for inducing NAFLD may also be reflected in differential miRNA expression. For example, high fat diet induced NAFLD has substantial differences with a choline- and folate-deficient diet that are very different than dyslipidemic mouse knockout models. Indeed, evidence suggests strain background also has substantial effect on miRNA levels, further implicating complex regulation.

The discrepancy between a decrease observed for human liver and an increase in circulating miR-122 is more difficult to reconcile, although little is known about the metabolism of miRNAs in circulation. In addition, serum levels will reflect the total body levels of the miRNA, thus contributions from other tissues may compensate for decreased levels in the liver. The relative severity of NAFLD may also be related to the levels in the serum, particularly if there is significant ballooning degeneration and hepatocyte cell death with subsequent release of miRNAs.

Despite the discrepant results, the change in expression with NAFLD of 10 of the 12 miRNAs were found to be in the same direction, although seven were only studied in rats and mice. However, three zebrafish miRNAs were concordant with those found in either mice or rats. The general agreement in results between two rodent species, as well as between rodents and zebrafish, suggests that the biology may be conserved. Cross-model concordance between evolutionarily distant species

Table 4 Cross-study comparison of miRNA changes with nonalcoholic fatty liver disease

	Cell lines	Mice	Rats	Zebrafish	Human liver	Human serum
MiR-122		INC/NC/DEC	DEC	INC	DEC	INC
miR-34a	INC/DEC	INC	INC		INC	
miRNA-107		INC	INC			
miR-29c		INC	INC			
miR-200b		INC	INC			
miR-103		INC	INC			
mir-106b		INC	INC			
miR-15b		INC	INC			
miR-126		DEC		DEC		
MiR-145		DEC	DEC			
miR-451			DEC		DEC	
miR-27b			INC	INC		

also suggests functional relevancy. Unfortunately, few human data are available for comparison with animal model results. The need for additional human data is largely limited by the availability of tissue samples. Future studies based on large cohorts of well annotated and high quality biological samples and clinical data are needed.

CONCLUSION

miRNAs may act either independently or interactively with environmental exposures and lifestyle factors to affect susceptibility to hepatic fat accumulation. Thus, the link between miRNA and progression of fatty liver disease represents a key area of focus for research endeavors in the development of novel therapies targeting control and prevention of NASH. Given the substantial public health burden of NAFLD, which is increasing at alarming rates due to the rising prevalence of obesity, novel therapeutic targets are urgently needed to facilitate the development of improved pharmacological therapies for the treatment and prevention of the disease.

In NAFLD, a small number of miRNAs, most notably miR-122, have emerged as potential participants in the regulation of biological processes relevant to the disease. However, much less is known of the specific targets of candidate miRNAs, and how, in fact, they affect disease development and progression and variability in liver-related outcomes in affected individuals. Studies aimed at delineating specific miRNA/mRNA networks will enhance our understanding of the complex pathogenesis of NAFLD and enable exploitation of relevant miRNAs as novel targets for therapeutic interventions. Notably, identification and validation of circulating miRNA signatures may facilitate the development of improved methods for diagnosis and clinical monitoring of disease progression. At present, current findings, combined with the rapidly expanding field of miRNA research, are expected to yield new insights into the complex pathogenesis of NAFLD and may eventually lead to the identification of novel, noninvasive biomarkers for the disease.

REFERENCES

1 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

2 **Starley BQ**, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820-1832 [PMID: 20432259 DOI: 10.1002/hep.23594]

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

4 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

5 **Pagadala MR**, McCullough AJ. The relevance of liver histology to predicting clinically meaningful outcomes in nonalcoholic steatohepatitis. *Clin Liver Dis* 2012; **16**: 487-504 [PMID: 22824477 DOI: 10.1016/j.cld.2012.05.006]

6 **Younossi ZM**, Stepanova M, Rafiq N, Makhlouf H, Younoszai Z, Agrawal R, Goodman Z. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. *Hepatology* 2011; **53**: 1874-1882 [PMID: 21360720 DOI: 10.1002/hep.24268]

7 **Jin X**, Chen YP, Kong M, Zheng L, Yang YD, Li YM. Transition from hepatic steatosis to steatohepatitis: unique microRNA patterns and potential downstream functions and pathways. *J Gastroenterol Hepatol* 2012; **27**: 331-340 [PMID: 21793903 DOI: 10.1111/j.1440-1746.2011.06864.x]

8 **Gori M**, Arciello M, Balsano C. MicroRNAs in nonalcoholic fatty liver disease: novel biomarkers and prognostic tools during the transition from steatosis to hepatocarcinoma. *Biomed Res Int* 2014; **2014**: 741465 [PMID: 24745023 DOI: 10.1155/2014/741465]

9 **Alvarez ML**, DiStefano JK. Towards microRNA-based therapeutics for diabetic nephropathy. *Diabetologia* 2013; **56**: 444-456 [PMID: 23135222 DOI: 10.1007/s00125-012-2768-x]

10 **Zheng L**, Lv GC, Sheng J, Yang YD. Effect of miRNA-10b in regulating cellular steatosis level by targeting PPAR-alpha

expression, a novel mechanism for the pathogenesis of NAFLD. *J Gastroenterol Hepatol* 2010; **25**: 156-163 [PMID: 19780876 DOI: 10.1111/j.1440-1746.2009.05949.x]

11 **Whittaker R**, Loy PA, Sisman E, Suyama E, Aza-Blanc P, Ingermanson RS, Price JH, McDonough PM. Identification of MicroRNAs that control lipid droplet formation and growth in hepatocytes via high-content screening. *J Biomol Screen* 2010; **15**: 798-805 [PMID: 20639500 DOI: 10.1177/1087057110374991]

12 **Fujimoto Y**, Itabe H, Sakai J, Makita M, Noda J, Mori M, Higashi Y, Kojima S, Takano T. Identification of major proteins in the lipid droplet-enriched fraction isolated from the human hepatocyte cell line HuH7. *Biochim Biophys Acta* 2004; **1644**: 47-59 [PMID: 14741744]

13 **Shibata C**, Kishikawa T, Otsuka M, Ohno M, Yoshikawa T, Takata A, Yoshida H, Koike K. Inhibition of microRNA122 decreases SREBP1 expression by modulating suppressor of cytokine signaling 3 expression. *Biochem Biophys Res Commun* 2013; **438**: 230-235 [PMID: 23891753 DOI: 10.1016/j.bbrc.2013.07.064]

14 **Yoshikawa T**, Takata A, Otsuka M, Kishikawa T, Kojima K, Yoshida H, Koike K. Silencing of microRNA-122 enhances interferon-α signaling in the liver through regulating SOCS3 promoter methylation. *Sci Rep* 2012; **2**: 637 [PMID: 22957141 DOI: 10.1038/srep00637]

15 **Zhong D**, Zhang Y, Zeng YJ, Gao M, Wu GZ, Hu CJ, Huang G, He FT. MicroRNA-613 represses lipogenesis in HepG2 cells by downregulating LXRα. *Lipids Health Dis* 2013; **12**: 32 [PMID: 23496987 DOI: 10.1186/1476-511X-12-32]

16 **Bhatia H**, Verma G, Datta M. miR-107 orchestrates ER stress induction and lipid accumulation by post-transcriptional regulation of fatty acid synthase in hepatocytes. *Biochim Biophys Acta* 2014; **1839**: 334-343 [PMID: 24560669 DOI: 10.1016/j.bbagrm.2014.02.009]

17 **Pogribny IP**, Starlard-Davenport A, Tryndyak VP, Han T, Ross SA, Rusyn I, Beland FA. Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Lab Invest* 2010; **90**: 1437-1446 [PMID: 20548288 DOI: 10.1038/labinvest.2010.113]

18 **Alisi A**, Da Sacco L, Bruscalupi G, Piemonte F, Panera N, De Vito R, Leoni S, Bottazzo GF, Masotti A, Nobili V. Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. *Lab Invest* 2011; **91**: 283-293 [PMID: 20956972 DOI: 10.1038/labinvest.2010.166]

19 **Zhang Y**, Cheng X, Lu Z, Wang J, Chen H, Fan W, Gao X, Lu D. Upregulation of miR-15b in NAFLD models and in the serum of patients with fatty liver disease. *Diabetes Res Clin Pract* 2013; **99**: 327-334 [PMID: 23287814 DOI: 10.1016/j.diabres.2012.11.025]

20 **Ahn J**, Lee H, Chung CH, Ha T. High fat diet induced downregulation of microRNA-467b increased lipoprotein lipase in hepatic steatosis. *Biochem Biophys Res Commun* 2011; **414**: 664-669 [PMID: 21986524 DOI: 10.1016/j.bbrc.2011.09.120]

21 **Tian GP**, Tang YY, He PP, Lv YC, Ouyang XP, Zhao GJ, Tang SL, Wu JF, Wang JL, Peng J, Zhang M, Li Y, Cayabyab FS, Zheng XL, Zhang DW, Yin WD, Tang CK. The effects of miR-467b on lipoprotein lipase (LPL) expression, pro-inflammatory cytokine, lipid levels and atherosclerotic lesions in apolipoprotein E knockout mice. *Biochem Biophys Res Commun* 2014; **443**: 428-434 [PMID: 24309104 DOI: 10.1016/j.bbrc.2013.11.109]

22 **Joven J**, Espinel E, Rull A, Aragonès G, Rodríguez-Gallego E, Camps J, Micol V, Herranz-López M, Menéndez JA, Borrás I, Segura-Carretero A, Alonso-Villaverde C, Beltrán-Debón R. Plant-derived polyphenols regulate expression of miRNA paralogs miR-103/107 and miR-122 and prevent diet-induced fatty liver disease in hyperlipidemic mice. *Biochim Biophys Acta* 2012; **1820**: 894-899 [PMID: 22503922 DOI: 10.1016/j.bbagen.2012.03.020]

23 **Ahn J**, Lee H, Jung CH, Ha T. Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet. *Mol Nutr Food Res* 2012; **56**: 1665-1674 [PMID: 22968990 DOI: 10.1002/mnfr.201200182]

24 **Tryndyak VP**, Latendresse JR, Montgomery B, Ross SA, Beland FA, Rusyn I, Pogribny IP. Plasma microRNAs are sensitive indicators of inter-strain differences in the severity of liver injury induced in mice by a choline- and folate-deficient diet. *Toxicol Appl Pharmacol* 2012; **262**: 52-59 [PMID: 22561871 DOI: 10.1016/j.taap.2012.04.018]

25 **Hsu SH**, Wang B, Kota J, Yu J, Costinean S, Kutay H, Yu L, Bai S, La Perle K, Chivukula RR, Mao H, Wei M, Clark KR, Mendell JR, Caligiuri MA, Jacob ST, Mendell JT, Ghoshal K. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest* 2012; **122**: 2871-2883 [PMID: 22820288 DOI: 10.1172/JCI63539]

26 **Miller AM**, Gilchrist DS, Nijjar J, Araldi E, Ramirez CM, Lavery CA, Fernández-Hernando C, McInnes IB, Kurowska-Stolarska M. MiR-155 has a protective role in the development of non-alcoholic hepatosteatosis in mice. *PLoS One* 2013; **8**: e72324 [PMID: 23991091 DOI: 10.1371/journal.pone.0072324]

27 **Liang T**, Liu C, Ye Z. Deep sequencing of small RNA repertoires in mice reveals metabolic disorders-associated hepatic miRNAs. *PLoS One* 2013; **8**: e80774 [PMID: 24260478 DOI: 10.1371/journal.pone.0080774]

28 **Hölttä-Vuori M**, Salo VT, Nyberg L, Brackmann C, Enejder A, Panula P, Ikonen E. Zebrafish: gaining popularity in lipid research. *Biochem J* 2010; **429**: 235-242 [PMID: 20578994 DOI: 10.1042/BJ20100293]

29 **Her GM**, Hsu CC, Hong JR, Lai CY, Hsu MC, Pang HW, Chan SK, Pai WY. Overexpression of gankyrin induces liver steatosis in zebrafish (Danio rerio). *Biochim Biophys Acta* 2011; **1811**: 536-548 [PMID: 21722753 DOI: 10.1016/j.bbalip.2011.06.011]

30 **Xin X**, Zhang Y, Liu X, Xin H, Cao Y, Geng M. MicroRNA in hepatic fibrosis and cirrhosis. *Front Biosci* (Landmark Ed) 2014; **19**: 1418-1424 [PMID: 24896361]

31 **Cheung O**, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, Kellum JM, Min H, Luketic VA, Sanyal AJ. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology* 2008; **48**: 1810-1820 [PMID: 19030170 DOI: 10.1002/hep.22569]

32 **Castro RE**, Ferreira DM, Afonso MB, Borralho PM, Machado MV, Cortez-Pinto H, Rodrigues CM. miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. *J Hepatol* 2013; **58**: 119-125 [PMID: 22902550 DOI: 10.1016/j.jhep.2012.08.008]

33 **Estep M**, Armistead D, Hossain N, Elarainy H, Goodman Z, Baranova A, Chandhoke V, Younossi ZM. Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2010; **32**: 487-497 [PMID: 20497147 DOI: 10.1111/j.1365-2036.2010.04366.x]

34 **Sharma H**, Estep M, Birerdinc A, Afendy A, Moazzez A, Elariny H, Goodman Z, Chandhoke V, Baranova A, Younossi ZM. Expression of genes for microRNA-processing enzymes is altered in advanced non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2013; **28**: 1410-1415 [PMID: 23663110 DOI: 10.1111/jgh.12268]

35 **Miyaaki H**, Ichikawa T, Kamo Y, Taura N, Honda T, Shibata H, Milazzo M, Fornari F, Gramantieri L, Bolondi L, Nakao K. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. *Liver Int* 2014; **34**: e302-e307 [PMID: 24313922 DOI: 10.1111/liv.12429]

36 **Cermelli S**, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS One* 2011; **6**: e23937 [PMID: 21886843 DOI: 10.1371/journal.pone.0023937]

37 **Pirola CJ,** Fernandez Gianotti T, Castano GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, Flichman D, Mirshahi F, Sanyal AJ, Sookoian S. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2014

[PMID: 24973316 DOI: 10.1136/gutjnl-2014-306996]
38 **Szabo G**, Bala S. MicroRNAs in liver disease. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 542-552 [PMID: 23689081 DOI: 10.1038/nrgastro.2013.87]

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MINIREVIEWS

Image-guided therapies in the treatment of hepatocellular carcinoma: A multidisciplinary perspective

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Abstract

A multidisciplinary approach to the treatment of patients with unresectable hepatocellular carcinoma (HCC) has led to improvements in screening, detection, and treatments. Interventional techniques include thermal ablation, transarterial chemoembolization, and radioembolization whilst stereotactic body radiation therapy also uses imaging to target the radiation. Both survival rates and cure rates have improved markedly since the introduction of these techniques. This review article describes the image guided techniques used for the treatment of HCC.

Key words: Ablation; Chemoembolization; Radiation; Hepatocellular carcinoma

Core tip: This review article provides an updated description of the image guided therapies for hepatocellular carcinoma including stereotactic radiation, set in the context of a multidisciplinary approach.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer related deaths[1]. Treatment depends on the stage of the tumor, performance status, and liver function, as well as on the multidisciplinary capabilities of the managing team of hepatologists, gastroenterologists, surgeons, radiologists and oncologists. Curative resection, liver transplantation, ablative therapies, trans-arterial chemoembolization (TACE), radioembolization and systemic therapy all

lie within the range of treatments available to this team[2,3].

In recent years surveillance strategies for patients with viral hepatititis or with cirrhosis have improved, leading to earlier diagnosis in many patients. These patients have a chance of gaining a curative response to treatment[4,5]. In contrast, delay in treatment leads to worse survival[6]. Resection remains the first option for patients who are suitable for surgery, as defined by the Barcelona Cancer of the Liver Clinic (BCLC) staging system. However, several different image guided minimally invasive therapies have emerged and evolved to improve the treatment of HCC at an early stage. These complement therapies provided by surgical and radiation/oncology services. Selection of treatment pathways is determined by a multidisciplinary approach[7-9] and is most commonly based on the BCLC staging system[10]. For early and intermediate stage hepatocellular carcinoma (HCC) (stages A and B) locoregional treatments including ablative therapies and TACE are used. Radioembolization is used for intermediate and advanced stage HCC who are poor candidates for TACE, and who have portal vascular invasion[11]. It is also used with limited evidence base for the downstaging of tumors so that more curative treatments can be employed[12,13].

For patients who have either failed locoregional therapies or who present with more advanced HCC, Sorafenib induces a clinically relevant improvement in time to progression and in survival.

ABLATIVE THERAPIES

Liver transplantation and surgical resection remain the primary options for curative treatment in appropriate patients. The Milan criteria[14] provide strict guidelines for transplantation eligibility, whilst surgical resection is suitable only for patients with single nodules and Child Pugh class A liver function[15]. The limitations on these treatment options offer up a substantial number of patients who can benefit from locoregional therapies. Radiofrequency ablation has become the most accepted treatment for patients with very early and early stage (BCLC 0 and A) disease who are not eligible for surgery[16,17]. In three independent meta-analyses[17-19] which include five randomized controlled trials, better local control and increased survival has been demonstrated in comparison with percutaneous ethanol ablation. When compared with surgical resection, there is conflicting evidence. In randomized controlled trials Huang *et al*[16] indicate improved results for surgery over RFA who were followed up for 5 years while Feng *et al*[20] showed that although there was a greater risk of local recurrence with RFA, there was no significant difference in overall survival. Similar conflicts are demonstrated in meta-analyses. Liu *et al*[21] found equivalent survival rates despite higher rates of local recurrence with RFA, whilst Zhou *et al*[22] found better survival rates in surgical patients for tumors measuring greater than 3 cm, and equivalent rates in smaller tumors. Survival ranges from 78%-94% at 1 year and 58%-96% at 3 years[17-22].

RFA employs low-voltage alternating current to provide sufficient heat to kill cells[21-24]. The probes are inserted under ultrasound or computed tomography (CT) guidance. The procedure is performed under moderate sedation or general anesthesia and patients can be discharged on the same day or the following day. Complication rates are lower than those of surgery and include abscess formation, tumor seeding along the electrode track, burns from the grounding pads, bile duct injury and thermal injury to adjacent organs[25]. The procedure is also less expensive than surgery.

Cyroablation is similar in terms of technical approach to RFA, but creates tissue injury from low temperatures of -20 ℃ to -60 ℃[26]. More than one needle is usually required. The procedure can be applied with lower rates of complication than RFA when close to the gall bladder[27] or bowel loops, and is less painful when employed for lesions which are contiguous with the diaphragm[28]. The procedure can be performed under moderate sedation[29]. It is possible to follow the ablative effect on CT by visualization of the ice ball[23,28-30]. One and 3 year survival rates are demonstrated at 81.4% and 60.3%, similar to those of RFA[30]. A single meta-analysis shows an advantage for RFA over cryotherapy in terms of recurrence rate[28]. There is no study comparing the survival rates.

Microwave therapy also works by heating the local tissues. It achieves a larger ablation zone in a shorter period of time than RFA[31]. Early studies have shown that there is a larger rate of local recurrence with microwave than with RFA, but there are no large studies or randomized studies to support this.

With the advent of RFA, percutaneous ethanol injection has decreased in popularity. The procedure is low cost, but requires several sessions of treatment. It is performed with a fine needle under ultrasound guidance. Tumor recurrence rates and survival rates are inferior in comparison with those of RFA[32].

CHEMOEMBOLIZATION

HCC is preferentially supplied by the hepatic arterial inflow, in contrast to the normal liver parenchyma which is largely supplied by the portal vein. The TACE procedure exploits these blood supply dynamics. Techniques vary according to resources and expense, but the principal is that an intra-arterial catheter is placed in the vessel(s) supplying the tumor(s) and high concentrations of a chemotherapeutic agent is delivered along with an embolic agent to achieve the dual purposes of targeted chemotherapy and reduction in arterial supply to the tumor.

TACE has been performed since 1980. Chemotherapeutic drugs, most commonly doxorubicin, cisplatin and mitomycin, are delivered locally along with an embolic agent, normally lipiodol, an oil emulsifying agent, thereby avoiding systemic toxicity. Other embolic agents used are gelatin sponge and PVA particles.

Drug eluting beads (DEB-TACE), although not yet the standard, are becoming increasingly popular largely due to the decreased side effect profile in comparison with the standard TACE cocktail of drugs. DEB-TACE delivers small beads which have been soaked for several hours, normally in doxorubicin. The loaded beads occlude the feeding vessels of HCC, while the anticancer drug is released gradually, creating tumor necrosis and increasing chemotherapeutic concentrations locally. Bead size varies from 75 micron to 700 micron, the choice of size being dependent on tumor size and the desired level of concentration within the treated volume. Improved results are achieved when chemoembolization is performed selectively to segmental or subsegmental arteries feeding the tumor(s)[33].

TACE is recommended as the standard of care for intermediate stage HCC without vascular invasion or distant metastases. Although there has been some heterogeneity in the results of several randomized controlled trials, TACE has been shown to achieve at least a partial response in 15% to 62% of patients, and improves survival from 16 mo to 20 mo[34-40]. The variability in results is likely explained by the fact that intermediate stage HCC covers a broad spectrum of disease burden, that there is variability in the chemotherapeutic agents and embolization materials administered to patients, and that the procedure is performed on both Childs A and Childs B liver disease populations. DEB-TACE has been shown to achieve improved outcomes in patients with Child-Pugh B, bi-lobar disease and recurrent disease[41].

There remains debate about the optimal degree of arterial embolization to achieve tumor ischemia[42,43]. There is some evidence which indicates that complete tumor ischemia may stimulate angiogenesis, resulting in an increased susceptibility to tumor growth rather than suppression. It is therefore suggested that arterial patency be maintained, not only to prevent this angiogenic effect, but also so that patients can receive repeated treatments[44].

DEB-TACE causes fewer side effects than conventional TACE. Side effects associated with both DEB-TACE and conventional TACE include nausea, vomiting and right upper quadrant pain (post embolization syndrome), cardiac toxicity related to the doxorubicin, bone marrow aplasia, hepatic abscess and cholecystitis[36,38,45]. Two recent randomized controlled trials have shown improved side effect profiles[46,47]. One trial showed equivalent survival rates[42,46] whilst the other showed longer time to progression for DEB-TACE in comparison with conventional TACE[47]. A single meta-analysis demonstrated equivalent tumor response rates[48].

RADIOEMBOLIZATION

Radioembolization for primary hepatic cancer with Yttrium-90 (90Y) was first described in 1965 who used isotope embedded 50 μm ceramic microspheres to embolize hepatic cancer *via* a surgically placed catheter based in the hepatic artery[49]. Today, the technique has evolved away from an open surgical approach to a minimally invasive fluoroscopically guided microcatheter based technique using either 90Y embedded non-biodegradable glass microspheres measuring 25 ± 10 μm (Theraspheres, Nordion Incorporated, Ottawa, Ontario, Canada) or 90Y embedded non-biodegradable glass resin based microspheres measuring 29-35 μm (SIR-Spheres, Sirtex Medical Incorporated, Lake Forest, Illinois). Radioembolization, similar to TACE, exploits the preferential arterial blood supply of an HCC by delivering radiotherapy in an embolic agent directly to the tumor bed while preserving the blood flow to the normal liver parenchyma, which is supplied primarily by the portal vein. Unlike TACE, which uses 75-700 μm beads to occlude medium to large sized arteries leading to tumor ischemia, 90Y radioembolization uses these smaller beads to act as a microembolic agent to deposit radiotherapy directly within the tumor *via* an intratumoral vessel. Once deposited at the target lesion, 90Y delivers tumoricidal doses of a pure high energy beta emitter (937 KeV) with a short tissue penetration (mean 2.5 mm and maximum 11 mm) and short half-life of 2.67 d. The short tissue penetration and half-life of 90Y make it an ideal radioisotope for intra-arterial radiotherapy as there is minimal dose deposited in the adjacent liver parenchyma and the patient can immediately be safely discharged home without fear of radiation being delivered to others.

Radioembolization with 90Y has generally been reserved for patients who have intermediate/advanced BCLC stage hepatocellular carcinoma and who are not candidates for TACE due to portal vein invasion[10,50,51]. Sorafenib is generally considered the treatment of choice for advanced HCC[52]. However, Sorafenib is often not well tolerated[53], and 90Y radioembolization is a suitable alternative for patients with advanced HCC given the equivalent median overall survival of 13.2 mo in the radioembolization group *vs* 14.4 mo in the Sorafenib group[54]. 90Y radioembolization has also been proposed as an alternative treatment option to prevent progression of disease in eligible transplant patients and to downstage patients in order to become eligible transplant recipients based on the Milan criteria[12-14].

According to the Radioembolization Brachytherapy

Oncology Consortium, it is recommend that patients undergo preembolization planning and treatment simulation with intrarterial injection of technectium-99m labeled macroaggregated albumin (^{99m}Tc-MAA) and CT to rule out > 30 Gy radiation exposure to the lung from hepatopulmonary shunting and to measure liver volumes[51]. There is currently no consensus on the recommend radiation dose to deliver to effectively treat HCC. In patients with advanced stage inoperable HCC, however, Lau *et al*[55] did demonstrate a median survival benefit of 55.9 wk *vs* 26.2 wk in patients receiving > 120 Gy *vs* < 120 Gy, respectively. A randomized controlled trial is underway examining the efficacy of radioembolization when compared with chemo-embolization (Seinstra)[56].

COMBINATION TACE AND RFA

Ablative techniques demonstrate diminished efficacy when tumor diameter is greater than 3 cm[57,58]. This failure to achieve complete tumor necrosis is largely attributed to the "heat sink" effect: cooling by blood flow resulting in a reduction in temperature adjacent to vessels within or adjacent to the ablation zone[59].

Adjuvant locoregional therapies have been employed to achieve higher rates of efficacy in the treatment of larger tumors (3-5 cm). The most common of these is chemoembolization. The embolic effect of the lipiodol or beads decreases the "heat sink" effect caused by local vessels, whilst the addition of the chemotherapeutic drug improves overall tumor kill efficacy[60-62]. A single randomized controlled trial has shown decreased rates of tumor progression in the combination group in comparison with the RFA only group[63], although no significant difference in survival was demonstrated.

SURGICALLY ASSISTED RFA

Radiofrequency ablation was widely adapted in the 1990's as a method to treat lesions deemed unresectable at the time of open hepatectomy. As technology improved, RFA moved from the operating room to the IR suite where percutaneous ablations could be performed without the morbidity of a laparotomy. While percutaneous image guided ablative therapies are a useful tool in the armamentarium for the loco-regional treatment of liver lesions, there are some limitations. These include difficulty in localizing lesions, potential for injury to extra hepatic structures and decreased efficacy in close proximity to liver vasculature. Many of these limitations can be addressed by performing surgically assisted RFA using a laparoscopic approach[64].

Because percutaneous ablation relies on the ability to localize lesions with ultrasound, obese patients with thick abdominal walls can provide a challenge, particularly with lesions in the dome. While this has in large part been abrogated by use of CT and magnetic resonance imaging (MRI) guidance, some tumors are difficult to localize on cross sectional imaging. The ability to perform ultrasound directly on the liver surface allows for more accurate tumor localization and may result in more efficacious tumor treatment when compared to percutaneous ablation[65,66]. The most widely used technique for laparoscopic assisted RFA is with insufflation of the abdomen after induction of general anesthesia. A laparoscopic ultrasound probe is then introduced and used to guide a percutaneously placed RFA needle. Additional laparoscopic ports can be introduced to manipulate the liver as well as other extra-hepatic structures. The ability to manipulate the peri-hepatic environment can protect structures such as the colon, stomach, small bowel and diaphragm from transmitted heat. It also allows for potential removal of the gallbladder prior to RFA, preventing injury and heat sink. Other techniques to protect peri-hepatic structures include instillation of artificial ascites which can absorb heat without transmission to surrounding viscera[67].

An additional benefit of surgically assisted RFA is the ability to occlude hepatic vascular inflow, which in theory reduces the heat sink from major vessels. With minimal mobilization of the liver, a temporary ligature can be placed around the porta hepatis and tightened immediately prior to application of energy. In-vivo animal studies have indicated an increase in tumor necrosis around blood vessels, although human data is lacking[68,69].

TACE + RADIOTHERAPY FOR HCC

Locoregional relapse remains an important issue for HCC. In early stage HCC stereotactic body radiotherapy (SBRT) has been used in conjunction with TACE in an effort to improve cure rates[70,71]. In the locally advanced setting, three dimensional conventional radiation therapy (3DCRT) has shown promising results following TACE in promoting tumor necrosis and reducing local relapse.

SBRT entails the delivery of highly conformal, high dose, ablative radiotherapy to a liver lesion in a short period of time (typically over 1-2 wk). Selection criteria for liver SBRT is similar to that of TACE: Childs A liver function, 1-3 lesions, more than 700 cc uninvolved liver, tumors less than 5 cm, and well controlled extrahepatic disease[72]. Prior to SBRT patients undergo a 4 dimensional CT planning scan to delineate the target lesion and its real time movement across several phases of respiration. Ultrasound guided insertion of tumor fiducial markers is often useful for image guided radiotherapy where the markers act as a surrogate for tracking the lesion's location for radiation delivery. Liver SBRT prescriptions can vary from 50 Gy/5 fractions to

60 Gy/3 fractions, in contrast to 3DCRT where the conventional daily dose of radiation is 1.8 to 2.0 Gy/fraction and the total dose is 45-50 Gy in 25 fractions. Care is taken to avoid excess dose to adjacent bowel and the remaining liver during SBRT given the potential for severe complications with high doses.

Data is also emerging that SBRT may be an effective salvage strategy for patients who experience local failure post TACE. Patients with Childs A disease and tumors measuring less than 10 cm who have undergone partial or incomplete TACE may have 2 year local control rates as high as 94.6% when salvaged with SBRT[73-75]. High grade toxicity resulting in duodenal or gastric perforation is rare (approximately 5%) if dose constraints are respected[73]. However, the presence of tumor vascular thrombosis is a risk factor for severe and even mortal toxicity[76,77]. Combination TACE + SBRT appears to be a potentially promising treatment for early stage HCC and likely merits a multi-institutional phase Ⅲ study as the existing literature consists of single institution retrospective data or small phase Ⅰ/Ⅱ trials[78].

In locally advanced HCC, 3DCRT or chemo-radiation post TACE or partial TACE may confer better outcomes than Sorafenib. One study compared 67 patients with BCLC stage C disease who received TACE + 3DCRT with a cohort that was given Sorafenib as first line treatment. While this study did not examine local control, the median survival of the TACE + RT group was 14.1 mo compared to 3.1 mo in the Sorafenib group[79,80]. Combining TACE and conventional radiation treatments for locally advanced HCC may also be an effective treatment in patients with extensive portal vein thrombosis[74,81-85]. One year progression free rates in patients who receive TACE + 3DCRT for unresectable HCC can be as high as 70% compared to TACE alone (40%)[85]. Patients who have failed 1-2 TACE treatments and who received subsequent 3DCRT have been reported to have as high as 68% response rate post radiotherapy with 70% achieving stable disease at 1 year[86,87]. As prognosis for locally advanced HCC remains poor, the use of local therapies in conjunction with chemotherapy is also being explored. Clinical trials of concurrent chemoradiation and TACE in advanced disease have shown promise in improving local control and progression free survival[88,89].

IMAGING AFTER IMAGE-GUIDED THERAPIES

With the variety of image-guided liver directed therapies available, it is important to know the different expected post-therapy appearances and be able to differentiate these from abnormal imaging findings. Contrast-enhanced CT or MRI are the preferred imaging modalities for post therapy surveillance. Post therapy imaging should be performed at scheduled intervals, although a standard interval has not been established. At our institution, we perform contrast enhanced CT or MRI at 6 wk and then at 3, 6, 9 and 12 mo intervals.

ABLATION

An ablation zone encompasses the tumor with a variable margin, and is therefore usually larger than the tumor on initial imaging. Unenhanced CT and MRI images are obligatory as the ablation zone may be hyperattenuating or have intrinsic hyperintensity on pre-contrast T1-weighted images due to coagulative necrosis and hemorrhage making evaluation for arterial enhancement more difficult. Subtraction MRI is particularly useful when there is T1 hyperintensity on unenhanced images. In a completely treated lesion, contrast enhanced images demonstrate a non-enhancing well-defined ablation zone.

Familiarity with normal periablation changes is also important. Transient hyperemia and edema can be present around the ablation zone due to thermal injury to the surrounding parenchyma, manifesting as a concentric thin rim of enhancement on arterial and sometimes portal venous phase contrast enhanced CT and MRI and a hyperintense rim on T2-weighted images. Peripheral geographic arterial enhancement can also be seen post ablation, often related to injury to the portal vein branches and subsequent increase in perfusion from the hepatic artery. These changes usually resolve within several months[90,91]. Residual disease in contrast demonstrates an area of irregular or thick, peripheral arterial enhancement.

MRI is particularly helpful for residual disease evaluation, as this demonstrates focal hyperintensity on T2-weighted images and often increased signal on diffusion-weighted images. Recurrent disease has similar imaging characteristics to residual disease, but can occur within or adjacent to the ablation zone. New disease occurs in other areas of the liver or in extra-hepatic locations. In some cases, it may be difficult to differentiate expected a post ablation peripheral rim of enhancement from residual or recurrent tumor. In these cases, closer follow-up imaging may be necessary. Risk factors for residual or recurrent disease include large tumor size, aggressive histology, difficult location, and heat sink effect, specifically in radiofrequency ablation[92].

Transient bile duct dilatation peripheral to the ablation zone is often seen. Leakage of bile from injured ducts can result in a biloma, which appear as a non-enhancing fluid collection.

If there is injury to larger vessels, parenchymal or intraperitoneal hemorrhage can occur and be detected on CT or MRI. If both the portal and hepatic arteries are injured, hepatic infarction can occur, which

appears as non-enhancing parenchyma peripheral to the ablation zone. Other vascular complications, such as arteriovenous fistula or pseudoaneurysm can also be identified on arterial phase imaging.

Hepatic abscess is an additional complication which can be seen after ablation. This usually presents a few weeks after the procedure and demonstrates peripheral enhancement and development of gas within or adjacent to the ablation zone. Injury to adjacent structures is an additional complication to be aware of after ablation: for example, adjacent bowel or the diaphragm[92].

TACE, CONVENTIONAL TACE, AND RADIOEMBOLIZATION

Imaging following TACE and transarterial radioembolization is similar to ablation with a few additional caveats. The treated lesion again should demonstrate lack of enhancement, but also may demonstrate a peripheral rim of enhancement, geographic arterial enhancement or both. After transarterial radioembolization, there may be heterogeneous parenchymal enhancement in a perivascular distribution due to radiation effect. This can mimic tumor and may need shorter term follow-up. In patients treated with lipiodol MRI has been shown to be superior to CT given the ability to perform diffuse weighted images and image subtraction[93]. Residual or recurrent disease appears as nodular arterially enhancing tumor, often in the periphery, similar to ablation.

SBRT

Treatment response assessment for SBRT is evolving. As with other image guided therapies, tumor response after SBRT is recognized as non-enhancement of tumor. However there are other unique imaging characteristics. After SBRT, recurrence can occur within the planned target volume, suggesting that an inadequate dose was used, or can occur along the margin of the high dose region, suggesting incomplete coverage of the tumor margin. This marginal recurrence may be due to patient respiratory motion. Additionally, focal peritumoral enhancement may be seen on any phase of imaging, likely representing radiation induced changes and inflammation of the surrounding normal liver parenchyma. These areas of enhancement can persist for months and should not be confused with recurrent tumor. Additionally, it can take time for the initial tumor enhancement to disappear, and therefore continued follow-up is necessary. Sanuki *et al*[94] demonstrated in 38 patients a median time of 5.9 mo to reach complete treatment response with a range of 1.2 to 34.2 mo.

RESPONSE ASSESSMENT

Different criteria have been developed to evaluate tumor treatment response. Conventional size measurement, such as Response Evaluation Criteria in Solid Tumors (RECIST), is predominantly useful for evaluation of cytotoxic systemic agents but does not work well for evaluation after locoregional therapy, as tumor necrosis is the goal and may not always manifest as a decrease in lesion size. The European Association for the Study of the Liver (EASL) measures the arterially enhancing area in two dimensions, while a modified RECIST classification uses a single largest diameter of arterially enhancing tumor. The modified RECIST criteria has been recommended as the preferred criteria for tumor response by the EASL and European Organisation for Research and Treatment of Cancer[95].

CONCLUSION

Most patients presenting with HCC are ineligible for surgical curative treatment. Advances In locoregional therapy, both catheter based and ablative, have led to improvements both in cure rates and in survival. A multidisciplinary approach is optimal for the planning of treatment given that there are treatment contributions from gastroenterology, surgery, interventional radiology, and oncology.

REFERENCES

1 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432]

2 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/S0140 6736(11)61347-0]

3 **Ye SL**, Takayama T, Geschwind J, Marrero JA, Bronowicki JP. Current approaches to the treatment of early hepatocellular carcinoma. *Oncologist* 2010; **15** Suppl 4: 34-41 [PMID: 21115579 DOI: 10.1634/theoncologist.2010-S4-34]

4 **Padhya KT**, Marrero JA, Singal AG. Recent advances in the treatment of hepatocellular carcinoma. *Curr Opin Gastroenterol* 2013; **29**: 285-292 [PMID: 23507917 DOI: 10.1097/MOG.0b013e32835ff1cf]

5 **Kanwal F**, Befeler A, Chari RS, Marrero J, Kahn J, Afdhal N, Morgan T, Roberts L, Mohanty SR, Schwartz J, VanThiel D, Li J, Zeringue A, Di'Bisceglie A. Potentially curative treatment in patients with hepatocellular cancer--results from the liver cancer research network. *Aliment Pharmacol Ther* 2012; **36**: 257-265 [PMID: 22670798 DOI: 10.1111/j.1365-2036.2012.05174.x]

6 **Singal AG**, Waljee AK, Patel N, Chen EY, Tiro JA, Marrero JA, Yopp AC. Therapeutic delays lead to worse survival among patients with hepatocellular carcinoma. *J Natl Compr Canc Netw* 2013; **11**: 1101-1108 [PMID: 24029125]

7 **Bolondi L**, Cillo U, Colombo M, Craxì A, Farinati F, Giannini EG, Golfieri R, Levrero M, Pinna AD, Piscaglia F, Raimondo G, Trevisani F, Bruno R, Caraceni P, Ciancio A, Coco B, Fraquelli M, Rendina M, Squadrito G, Toniutto P. Position paper of the Italian Association for the Study of the Liver (AISF): the multidisciplinary clinical approach to hepatocellular carcinoma. *Dig Liver Dis* 2013; **45**: 712-723 [PMID: 23769756 DOI: 10.1016/j.dld.2013.01.012]

8 **Cohen GS**, Black M. Multidisciplinary management of hepatocellular carcinoma: a model for therapy. *J Multidiscip*

Healthc 2013; **6**: 189-195 [PMID: 23690690 DOI: 10.2147/JMDH.S41206]

9 **Yopp AC**, Mansour JC, Beg MS, Arenas J, Trimmer C, Reddick M, Pedrosa I, Khatri G, Yakoo T, Meyer JJ, Shaw J, Marrero JA, Singal AG. Establishment of a multidisciplinary hepatocellular carcinoma clinic is associated with improved clinical outcome. *Ann Surg Oncol* 2014; **21**: 1287-1295 [PMID: 24318095 DOI: 10.1245/s10434-013-3413-8]

10 **Sherman M**. Hepatocellular carcinoma: screening and staging. *Clin Liver Dis* 2011; **15**: 323-324, vii-x [PMID: 21689616]

11 **Sangro B**, Iñarrairaegui M, Bilbao JI. Radioembolization for hepatocellular carcinoma. *J Hepatol* 2012; **56**: 464-473 [PMID: 21816126 DOI: 10.1016/j.jhep.2011.07.012]

12 **Ettorre GM**, Laurenzi A, Vennarecci G. Downstaging Hepatocellular Carcinoma with Yttrium-90 radioembolization: resection or transplantation? *Eur J Surg Oncol* 2014; **40**: 789-790 [PMID: 24572481 DOI: 10.1016/j.ejso.2014.01.017]

13 **Tohme S**, Sukato D, Chen HW, Amesur N, Zajko AB, Humar A, Geller DA, Marsh JW, Tsung A. Yttrium-90 radioembolization as a bridge to liver transplantation: a single-institution experience. *J Vasc Interv Radiol* 2013; **24**: 1632-1638 [PMID: 24160821 DOI: 10.1016/j.jvir.2013.07.026]

14 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

15 **Bhoori S**, Schiavo M, Russo A, Mazzaferro V. First-line treatment for hepatocellular carcinoma: resection or transplantation? *Transplant Proc* 2007; **39**: 2271-2273 [PMID: 17889160 DOI: 10.1016/j.transproceed.2007.06.015]

16 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100 DOI: 10.1097/SLA.0b013e3181efc656]

17 **Shen A**, Zhang H, Tang C, Chen Y, Wang Y, Zhang C, Wu Z. Systematic review of radiofrequency ablation versus percutaneous ethanol injection for small hepatocellular carcinoma up to 3 cm. *J Gastroenterol Hepatol* 2013; **28**: 793-800 [PMID: 23432154 DOI: 10.1111/jgh.12162]

18 **Orlando A**, Leandro G, Olivo M, Andriulli A, Cottone M. Radiofrequency thermal ablation vs. percutaneous ethanol injection for small hepatocellular carcinoma in cirrhosis: meta-analysis of randomized controlled trials. *Am J Gastroenterol* 2009; **104**: 514-524 [PMID: 19174803 DOI: 10.1038/ajg.2008.80]

19 **Cho YK**, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009; **49**: 453-459 [PMID: 19065676 DOI: 10.1002/hep.22648]

20 **Feng K**, Yan J, Li X, Xia F, Ma K, Wang S, Bie P, Dong J. A randomized controlled trial of radiofrequency ablation and surgical resection in the treatment of small hepatocellular carcinoma. *J Hepatol* 2012; **57**: 794-802 [PMID: 22634125 DOI: 10.1016/j.jhep.2012.05.007]

21 **Liu Z**, Zhou Y, Zhang P, Qin H. Meta-analysis of the therapeutic effect of hepatectomy versus radiofrequency ablation for the treatment of hepatocellular carcinoma. *Surg Laparosc Endosc Percutan Tech* 2010; **20**: 130-140 [PMID: 20551807 DOI: 10.1097/SLE.0b013e3181d823df]

22 **Zhou Y**, Zhao Y, Li B, Xu D, Yin Z, Xie F, Yang J. Meta-analysis of radiofrequency ablation versus hepatic resection for small hepatocellular carcinoma. *BMC Gastroenterol* 2010; **10**: 78 [PMID: 20618937 DOI: 10.1186/1471-230X-10-78]

23 **Buscarini L**, Buscarini E, Di Stasi M, Vallisa D, Quaretti P, Rocca A. Percutaneous radiofrequency ablation of small hepatocellular carcinoma: long-term results. *Eur Radiol* 2001; **11**: 914-921 [PMID: 11419162 DOI: 10.1007/s003300000659]

24 **McGahan JP**, Browning PD, Brock JM, Tesluk H. Hepatic ablation using radiofrequency electrocautery. *Invest Radiol* 1990; **25**: 267-270 [PMID: 2185179 DOI: 10.1097/00004424-199003000-00011]

25 **Livraghi T**, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN. Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. *Radiology* 2003; **226**: 441-451 [PMID: 12563138 DOI: 10.1148/radiol.2262012198]

26 **Permpongkosol S**, Nicol TL, Khurana H, Link RE, Zhai QJ, Kavoussi LR, Solomon SB. Thermal maps around two adjacent cryoprobes creating overlapping ablations in porcine liver, lung, and kidney. *J Vasc Interv Radiol* 2007; **18**: 283-287 [PMID: 17327563 DOI: 10.1016/j.jvir.2006.12.008]

27 **Fairchild AH**, Tatli S, Dunne RM, Shyn PB, Tuncali K, Silverman SG. Percutaneous cryoablation of hepatic tumors adjacent to the gallbladder: assessment of safety and effectiveness. *J Vasc Interv Radiol* 2014; **25**: 1449-1455 [PMID: 24906627]

28 **Huang YZ**, Zhou SC, Zhou H, Tong M. Radiofrequency ablation versus cryosurgery ablation for hepatocellular carcinoma: a meta-analysis. *Hepatogastroenterology* 2013; **60**: 1131-1135 [PMID: 23321123 DOI: 10.5754/hge121142]

29 **Molla N**, AlMenieir N, Simoneau E, Aljiffry M, Valenti D, Metrakos P, Boucher LM, Hassanain M. The role of interventional radiology in the management of hepatocellular carcinoma. *Curr Oncol* 2014; **21**: e480-e492 [PMID: 24940108 DOI: 10.3747/co.21.1829]

30 **Chen HW**, Lai EC, Zhen ZJ, Cui WZ, Liao S, Lau WY. Ultrasound-guided percutaneous cryotherapy of hepatocellular carcinoma. *Int J Surg* 2011; **9**: 188-191 [PMID: 21093616 DOI: 10.1016/j.ijsu.2010.11.008]

31 **Lee KF**, Hui JW, Cheung YS, Wong JS, Chong CN, Wong J, Yu SC, Lai PB. Surgical ablation of hepatocellular carcinoma with 2.45-GHz microwave: a critical appraisal of treatment outcomes. *Hong Kong Med J* 2012; **18**: 85-91 [PMID: 22477730]

32 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54**: 1151-1156 [PMID: 16009687 DOI: 10.1136/gut.2004.045203]

33 **Golfieri R**, Cappelli A, Cucchetti A, Piscaglia F, Carpenzano M, Peri E, Ravaioli M, D'Errico-Grigioni A, Pinna AD, Bolondi L. Efficacy of selective transarterial chemoembolization in inducing tumor necrosis in small (& lt; 5 cm) hepatocellular carcinomas. *Hepatology* 2011; **53**: 1580-1589 [PMID: 21351114 DOI: 10.1002/hep.24246]

34 **Geschwind JF**. Chemoembolization for hepatocellular carcinoma: where does the truth lie? *J Vasc Interv Radiol* 2002; **13**: 991-994 [PMID: 12397119 DOI: 10.1016/S1051-0443(07)61862-4]

35 **Geschwind JF**, Ramsey DE, Choti MA, Thuluvath PJ, Huncharek MS. Chemoembolization of hepatocellular carcinoma: results of a metaanalysis. *Am J Clin Oncol* 2003; **26**: 344-349 [PMID: 12902882 DOI: 10.1097/01.COC.0000020588.20717.BB]

36 **Lo CM**, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]

37 **Boily G,** Villeneuve JP, Lacoursiere L, Chaudhury P, Couture F, Ouellet JF, Lapointe R, Goulet S, Gervais N. Transarterial embolization therapies for the treatment of hepatocellular carcinoma: CEPO review and clinical recommendations. *HPB* (Oxford) 2014 [PMID: 24961288]

38 **Forner A**, Llovet JM, Bruix J. Chemoembolization for intermediate HCC: is there proof of survival benefit? *J Hepatol* 2012; **56**: 984-986 [PMID: 22008737 DOI: 10.1016/j.jhep.2011.08.017]

39 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]

40 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]

41 **Lammer J**, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Pitton M, Sergent G, Pfammatter T, Terraz S, Benhamou Y, Avajon Y, Gruenberger T, Pomoni M, Langenberger H, Schuchmann M, Dumortier J, Mueller C, Chevallier P, Lencioni R. Prospective randomized study of doxorubicin-eluting-bead embolization in the treatment of hepatocellular carcinoma: results of the PRECISION V study. *Cardiovasc Intervent Radiol* 2010; **33**: 41-52 [PMID: 19908093 DOI: 10.1007/s00270-009-9711-7]

42 **Xiong ZP**, Yang SR, Liang ZY, Xiao EH, Yu XP, Zhou SK, Zhang ZS. Association between vascular endothelial growth factor and metastasis after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 386-390 [PMID: 15313674]

43 **Kobayashi N**, Ishii M, Ueno Y, Kisara N, Chida N, Iwasaki T, Toyota T. Co-expression of Bcl-2 protein and vascular endothelial growth factor in hepatocellular carcinomas treated by chemoembolization. *Liver* 1999; **19**: 25-31 [PMID: 9928762 DOI: 10.1111/j.1478-3231.1999.tb00005.x]

44 **Jaeger HJ**, Mehring UM, Castañeda F, Hasse F, Blumhardt G, Loehlein D, Mathias KD. Sequential transarterial chemoembolization for unresectable advanced hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 1996; **19**: 388-396 [PMID: 8994703 DOI: 10.1007/BF02577625]

45 **Varela M**, Real MI, Burrel M, Forner A, Sala M, Brunet M, Ayuso C, Castells L, Montañá X, Llovet JM, Bruix J. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; **46**: 474-481 [PMID: 17239480 DOI: 10.1016/j.jhep.2006.10.020]

46 **Golfieri R**, Giampalma E, Renzulli M, Cioni R, Bargellini I, Bartolozzi C, Breatta AD, Gandini G, Nani R, Gasparini D, Cucchetti A, Bolondi L, Trevisani F. Randomised controlled trial of doxorubicin-eluting beads vs conventional chemoembolisation for hepatocellular carcinoma. *Br J Cancer* 2014; **111**: 255-264 [PMID: 24937669 DOI: 10.1038/bjc.2014.199]

47 **Song MJ**, Chun HJ, Song do S, Kim HY, Yoo SH, Park CH, Bae SH, Choi JY, Chang UI, Yang JM, Lee HG, Yoon SK. Comparative study between doxorubicin-eluting beads and conventional transarterial chemoembolization for treatment of hepatocellular carcinoma. *J Hepatol* 2012; **57**: 1244-1250 [PMID: 22824821 DOI: 10.1016/j.jhep.2012.07.017]

48 **Gao S**, Yang Z, Zheng Z, Yao J, Deng M, Xie H, Zheng S, Zhou L. Doxorubicin-eluting bead versus conventional TACE for unresectable hepatocellular carcinoma: a meta-analysis. *Hepatogastroenterology* 2013; **60**: 813-820 [PMID: 23282741]

49 **Ariel IM**. Treatment of inoperable primary pancreatic and liver cancer by the intra-arterial administration of radioactive isotopes (Y90 radiating microspheres). *Ann Surg* 1965; **162**: 267-278 [PMID: 14327011 DOI: 10.1097/00000658-196508000-00018]

50 **Hong K**, Georgiades CS, Geschwind JF. Technology insight: Image-guided therapies for hepatocellular carcinoma--intra-arterial and ablative techniques. *Nat Clin Pract Oncol* 2006; **3**: 315-324 [PMID: 16757969 DOI: 10.1038/ncponc0512]

51 **Kennedy A**, Nag S, Salem R, Murthy R, McEwan AJ, Nutting C, Benson A, Espat J, Bilbao JI, Sharma RA, Thomas JP, Coldwell D. Recommendations for radioembolization of hepatic malignancies using yttrium-90 microsphere brachytherapy: a consensus panel report from the radioembolization brachytherapy oncology consortium. *Int J Radiat Oncol Biol Phys* 2007; **68**: 13-23 [PMID: 17448867 DOI: 10.1016/j.ijrobp.2006.11.060]

52 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

53 **Iavarone M**, Cabibbo G, Piscaglia F, Zavaglia C, Grieco A, Villa E, Cammà C, Colombo M. Field-practice study of sorafenib therapy for hepatocellular carcinoma: a prospective multicenter study in Italy. *Hepatology* 2011; **54**: 2055-2063 [PMID: 21898496 DOI: 10.1002/hep.24644]

54 **Gramenzi A,** Golfieri R, Mosconi C, Cappelli A, Granito A, Cucchetti A, Marinelli S, Pettinato C, Erroi V, Fiumana S, Bolondi L, Bernardi M, Trevisani F. Yttrium-90 radioembolization vs sorafenib for intermediate-locally advanced hepatocellular carcinoma: a cohort study with propensity score analysis. *Liver Int* 2014 Apr 22; Epub ahead of print [PMID: 24750853]

55 **Lau WY**, Leung WT, Ho S, Leung NW, Chan M, Lin J, Metreweli C, Johnson P, Li AK. Treatment of inoperable hepatocellular carcinoma with intrahepatic arterial yttrium-90 microspheres: a phase I and II study. *Br J Cancer* 1994; **70**: 994-999 [PMID: 7947110 DOI: 10.1038/bjc.1994.436]

56 **Seinstra BA**, Defreyne L, Lambert B, Lam MG, Verkooijen HM, van Erpecum KJ, van Hoek B, van Erkel AR, Coenraad MJ, Al Younis I, van Vlierberghe H, van den Bosch MA. Transarterial radioembolization versus chemoembolization for the treatment of hepatocellular carcinoma (TRACE): study protocol for a randomized controlled trial. *Trials* 2012; **13**: 144 [PMID: 22913492 DOI: 10.1186/1745-6215-13-144]

57 **Xu HX**, Lu MD, Xie XY, Yin XY, Kuang M, Chen JW, Xu ZF, Liu GJ. Prognostic factors for long-term outcome after percutaneous thermal ablation for hepatocellular carcinoma: a survival analysis of 137 consecutive patients. *Clin Radiol* 2005; **60**: 1018-1025 [PMID: 16124984 DOI: 10.1016/j.crad.2005.04.009]

58 **Livraghi T**, Goldberg SN, Lazzaroni S, Meloni F, Ierace T, Solbiati L, Gazelle GS. Hepatocellular carcinoma: radio-frequency ablation of medium and large lesions. *Radiology* 2000; **214**: 761-768 [PMID: 10715043 DOI: 10.1148/radiology.214.3.r00mr02761]

59 **Lu DS**, Raman SS, Vodopich DJ, Wang M, Sayre J, Lassman C. Effect of vessel size on creation of hepatic radiofrequency lesions in pigs: assessment of the "heat sink" effect. *AJR Am J Roentgenol* 2002; **178**: 47-51 [PMID: 11756085 DOI: 10.2214/ajr.178.1.1780047]

60 **Takaki H**, Yamakado K, Nakatsuka A, Fuke H, Murata K, Shiraki K, Takeda K. Radiofrequency ablation combined with chemoembolization for the treatment of hepatocellular carcinomas 5 cm or smaller: risk factors for local tumor progression. *J Vasc Interv Radiol* 2007; **18**: 856-861 [PMID: 17609444 DOI: 10.1016/j.jvir.2007.04.022]

61 **Kim JH**, Won HJ, Shin YM, Kim SH, Yoon HK, Sung KB, Kim PN. Medium-sized (3.1-5.0 cm) hepatocellular carcinoma: transarterial chemoembolization plus radiofrequency ablation versus radiofrequency ablation alone. *Ann Surg Oncol* 2011; **18**: 1624-1629 [PMID: 21445671 DOI: 10.1245/s10434-011-1673-8]

62 **Peng ZW**, Chen MS, Liang HH, Gao HJ, Zhang YJ, Li JQ, Zhang YQ, Lau WY. A case-control study comparing percutaneous radiofrequency ablation alone or combined with transcatheter arterial chemoembolization for hepatocellular carcinoma. *Eur J Surg Oncol* 2010; **36**: 257-263 [PMID: 19643561 DOI: 10.1016/j.ejso.2009.07.007]

63 **Morimoto M**, Numata K, Kondou M, Nozaki A, Morita S, Tanaka K. Midterm outcomes in patients with intermediate-sized hepatocellular carcinoma: a randomized controlled trial for determining the efficacy of radiofrequency ablation combined with transcatheter arterial chemoembolization. *Cancer* 2010; **116**: 5452-5460 [PMID: 20672352 DOI: 10.1002/cncr.25314]

64 **Berber E**, Siperstein AE. Perioperative outcome after laparoscopic radiofrequency ablation of liver tumors: an analysis of 521 cases. *Surg Endosc* 2007; **21**: 613-618 [PMID: 17287917 DOI: 10.1007/s00464-006-9139-y]

65 **Mulier S**, Ni Y, Jamart J, Ruers T, Marchal G, Michel L. Local recurrence after hepatic radiofrequency coagulation: multivariate meta-analysis and review of contributing factors. *Ann Surg* 2005; **242**: 158-171 [PMID: 16041205 DOI: 10.1097/01.sla.0000171032.99149.fe]

66 **Bleicher RJ**, Allegra DP, Nora DT, Wood TF, Foshag LJ, Bilchik AJ. Radiofrequency ablation in 447 complex unresectable liver

tumors: lessons learned. *Ann Surg Oncol* 2003; **10**: 52-58 [PMID: 12513961 DOI: 10.1245/ASO.2003.03.018]

67 **Hirooka M**, Kisaka Y, Uehara T, Ishida K, Kumagi T, Watanabe Y, Abe M, Matsuura B, Hiasa Y, Onji M. Efficacy of laparoscopic radiofrequency ablation for hepatocellular carcinoma compared to percutaneous radiofrequency ablation with artificial ascites. *Dig Endosc* 2009; **21**: 82-86 [PMID: 19691779 DOI: 10.1111/j.1443-1661.2009.00836.x]

68 **Chinn SB**, Lee FT, Kennedy GD, Chinn C, Johnson CD, Winter TC, Warner TF, Mahvi DM. Effect of vascular occlusion on radiofrequency ablation of the liver: results in a porcine model. *AJR Am J Roentgenol* 2001; **176**: 789-795 [PMID: 11222227 DOI: 10.2214/ajr.176.3.1760789]

69 **Shen P**, Fleming S, Westcott C, Challa V. Laparoscopic radiofrequency ablation of the liver in proximity to major vasculature: effect of the Pringle maneuver. *J Surg Oncol* 2003; **83**: 36-41 [PMID: 12722095 DOI: 10.1002/jso.10235]

70 **Liao M**, Huang J, Zhang T, Wu H. Transarterial chemoembolization in combination with local therapies for hepatocellular carcinoma: a meta-analysis. *PLoS One* 2013; **8**: e68453 [PMID: 23844203 DOI: 10.1371/journal.pone.0068453]

71 **Jacob R,** Turley F, Redden DT, Saddekni S, Aal AK, Keene K, Yang E, Zarzour J, Bolus D, Smith JK, Gray S, White J, Eckhoff DE, DuBay DA. Adjuvant stereotactic body radiotherapy following transarterial chemoembolization in patients with non-resectable hepatocellular carcinoma tumours of ≥3 cm. *HPB* (Oxford) 2014; **17**: 140-149 [PMID: 25186290 DOI: 10.1111/hpb.12331]

72 **Dawson LA**, Hashem S, Bujold A. Stereotactic body radiation therapy for hepatocellular carcinoma. *Am Soc Clin Oncol Educ Book* 2012: 261-264 [PMID: 24451745]

73 **Kang JK**, Kim MS, Cho CK, Yang KM, Yoo HJ, Kim JH, Bae SH, Jung da H, Kim KB, Lee DH, Han CJ, Kim J, Park SC, Kim YH. Stereotactic body radiation therapy for inoperable hepatocellular carcinoma as a local salvage treatment after incomplete transarterial chemoembolization. *Cancer* 2012; **118**: 5424-5431 [PMID: 22570179 DOI: 10.1002/cncr.27533]

74 **Choi BO**, Choi IB, Jang HS, Kang YN, Jang JS, Bae SH, Yoon SK, Chai GY, Kang KM. Stereotactic body radiation therapy with or without transarterial chemoembolization for patients with primary hepatocellular carcinoma: preliminary analysis. *BMC Cancer* 2008; **8**: 351 [PMID: 19038025]

75 **Shin YJ**, Kim MS, Yoo SY, Cho CK, Seo YS, Kang JK, Park SC, Han CJ, Kim SB, Lee BH, Lees DH. Pilot study of stereotactic body radiotherapy for huge hepatocellular carcinoma unsuitable for other therapies. *Tumori* 2010; **96**: 65-70 [PMID: 20437860]

76 **Bujold A**, Massey CA, Kim JJ, Brierley J, Cho C, Wong RK, Dinniwell RE, Kassam Z, Ringash J, Cummings B, Sykes J, Sherman M, Knox JJ, Dawson LA. Sequential phase I and II trials of stereotactic body radiotherapy for locally advanced hepatocellular carcinoma. *J Clin Oncol* 2013; **31**: 1631-1639 [PMID: 23547075 DOI: 10.1200/JCO.2012.44.1659]

77 **Bujold A**, Dawson LA. Stereotactic radiation therapy and selective internal radiation therapy for hepatocellular carcinoma. *Cancer Radiother* 2011; **15**: 54-63 [PMID: 21239204 DOI: 10.1016/j.canrad.2010.11.003]

78 **Honda Y**, Kimura T, Aikata H, Kobayashi T, Fukuhara T, Masaki K, Nakahara T, Naeshiro N, Ono A, Miyaki D, Nagaoki Y, Kawaoka T, Takaki S, Hiramatsu A, Ishikawa M, Kakizawa H, Kenjo M, Takahashi S, Awai K, Nagata Y, Chayama K. Stereotactic body radiation therapy combined with transcatheter arterial chemoembolization for small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013; **28**: 530-536 [PMID: 23216217 DOI: 10.1111/jgh.12087]

79 **Cho JY**, Paik YH, Park HC, Yu JI, Sohn W, Gwak GY, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC. The feasibility of combined transcatheter arterial chemoembolization and radiotherapy for advanced hepatocellular carcinoma. *Liver Int* 2014; **34**: 795-801 [PMID: 24350564 DOI: 10.1111/liv.12445]

80 **Kim KH**, Kim MS, Chang JS, Han KH, Kim do Y, Seong J. Therapeutic benefit of radiotherapy in huge (≥10 cm) unresectable hepatocellular carcinoma. *Liver Int* 2014; **34**: 784-794 [PMID: 24330457 DOI: 10.1111/liv.12436]

81 **Ishikura S**, Ogino T, Furuse J, Satake M, Baba S, Kawashima M, Nihei K, Ito Y, Maru Y, Ikeda H. Radiotherapy after transcatheter arterial chemoembolization for patients with hepatocellular carcinoma and portal vein tumor thrombus. *Am J Clin Oncol* 2002; **25**: 189-193 [PMID: 11943901 DOI: 10.1097/00000421-200204000-00019]

82 **Kim TH**, Kim DY, Park JW, Kim YI, Kim SH, Park HS, Lee WJ, Park SJ, Hong EK, Kim CM. Three-dimensional conformal radiotherapy of unresectable hepatocellular carcinoma patients for whom transcatheter arterial chemoembolization was ineffective or unsuitable. *Am J Clin Oncol* 2006; **29**: 568-575 [PMID: 17148993 DOI: 10.1097/01.coc.0000239147.60196.11]

83 **Zhou ZH**, Liu LM, Chen WW, Men ZQ, Lin JH, Chen Z, Zhang XJ, Jiang GL. Combined therapy of transcatheter arterial chemoembolisation and three-dimensional conformal radiotherapy for hepatocellular carcinoma. *Br J Radiol* 2007; **80**: 194-201 [PMID: 17038412 DOI: 10.1259/bjr/33521596]

84 **Yoon SM**, Lim YS, Won HJ, Kim JH, Kim KM, Lee HC, Chung YH, Lee YS, Lee SG, Park JH, Suh DJ. Radiotherapy plus transarterial chemoembolization for hepatocellular carcinoma invading the portal vein: long-term patient outcomes. *Int J Radiat Oncol Biol Phys* 2012; **82**: 2004-2011 [PMID: 21621346 DOI: 10.1016/j.ijrobp.2011.03.019]

85 **Koo JE**, Kim JH, Lim YS, Park SJ, Won HJ, Sung KB, Suh DJ. Combination of transarterial chemoembolization and three-dimensional conformal radiotherapy for hepatocellular carcinoma with inferior vena cava tumor thrombus. *Int J Radiat Oncol Biol Phys* 2010; **78**: 180-187 [PMID: 19926229 DOI: 10.1016/j.ijrobp.2009.07.1730]

86 **Oh D**, Lim do H, Park HC, Paik SW, Koh KC, Lee JH, Choi MS, Yoo BC, Lim HK, Lee WJ, Rhim H, Shin SW, Park KB. Early three-dimensional conformal radiotherapy for patients with unresectable hepatocellular carcinoma after incomplete transcatheter arterial chemoembolization: a prospective evaluation of efficacy and toxicity. *Am J Clin Oncol* 2010; **33**: 370-375 [PMID: 20142728 DOI: 10.1097/COC.0b013e3181b0c298]

87 **Ni S**, Liu L, Shu Y. Sequential transcatheter arterial chemoembolization, three-dimensional conformal radiotherapy, and high-intensity focused ultrasound treatment for unresectable hepatocellular carcinoma patients. *J Biomed Res* 2012; **26**: 260-267 [PMID: 23554758 DOI: 10.7555/JBR.26.20120016]

88 **Park MS**, Kim SU, Park JY, Kim do Y, Ahn SH, Han KH, Chon CY, Seong J. Combination treatment of localized concurrent chemoradiation therapy and transarterial chemoembolization in locally advanced hepatocellular carcinoma with intrahepatic metastasis. *Cancer Chemother Pharmacol* 2013; **71**: 165-173 [PMID: 23079897 DOI: 10.1007/s00280-012-1993-9]

89 **Cupino AC**, Hair CD, Angle JF, Caldwell SH, Rich TA, Berg CL, Northup PG, Al-Osaimi AM, Argo CK. Does external beam radiation therapy improve survival following transarterial chemoembolization for unresectable hepatocellular carcinoma? *Gastrointest Cancer Res* 2012; **5**: 13-17 [PMID: 22574232]

90 **Sainani NI**, Gervais DA, Mueller PR, Arellano RS. Imaging after percutaneous radiofrequency ablation of hepatic tumors: Part 1, Normal findings. *AJR Am J Roentgenol* 2013; **200**: 184-193 [PMID: 23255761 DOI: 10.2214/AJR.12.8478]

91 **Lim HS**, Jeong YY, Kang HK, Kim JK, Park JG. Imaging features of hepatocellular carcinoma after transcatheter arterial chemoembolization and radiofrequency ablation. *AJR Am J Roentgenol* 2006; **187**: W341-W349 [PMID: 16985104 DOI: 10.2214/AJR.04.1932]

92 **Sainani NI**, Gervais DA, Mueller PR, Arellano RS. Imaging after percutaneous radiofrequency ablation of hepatic tumors: Part 2, Abnormal findings. *AJR Am J Roentgenol* 2013; **200**: 194-204 [PMID: 23255762 DOI: 10.2214/AJR.12.8479]

93 **Kloeckner R**, Otto G, Biesterfeld S, Oberholzer K, Dueber C, Pitton MB. MDCT versus MRI assessment of tumor response after transarterial chemoembolization for the treatment of hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2010; **33**: 532-540 [PMID: 19847482 DOI: 10.1007/s00270-009-9728-y]

94 **Sanuki N**, Takeda A, Mizuno T, Oku Y, Eriguchi T, Iwabuchi S, Kunieda E. Tumor response on CT following hypofractionated stereotactic ablative body radiotherapy for small hypervascular hepatocellular carcinoma with cirrhosis. *AJR Am J Roentgenol* 2013; **201**: W812-W820 [PMID: 24261388 DOI: 10.2214/AJR.12.10169]

95 EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *Eur J Cancer* 2012; **48**: 599-641 [PMID: 22424278 DOI: 10.1016/j.ejca.2011.12.021]

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MINIREVIEWS

Surgical management of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the second most common cause of death from cancer worldwide. Standard potentially curative treatments are either resection or transplantation. The aim of this paper is to provide an overview of the surgical management of HCC, as well as highlight current issues in hepatic resection and transplantation. In summary, due to the relationship between HCC and chronic liver disease, the management of HCC depends both on tumour-related and hepatic function-related considerations. As such, HCC is currently managed largely through non-surgical means as the criteria, in relation to the above considerations, for surgical management is still largely restrictive. For early stage tumours, both resection and transplantation offer fairly good survival outcomes (5 years overall survival of around 50%). Selection therefore would depend on the level of hepatic function derangement, organ availability and local expertise. Patients with intermediate stage cancers have limited options, with resection being the only potential for cure. Otherwise, locoregional therapy with transarterial chemoembolization or radiofrequency ablation are viable options. Current issues in resection and transplantation are also briefly discussed such as laparoscopic resection, ablation *vs* resection, anatomical vs non-anatomical resection, transplantation vs resection, living donor liver transplantation and salvage liver transplantation.

Key words: Hepatocellular carcinoma; Liver surgery; Liver resection; Liver transplantation; Laparoscopic liver surgery

Core tip: Surgical management through either resection or transplantation are the only potentially curative treatment for hepatocellular carcinoma. The decision for the management strategy depends on tumour factors, hepatic functional reserve, organ availability, wait time as well as local expertise and resources.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a prevalent cancer. It is estimated, by the World Health Organisation, to affect 782000 people and caused 746000 deaths worldwide in 2012. In fact, it is the second most

common cause of death from cancer worldwide[1]. Its incidence is the highest in East and South East Asia, which is related to the prevalence of chronic hepatitis B in these regions. Standard potentially curative treatments for this cancer are either resection or transplantation, although radiofrequency ablation is considered curative therapy in some cases[2]. Because of its relationship with liver cirrhosis, the assessment of hepatic function is an important consideration in determining management. It is now well established that liver transplantation is the treatment of choice for early stage HCC in patients with decompensated cirrhosis[3-5].

The aim of this paper is to review the surgical management of hepatocellular carcinoma, as well as highlight current issues in this area.

SURGICAL RESECTION

Only 10%-37% of patients with HCC are amenable to liver resection at the time of diagnosis[6-8]. An Australian study of 235 patients demonstrated that only 17% and 16% of HCC patients were treated with liver resection or transplantation respectively[9]. In fact, best supportive treatment was the most common management strategy employed for this cohort. An important aspect of diagnosis in HCC is that there are risk factors which are known to increase the risk of its development. Both the AASLD and EASL-EORTC guidelines recommend 6 monthly surveillance using abdominal ultrasonography in high risk patients, although the definition of which patients are considered high risk varied[10,11] Interestingly, a systematic review of the benefits and harms of HCC screening in patients with chronic liver disease found only poor quality evidence to support the benefits of screening. Of the two randomised controlled trials reviewed, only one demonstrated a survival benefit (ultrasound screening) and one found no difference in all-cause mortality (alpha-feto protein screening)[12].

Improvements in surgical technique and perio-perative care have led to a fall in the morbidity and mortality of liver resection over the last two decades. Currently, in high volume centres, the mortality of liver resection is expected to be less than 4%[13-16]. There is also evidence to suggest that there is a volume-outcome relationship in hepatic resection surgery[17]. Whilst the 5 years overall survival rates of around 50% is now achievable, the recurrence rate remains high which remains an important cause of late deaths[18,19].

The decision for liver resection depends on the assessment of tumour factors, hepatic function and remnant size. With regards to tumour factors, one of the most commonly used staging system in Western countries is the Barcelona Clinic liver Cancer (BCLC) system which classifies patients into early, intermediate, advanced and terminal stages[20]. This system is utilised by both the American Association for the Study of Liver Disease and the European Association for the Study of Liver guidelines on the management of HCC[21,22]. According to this system, the surgical treatment of HCC is limited to the early stage cancers, that is, those which satisfy the Milan Criteria (a single HCC $\leqslant$ 5 cm in diameter or up to 3 HCCs $\leqslant$ 3 cm in diameter[5]) and have good hepatic function (Child-Pugh class A in the absence of portal hypertension) and performance status. However, such a criteria for resection is considered restrictive, for size and number of tumours is not a contraindication for resection provided there is adequate hepatic reserve and that the tumour is resectable. Certainly, long term disease-free survival is possible in these patients[23]. Tumour size and number are not the most important factors influencing survival[9,19]. In fact, for those patients with HCCs who do not satisfy the Milan Criteria, the only hope of cure is through hepatic resection. Ng *et al*[24] demonstrated that large or multinodular HCC could be safely resected, with a five-year overall survival of 39% and disease-free survival of 26% being achievable. In general, tumours which are extensively multifocal and bilateral, involve the main portal vein or inferior vena cava are considered contraindicated for surgery.

Hepatic function can be classified using a variety of measures. The simplest and most commonly used is the Child-Pugh Score[25]. Resection is really only considered in patients with Child A cirrhosis and early Child B cirrhosis. In the former, up to 50% resection may be considered, whilst in the latter, up to 25% resection may be performed. On the other hand, in patients with entirely normal hepatic function with no history of cirrhosis could tolerate the resection of up to 75% of liver parenchyma[26]. In Asian countries, the use of ICG clearance at 15 min is also prevalent, with a cut-off of greater than 20% precluding major liver resection[27,28]. Model of End-Stage Liver Disease (MELD) score is an alternative score used to classify patients into risk groups. A MELD score of < 9 is associated with minimal perioperative mortality[29,30]. In addition to hepatic function, the other aspect which precludes hepatic resection is significant portal hypertension. This can be objectively measured using a transhepatic caval approach (hepatic vein pressure gradient). This is a measure of the pressure difference between the wedged hepatic venous pressure (an estimation of portal venous pressure) and the free hepatic venous pressure (inferior vena caval pressure). A pressure gradient of greater than 10 mmHg is associated with poorer outcomes post resection[31]. Other indicators of clinically relevant portal hypertension include splenomegaly, oesophageal varices and thrombocytopaenia.

Given the relevance of liver function on the permitted resection size, the size of the liver remnant is important. This can be measured using CT volumetry[32]. If adequate future liver remnant is not achievable, then portal vein embolization (PVE) should be considered. The aim of PVE is to induce compensatory hypertrophy in the non-embolised side. Generally, this is performed by the percutaneous transhepatic approach. A recent meta-analysis has demonstrated that PVE is safe and effective in inducing liver hypertrophy and preventing liver failure[33]. It has also been shown to increase resectability[34,35].

It should be noted that the recurrence rate after hepatic resection is high. In a systematic review and meta-analysis of resection vs transplantation, the 5 year disease-free survival rate of resection varied from 18%-51% compared to 54%-84% for transplanted patients[18]. In patients with intermediate and advanced stage HCC (multiple tumours or macrovascular invasion), 5 year disease free survival range from 0%-31%[36]. Follow-up for recurrence is therefore mandatory and recurrence should be managed using a multimodal approach including re-resection, TACE and ablative therapy.

CURRENT ISSUES IN HEPATIC RESECTION

Laparoscopic liver resection

With the advent of minimally invasive surgery, there is increasing uptake of the laparoscopic techniques for liver resection. Initially, the experience of laparoscopic liver resection was restricted to benign pathologies, and peripheral lesions/left lateral sectionectomy, although now major resections are being conducted laparoscopically[37]. There have been several systematic reviews with meta-analyses on this topic. The most recent and the largest, a meta-analysis of 32 studies by Rao *et al*[37], found that laparoscopic hepatic resection was associated with significantly lower blood transfusion requirements, blood loss and length of stay but longer operating time. The overall complication rate was significantly lower (OR = 0.35, $P < 0.001$) in the laparoscopic group. Whilst overall survival was not different between the two groups, the rate of positive resection margins were found to be lower in the laparoscopic group. Note however, that the vast majority of studies were retrospective studies, with no randomised controlled trials, and therefore there may be significant selection bias. Unsurprisingly, these findings echo those of an earlier meta-analysis of 26 studies[38]. However, in relation to oncological outcomes, this meta-analysis analysed HCC outcomes separately to other malignant diseases and found that there was a significant trend for improved overall survival (OR: 1.5 - 1.0-2.2; $P = 0.049$) in the laparoscopic group. Another meta-analysis restricted only to studies evaluating laparoscopic resection for patients with HCC has demonstrated similar findings - lesser blood loss and blood transfusion requirements, lesser overall morbidity, cirrhotic decompensation and shorter length of stay[39]. However, no differences in oncological outcomes (margins and survival) were found. Whilst the above studies point to potential advantages of performing laparoscopic hepatic resection, the major weakness of this systematic review is that the majority of studies included only patients who underwent minor hepatic resections. Their findings therefore may not be applicable to major laparoscopic hepatic resections. The efficacy of major liver resections is still under evaluation although early reports would suggest that they are comparable to the open procedure in terms of short and long term outcomes. For instance, Martin *et al*[40] compared 90 laparoscopic hepatectomies (left or right) to case-matched open hepatectomies and found lesser blood loss, lesser use of Pringle manoeuvre, lesser operative time, and lesser incidence of any type of complication. At present there exist only a few case series on robotic major hepatic resections-so while it is possible, the limited experience makes any conclusion about its comparative efficacy and risks difficult to make at the present time[41].

Whilst the above results are encouraging, these should be interpreted with caution as there is likely significant publication and selection biases in the above studies. Also, laparoscopic liver resection has a learning curve, both for the surgeon and the institution[42]. Results in centres where such expertise is available may not be generalizable to other centres. For instance, Vigano *et al*[43] demonstrated over the course of a 12 year period, operative time, pedicle clamping, blood loss, morbidity and hospital stay all decreased[43]. They estimated the learning curve for minor laparoscopic liver resection is 60 based upon cumulative sum analysis on conversion rates. The same group reviewed the major hepatectomies performed laparoscopically at 6 experienced centres around the world and found similar improvements in operative time, conversion rate, blood loss and pedicle clamping[42]. A larger study of 365 patients over a 14 year period estimated the learning curve for laparoscopic liver surgery to be in the order of 30-40 cases[44].

Ablation vs resection

The alternative to resection in early HCC (satisfying Milan criteria) is ablation, either percutaneous ethanol injection (PEI), radiofrequency (RFA) or microwave ablation. In theory, ablation could treat a tumour of up to 5 cm in diameter - a size which correlates with the Milan criteria. Indeed, the European Association for the Study of the Liver

(EASL) and the European Organisation for Research and Treatment of Cancer (EORTC) guidelines suggest that ablation with either radiofrequency ablation or percutaneous ethanol injection is recommended as standard of care for patients with BCLC stage 0 or A who are unsuitable for surgery[11]. A recent meta-analysis analysing outcomes from three randomised controlled trials and 25 non randomised studies has suggested little difference in survival or recurrence early after intervention but at 5 years, significantly different survival began to be observed. This appeared to be more pronounced in larger tumours than smaller ones and more in the non-randomised studies than the randomised trials. Consistently however, the complication rate and length of stay favoured RFA[45]. Another recent systematic review which pooled the findings of 6 randomised controlled trials and 4 cohort studies comparing RFA/PEI to resection came to the same conclusion - that early outcomes (survival and recurrence) were equivalent (at one-year) but the differences became more pronounced with longer duration of follow-up. This seemed to apply even for small cancers (tumour size $\leqslant$ 3 cm). Complication rates however significantly favoured ablative therapy[46]. Therefore one can conclude that in appropriately selected patients, surgical resection is the preferred management even for small cancers although ablative treatment had the advantage of lower morbidity.

Anatomical vs non-anatomical resection

The debate surrounding anatomical *vs* non-anatomical resection remain a controversial one. In theory, hepatocellular carcinomas recurrence is strongly related to microvascular tumour emboli, therefore, resection of the vascular territory of the tumour makes oncological sense[47]. On the other hand, HCC often occurs in cirrhotic livers and the preservation of hepatic parenchyma to prevent postoperative liver failure suggests a non-anatomical approach. Indeed, numerous studies have been performed to elucidate the benefits of either approach, but none in a randomised fashion[48]. Two meta-analysis of these non-randomised studies favoured anatomical resection although only one found statistically significant difference between the two groups in terms of both overall and disease-free survival[49,50]. To further complicate one's understanding of this debate, a meta-regression performed by Cucchetti *et al*[48] demonstrated that much of the heterogeneity of overall and disease-free survival results arose from the presence or absence of cirrhosis as a covariate. That is, non-anatomical resection had poorer outcomes because of the higher prevalence of cirrhosis in that group[48]. Hence, only a randomised trial whereby the baseline characteristics are randomised can we make any final conclusions regarding this ongoing debate. An interesting addition to the debate comes from the development of preoperative 3D simulation, which facilitates subsegmental and segmental anatomical resection, potentially allowing anatomical resection to be performed in patients who have limited hepatic reserve and allowing for a quality indicator of success or otherwise of anatomical resection[51].

LIVER TRANSPLANTATION

The first successful liver transplantation in humans was performed in 1967[52]. The attraction of using liver resection to manage HCC is that not only is the HCC treated with maximal resection margins, the underlying liver disease (and hence, premalignant field change) is also treated. In a landmark paper by Mazzaferro *et al*[5], the Milan criteria were established in 1996. Mazzaferro *et al*[5] described that patients operated within this criteria had excellent outcomes post liver transplantation which were comparable to those of patients operated on for non-cancer indications. The overall and recurrence-free survival rates at 4 years were 85% and 92% respectively[5]. This criteria was subsequently expanded by the University of California San Francisco (UCSF) group who nonetheless demonstrated equivalent excellent outcomes - 5 year survival of 72%[53]. These results have been validated in the Australian/New Zealand cohort by Chen *et al*[54], who demonstrated a 5 year survival rate of 74% and 73% in those satisfying the Milan and UCSF criteria respectively[54]. Those outside Milan or UCSF criteria were found to have significantly poorer outcomes. On the other hand, the use of UCSF criteria as preoperative selection criteria was found by a French group to have resulted in a 5 year survival of less than 50% despite a short waiting time[55].

Other complications of transplantation include rejection as well as complications from immunosuppression may limit the long term survival of transplant recipients[56]. The major limitation of liver transplantation in the treatment of HCC is the limited availability of donor livers. These patients with HCC also compete with non-cancer patients for transplants. As a result, strict listing criteria are used, such as mentioned above, to limit transplantation to those patients whose outcomes are comparable to those who do not have HCC. In the context of donor shortage, it is often accepted that transplanted patients should have 5 year survival rates of at least 50%. Unfortunately, whilst outcomes of transplantation are good, the potentially significant period on the waiting list may lead to dropout due to disease progression. This could be as high as 25%-38% in 12 mo, although it is highly variable[55,57,58]. In fact, studies included in a recent systematic review reported median time to transplantation varying from 30 to 231 d[18].

CURRENT ISSUES IN LIVER TRANSPLANTATION

Liver resection vs liver transplantation

The question of whether liver transplantation or liver resection is more efficacious in the treatment of HCC depend very much on the clinical scenario. For instance, for the patient with early HCC with inadequate hepatic reserve for resection, transplantation may be the only potentially curative option. On the other hand, a patient with a large HCC but with preserved hepatic function would only have resection as the curative option. The controversial case is of patients with early HCC with well compensated cirrhosis. In this case, not only are the outcomes of resection and transplantation important, the availability of donor livers, and therefore the dropout rate is highly relevant. Analysis of survival on an intention-to-treat basis would be more reflective of the relative efficacy of each treatment strategy in the real world. There have been some recent meta-analyses conducted to evaluate the question of relative efficacy. Rahman *et al*[18] in 2012 looked at nine studies comparing liver resection and transplantation for early stage HCC[18]. The key finding was that all these studies were retrospective and only a few reported intention-to-treat survival data for the transplantation group. Five-year overall survival ranged from 40%-70% for resection and 52%-81% for transplantation. Pooling of data from studies which conducted intention-to-treat survival analysis demonstrated no significant difference in survival between the two treatment strategies at 5 years. Another meta-analysis on an intention-to-treat basis also found no significant difference between the outcomes of the two groups[59]. The other key finding highlighted by these two systematic reviews is the lack of prospective/randomised or even simply well-matched studies in the literature. Certainly, as yet there are no randomised controlled trials to guide treatment[60].

Living donor liver transplantation

With the limitation of the availability of cadaveric liver transplants, there is increasing interest in the use of living donor liver transplant (LDLT). Clearly this requires the donation of a liver graft from a donor - a procedure not without its risks. The risk of mortality is estimated to be 0.1% for donor left hepatectomy and 0.5% for donor right hepatectomy; with a morbidity rate of up to 20%[61,62]. This risk to the donor, without direct beneficial effects to the person also brings about an ethical dilemma to transplant surgeons and physicians alike - "first do no harm". However, the advantage of LDLT is that as the liver is obtained outside the usual donor pool, this strategy expands the number of organs available for transplantation. As a result, the criteria for liver transplantation can often be extended beyond the usual Milan or UCSF criteria. The Kyoto criteria ($\leqslant$ 10 tumours, less than 5 cm, PIVKA-Ⅱ $\leqslant$ 400) and "up-to-seven" criteria are examples of extended transplant criteria which have been used in the context of living donor transplants[63,64]. Using a decision analytical model taking into account the risk of dropout while waiting (4% per month), the expected survival of the recipient (70% at five years) and the risk for the donor (0.3% to 0.5% mortality), Sarasin *et al*[65] demonstrated that patients with HCC waiting more than seven months for a deceased donor liver would benefit from LDLT. Early reports have suggested a higher recurrence rate in LDLT as compared to Deceased donor liver transplantation (DDLT), however, this is hypothesised to be due to the "fast-tracking" of LDLT which therefore allowed transplantation of patients with more aggressive HCC[61]. Two recent meta-analysis of LDLT *vs* DDLT were reported. These found overall survival rates to be similar between the two groups. One of these, by Grant *et al*[66] found LDLTs to be associated with decreased disease-free survival rates. On the other hand, Liang *et al*[67] performed a subgroup analysis of patients within the Milan criteria and found similar survival outcomes between the two groups.

Bridging therapy and salvage liver transplantation

Out of the need to minimise dropout during waiting, strategies such as bridging therapy or resection with salvage transplantation has been developed. Bridging therapies such as RFA or TACE are frequently used. Whilst bridging therapy does seem to be useful in decreasing dropout rate whilst awaiting transplant, its role in improving survival after transplantation has not been established[68]. An alternative strategy to primary transplantation is primary resection followed by salvage transplantation. The advantage to this is to minimise the need for organs and to use resection as the ultimate bridging therapy to prevent progression whilst waiting for transplantation. A recent systematic review of 16 studies found that of those 7 studies which reported salvage transplant rates, the median rate of salvage transplantation was 41% after a median time to recurrence of 21 mo[69]. Whilst a meta-analysis was not performed, they found a median 5 year survival to be 67%. Interestingly, half of studies reported a mortality rate of higher than 5% and two studies reported mortality rates of greater than 10%[70,71]. A meta-analysis by Zhu *et al*[72] analysed 14 studies, of which 10 overlapped with Chan's systematic review. Zhu *et al*[72] found that compared to primary liver transplantation, salvage liver transplantation was associated with longer operative time, greater blood loss but failed to find a significant difference in postoperative mortality. With regards to long term survival, primary liver transplant was found to have

better five-year disease free survival but not overall survival[72]. These results would suggest that salvage liver transplant is a viable strategy in appropriately resourced transplant centres.

CONCLUSION

HCC is currently largely managed through non-surgical means as the tumour-related and hepatic function considerations for surgical management is still largely restrictive. For those who have tumours eligible for surgical therapy from the tumour point of view (early stage tumours), those with good hepatic function and significant functional liver remnant would be candidates for either resection or transplantation depending on local resources. Those with poor hepatic function may be placed on the liver transplant list, with or without bridging therapy. Patients with intermediate stage cancers have limited options, with resection being the only potential for cure. Otherwise, regional therapy with TACE or RFA are viable options. With further development of surgical techniques, including salvage liver transplantation, the indications for surgical management of HCC may continue to expand. With this, the outcomes of HCC may further improve.

Surgical therapy is the only curative hope for patients with HCC. The selection of patients for transplantation and resection will depend on local resources, but both have potentially good outcomes in appropriately selected patients.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin D, Forman D, Bray F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer, 2013. Available from: URL: http://globocan.iarc.fr

2 **Lau WY**, Lai EC. Hepatocellular carcinoma: current management and recent advances. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 237-257 [PMID: 18522878]

3 **Poon RT**, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002; **235**: 373-382 [PMID: 11882759]

4 **Fan ST**, Cheung ST, Lo CM. Indications for liver transplantation in patients with chronic hepatitis B and C virus infection and hepatocellular carcinoma. *J Gastroenterol Hepatol* 2000; **15** Suppl: E181-E186 [PMID: 10921405]

5 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

6 **Fan ST**, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg* 1999; **229**: 322-330 [PMID: 10077043]

7 **Colella G**, Bottelli R, De Carlis L, Sansalone CV, Rondinara GF, Alberti A, Belli LS, Gelosa F, Iamoni GM, Rampoldi A, De Gasperi A, Corti A, Mazza E, Aseni P, Meroni A, Slim AO, Finzi M, Di Benedetto F, Manochehri F, Follini ML, Ideo G, Forti D. Hepatocellular carcinoma: comparison between liver transplantation, resective surgery, ethanol injection, and chemoembolization. *Transpl Int* 1998; **11** Suppl 1: S193-S196 [PMID: 9664977]

8 **Fong Y**, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a Western center. *Ann Surg* 1999; **229**: 790-799; discussion 790-799 [PMID: 10363892]

9 **Perry JF**, Charlton B, Koorey DJ, Waugh RC, Gallagher PJ, Crawford MD, Verran DJ, McCaughan GW, Strasser SI. Outcome of patients with hepatocellular carcinoma referred to a tertiary centre with availability of multiple treatment options including cadaveric liver transplantation. *Liver Int* 2007; **27**: 1240-1248 [PMID: 17919236 DOI: 10.1111/j.1478-3231.2007.01569.x]

10 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

11 **European Association For The Study Of The L, European Organisation For R, Treatment Of C.** EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

12 **Kansagara D**, Papak J, Pasha AS, O'Neil M, Freeman M, Relevo R, Quiñones A, Motu'apuaka M, Jou JH. Screening for hepatocellular carcinoma in chronic liver disease: a systematic review. *Ann Intern Med* 2014; **161**: 261-269 [PMID: 24934699 DOI: 10.7326/M14-0558]

13 **Andreou A**, Vauthey JN, Cherqui D, Zimmitti G, Ribero D, Truty MJ, Wei SH, Curley SA, Laurent A, Poon RT, Belghiti J, Nagorney DM, Aloia TA. Improved long-term survival after major resection for hepatocellular carcinoma: a multicenter analysis based on a new definition of major hepatectomy. *J Gastrointest Surg* 2013; **17**: 66-77; discussion p.77 [PMID: 22948836 DOI: 10.1007/s11605-012-2005-4]

14 **Fan ST**, Mau Lo C, Poon RT, Yeung C, Leung Liu C, Yuen WK, Ming Lam C, Ng KK, Ching Chan S. Continuous improvement of survival outcomes of resection of hepatocellular carcinoma: a 20-year experience. *Ann Surg* 2011; **253**: 745-758 [PMID: 21475015 DOI: 10.1097/SLA.0b013e3182111195]

15 **Li GZ**, Speicher PJ, Lidsky ME, Darrabie MD, Scarborough JE, White RR, Turley RS, Clary BM. Hepatic resection for hepatocellular carcinoma: do contemporary morbidity and mortality rates demand a transition to ablation as first-line treatment? *J Am Coll Surg* 2014; **218**: 827-834 [PMID: 24655879]

16 **Yang T**, Zhang J, Lu JH, Yang GS, Wu MC, Yu WF. Risk factors influencing postoperative outcomes of major hepatic resection of hepatocellular carcinoma for patients with underlying liver diseases. *World J Surg* 2011; **35**: 2073-2082 [PMID: 21656309 DOI: 10.1007/s00268-011-1161-0]

17 **Richardson AJ**, Pang TC, Johnston E, Hollands MJ, Lam VW, Pleass HC. The volume effect in liver surgery--a systematic review and meta-analysis. *J Gastrointest Surg* 2013; **17**: 1984-1996 [PMID: 24002759 DOI: 10.1007/s11605-013-2314-2]

18 **Rahman A**, Assifi MM, Pedroso FE, Maley WR, Sola JE, Lavu H, Winter JM, Yeo CJ, Koniaris LG. Is resection equivalent to transplantation for early cirrhotic patients with hepatocellular carcinoma? A meta-analysis. *J Gastrointest Surg* 2012; **16**: 1897-1909 [PMID: 22836922]

19 **Poon RT**, Fan ST, Lo CM, Ng IO, Liu CL, Lam CM, Wong J. Improving survival results after resection of hepatocellular carcinoma: a prospective study of 377 patients over 10 years. *Ann Surg* 2001; **234**: 63-70 [PMID: 11420484]

20 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750]

21 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607]

22 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/

hep.20933]
23 **Poon RT**, Ng IO, Fan ST, Lai EC, Lo CM, Liu CL, Wong J. Clinicopathologic features of long-term survivors and disease-free survivors after resection of hepatocellular carcinoma: a study of a prospective cohort. *J Clin Oncol* 2001; **19**: 3037-3044 [PMID: 11408499]
24 **Ng KK**, Vauthey JN, Pawlik TM, Lauwers GY, Regimbeau JM, Belghiti J, Ikai I, Yamaoka Y, Curley SA, Nagorney DM, Ng IO, Fan ST, Poon RT. Is hepatic resection for large or multinodular hepatocellular carcinoma justified? Results from a multi-institutional database. *Ann Surg Oncol* 2005; **12**: 364-373 [PMID: 15915370]
25 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913]
26 **de Baere T**, Roche A, Elias D, Lasser P, Lagrange C, Bousson V. Preoperative portal vein embolization for extension of hepatectomy indications. *Hepatology* 1996; **24**: 1386-1391 [PMID: 8938166 DOI: 10.1053/jhep.1996.v24.pm0008938166]
27 **Lam CM**, Fan ST, Lo CM, Wong J. Major hepatectomy for hepatocellular carcinoma in patients with an unsatisfactory indocyanine green clearance test. *Br J Surg* 1999; **86**: 1012-1017 [PMID: 10460635 DOI: 10.1046/j.1365-2168.1999.01204.x]
28 **Poon RT**, Fan ST. Hepatectomy for hepatocellular carcinoma: patient selection and postoperative outcome. *Liver Transpl* 2004; **10**: S39-S45 [PMID: 14762838]
29 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, La Barba G, Zanello M, Grazi GL, Pinna AD. Impact of model for end-stage liver disease (MELD) score on prognosis after hepatectomy for hepatocellular carcinoma on cirrhosis. *Liver Transpl* 2006; **12**: 966-971 [PMID: 16598792 DOI: 10.1002/lt.20761]
30 **Teh SH**, Christein J, Donohue J, Que F, Kendrick M, Farnell M, Cha S, Kamath P, Kim R, Nagorney DM. Hepatic resection of hepatocellular carcinoma in patients with cirrhosis: Model of End-Stage Liver Disease (MELD) score predicts perioperative mortality. *J Gastrointest Surg* 2005; **9**: 1207-1215; discussion 1215 [PMID: 16332475 DOI: 10.1016/j.gassur.2005.09.008]
31 **Bruix J**, Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597]
32 **Vauthey JN**, Chaoui A, Do KA, Bilimoria MM, Fenstermacher MJ, Charnsangavej C, Hicks M, Alsfasser G, Lauwers G, Hawkins IF, Caridi J. Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations. *Surgery* 2000; **127**: 512-519 [PMID: 10819059 DOI: 10.1067/msy.2000.105294]
33 **Abulkhir A**, Limongelli P, Healey AJ, Damrah O, Tait P, Jackson J, Habib N, Jiao LR. Preoperative portal vein embolization for major liver resection: a meta-analysis. *Ann Surg* 2008; **247**: 49-57 [PMID: 18156923 DOI: 10.1097/SLA.0b013e31815f6e5b]
34 **Azoulay D**, Castaing D, Krissat J, Smail A, Hargreaves GM, Lemoine A, Emile JF, Bismuth H. Percutaneous portal vein embolization increases the feasibility and safety of major liver resection for hepatocellular carcinoma in injured liver. *Ann Surg* 2000; **232**: 665-672 [PMID: 11066138]
35 **Wakabayashi H**, Ishimura K, Okano K, Izuishi K, Karasawa Y, Goda F, Maeba T, Maeta H. Is preoperative portal vein embolization effective in improving prognosis after major hepatic resection in patients with advanced-stage hepatocellular carcinoma? *Cancer* 2001; **92**: 2384-2390 [PMID: 11745294]
36 **Zhong JH,** Ke Y, Wang YY, Li LQ. Liver resection for patients with hepatocellular carcinoma and macrovascular invasion, multiple tumours, or portal hypertension. *Gut* 2014 Sep 3; Epub ahead of print [PMID: 25187522]
37 **Rao A**, Rao G, Ahmed I. Laparoscopic or open liver resection? Let systematic review decide it. *Am J Surg* 2012; **204**: 222-231 [PMID: 22245507 DOI: 10.1016/j.amjsurg.2011.08.013]
38 **Mirnezami R**, Mirnezami AH, Chandrakumaran K, Abu Hilal M, Pearce NW, Primrose JN, Sutcliffe RP. Short- and long-term outcomes after laparoscopic and open hepatic resection: systematic review and meta-analysis. *HPB* (Oxford) 2011; **13**: 295-308 [PMID: 21492329 DOI: 10.1111/j.1477-2574.2011.00295.x]
39 **Zhou YM**, Shao WY, Zhao YF, Xu DH, Li B. Meta-analysis of laparoscopic versus open resection for hepatocellular carcinoma. *Dig Dis Sci* 2011; **56**: 1937-1943 [PMID: 21259071 DOI: 10.1007/s10620-011-1572-7]
40 **Martin RC**, Scoggins CR, McMasters KM. Laparoscopic hepatic lobectomy: advantages of a minimally invasive approach. *J Am Coll Surg* 2010; **210**: 627-634, 634-636 [PMID: 20421019 DOI: 10.1016/j.jamcollsurg.2009.12.022]
41 **Boggi U**, Caniglia F, Amorese G. Laparoscopic robot-assisted major hepatectomy. *J Hepatobiliary Pancreat Sci* 2014; **21**: 3-10 [PMID: 24115394 DOI: 10.1002/jhbp.34]
42 **Kluger MD**, Vigano L, Barroso R, Cherqui D. The learning curve in laparoscopic major liver resection. *J Hepatobiliary Pancreat Sci* 2013; **20**: 131-136 [PMID: 23064988]
43 **Vigano L**, Laurent A, Tayar C, Tomatis M, Ponti A, Cherqui D. The learning curve in laparoscopic liver resection: improved feasibility and reproducibility. *Ann Surg* 2009; **250**: 772-782 [PMID: 19801926 DOI: 10.1097/SLA.0b013e3181bd93b2]
44 **Cai X**, Li Z, Zhang Y, Yu H, Liang X, Jin R, Luo F. Laparoscopic liver resection and the learning curve: a 14-year, single-center experience. *Surg Endosc* 2014; **28**: 1334-1341 [PMID: 24399518]
45 **Wang Y**, Luo Q, Li Y, Deng S, Wei S, Li X. Radiofrequency ablation versus hepatic resection for small hepatocellular carcinomas: a meta-analysis of randomized and nonrandomized controlled trials. *PLoS One* 2014; **9**: e84484 [PMID: 24404166 DOI: 10.1371/journal.pone.0084484]
46 **Ni JY**, Xu LF, Sun HL, Zhou JX, Chen YT, Luo JH. Percutaneous ablation therapy versus surgical resection in the treatment for early-stage hepatocellular carcinoma: a meta-analysis of 21,494 patients. *J Cancer Res Clin Oncol* 2013; **139**: 2021-2033 [PMID: 24072235 DOI: 10.1007/s00432-013-1530-1]
47 **Park JH**, Koh KC, Choi MS, Lee JH, Yoo BC, Paik SW, Rhee JC, Joh JW. Analysis of risk factors associated with early multinodular recurrences after hepatic resection for hepatocellular carcinoma. *Am J Surg* 2006; **192**: 29-33 [PMID: 16769271 DOI: 10.1016/j.amjsurg.2005.11.010]
48 **Cucchetti A**, Cescon M, Ercolani G, Bigonzi E, Torzilli G, Pinna AD. A comprehensive meta-regression analysis on outcome of anatomic resection versus nonanatomic resection for hepatocellular carcinoma. *Ann Surg Oncol* 2012; **19**: 3697-3705 [PMID: 22722807 DOI: 10.1245/s10434-012-2450-z]
49 **Zhou Y**, Xu D, Wu L, Li B. Meta-analysis of anatomic resection versus nonanatomic resection for hepatocellular carcinoma. *Langenbecks Arch Surg* 2011; **396**: 1109-1117 [PMID: 21476060 DOI: 10.1007/s00423-011-0784-9]
50 **Chen J**, Huang K, Wu J, Zhu H, Shi Y, Wang Y, Zhao G. Survival after anatomic resection versus nonanatomic resection for hepatocellular carcinoma: a meta-analysis. *Dig Dis Sci* 2011; **56**: 1626-1633 [PMID: 21082347 DOI: 10.1007/s10620-010-1482-0]
51 **Takamoto T**, Hashimoto T, Ogata S, Inoue K, Maruyama Y, Miyazaki A, Makuuchi M. Planning of anatomical liver segmentectomy and subsegmentectomy with 3-dimensional simulation software. *Am J Surg* 2013; **206**: 530-538 [PMID: 23809675 DOI: 10.1016/j.amjsurg.2013.01.041]
52 **Starzl TE**, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, Blanchard H, Martin AJ, Porter KA. Orthotopic homotransplantation of the human liver. *Ann Surg* 1968; **168**: 392-415 [PMID: 4877589]
53 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403 [PMID: 11391528 DOI: 10.1053/jhep.2001.24563]
54 **Chen JW**, Kow L, Verran DJ, McCall JL, Munn S, Balderson GA, Fawcett JW, Gow PJ, Jones RM, Jeffrey GP, House AK, Strasser SI. Poorer survival in patients whose explanted hepatocellular carcinoma (HCC) exceeds Milan or UCSF Criteria. An analysis

of liver transplantation in HCC in Australia and New Zealand. *HPB (Oxford)* 2009; **11**: 81-89 [PMID: 19590628 DOI: 10.1111/j.1477-2574.2009.00022.x]

55 **Decaens T**, Roudot-Thoraval F, Hadni-Bresson S, Meyer C, Gugenheim J, Durand F, Bernard PH, Boillot O, Sulpice L, Calmus Y, Hardwigsen J, Ducerf C, Pageaux GP, Dharancy S, Chazouilleres O, Cherqui D, Duvoux C. Impact of UCSF criteria according to pre- and post-OLT tumor features: analysis of 479 patients listed for HCC with a short waiting time. *Liver Transpl* 2006; **12**: 1761-1769 [PMID: 16964590 DOI: 10.1002/lt.20884]

56 **Conti F**, Morelon E, Calmus Y. Immunosuppressive therapy in liver transplantation. *J Hepatol* 2003; **39**: 664-678 [PMID: 14568246]

57 **Maddala YK**, Stadheim L, Andrews JC, Burgart LJ, Rosen CB, Kremers WK, Gores G. Drop-out rates of patients with hepatocellular cancer listed for liver transplantation: outcome with chemoembolization. *Liver Transpl* 2004; **10**: 449-455 [PMID: 15004776 DOI: 10.1002/lt.20099]

58 **Yao FY**, Bass NM, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: lessons from the first year under the Model of End-Stage Liver Disease (MELD) organ allocation policy. *Liver Transpl* 2004; **10**: 621-630 [PMID: 15108253 DOI: 10.1002/lt.20159]

59 **Dhir M**, Lyden ER, Smith LM, Are C. Comparison of outcomes of transplantation and resection in patients with early hepatocellular carcinoma: a meta-analysis. *HPB* (Oxford) 2012; **14**: 635-645 [PMID: 22882201 DOI: 10.1111/j.1477-2574.2012.00500.x]

60 **Taefi A**, Abrishami A, Nasseri-Moghaddam S, Eghtesad B, Sherman M. Surgical resection versus liver transplant for patients with hepatocellular carcinoma. *Cochrane Database Syst Rev* 2013; **6**: CD006935 [PMID: 23813393]

61 **Chan SC**. Section 2. Small-for-size liver graft and hepatocellular carcinoma recurrence. *Transplantation* 2014; **97** Suppl 8: S7-S10 [PMID: 24849840 DOI: 10.1097/01.tp.0000446266.42019.28]

62 **Chan SC**, Fan ST, Lo CM, Liu CL, Wong J. Toward current standards of donor right hepatectomy for adult-to-adult live donor liver transplantation through the experience of 200 cases. *Ann Surg* 2007; **245**: 110-117 [PMID: 17197973 DOI: 10.1097/01.sla.0000225085.82193.08]

63 **Ito T**, Takada Y, Ueda M, Haga H, Maetani Y, Oike F, Ogawa K, Sakamoto S, Ogura Y, Egawa H, Tanaka K, Uemoto S. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007; **13**: 1637-1644 [PMID: 18044766 DOI: 10.1002/lt.21281]

64 **Xiaolu D**, Jing P, Fang H, Lifen Y, Liwen W, Ciliu Z, Fei Y. Role of p115RhoGEF in lipopolysaccharide-induced mouse brain microvascular endothelial barrier dysfunction. *Brain Res* 2011; **1387**: 1-7 [PMID: 21354111 DOI: 10.1016/j.brainres.2011.02.059]

65 **Sarasin FP**, Majno PE, Llovet JM, Bruix J, Mentha G, Hadengue A. Living donor liver transplantation for early hepatocellular carcinoma: A life-expectancy and cost-effectiveness perspective. *Hepatology* 2001; **33**: 1073-1079 [PMID: 11343234 DOI: 10.1053/jhep.2001.23311]

66 **Grant RC**, Sandhu L, Dixon PR, Greig PD, Grant DR, McGilvray ID. Living vs. deceased donor liver transplantation for hepatocellular carcinoma: a systematic review and meta-analysis. *Clin Transplant* 2013; **27**: 140-147 [PMID: 23157398 DOI: 10.1111/ctr.12031]

67 **Liang W**, Wu L, Ling X, Schroder PM, Ju W, Wang D, Shang Y, Kong Y, Guo Z, He X. Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: a meta-analysis. *Liver Transpl* 2012; **18**: 1226-1236 [PMID: 22685095 DOI: 10.1002/lt.23490]

68 **Pompili M**, Francica G, Ponziani FR, Iezzi R, Avolio AW. Bridging and downstaging treatments for hepatocellular carcinoma in patients on the waiting list for liver transplantation. *World J Gastroenterol* 2013; **19**: 7515-7530 [PMID: 24282343 DOI: 10.3748/wjg.v19.i43.7515]

69 **Chan DL**, Alzahrani NA, Morris DL, Chua TC. Systematic review of efficacy and outcomes of salvage liver transplantation after primary hepatic resection for hepatocellular carcinoma. *J Gastroenterol Hepatol* 2014; **29**: 31-41 [PMID: 24117517 DOI: 10.1111/jgh.12399]

70 **Adam R**, Azoulay D, Castaing D, Eshkenazy R, Pascal G, Hashizume K, Samuel D, Bismuth H. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003; **238**: 508-518; discussion 518-519 [PMID: 14530722 DOI: 10.1097/01.sla.0000090449.87109.44]

71 **Vennarecci G**, Ettorre GM, Antonini M, Santoro R, Maritti M, Tacconi G, Spoletini D, Tessitore L, Perracchio L, Visco G, Puoti C, Santoro E. First-line liver resection and salvage liver transplantation are increasing therapeutic strategies for patients with hepatocellular carcinoma and child a cirrhosis. *Transplant Proc* 2007; **39**: 1857-1860 [PMID: 17692633 DOI: 10.1016/j.transproceed.2007.05.073]

72 **Zhu Y**, Dong J, Wang WL, Li MX, Lu Y. Short- and long-term outcomes after salvage liver transplantation versus primary liver transplantation for hepatocellular carcinoma: a meta-analysis. *Transplant Proc* 2013; **45**: 3329-3342 [PMID: 24182812 DOI: 10.1016/j.transproceed.2013.06.004]

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MINIREVIEWS

Occult hepatitis B virus co-infection in human immunodeficiency virus-positive patients: A review of prevalence, diagnosis and clinical significance

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Abstract

The prevalence of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) co-infection is high as they share similar mechanisms of transmission. The development and widespread use of highly sensitive tests for HBV diagnosis has demonstrated that a significant proportion of apparently healthy individuals with evidence of exposure to HBV continue to carry fully functional HBV DNA in their hepatocytes, a situation that predisposes them to the development of progressive liver disease and hepatocellular carcinoma. The presence of co-infections frequently influences the natural evolution of each of the participating infections present by either facilitating their virulence or competing for resources. Furthermore, the drugs used to treat these infections may also contribute to changes in the natural course of these infections, making the analysis of the impact of co-infection more difficult. The majority of studies has examined the impact of HIV on overt chronic hepatitis B, finding that co-infection carries an increased risk of progressive liver disease and the development of hepatocellular carcinoma. Although the effect of HIV on the natural history of occult hepatitis B infection (OBI) has not been fully assessed, all available data suggest a persisting risk of repeated flares of hepatitis and progressive liver disease. We describe studies regarding the diagnosis, prevalence and clinical significance of OBI in HIV-positive patients in this short review. Discrepancies in worldwide prevalence show the urgent need for the standardization of diagnostic criteria, as established by the Taormina statements. Ideally, standardized protocols for testing should be employed to enable the comparison of data from different groups. Additional studies are needed to define the differences in risk for OBI without HIV and in HIV-HBV co-infected patients with or without overt disease.

Key words: Hepatitis B virus; Occult hepatitis B; Human immunodeficiency virus; Prevalence; Diagnosis; Clinical significance

Core tip: The prevalence of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) co-infection is high. However, as HBV infection may be occult, its diagnosis requires the routine use of highly sensitive tests. Although viral load or replication in these patients is low, they still have an increased risk of viral reactivation, chronic liver disease and hepatocellular carcinoma development. The majority of our knowledge on occult hepatitis B infection is derived from studies performed in patients with mono-infection or with HIV co-infection. This review summarizes the latest contributions in the field, clearly revealing that more studies are needed to evaluate the full impact of HIV in patients with occult HBV disease.

Maldonado-Rodriguez A, Cevallos AM, Rojas-Montes O, Enriquez-Navarro K, Alvarez-Muñoz MT, Lira R. Occult hepatitis B virus co-infection in human immunodeficiency virus-positive patients: A review of prevalence, diagnosis and clinical significance. *World J Hepatol* 2015; 7(2): 253-260 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i2/253.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i2.253

INTRODUCTION

The presence of co-infections frequently influences the natural evolution of each of the participating infections present, either by facilitating their virulence or competing for resources[1]. Furthermore, the drugs used to treat these infections may also contribute to changes in the natural history of these infections[2], complicating the analysis of the impact of co-infection.

For overt chronic hepatitis B virus (HBV) infection, it is known that the virus does not substantially alter the progression of human immunodeficiency virus (HIV) disease, nor does it influence HIV suppression or CD4 cell responses following the initiation of antiretroviral treatment[3-5]. In contrast, it is clear that HIV infection has a negative effect on HBV disease, for both acute and chronic infections. In cases of acute hepatitis, progression to chronic infection is approximately 4 times more frequent in patients with HIV than in those without HIV infection (20% *vs* 5% and most likely higher if the CD4 count is low)[5]. For chronic hepatitis, HIV infection results in a faster progression of fibrosis, a faster development of cirrhosis and hepatocellular carcinoma, and a lower rate of spontaneous hepatitis B e antigen (HBeAg) or hepatitis B surface antigen (HBsAg) seroconversion[5]. There is also a greater risk of HBV reactivation in inactive carriers.

Occult HBV infection (OBI) occurs when viral DNA persists in the liver (with detectable or undetectable HBV DNA in serum, with or without HBV antibodies) in individuals testing negative for HBsAg[6]. By convention, OBI has been defined as the persistence of isolated anti-HBc in patients who may or may not have detectable serum HBV DNA. However, OBI may also occur in patients without anti-HBc antibodies. Thus, in a workshop of the European Association for the Study of Liver held in Taormina (Italy) in 2008, experts joined together to re-define and standardize diagnostic criteria, which resulted in a series of new criteria known as the Taormina statements[7]. It is expected that the universal use of these criteria will allow the comparison and proper evaluation of studies by different research groups.

In patients with HBV infection, the absence of HBsAg can occur in two main scenarios: firstly, in the early phases of acute infection prior to the development of antibodies and the detection of HBsAg in serum; secondly, during chronic HBV infection following the decline of HBsAg to an undetectable level, which is sometimes associated with the appearance of anti-HBs[8]. In chronic occult infections, viral covalently closed circular DNA (cccDNA) persists as a stable chromatinized episome in the nucleus of infected cells. This viral genome remains competent for replication and able to synthesize minute amounts of antigens, which are undetectable by the available technical approaches but sufficient to maintain an HBV-specific T cell response[6]. Fragments of the HBV genome may integrate into the host hepatic cell genome but this integration does not have a role in the replicative cycle of HBV and should not be strictly considered as occult infection[6]. In addition, chronic occult infection may be associated with the presence of one or more specific antibodies in serum; therefore, individuals with occult infections are conventionally divided into seropositive [anti-hepatitis B core antigen (anti-HBcAg) and/or anti-hepatitis B surface antigen (anti-HBsAg) positive] and seronegative (anti-HBc and anti-HBs negative) groups[6,7]. More than 20% of occult-infected individuals are negative for all HBV serum markers[9]. The development of occult infections is mainly determined by host factors, such as the immune response, and the status of the infection can be modified by the presence of co-infections, such as with hepatitis C virus (HCV) or HIV, or the administration of drugs, including immune suppressors and/or antiretrovirals[10]. Multiple viral variants have been identified in the liver of occult HBV-infected patients and it is believed that viral factors are not major determinants for the development of occult infections[6,11].

We describe the diagnosis, prevalence and clinical significance of OBI in HIV-positive patients in this short review.

OBI DIAGNOSIS

The presence or absence of full infective virions is not assessed in clinical practice. Surrogate markers

for the detection of circulating virus are either the presence of HBV DNA or HBV proteins (HBsAg, HBcAg and HBeAg). Typically, the presence of anti-HBs antibodies and the absence of HBsAg suggests a resolved infection; however, the persistence of only anti-HBc may be associated with OBI[12]. Overt HBV infection (acute or chronic) is defined as the presence of circulating HBsAg; according to the Taormina statements, OBI is defined as the absence of circulating HBsAg and the presence of HBV DNA[7]. However, the sensitivity and specificity of diagnosis strongly depend on the sensitivity of the assays used; indeed, for many years, HBV DNA tests were not very sensitive. Thus, the presence of anti-HBc was used as a surrogate marker and according to the Taormina statements, it still can be used in regions where modern molecular assays are not available[13,14]. The gold standard for OBI diagnosis is the demonstration of the presence of HBV DNA in the liver. To rule out the possibility that amplified fragments correspond to partial regions of the viral genome integrated into the host genome, several regions of the genome must be identified to suggest that full-length cccDNA is present. It is accepted that the diagnosis is frequently underestimated as liver biopsies are only rarely available and therefore diagnosis is usually based on blood samples. There is no evidence to date that HIV infection modifies the sensitivity or specificity of these tests.

Markers for screening OBI

HBsAg: It is of crucial importance to define the best methodology to test HBsAg to prevent false positive results, which is dependent on the HBsAg assay sensitivity. Several problems of this aspect are associated with the virus, the host or the test kits employed in practice. The quantification of HBsAg should be performed by comparing the sample with a standard curve generated with the second International Standard for HBsAg (World Health Organization code number 00/588, document WHO/BS/03.1987); 1 international unit is equivalent to 5.6 Abbott ng, 1.9 French ng and 0.43 PEI units[8]. Unfortunately, some HBV variants have mutations in the *HBs* gene and the encoded proteins are not detected with conventional commercial kits. Accordingly, alternative methods should be tested to detect common mutants[15].

HBV DNA: It has been established that HVB-DNA is the only reliable diagnostic marker for OBI. The experts meeting at Taormina recommended that new generation assays with detection limits of less than 10 copies of HBV DNA per reaction should be employed[7]. The estimated viral load in OBI is usually below 200 IU/mL.

New generation assays for DNA detection include nested-polymerase chain reaction (PCR), real-time PCR and transcription-based mediated amplification. Indeed, advances in the development of these DNA detection technologies has allowed a decrease in the lower detection limit (< 5 IU/mL of HBV DNA), which is particularly important in OBI because DNA levels vary at ~5-10 IU/mL (range < 10 to 425 copies/mL)[8].

HBV genome regions for diagnosis: According to the Taormina statements[7], the primers used must be specific for different HBV genomic regions and be complementary to highly conserved nucleotide sequences. The *S* and *X* genes are the regions most commonly amplified by PCR for diagnosis; it has been found that the *X* gene is the most sensitive for the liver, whereas the S gene is better for serum samples[9]. To avoid the problems of cross-contamination, appropriate controls in each PCR assay as well as amplicon sequencing are recommended.

PREVALENCE

The prevalence of infections in open populations is frequently estimated using data obtained from serological testing performed using blood donor samples; however, HBV tests can detect either overt infections or previous exposure to the virus in blood donors. Therefore, the available data reflect only the prevalence of overt HBV infections. OBI is only screened in specific scenarios, such as in areas of high endemicity of HBV, intravenous drug users, organ transplant patients, patients on maintenance hemodialysis or patients with HIV and/or HCV. For this review, we analyzed 34 papers that examined the prevalence of OBI in HIV patients during the period 2003-2014 (Table 1). The range of reported prevalence in these studies varied from 0.63% to 88.4%, which is similar to the prevalence reported in a previous review (0%-89.5%)[16]. This extremely wide range of prevalence reflects the diverse nature of published studies. Some of the differences are explained by the individual prevalence of HIV and HBV in the different populations studied. Although there are reports from central and south America[17-22], the majority of the studies are from regions of Africa, India and the Far East, regions where the prevalence of both HIV and HBV is high[23-27]. Differences also arise from the type of high-risk group to which the co-infection patients studied belong (*e.g.*, hemodialysis patients, homosexuals, intravenous drug users). Another source of variability depends on differences in the sensitivity of the diagnostic test used, with more recent studies using more sensitive HBV DNA detection assays than earlier studies[28]. Clearly, prospective, longitudinal studies in well-defined populations are needed to fully evaluate the prevalence and impact of HIV in the natural history of OBI. These studies should contain detailed clinical and demographic data, including age, sex, risk group liver function tests and, whenever possible, the evaluation

Table 1 Reported prevalence of occult hepatitis B virus infection in HIV-infected subjects *n* (%)

Ref.	Country	OBI prevalence			
		Overall	Anti-HBsAg-/anti-HBcAg+	Anti-HBsAg+/anti-HBcAg+	Anti-HBsAg-/anti-HBcAg-
Alvarez-Muñoz *et al*[17]	Mexico	24 (49.0)	5 (10.2)	8 (16.3)	11 (22.4)
Araujo *et al*[18]	Brazil	6 (14.0)	1 (2.3)	5 (10.2)	ND
Attia *et al*[45]	Africa	40 (21.3)	40 (21.3)	ND	ND
Azadmanesh *et al*[46]	Iran	3 (13.6)	2 (9.1)	ND	1 (4.5)
Bagaglio *et al*[47]	Italy	9 (31.0)	9 (31.0)	ND	ND
Bell *et al*[48]	Africa	45 (15.1)	16 (5.4)	17 (5.7)	12 (4.0)
Bloquel *et al*[38]	France	3 (0.8)	2 (0.5)	ND	1 (0.3)
Chadwick *et al*[49]	England	15 (4.5)	5 (1.5)	10[1] (3.0)	ND
Coffin *et al*[50]	Canada	19 (42.0)	ND	19 (42.2)	ND
Dapena *et al*[51]	Spain	6 (2.4)	2 (0.8)	4 (1.6)	ND
Filippini *et al*[13]	Italy	17 (20.0)	11 (12.8)	3 (3.5)	3 (3.5)
Firnhaber *et al*[23]	Africa	38 (88.4)	38 (88.4)	ND	ND
Gupta *et al*[30]	India	24 (45.3)	13 (24.5)	11 (20.8)	ND
Hakeem *et al*[52]	Scotland	2 (2.8)	2(2.9)	ND	ND
Jardim *et al*[19]	Brazil	8 (5.0)	2 (1.3)	6 (3.8)	ND
Khamduang *et al*[35]	Thailand	47 (23.5)	47 (23.5)	ND	ND
Liang *et al*[53]	Taiwan	3 (2.3)	3 (2.3)	ND	ND
Lo Re *et al*[54]	United States	17 (10.0)	10 (5.6)	7 (3.9)	ND
Loustaud-Ratti *et al*[55]	France	31 (44.3)	20 (28.6)	11 (15.7)	ND
Morsica *et al*[56]	Italy	27 (15.4)	9 (5.1)	18 (10.3)	ND
Mphahlele *et al*[57]	Africa	31 (18.6)[2]	5 (3.0)	26 (15.6)	ND
N'Dri-Yoman *et al*[24]	Africa	51 (10.0)	51 (11.8)	ND	ND
Neau *et al*[58]	France	1 (0.6)	1 (0.6)	ND	ND
Nebbia *et al*[59]	England	48 (14.0)	48 (14.0)	ND	ND
Opaleye *et al*[25]	Nigeria	21 (11.2)	8 (4.3)	9 (4.8)	2 (1.1)
Panigrahi *et al*[26]	India	12 (10.7)	9 (8.0)	3 (2.7)	ND
Santos *et al*[20]	Brazil	16 (15.8)[2]	4 (4.0)	12 (11.9)	ND
Sen *et al*[27]	India	1 (5.6)[2]	1 (5.6)	ND	ND
Shire *et al*[60]	United States	4 (10.5)	4 (10.5)	ND	ND
Shire *et al*[61]	United States	12 (30.2)	3 (7.0)	5[1] (11.6)	5 (11.6)
Sucupira *et al*[21]	Brazil	6 (18.8)[2]	3 (9.4)	3 (9.4)	ND
Torres Barranda *et al*[22]	Mexico	7 (18.4)	1 (2.6)	1 (2.6)	5 (13.2)
Tramuto *et al*[62]	Italy	24 (5.9)	8 (2.0)	7[1] (1.7)	9 (2.2)
Tsui *et al*[63]	United States	8 (2.0)	8 (2.0)	ND	ND

[1]In some studies the anti-HBsAg positive group was also included; [2]Prevalence calculated using the reported data; anti-HBsAg+, antibodies against hepatitis B surface antigen positive; anti-HBcAg+ antibodies against hepatitis B core antigen positive. Prevalence (%) were included for each group of patients studied according the HBV serological markers (Anti-HBsAg-/anti-HBcAg+, Anti-HBsAg+/anti-HBcAg+, Anti-HBsAg-/anti-HBcAg-). ND: Not determined because this group was not included in the study; OBI: Occult hepatitis B infection; anti-HBsAg: Anti-hepatitis B surface antigen; anti-HBcAg: Anti-hepatitis B core antigen.

of liver damage by biopsy. Ideally, standardized protocols for testing should be employed so that studies from different groups can be compared.

In general, OBI prevalence appears to be higher among patients at high risk for HBV infection and with liver disease than among individuals at low risk of infection and without liver disease[9,29-31]. As mentioned above, patients with OBI may be negative for all HBV serum markers and there is also evidence that levels of viremia are correlated with levels of anti-HBc and/or anti-HBs[8]. Unfortunately, the majority of studies have been performed in seropositive patients (usually isolated anti-HBc), whereas seronegative patients who are more difficult to identify have not been fully assessed. Furthermore, the available data suggest seronegative patients have a different clinical evolution and should therefore be evaluated separately. Another factor that is common in HIV patients and that is known to affect the evolution of HBV infection is HCV co-infection; the presence of HCV should always be screened and those with OBI/HCV should be analyzed separately.

CLINICAL SIGNIFICANCE

The significance of chronic OBI relates to the risk of transmission, reactivation, progression to chronic liver disease and development of hepatocellular cancer[6,8,32,33]. The rate of transmission is directly proportional to the number of viable virions in blood. Unfortunately, the true level of viremia, *i.e.*, the number of infectious HBV particles within 1 mL of serum or plasma, is difficult to measure in a bioassay[34]. Therefore, surrogate markers of HBV infectivity are being used to measure the risk of transmission. The best available marker for the presence and number of infectious HBV particles is the number of HBV DNA molecules. However, it is

important to note that the detection of HBV DNA in serum does not always correspond to infectivity or to the number of HBV progeny viruses released from hepatocytes. Indeed, in the majority of viral infections, the number of physical virus particles is much larger than the number of fully infectious virions[34]. There also appears to be a correlation between levels of HBV DNA and serological status among patients with OBI: HBV DNA levels are lowest in seronegative patients, intermediate in anti-HBc negative and anti-HBs positive patients, and highest in subjects who are anti HBc-positive but anti-HBs negative[8]. This last group is more likely to be infectious. The possibility of transmission is crucial for non-immunocompromised individuals who are potential donors of blood or other organs, for health care providers who can infect their patients, and for pregnant women *via* vertical transmission. Although known HIV patients are not candidates for organ donation, there remains a risk of HIV/HBV transmission either through sexual encounters, needle sharing or other risky behaviors. The potential risk of vertical transmission in pregnant HIV/OBI women is clear but the prevalence rate has not been fully evaluated. In a recent study of 1682 HIV-infected pregnant women in Thailand who were fully evaluated for HBV infection, 216 were HBsAg negative and anti-HBc positive (14%). It was also possible to assess the levels of HBV DNA in 200 of these women with OBI; all 200 women had HBV DNA < 1000 IU/mL, with 153 showing HBV DNA below the limit of detection (15 IU/mL), 44 with an HBV DNA level between 15-100 IU/mL, and 3 showing HBV DNA between 101 and 1000 IU/mL. However, none of these women transmitted the disease to their infants[35]. Based on the available information, a group of experts in the United States has provided guidelines to manage pregnant women with HIV-HBV co-infection; the full guidelines have been published at the AidsInfo site of the National Institute of Health (http://aidsinfo.nih.gov/guidelines/html/3/perinatal-guidelines/159/hiv-hepatitis-b-virus-coinfection). It is recommended that all pregnant women should have full screens for both HBV and HCV; these tests would identify most cases of OBI in HIV patients. They also strongly recommend that the management of HIV/HBV co-infection in pregnancy should be performed with the advice of an expert in HIV and HBV, with close monitoring of the viral activities in pregnant woman. In their review, the experts conclude that women who screen negative for HBV (*i.e.,* HBsAg-negative, anti-HBc-negative and anti-HBs-negative) should be vaccinated for HBV, stating that the presence of isolated anti-HBc can represent a false-positive case or can indicate a previous exposure and a posterior loss of anti-HBs or most likely an "occult" HBV infection. The possibility of OBI needs to be confirmed by HBV DNA detection. These experts also recognize that the clinical significance of isolated anti-HBc is still unknown. For that, they recommend, with the panel of their peers on opportunistic infections in HIV-infected adults and adolescents, to test for HBV DNA in HIV+/anti-HBc+ patients before HBV vaccination and treatment initiation or prophylaxis to avoid the risk of a paradoxical exacerbation of HBV infection and the incidence of immune reconstitution inflammatory syndrome (http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf). The risk of vertical HBV transmission in mothers with OBI is most likely small due to the low levels of HBV viremia. Clearly, the decision to treat a pregnant woman with OBI should be made by an expert.

The natural history of chronic hepatitis B is highlighted by spontaneous flares of the disease. The reasons for HBV reactivation are not clear but most likely can be explained by subtle modifications in virus replicative fitness due to host immunological control, such as that which occurs with herpes virus[36]. The flares are potentially important clinically because they can have severe or even fatal consequences. Most frequently, the reactivation of HBV replication occurs in patients with overt chronic HBV infection (HBsAg-positive) who receive cytotoxic or immunosuppressive therapy[10,33]. In contrast, HBV reactivation occurs more rarely in patients with OBI. Both in overt and occult infection, the risk of HBV reactivation is high, particularly in patients with hematological malignancies, in those receiving hematopoietic stem cell transplantation and in those treated with either anti-CD20 (rituximab, which destroys B-cells) or anti-CD52 (alemtuzumab, which targets mature lymphocytes) monoclonal antibodies (reviewed in[10]). In these cases, HBV reactivation is associated with a mortality rate of approximately 20% due to hepatic failure or to progression of the underlying disease owing to the discontinuation of treatment.

There are multiple anecdotal reports of the reactivation of OBI in HIV-patients, with the majority of cases being identified in seropositive patients. Many factors, similar to those demonstrated for overt infection, have been implicated in the recurrence of HBV replication in HIV/OBI-co-infected patients, including the interruption of HAART[37-39], recovery of immune responses after HIV-treatment[14], development of resistance to lamivudine therapy[13], and appearance of HBV immune-escape[40].

The overall prevalence of OBI reactivation in HIV patients and the frequency of the specific triggers of reactivation need to be assessed in prospective longitudinal studies[14]. In an effort to evaluate the full impact of HIV-infection in OBI, a multicenter prospective study on 115 consecutive anti-HIV+, HBsAg-negative, treatment-naïve patients was performed[13]. Of the 86 patients with at least 6 mo of follow-up, 13 were HBV DNA positive on

admission and four in the subsequent testing. The HBV DNA+ frequency for the anti-HBs-negative/anti-HBc-positive group was 36% and 21% for the anti-HBs-positive/anti-HBc-positive group; the lowest frequency was reported in the anti-HBs-negative/anti-HBc-negative group (9%). Episodes of reactivation were detected in 32% of the patients and were more common in patients with detectable HBV DNA than in those without it (65% *vs* 25%). These preliminary data await confirmation in larger studies[10].

HBV infection causes liver inflammation and hepatocellular carcinoma. The lifetime risk of developing HBV-related cirrhosis or hepatocellular carcinoma has been estimated to be between 15% and 40% for males who acquire infection during early life[36]. There is evidence to suggest that the risk of progression of liver disease from inflammation, to fibrosis, to cirrhosis and to cancer is directly related to the level of transcription of HBV DNA[41-43]. However, the prevalence and rate of progression to cirrhosis are also related to differences in the clinical and serological features of the disease, such as repeated flares, age and HBV genotype[36]. There is an increased risk of hepatocellular carcinoma even in patients with inactive HBV infection[44]. It is widely accepted that the risk of progression is particularly high in patients with OBI/HCV co-infection[8]. However, the impact of HIV infection in the oncogenicity of OBI HIV patients has not been studied and requires specific evaluation[10].

CONCLUSION

The real impact of co-infection can only be fully established when the presence of OBI is routinely assessed in all patients with HIV. As routine liver biopsy of all HIV patients is not possible, at least all HIV patients should be screened initially with a complete panel of HBV tests. As both the reactivation of hepatitis (with or without the emergence of drug resistance) and an increase in the risk of transmission are associated with higher levels of HBV DNA, the monitoring of OBI should always include regular highly sensitive measurements of circulating HBV DNA. More studies are needed to define when to repeat the full set of HBV tests and when a liver biopsy is indicated in these patients.

REFERENCES

1 **Eswarappa SM**, Estrela S, Brown SP. Within-host dynamics of multi-species infections: facilitation, competition and virulence. *PLoS One* 2012; **7**: e38730 [PMID: 22737220 DOI: 10.1371/journal.pone.0038730]

2 **Gürtler LG**. Effect of antiretroviral HIV therapy on hepatitis B virus replication and pathogenicity. *Intervirology* 2014; **57**: 212-217 [PMID: 25034490 DOI: 10.1159/000360942]

3 **Konopnicki D**, Mocroft A, de Wit S, Antunes F, Ledergerber B, Katlama C, Zilmer K, Vella S, Kirk O, Lundgren JD. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. *AIDS* 2005; **19**: 593-601 [PMID: 15802978 DOI: 10.1097/01.aids.0000163936.99401.fe]

4 **Hoffmann CJ**, Seaberg EC, Young S, Witt MD, D'Acunto K, Phair J, Thio CL. Hepatitis B and long-term HIV outcomes in coinfected HAART recipients. *AIDS* 2009; **23**: 1881-1889 [PMID: 19550291 DOI: 10.1097/QAD.0b013e32832e463a]

5 **Mallet V**, Vallet-Pichard A, Pol S. The impact of human immunodeficiency virus on viral hepatitis. *Liver Int* 2011; **31** Suppl 1: 135-139 [PMID: 21205151 DOI: 10.1111/j.1478-3231.2010.02394.x]

6 **Raimondo G**, Caccamo G, Filomia R, Pollicino T. Occult HBV infection. *Semin Immunopathol* 2013; **35**: 39-52 [PMID: 22829332 DOI: 10.1007/s00281-012-0327-7]

7 **Raimondo G**, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxì A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trépo C, Villa E, Will H, Zanetti AR, Zoulim F. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008; **49**: 652-657 [PMID: 18715666 DOI: 10.1016/j.jhep.2008.07.014]

8 **Hollinger FB**, Sood G. Occult hepatitis B virus infection: a covert operation. *J Viral Hepat* 2010; **17**: 1-15 [PMID: 20002296 DOI: 10.1111/j.1365-2893.2009.01245.x]

9 **Torbenson M**, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002; **2**: 479-486 [PMID: 12150847 DOI: 10.1016/S1473-3099(02)00345-6]

10 **Sagnelli E**, Pisaturo M, Martini S, Filippini P, Sagnelli C, Coppola N. Clinical impact of occult hepatitis B virus infection in immunosuppressed patients. *World J Hepatol* 2014; **6**: 384-393 [PMID: 25018849 DOI: 10.4254/wjh.v6.i6.384]

11 **Pollicino T**, Raffa G, Costantino L, Lisa A, Campello C, Squadrito G, Levrero M, Raimondo G. Molecular and functional analysis of occult hepatitis B virus isolates from patients with hepatocellular carcinoma. *Hepatology* 2007; **45**: 277-285 [PMID: 17256766 DOI: 10.1002/hep.21529]

12 **Gerlich WH**. Medical virology of hepatitis B: how it began and where we are now. *Virol J* 2013; **10**: 239 [PMID: 23870415 DOI: 10.1186/1743-422X-10-239]

13 **Filippini P**, Coppola N, Pisapia R, Scolastico C, Marrocco C, Zaccariello A, Nacca C, Sagnelli C, De Stefano G, Ferraro T, De Stefano C, Sagnelli E. Impact of occult hepatitis B virus infection in HIV patients naive for antiretroviral therapy. *AIDS* 2006; **20**: 1253-1260 [PMID: 16816553 DOI: 10.1097/01.aids.0000232232.41877.2a]

14 **Jain MK**, Parekh NK, Hester J, Lee WM. Aminotransferase elevation in HIV/hepatitis B virus co-infected patients treated with two active hepatitis B virus drugs. *AIDS Patient Care STDS* 2006; **20**: 817-822 [PMID: 17192146 DOI: 10.1089/apc.2006.20.817]

15 **Hollinger FB**. Hepatitis B virus genetic diversity and its impact on diagnostic assays. *J Viral Hepat* 2007; **14** Suppl 1: 11-15 [PMID: 17958637 DOI: 10.1111/j.1365-2893.2007.00910.x]

16 **Ramezani A**, Banifazl M, Mohraz M, Rasoolinejad M, Aghakhani A. Occult hepatitis B virus infection: A major concern in HIV-infected patients: Occult HBV in HIV. *Hepat Mon* 2011; **11**: 7-10 [PMID: 22087108]

17 **Alvarez-Muñoz MT**, Maldonado-Rodriguez A, Rojas-Montes O, Torres-Ibarra R, Gutierrez-Escolano F, Vazquez-Rosales G, Gomez A, Muñoz O, Torres J, Lira R. Occult hepatitis B virus infection among Mexican human immunodeficiency virus-1-infected patients. *World J Gastroenterol* 2014; **20**: 13530-13537 [PMID: 25309083 DOI: 10.3748/wjg.v20.i37.13530]

18 **Araujo NM**, Branco-Vieira M, Silva AC, Pilotto JH, Grinsztejn B, de Almeida AJ, Trepo C, Gomes SA. Occult hepatitis B virus infection in HIV-infected patients: Evaluation of biochemical, virological and molecular parameters. *Hepatol Res* 2008; **38**: 1194-1203 [PMID: 18624719 DOI: 10.1111/j.1872-034X.2008.00392.x]

19 **Jardim RN**, Gonçales NS, Pereira JS, Fais VC, Gonçales Junior

FL. Occult hepatitis B virus infection in immunocompromised patients. *Braz J Infect Dis* 2008; **12**: 300-305 [PMID: 19030729 DOI: 10.1590/S1413-86702008000400008]

20 **Santos EA**, Yoshida CF, Rolla VC, Mendes JM, Vieira IF, Arabe J, Gomes SA. Frequent occult hepatitis B virus infection in patients infected with human immunodeficiency virus type 1. *Eur J Clin Microbiol Infect Dis* 2003; **22**: 92-98 [PMID: 12627282]

21 **Sucupira MV**, Mello FC, Santos EA, Niel C, Rolla VC, Arabe J, Gomes SA. Patterns of hepatitis B virus infection in Brazilian human immunodeficiency virus infected patients: high prevalence of occult infection and low frequency of lamivudine resistant mutations. *Mem Inst Oswaldo Cruz* 2006; **101**: 655-660 [PMID: 17072479 DOI: 10.1590/S0074-02762006000600013]

22 **Torres-Baranda R**, Bastidas-Ramírez BE, Maldonado-González M, Sánchez-Orozco LV, Vázquez-Vals E, Rodríguez-Noriega E, Panduro A. Occult hepatitis B in Mexican patients with HIV, an analysis using nested polymerase chain reaction. *Ann Hepatol* 2006; **5**: 34-40 [PMID: 16531963]

23 **Firnhaber C**, Viana R, Reyneke A, Schultze D, Malope B, Maskew M, Di Bisceglie A, MacPhail P, Sanne I, Kew M. Occult hepatitis B virus infection in patients with isolated core antibody and HIV co-infection in an urban clinic in Johannesburg, South Africa. *Int J Infect Dis* 2009; **13**: 488-492 [PMID: 19081280 DOI: 10.1016/j.ijid.2008.08.018]

24 **N'Dri-Yoman T**, Anglaret X, Messou E, Attia A, Polneau S, Toni T, Chenal H, Seyler C, Gabillard D, Wakasugi N, Eholié S, Danel C. Occult HBV infection in untreated HIV-infected adults in Côte d' Ivoire. *Antivir Ther* 2010; **15**: 1029-1034 [PMID: 21041918 DOI: 10.3851/IMP1641]

25 **Opaleye OO**, Oluremi AS, Atiba AB, Adewumi MO, Mabayoje OV, Donbraye E, Ojurongbe O, Olowe OA. Occult Hepatitis B Virus Infection among HIV Positive Patients in Nigeria. *J Trop Med* 2014; **2014**: 796121 [PMID: 24868208 DOI: 10.1155/2014/796121]

26 **Panigrahi R**, Majumder S, Gooptu M, Biswas A, Datta S, Chandra PK, Banerjee A, Chakrabarti S, Bandopadhyay D, De BK, Chakravarty R. Occult HBV infection among anti-HBc positive HIV-infected patients in apex referral centre, Eastern India. *Ann Hepatol* 2012; **11**: 870-875 [PMID: 23109450]

27 **Sen S**, Panda SP, Shanmuganandan K, Gupta R, Praharaj A. Prevalence of occult hepatitis B amongst Indian human immunodeficiency virus type 1 infected individuals-a pilot study. *Med J Armed Forces India* 2012; **68**: 16-19 [PMID: 24669033 DOI: 10.1016/S0377-1237(11)60127-5]

28 **Shire AM**, Roberts LR. Occult hepatitis B virus infection: bit player or role player? *Hepatology* 2011; **54**: 760-763 [PMID: 21735471 DOI: 10.1002/hep.24528]

29 **Bréchot C**, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; **34**: 194-203 [PMID: 11431751 DOI: 10.1053/jhep.2001.25172]

30 **Gupta S**, Singh S. Occult hepatitis B virus infection in ART-naive HIV-infected patients seen at a tertiary care centre in north India. *BMC Infect Dis* 2010; **10**: 53 [PMID: 20205948 DOI: 10.1186/1471-2334-10-53]

31 **Raimondo G**, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. *J Hepatol* 2007; **46**: 160-170 [PMID: 17112622 DOI: 10.1016/j.jhep.2006.10.007]

32 **Schmeltzer P**, Sherman KE. Occult hepatitis B: clinical implications and treatment decisions. *Dig Dis Sci* 2010; **55**: 3328-3335 [PMID: 20927592 DOI: 10.1007/s10620-010-1413-0]

33 **Squadrito G,** Spinella R and Raimondo G. The clinical significance of occult HBV infection. *Ann Gastroenterol* 2014; **27**: 15-19 [PMID: 24714731]

34 **Gerlich WH**. Reduction of infectivity in chronic hepatitis B virus carriers among healthcare providers and pregnant women by antiviral therapy. *Intervirology* 2014; **57**: 202-211 [PMID: 25034489 DOI: 10.1159/000360949]

35 **Khamduang W**, Ngo-Giang-Huong N, Gaudy-Graffin C, Jourdain G, Suwankornsakul W, Jarupanich T, Chalermpolprapa V, Nanta S, Puarattana-Aroonkorn N, Tonmat S, Lallemant M, Goudeau A, Sirirungsi W. Prevalence, risk factors, and impact of isolated antibody to hepatitis B core antigen and occult hepatitis B virus infection in HIV-1-infected pregnant women. *Clin Infect Dis* 2013; **56**: 1704-1712 [PMID: 23487379 DOI: 10.1093/cid/cit166]

36 **Perrillo RP**. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. *Gastroenterology* 2001; **120**: 1009-1022 [PMID: 11231956 DOI: 10.1053/gast.2001.22461]

37 **Chamorro AJ,** Casado JL, Bellido D, Moreno S. Reactivation of hepatitis B in an HIV-infected patient with antibodies against hepatitis B core antigen as the only serological marker. *Eur J Clin Microbiol Infect Dis* 2005; **24:** 492-494 [PMID: 15990987]

38 **Bloquel B**, Jeulin H, Burty C, Letranchant L, Rabaud C, Venard V. Occult hepatitis B infection in patients infected with HIV: report of two cases of hepatitis B reactivation and prevalence in a hospital cohort. *J Med Virol* 2010; **82**: 206-212 [PMID: 20029819 DOI: 10.1002/jmv.21685]

39 **Puoti M**, Torti C, Bruno R, Filice G, Carosi G. Natural history of chronic hepatitis B in co-infected patients. *J Hepatol* 2006; **44**: S65-S70 [PMID: 16338021 DOI: 10.1016/j.jhep.2005.11.015]

40 **Pei R**, Grund S, Verheyen J, Esser S, Chen X, Lu M. Spontaneous reactivation of hepatitis B virus replication in an HIV coinfected patient with isolated anti-Hepatitis B core antibodies. *Virol J* 2014; **11**: 9 [PMID: 24444423 DOI: 10.1186/1743-422X-11-9]

41 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174 [PMID: 12124405 DOI: 10.1056/NEJMoa013215]

42 **Iloeje UH**, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678-686 [PMID: 16530509 DOI: 10.1053/j.gastro.2005.11.016]

43 **Hollinger FB**, Dodd RY. Hepatitis B virus traceback and lookback: factors to consider. *Transfusion* 2009; **49**: 176-184 [PMID: 19140810 DOI: 10.1111/j.1537-2995.2008.01961.x]

44 **Chen JD**, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, Su J, Sun CA, Liaw YF, Chen CJ. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. *Gastroenterology* 2010; **138**: 1747-1754 [PMID: 20114048 DOI: 10.1053/j.gastro.2010.01.042]

45 **Attia KA**, Eholié S, Messou E, Danel C, Polneau S, Chenal H, Toni T, Mbamy M, Seyler C, Wakasugi N, N'dri-Yoman T, Anglaret X. Prevalence and virological profiles of hepatitis B infection in human immunodeficiency virus patients. *World J Hepatol* 2012; **4**: 218-223 [PMID: 22855697 DOI: 10.4254/wjh.v4.i7.218]

46 **Azadmanesh K**, Mohraz M, Aghakhani A, Edalat R, Jam S, Eslamifar A, Banifazl M, Moradmand-Badie B, Ramezani A. Occult hepatitis B virus infection in HIV-infected patients with isolated hepatitis B core antibody. *Intervirology* 2008; **51**: 270-274 [PMID: 18841029 DOI: 10.1159/000160217]

47 **Bagaglio S**, Bianchi G, Danise A, Porrino L, Uberti-Foppa C, Lazzarin A, Castagna A, Morsica G. Longitudinal evaluation of occult hepatitis B infection in HIV-1 infected individuals during highly active antiretroviral treatment interruption and after HAART resumption. *Infection* 2011; **39**: 121-126 [PMID: 21424854 DOI: 10.1007/s15010-011-0093-9]

48 **Bell TG**, Makondo E, Martinson NA, Kramvis A. Hepatitis B virus infection in human immunodeficiency virus infected southern African adults: occult or overt--that is the question. *PLoS One* 2012; **7**: e45750 [PMID: 23049685 DOI: 10.1371/journal.pone.0045750]

49 **Chadwick D**, Doyle T, Ellis S, Price D, Abbas I, Valappil M, Geretti AM. Occult hepatitis B virus coinfection in HIV-positive African migrants to the UK: a point prevalence study. *HIV Med* 2014; **15**: 189-192 [PMID: 24118868 DOI: 10.1111/hiv.12093]

50 **Coffin CS**, Mulrooney-Cousins PM, Osiowy C, van der Meer F, Nishikawa S, Michalak TI, van Marle G, Gill MJ. Virological characteristics of occult hepatitis B virus in a North American cohort of human immunodeficiency virus type 1-positive patients on dual active anti-HBV/HIV therapy. *J Clin Virol* 2014; **60**: 347-353

[PMID: 24881491 DOI: 10.1016/j.jcv.2014.04.021]
51 **Dapena M**, Figueras C, Noguera-Julian A, Fortuny C, de José MI, Mellado MJ, Gavilán C, Falcón-Neyra MD, Navarro ML, de Ory SJ, López C, Mayol L, Méndez M, Ciria LM, Coll MT, García L, Nuñez E, Espiau M, Soler-Palacín P. Implementation of occult hepatitis screening in the Spanish cohort of HIV-infected pediatric patients. *Pediatr Infect Dis J* 2013; **32**: e377-e379 [PMID: 23446444 DOI: 10.1097/INF.0b013e31828e9b99]
52 **Hakeem L**, Thomson G, McCleary E, Bhattacharyya D, Banerjee I. Prevalence and Immunization Status of Hepatitis B Virus in the HIV Cohort in Fife, Scotland. *J Clin Med Res* 2010; **2**: 34-38 [PMID: 22457699 DOI: 10.4021/jocmr2009.12.1282]
53 **Liang SH**, Chen TJ, Lee SS, Tseng FC, Huang CK, Lai CH, Chiou CP, Wang JL, Chung HC, Lin JN, Kuo YC, Lin HH. Risk factors of isolated antibody against core antigen of hepatitis B virus: association with HIV infection and age but not hepatitis C virus infection. *J Acquir Immune Defic Syndr* 2010; **54**: 122-128 [PMID: 20386111 DOI: 10.1097/QAI.0b013e3181daafd5]
54 **Lo Re V**, Frank I, Gross R, Dockter J, Linnen JM, Giachetti C, Tebas P, Stern J, Synnestvedt M, Localio AR, Kostman JR, Strom BL. Prevalence, risk factors, and outcomes for occult hepatitis B virus infection among HIV-infected patients. *J Acquir Immune Defic Syndr* 2007; **44**: 315-320 [PMID: 17159655 DOI: 10.1097/qai.0b013e31802ea499]
55 **Loustaud-Ratti V**, Wagner A, Carrier P, Marczuk V, Chemin I, Lunel F, Fouchard-Hubert I, Ahmed SS, Abergel A, Rousseau A, Lefebvre A, Debette-Gratien M, Denis F, Alain S. Distribution of total DNA and cccDNA in serum and PBMCs may reflect the HBV immune status in HBsAg+ and HBsAg- patients coinfected or not with HIV or HCV. *Clin Res Hepatol Gastroenterol* 2013; **37**: 373-383 [PMID: 23477988 DOI: 10.1016/j.clinre.2012.11.002]
56 **Morsica G**, Ancarani F, Bagaglio S, Maracci M, Cicconi P, Cozzi Lepri A, Antonucci G, Bruno R, Santantonio T, Tacconi L, Baldelli F, Piscopo R, Santoro D, Lazzarin A, D'Arminio Monforte A. Occult hepatitis B virus infection in a cohort of HIV-positive patients: correlation with hepatitis C virus coinfection, virological and immunological features. *Infection* 2009; **37**: 445-449 [PMID: 19669092 DOI: 10.1007/s15010-008-8194-9]
57 **Mphahlele MJ**, Burnett RJ, Kyaw T, Schoeman HS, Aspinall S. Immunogenicity and safety of yeast-derived recombinant hepatitis B vaccine (Heberbiovac HB) in South African children. *S Afr Med J* 2004; **94**: 280-281 [PMID: 15150941]
58 **Neau D**, Winnock M, Jouvencel AC, Faure M, Castéra L, Legrand E, Lacoste D, Ragnaud JM, Dupon M, Fleury H, Lafon ME, Dabis F. Occult hepatitis B virus infection in HIV-infected patients with isolated antibodies to hepatitis B core antigen: Aquitaine cohort, 2002-2003. *Clin Infect Dis* 2005; **40**: 750-753 [PMID: 15714424 DOI: 10.1086/427882]
59 **Nebbia G**, Garcia-Diaz A, Ayliffe U, Smith C, Dervisevic S, Johnson M, Gilson R, Tedder R, Geretti AM. Predictors and kinetics of occult hepatitis B virus infection in HIV-infected persons. *J Med Virol* 2007; **79**: 1464-1471 [PMID: 17705185 DOI: 10.1002/jmv.20954]
60 **Shire NJ**, Rouster SD, Rajicic N, Sherman KE. Occult hepatitis B in HIV-infected patients. *J Acquir Immune Defic Syndr* 2004; **36**: 869-875 [PMID: 15213572 DOI: 10.1097/00126334-200407010-00015]
61 **Shire NJ**, Rouster SD, Stanford SD, Blackard JT, Martin CM, Fichtenbaum CJ, Sherman KE. The prevalence and significance of occult hepatitis B virus in a prospective cohort of HIV-infected patients. *J Acquir Immune Defic Syndr* 2007; **44**: 309-314 [PMID: 17159656 DOI: 10.1097/QAI.0b013e31802e29a9]
62 **Tramuto F**, Maida CM, Colomba GM, Di Carlo P, Vitale F. Prevalence of occult hepatitis B virus infection in a cohort of HIV-positive patients resident in Sicily, Italy. *Biomed Res Int* 2013; **2013**: 859583 [PMID: 24063015 DOI: 10.1155/2013/859583]
63 **Tsui JI**, French AL, Seaberg EC, Augenbraun M, Nowicki M, Peters M, Tien PC. Prevalence and long-term effects of occult hepatitis B virus infection in HIV-infected women. *Clin Infect Dis* 2007; **45**: 736-740 [PMID: 17712758 DOI: 10.1086/520989]

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MINIREVIEWS

Role of surgical resection for hepatocellular carcinoma based on Japanese clinical guidelines for hepatocellular carcinoma

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Abstract

In the Algorithm for Diagnosis and Treatment in the Japanese Evidence-Based Clinical Practice Guidelines for Hepatocellular Carcinoma, the treatment strategy is determined by three major factors: liver function and the number and size of tumors. The algorithm is quite simple, consisting of fewer components than the Barcelona-Clinic Liver Cancer staging system. In this article, we describe the roles of the treatment algorithm in hepatectomy and perioperative management of hepatocellular carcinoma.

Key words: Hepatocellular carcinoma; Guideline; Liver resection; Treatment algorithm

Core tip: In the Algorithm for Diagnosis and Treatment in the Japanese Evidence-Based Clinical Practice Guidelines for Hepatocellular Carcinoma, the treatment strategy is determined by three major factors: liver function and the number and size of tumors. The algorithm is quite simple, consisting of fewer components than the Barcelona-Clinic Liver Cancer staging system. In this article, we describe the roles of the treatment algorithm in hepatectomy and perioperative management of hepatocellular carcinoma.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common neoplasm worldwide and the third most frequent cause of cancer-related death. More than 0.7 million people were diagnosed with HCC in 2008, indicating an incidence of 16 per 0.1 million people[1]. The distribution of HCC is regional, with approximately 80% of HCC cases found in Eastern Asia and central Africa. The risk factors in these

Table 1 Staging systems for hepatocellular carcinoma

Classification	Year	Background	Variables		
			Tumor status	Liver function	Health status
Okuda staging	1985	850 Japanese patients	50% liver involvement	Ascites, bilirubin, albumin	-
BCLC staging	1999	Selected papers	Size, number, vascular invasion, Okuda stage	Child-Pugh, bilirubin, porta hypertension	Performance status
CLIP score	2000	435 Italian patients	50% liver involvement, vascular invasion, AFP	Child-Pugh	-
CUPI	2002	926 Chinese patients	TNM, AFP	Bilirubin, albumin, alkaline phosphatase	Presence of symptoms
JIS score	2003	3334 Japanese patients	TNM (Japanese)	Child-Pugh	-
m-JIS score	2006	42269 Japanese patients	TNM (Japanese)	Liver damage	-
Tokyo score	2005	403 Japanese patients	Size, number	Bilirubin, albumin	-

BCLC: Barcelona Clinic Liver Cancer; CLIP: Cancer of the liver Italian Program; CUPI: Chinese University Prognostic Index; JIS: Japan Integrated Staging; m-JIS: Modified Japan Integrated Staging; AFP: Alpha-fetoprotein. Revise from ref. [22].

areas are hepatitis B and aflatoxin, but those in North America, Europe, and Japan are hepatitis C and alcohol.

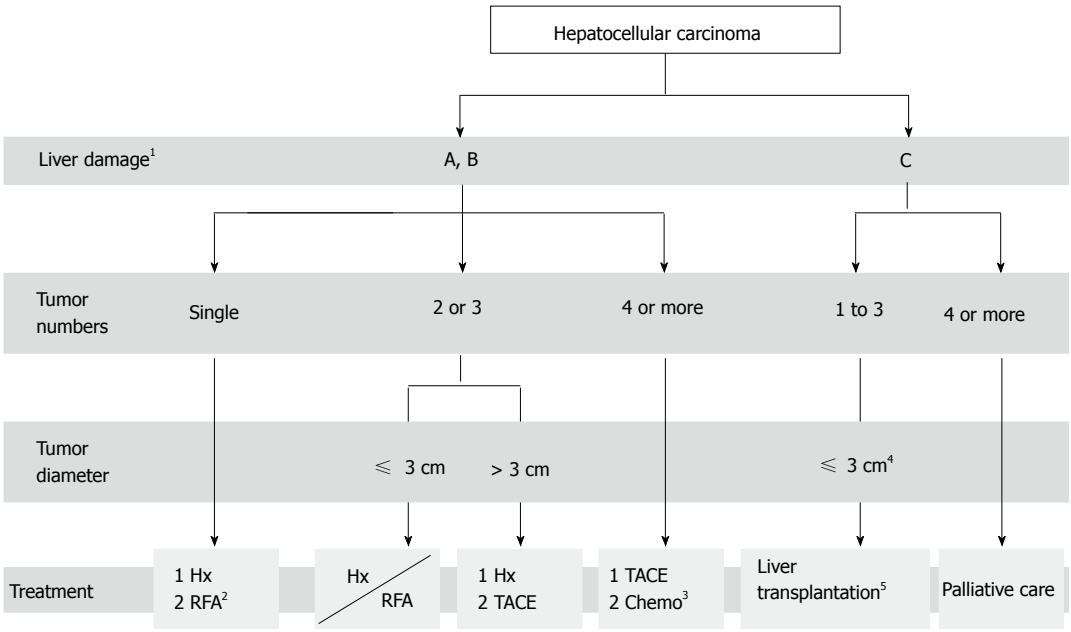
The spread of the concept of evidence-based medicine (EBM) has provided an opportunity for development of treatment guidelines. In Western countries, the Barcelona Clinic Liver Cancer (BCLC) staging system was published as practice guidelines in 2005 and updated in 2011, and is recommended for use by the American Association for the Study of Liver Diseases (AASLD)[2] and the European Association for the Study of the Liver (EASL). In Japan, the Clinical Practice Guidelines for Hepatocellular Carcinoma were published in 2005[3,4] and then revised in 2009 and 2013 to add new information[5]. The "treatment algorithm" listed in the guidelines has become well disseminated as a standard method for selection of optimal treatment based on liver function and tumor conditions[6]. Here, we describe the roles of the treatment algorithm in hepatectomy for HCC and we discuss current knowledge on hepatectomy in Japan.

STAGING SYSTEM FOR HCC

Staging systems for liver cancer have three elements: (1) tumor stage (TNM system); (2) hepatic functional reserve; and (3) integrated stage, a combination of (1) and (2). The International Union Against Cancer (UICC) published the UICC TNM classification of malignant tumors in 1968 and added liver cancer to the TNM classification in 1987. Now, the seventh edition is used from 2009[7]. The UICC-TNM classification is based on the staging system of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, with a database from multicenter research by the International Cooperative Study Group on Hepatocellular Carcinoma[8]. The UICC-TNM classification is a simplified version of the AJCC Manual, and the 7th edition set the cutoff for tumor size as 5 cm. In 1983, the Liver Cancer Study Group of Japan published the "General Rules for Clinical and Pathological Studies of Primary Liver Cancer" (henceforth referred to as the "General Rules"), which included the Japanese TNM classification[9] and was prepared based on a database developed by the Liver Cancer Study Group. In the latest edition, the stages are classified using a cutoff tumor size of 2 cm, single/multiple lesions, and vascular invasion. In a comparison of these two staging systems in Japanese patients, Minagawa *et al*[10] found that both systems allowed clear stratification of patients into prognostic groups, but that the General Rules were more appropriate for stratifying patients with early-stage HCC[10].

The Child-Pugh classification is most commonly used for evaluation of hepatic functional reserve[11,12]. This classification has five parameters: serum bilirubin, serum albumin, ascites, hepatic encephalopathy, and prothrombin activity, which are used to assess liver function in three classes: A, B and C. The indocyanine green retention rate at 15 min ($ICGR_{15}$) is also used in Japan, eastern Asia, and some European countries as a more detailed index for assessment of hepatic functional reserve. $ICGR_{15}$ is useful for prediction of postoperative mortality[13] and as a marker of liver function for determining the extent of hepatectomy[14]. The General Rules also have a liver damage classification system that uses $ICGR_{15}$, as well as serum bilirubin, serum albumin, ascites, and prothrombin activity[9,15]. The degree of liver damage has replaced the Child-Pugh classification to evaluate liver function in Japan. For serious liver failure patients, model for end stage liver disease (MELD) is used to indicate liver transplantation[16].

Integrated Stage score for liver function and tumor stage, including OKUDA[17], Cancer of the Liver Italian Program (CLIP)[18], Chinese University Prognostic Index (CUPI)[19], Japan Integrated Staging (JIS)[20], modified-JIS[15], and Tokyo[21], is effective for prognostic assessment in HCC (Table 1). Kudo *et al*[20] proposed the JIS score, which unified TNM staging in the General Rules and the



For patients with the severity of liver damage class A accompanied by vascular invasion, hepatectomy, chemotherapy, transcatheter arterial embolization may be selected. For patients with Child-Pugh class A with extrahepatic metastasi, chemotherapy is an option.

[1]When taking non-surgical treatment into consideration, using of Child-Pugh classification is possible; [2]Selected when the tumor measuring is 3 cm or less; [3]Oral or arterial infusion chemotherapy may be selected; [4]A single tumor measuring 5 cm or less in diameter; [5]Patients 65 years of age or younger.

Figure 1 Treatment algorithm for hepatocellular carcinoma. Revise from ref. [5]. Hx: Hepatectomy; RFA: Radiofrequency ablation; TACE: Transcatheter arterial chemo embolization; Chemo: Chemotherapy.

Child-Pugh classification[20]. The JIS is superior to the CLIP system [a combination of the Child-Pugh classification, tumor morphology, α-fetoprotein (AFP), and portal vein tumor thrombosis] in terms of (1) clear stratification of scores; (2) prognostic predictive power in HCC with a score of 0; and (3) differentiation of scores in patients with a poor prognosis. Thus, the JIS score is useful for prediction of prognosis of patients, but is not appropriate for comparison of treatment modalities or selection of optimal treatment.

CLINICAL GUIDELINES FOR HCC

The BCLC staging system, which is recommended by AASLD and EASL, is used worldwide to plan treatment for HCC. In contrast, in Japan, the treatment algorithm in the Clinical Practice Guidelines for Hepatocellular Carcinoma is commonly used for selection of optimal treatment based on liver function and tumor conditions (Figure 1). BCLC system links stage stratification to a treatment strategy and recommends standard care for a given patient, whereas the Japanese guidelines are not directly associated with clinical tumor stage, such as the JIS score[22]. The another major difference between the treatment algorithm used in Japan and the BCLC system is the indication of hepatectomy for HCC with ≤ 3 lesions and a diameter ≤ 3 cm on Child-Pugh A/B. The BCLC system recommends liver transplantation or radiofrequency ablation (RFA) for HCC with 2 or 3 nodules and a diameter ≤ 3 cm. In contrast, the treatment algorithm in Japan recommends hepatectomy for HCC with ≤ 3 lesions if liver function is good, regardless of the tumor size. The recommended treatment strategy also differs for HCC with portal hypertension (Table 2). The BCLC system states that liver transplantation or RFA, instead of hepatectomy, is indicated in such patients, but the treatment algorithm in Japan advises that aggressive hepatectomy based on $ICGR_{15}$ should be performed because the therapy must yield positive results[23].

DIAGNOSIS OF CLASSICAL HCC AND TREATMENT FOR EARLY HCC

Classical HCC is diagnosed based on CT images with early arterial enhancement and delayed washout (EASL criteria)[1,24]. Various guidelines have also adopted these criteria. Early HCC generally has stromal invasion in the portal region with remaining tumor[25] and has a macroscopically small nodular type with indistinct margins. Diagnostic imaging identifies this type as an ischemic mass. Prolongation of survival time by liver resection for early HCC is not significant and is limited due to the lead time bias[26]. This suggests that early HCC should be followed up

Table 2 Treatment options for hepatocellular carcinoma in barcelona clinic liver cancer system and Japanese guidelines

Tumor number	Tumor size (cm)	Child-Pugh class	Treatment	
			BCLC system	Japanese guidelines
Single	2	A, B	Resection	1 Resection 2 Ablation
	2.1-3	A, B	1 Resection 2 Transplantation or ablation	1 Resection 2 Ablation
	3.1-5	A, B	1 Resection 2 Transplantation	Resection
2 or 3 nodules	≤ 3	A, B	Transplantation or ablation	Resection or ablation
		C	Palliative care	Transplantation
	> 3	A, B	Chemoembolization	1 Resection 2 Chemoembolization
4 or more nodules		A, B	Chemoembolization	1 Chemoembolization 2 Chemotherapy
		C	Palliative care	Palliative care

Degree of Liver damage replaced Child-Pugh classification as liver function in Japan Revise from ref. [22].

without treatment based on the risk of a second primary cancer. This strategy is accepted according to the HCC management based on the consensus in the Japan Society of Hepatology[27].

EVIDENCE FOR EFFICACY OF HEPATECTOMY

The indication of hepatectomy for HCC is determined by the balance between liver function and tumor conditions. Excessive liver resection to completely remove lesions based on overestimation of hepatic functional reserve may cause hepatic failure, whereas minimal resection that does not correspond to the degree of tumor progression and focuses only on safety may increase the risk of early recurrence of HCC. Therefore, it is important to select an optimal approach that is appropriate for the degree of tumor progression based on the indication for hepatectomy. The major methods for preoperative assessment of liver function are the galactose tolerance test, ^{99m}Tc-GSA liver scintigraphy, and the ICG loading test. Makuuchi's criteria are particularly useful for patients with chronic hepatitis or hepatic cirrhosis[14]. These criteria are based on three factors: ascites, serum bilirubin, and $ICGR_{15}$. Patients who still have ascites after diuretic administration or those with a serum bilirubin level that is consistently > 2.0 mg/dL are not indicated for surgery. The patients with 1 < bilirubin level ≤ 2.0 mg/dL are indicated for limited liver resection. For eligible patients with serum bilirubin in the normal range of ≤ 1.0 mg/dL, the extent of resection is then determined based on $ICGR_{15}$ as resection of 2/3 of the total liver volume (TLV) (*e.g.*, right lobectomy) in patients with normal $ICGR_{15}$ of < 10%; 1/3 of the TLV (*e.g.*, left lobectomy) in those with $ICGR_{15}$ of 10%-19%; and 1/6 of the TLV (Couinaud segmentectomy) in those with $ICGR_{15}$ of 20%-29%. If $ICGR_{15}$ is ≥ 30%, limited resection or enucleation should be applied. A surgical mortality rate of 0% has been reported in 1056 consecutive hepatic resections performed in accordance with these criteria[28].

In portal venous invasion of HCC[29], the area supplied by the portal vein branches should be systemically removed as much as possible within the acceptable range of liver function. A new procedure of systematic subsegmentectomy has been developed to overcome the potential incompatibility between cure of cancer and preservation of liver function[30]. A study of survival after hepatectomy indicated a good prognosis in cases with a tumor diameter < 5 cm, a single lesion, capsule formation, no vascular invasion, serum albumin < 4.0 g/dL, and pathological TNM (pTNM) stage I or II. Of these parameters, pTNM stage is the most reliable prognostic factor[31]. A study of recurrence-free survival also identified the significant prognostic factors as the tumor stage, tumor size, number of tumors, and capsule formation, and also found that vascular invasion was a poor indicator of long-term survival[32]. Risk factors for early recurrence within 2 years postoperatively include non-anatomical resection, microscopic vascular invasion, and AFP ≥ 32 ng/mL[33]. A retrospective study showed that the cumulative survival rate was significantly greater after anatomical resection compared to that after non-anatomical resection, which suggests that the surgical technique can influence prognosis[34]. A future prospective study is required to clarify all of these findings.

Determination of the acceptable liver remnant volume after hepatectomy is an important task. In general, it is desirable to preserve the 20%-40% of the total liver volume (TLV) or the standard liver volume (SLV) in normal livers[35-42]. The MD Anderson group proposed that the smallest acceptable liver remnant volume is ≥ 20% of the SLV in cases

without chronic underlying liver disease[36], with the validity of this proposal supported by an analysis of 301 consecutive patients after extended right lobectomy[43]. On the other hand, there was a mortality rate on postoperative day 60 of 4.7% in this literature cited. However, HCC often develops in livers with chronic hepatitis or hepatic cirrhosis, and major hepatectomy such as lobectomy may induce hepatic failure due to insufficient liver remnant volume. Portal vein embolization (PE) prevents hepatic failure since the portal vein branches in hepatectomy are blocked to induce compensatory hypertrophy in the remnant liver area[44]. PE can be applied to cases with $ICGR_{15}$ < 10% and a ratio of nontumorous parenchymal volume of the resected liver to that of the whole liver (R2) ⩾ 60%, and those with $ICGR_{15}$ ⩾ 10% - < 20% and R2 of 40%-60%[35]. Three-dimensional CT permits simple and accurate determination of the relative positions of major blood vessels and the tumor, resection ranges, and liver remnant volume[45].

HEPATIC RESECTION

In liver surgery, hepatic parenchymal transection is associated with increased intraoperative blood loss, postoperative hemorrhage, and early complications such as bile leakage and surgical site infection (SSI). In addition to hemostasis, new devices have been developed to stop bleeding from the resection margin, which allows performance of safer and more secure hepatic resection. The Pringle maneuver, which blocks hepatic inflow once by manual compression of the hepatoduodenal ligament to minimize blood loss during hepatic resection, is also widely used. Several randomized clinical trials (RCTs) have shown that the Pringle maneuver reduces blood loss without affecting liver function[46,47]. Hemihepatic vascular occlusion has also been applied when resection is limited to one lobe[48,49].

Bleeding from the hepatic vein occurs most commonly during hepatic resection. Intraoperative hemorrhage is positively associated with central venous pressure (CVP) and several RCTs have shown that a decrease of CVP to ⩽ 5 cm H_2O during hepatectomy reduces intraoperative blood loss and stabilizes hemodynamics[50,51]. In contrast, infrahepatic inferior vena cava clamping with a low CVP has been shown not to reduce blood loss during hepatectomy[52], and thus the effects of low CVP require further study.

Hepatic parenchymal transection is performed using methods such as clamp crushing[53] and devices including the cavitron ultrasonic aspirator (CUSA)[54], Tissue Link[55], water jet scalpel[56], harmonic scalpel[57], floating ball[58], and LigaSure. In clamp crushing, a Pean clamp is used to ligate and resect remaining blood vessels after the hepatic parenchyma is crushed using the clamp. In RCTs, there were no differences in operating time, volume of blood loss, and incidence of postoperative complications between patients treated with clamp crushing and CUSA, but clamp crushing was superior in terms of complete appearance of landmark hepatic veins on the cut surface[53]. However, volume of blood loss and incidence of postoperative complications have also been reported to be lower using CUSA compared with clamp crushing[59]. A RCT comparing clamp crushing with Tissue Link found no differences in operating time, volume of blood loss and incidence of postoperative complications[60]. Another RCT showed the superiority of the LigaSure Vessel Sealing System for liver resection compared to vascular ligation based on clamp crushing[61], but a second RCT found no differences between these techniques[62].

PERIOPERATIVE MANAGEMENT

Since 1990, hepatectomy for HCC has been performed with acceptable blood loss of approximately 500 ml at many high-volume medical centers[28,63-67]. Allogenic blood transfusion in the perioperative period should be avoided when possible because it is likely to promote cancer recurrence and to induce hyperbilirubinemia and hepatic failure, and lower hematocrit is also desirable for microcirculation in the liver[68]. Autologous blood transfusion avoids homogenous red blood cell transfusion and does not increase the frequency of cancer recurrence[69]. The use of fresh frozen plasma (FFP) has been recommended for supplement of coagulation factors, maintenance of an effective plasma volume, and volume substitution[70]. However, FFP transfusion has also been reported to have no effect on the post-hepatectomy course[71] and to be unnecessary in Child-Pugh class A cases with intraoperative blood loss of < 1000 mL and serum albumin levels > 2.4 g/dL on postoperative day (POD) 2[72].

PREVENTION OF COMPLICATIONS

Bile leakage is a complication that is specific to hepatectomy and may be intractable. A RCT of the efficacy of a bile leakage test on prevention of bile leakage from the liver resection margin showed no difference in the incidence of bile leakage between patients who did and did not receive the test[73], whereas another RCT found that the test was able to prevent bile leakage and complications after hepatic resection[74]. Thus, more cases are required to evaluate the utility of this test.

Other post-hepatectomy complications include hemorrhage and intra-abdominal abscess, and these conditions may be fatal if diagnosis is delayed. Intraperitoneal drain placement is required for monitoring and treatment of these complications, but the efficacy of elective hepatectomy with standardized drain placement has been questioned and the

Centers for Disease Control and Prevention (CDC) guidelines state that such routine drain placement is not necessary: "If drainage is necessary, use a closed suction drain. Place a drain through a separate incision distant from the operative incision. Remove the drain as soon as possible"[75]. RCTs conducted in several countries on the need for drainage have also concluded that drain placement is not necessary[76-81]. Due to differences in the healthcare environment and health insurance system, drain placement has not been completely withdrawn in Japan, but early removal of drains has been recommended[82]. A RCT has also shown that subcutaneous drainage is not effective for prevention of SSI[83].

Immunity is weak after hepatectomy and this may result in hepatic failure and disseminated intravascular coagulation (DIC). A RCT of the efficacy of steroid administration for improvement of liver function after hepatectomy compared the post-hepatectomy liver function in patients treated with and without 500 mg/body hydrocortisone before hepatectomy[84]. Serum bilirubin levels were significantly lower in the steroid group on POD 2 compared with the non-steroid group and there were significant differences in serum bilirubin and prothrombin levels until POD 7, which shows the efficacy of steroid administration prior to hepatectomy. To unify the definition of post-hepatectomy liver failure (PHLF), in 2010 the International Study Group of Liver Surgery (ISGLS) proposed defining PHLF as an increased international normalized ratio (INR) and concomitant hyperbilirubinemia on or after POD 5[85]. PHLF seems to be the more efficient indicator comprehensively compared to 50-50 criteria[86] and MELD score because it is significantly associated with both of the incidence of post-hepatectomy complications and the post-hepatectomy mortality[87]. As for 50-50 criteria, it was not significantly related to the incidence of post-hepatectomy complications. As for MELD score, it revealed less strong association of the odds ratio (2.06) to the post-hepatectomy mortality.

CONCLUSION

In this article, we have described evidence-based techniques for hepatectomy and perioperative management of HCC. Improved assessment of liver function and development of surgical devices are likely to contribute to safe and effective hepatectomy and a good prognosis for patients.

REFERENCES

1 **Forner A**, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; **379**: 1245-1255 [PMID: 22353262 DOI: 10.1016/s0140-6736(11)61347-0]

2 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

3 **Kokudo N**, Makuuchi M. Evidence-based clinical practice guidelines for hepatocellular carcinoma in Japan: the J-HCC guidelines. *J Gastroenterol* 2009; **44** Suppl 19: 119-121 [PMID: 19148805 DOI: 10.1007/s00535-008-2244-z]

4 **Makuuchi M**, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, Saida Y, Takayama T, Yamaoka Y. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res* 2008; **38**: 37-51 [PMID: 18039202 DOI: 10.1111/j.1872-034X.2007.00216.x]

5 **Hepatology JSo**. Clinical practice guidelines for hepatocellular carcinoma. Tokyo, Japan: Kanehara, 2013

6 **Kokudo N**, Sasaki Y, Nakayama T, Makuuchi M. Dissemination of evidence-based clinical practice guidelines for hepatocellular carcinoma among Japanese hepatologists, liver surgeons and primary care physicians. *Gut* 2007; **56**: 1020-1021 [PMID: 17566034 DOI: 10.1136/gut.2007.119362]

7 **Sobin LH**, Compton CC. TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. *Cancer* 2010; **116**: 5336-5339 [PMID: 20665503 DOI: 10.1002/cncr.25537]

8 **Vauthey JN**, Lauwers GY, Esnaola NF, Do KA, Belghiti J, Mirza N, Curley SA, Ellis LM, Regimbeau JM, Rashid A, Cleary KR, Nagorney DM. Simplified staging for hepatocellular carcinoma. *J Clin Oncol* 2002; **20**: 1527-1536 [PMID: 11896101]

9 **Japan LCSGo.** General rules for the clinical and pathological study of primary liver cancer. 3rd ed. Tokyo: Kanehara, 2010

10 **Minagawa M**, Ikai I, Matsuyama Y, Yamaoka Y, Makuuchi M. Staging of hepatocellular carcinoma: assessment of the Japanese TNM and AJCC/UICC TNM systems in a cohort of 13,772 patients in Japan. *Ann Surg* 2007; **245**: 909-922 [PMID: 17522517 DOI: 10.1097/01.sla.0000254368.65878.da]

11 **Child CG.** The liver and portal hypertension. 3rd. MPCS. Philadelphia: W.B. Saunders, 1964

12 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913]

13 **Lau H**, Man K, Fan ST, Yu WC, Lo CM, Wong J. Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy. *Br J Surg* 1997; **84**: 1255-1259 [PMID: 9313707]

14 **Makuuchi M**, Kosuge T, Takayama T, Yamazaki S, Kakazu T, Miyagawa S, Kawasaki S. Surgery for small liver cancers. *Semin Surg Oncol* 1993; **9**: 298-304 [PMID: 8210909]

15 **Ikai I**, Takayasu K, Omata M, Okita K, Nakanuma Y, Matsuyama Y, Makuuchi M, Kojiro M, Ichida T, Arii S, Yamaoka Y. A modified Japan Integrated Stage score for prognostic assessment in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; **41**: 884-892 [PMID: 17048053 DOI: 10.1007/s00535-006-1878-y]

16 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]

17 **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 [PMID: 2990661]

18 A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* 1998; **28**: 751-755 [PMID: 9731568 DOI: 10.1002/hep.510280322]

19 **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 [PMID: 11920539]

20 **Kudo M**, Chung H, Haji S, Osaki Y, Oka H, Seki T, Kasugai H, Sasaki Y, Matsunaga T. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with

the CLIP score. *Hepatology* 2004; **40**: 1396-1405 [PMID: 15565571 DOI: 10.1002/hep.20486]
21 **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 [PMID: 15710994 DOI: 10.1136/gut.2003.035055]
22 **Takayama T.** Hepatocellular carcinoma. In: Clavien PA, editor. Malignant liver tumors: current and emerging therapies. 3rd ed. London: Wiley-Blackwell, 2010: 317-323
23 **Ishizawa T**, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, Imamura H, Sugawara Y, Kokudo N, Makuuchi M. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1908-1916 [PMID: 18549877 DOI: 10.1053/j.gastro.2008.02.091]
24 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
25 Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009; **49**: 658-664 [PMID: 19177576 DOI: 10.1002/hep.22709]
26 **Midorikawa Y**, Takayama T, Shimada K, Nakayama H, Higaki T, Moriguchi M, Nara S, Tsuji S, Tanaka M. Marginal survival benefit in the treatment of early hepatocellular carcinoma. *J Hepatol* 2013; **58**: 306-311 [PMID: 23063418 DOI: 10.1016/j.jhep.2012.09.026]
27 **Kudo M**, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, Kojiro M, Makuuchi M. Management of hepatocellular carcinoma in Japan: Consensus-Based Clinical Practice Guidelines proposed by the Japan Society of Hepatology (JSH) 2010 updated version. *Dig Dis* 2011; **29**: 339-364 [PMID: 21829027 DOI: 10.1159/000327577]
28 **Imamura H**, Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M. One thousand fifty-six hepatectomies without mortality in 8 years. *Arch Surg* 2003; **138**: 1198-1206; discussion 1206 [PMID: 14609867 DOI: 10.1001/archsurg.138.11.1198]
29 **Nakayama H**, Takayama T, Okubo T, Higaki T, Midorikawa Y, Moriguchi M, Itoh A. Proposal of objective morphological classification system for hepatocellular carcinoma using preoperative multiphase computed tomography. *J Gastroenterol* 2014; **49**: 1430-1437 [PMID: 24240709 DOI: 10.1007/s00535-013-0908-9]
30 **Makuuchi M**, Hasegawa H, Yamazaki S. Ultrasonically guided subsegmentectomy. *Surg Gynecol Obstet* 1985; **161**: 346-350 [PMID: 2996162]
31 **Poon RT**, Ng IO, Fan ST, Lai EC, Lo CM, Liu CL, Wong J. Clinicopathologic features of long-term survivors and disease-free survivors after resection of hepatocellular carcinoma: a study of a prospective cohort. *J Clin Oncol* 2001; **19**: 3037-3044 [PMID: 11408499]
32 **Arii S**, Tanaka J, Yamazoe Y, Minematsu S, Morino T, Fujita K, Maetani S, Tobe T. Predictive factors for intrahepatic recurrence of hepatocellular carcinoma after partial hepatectomy. *Cancer* 1992; **69**: 913-919 [PMID: 1310434]
33 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: 12547409]
34 **Hasegawa K**, Kokudo N, Imamura H, Matsuyama Y, Aoki T, Minagawa M, Sano K, Sugawara Y, Takayama T, Makuuchi M. Prognostic impact of anatomic resection for hepatocellular carcinoma. *Ann Surg* 2005; **242**: 252-259 [PMID: 16041216]
35 **Kubota K**, Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, Harihara Y, Takayama T. Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. *Hepatology* 1997; **26**: 1176-1181 [PMID: 9362359 DOI: 10.1053/jhep.1997.v26.pm0009362359]
36 **Abdalla EK**, Barnett CC, Doherty D, Curley SA, Vauthey JN. Extended hepatectomy in patients with hepatobiliary malignancies with and without preoperative portal vein embolization. *Arch Surg* 2002; **137**: 675-680; discussion 680-681 [PMID: 12049538]
37 **Schindl MJ**, Redhead DN, Fearon KC, Garden OJ, Wigmore SJ. The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. *Gut* 2005; **54**: 289-296 [PMID: 15647196 DOI: 10.1136/gut.2004.046524]
38 **Nagino M**, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y. Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. *Ann Surg* 2006; **243**: 364-372 [PMID: 16495702 DOI: 10.1097/01.sla.0000201482.11876.14]
39 **Sano T**, Shimada K, Sakamoto Y, Yamamoto J, Yamasaki S, Kosuge T. One hundred two consecutive hepatobiliary resections for perihilar cholangiocarcinoma with zero mortality. *Ann Surg* 2006; **244**: 240-247 [PMID: 16858186 DOI: 10.1097/01.sla.0000217605.66519.38]
40 **Ferrero A**, Viganò L, Polastri R, Muratore A, Eminefendic H, Regge D, Capussotti L. Postoperative liver dysfunction and future remnant liver: where is the limit? Results of a prospective study. *World J Surg* 2007; **31**: 1643-1651 [PMID: 17551779 DOI: 10.1007/s00268-007-9123-2]
41 **Giraudo G**, Greget M, Oussoultzoglou E, Rosso E, Bachellier P, Jaeck D. Preoperative contralateral portal vein embolization before major hepatic resection is a safe and efficient procedure: a large single institution experience. *Surgery* 2008; **143**: 476-482 [PMID: 18374044 DOI: 10.1016/j.surg.2007.12.006]
42 **Shimada K**, Nara S, Esaki M, Sakamoto Y, Kosuge T, Hiraoka N. Extended right hemihepatectomy for gallbladder carcinoma involving the hepatic hilum. *Br J Surg* 2011; **98**: 117-123 [PMID: 21136566 DOI: 10.1002/bjs.7262]
43 **Kishi Y**, Abdalla EK, Chun YS, Zorzi D, Madoff DC, Wallace MJ, Curley SA, Vauthey JN. Three hundred and one consecutive extended right hepatectomies: evaluation of outcome based on systematic liver volumetry. *Ann Surg* 2009; **250**: 540-548 [PMID: 19730239 DOI: 10.1097/SLA.0b013e3181b674df]
44 **Makuuchi M**, Thai BL, Takayasu K, Takayama T, Kosuge T, Gunvén P, Yamazaki S, Hasegawa H, Ozaki H. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990; **107**: 521-527 [PMID: 2333592]
45 **Saito S**, Yamanaka J, Miura K, Nakao N, Nagao T, Sugimoto T, Hirano T, Kuroda N, Iimuro Y, Fujimoto J. A novel 3D hepatectomy simulation based on liver circulation: application to liver resection and transplantation. *Hepatology* 2005; **41**: 1297-1304 [PMID: 15846773 DOI: 10.1002/hep.20684]
46 **Man K**, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Prospective evaluation of Pringle maneuver in hepatectomy for liver tumors by a randomized study. *Ann Surg* 1997; **226**: 704-711; discussion 711-713 [PMID: 9409569]
47 **Scatton O**, Zalinski S, Jegou D, Compagnon P, Lesurtel M, Belghiti J, Boudjema K, Lentschener C, Soubrane O. Randomized clinical trial of ischaemic preconditioning in major liver resection with intermittent Pringle manoeuvre. *Br J Surg* 2011; **98**: 1236-1243 [PMID: 21809337 DOI: 10.1002/bjs.7626]
48 **Makuuchi M**, Mori T, Gunvén P, Yamazaki S, Hasegawa H. Safety of hemihepatic vascular occlusion during resection of the liver. *Surg Gynecol Obstet* 1987; **164**: 155-158 [PMID: 3810429]
49 **Fu SY**, Lau WY, Li GG, Tang QH, Li AJ, Pan ZY, Huang G, Yin L, Wu MC, Lai EC, Zhou WP. A prospective randomized controlled trial to compare Pringle maneuver, hemihepatic vascular inflow occlusion, and main portal vein inflow occlusion in partial hepatectomy. *Am J Surg* 2011; **201**: 62-69 [PMID: 20409520 DOI: 10.1016/j.amjsurg.2009.09.029]
50 **Rahbari NN**, Koch M, Zimmermann JB, Elbers H, Bruckner T, Contin P, Reissfelder C, Schmidt T, Weigand MA, Martin E, Büchler MW, Weitz J. Infrahepatic inferior vena cava clamping for reduction of central venous pressure and blood loss during hepatic resection: a randomized controlled trial. *Ann Surg* 2011; **253**: 1102-1110 [PMID: 21412143 DOI: 10.1097/SLA.0b013e318214bee5]

51 **Ryu HG**, Nahm FS, Sohn HM, Jeong EJ, Jung CW. Low central venous pressure with milrinone during living donor hepatectomy. *Am J Transplant* 2010; **10**: 877-882 [PMID: 20420642 DOI: 10.1111/j.1600-6143.2010.03051.x]

52 **Kato M**, Kubota K, Kita J, Shimoda M, Rokkaku K, Sawada T. Effect of infra-hepatic inferior vena cava clamping on bleeding during hepatic dissection: a prospective, randomized, controlled study. *World J Surg* 2008; **32**: 1082-1087 [PMID: 18246387 DOI: 10.1007/s00268-007-9445-0]

53 **Takayama T**, Makuuchi M, Kubota K, Harihara Y, Hui AM, Sano K, Ijichi M, Hasegawa K. Randomized comparison of ultrasonic vs clamp transection of the liver. *Arch Surg* 2001; **136**: 922-928 [PMID: 11485528]

54 **Hodgson WJ**, Morgan J, Byrne D, DelGuercio LR. Hepatic resections for primary and metastatic tumors using the ultrasonic surgical dissector. *Am J Surg* 1992; **163**: 246-250 [PMID: 1739181]

55 **Nissen NN**, Grewal N, Lee J, Nawabi A, Korman J. Completely laparoscopic nonanatomic hepatic resection using saline-cooled cautery and hydrodissection. *Am Surg* 2007; **73**: 987-990 [PMID: 17983064]

56 **Papachristou DN**, Barters R. Resection of the liver with a water jet. *Br J Surg* 1982; **69**: 93-94 [PMID: 7059775]

57 **Cherqui D**, Husson E, Hammoud R, Malassagne B, Stéphan F, Bensaid S, Rotman N, Fagniez PL. Laparoscopic liver resections: a feasibility study in 30 patients. *Ann Surg* 2000; **232**: 753-762 [PMID: 11088070]

58 **Sakamoto Y**, Yamamoto J, Kokudo N, Seki M, Kosuge T, Yamaguchi T, Muto T, Makuuchi M. Bloodless liver resection using the monopolar floating ball plus ligasure diathermy: preliminary results of 16 liver resections. *World J Surg* 2004; **28**: 166-172 [PMID: 14708050 DOI: 10.1007/s00268-003-7167-5]

59 **Fan ST**, Lai EC, Lo CM, Chu KM, Liu CL, Wong J. Hepatectomy with an ultrasonic dissector for hepatocellular carcinoma. *Br J Surg* 1996; **83**: 117-120 [PMID: 8653334]

60 **Arita J**, Hasegawa K, Kokudo N, Sano K, Sugawara Y, Makuuchi M. Randomized clinical trial of the effect of a saline-linked radiofrequency coagulator on blood loss during hepatic resection. *Br J Surg* 2005; **92**: 954-959 [PMID: 16034832 DOI: 10.1002/bjs.5108]

61 **Saiura A**, Yamamoto J, Koga R, Sakamoto Y, Kokudo N, Seki M, Yamaguchi T, Yamaguchi T, Muto T, Makuuchi M. Usefulness of LigaSure for liver resection: analysis by randomized clinical trial. *Am J Surg* 2006; **192**: 41-45 [PMID: 16769273 DOI: 10.1016/j.amjsurg.2006.01.025]

62 **Ikeda M**, Hasegawa K, Sano K, Imamura H, Beck Y, Sugawara Y, Kokudo N, Makuuchi M. The vessel sealing system (LigaSure) in hepatic resection: a randomized controlled trial. *Ann Surg* 2009; **250**: 199-203 [PMID: 19638927 DOI: 10.1097/SLA.0b013e3181a334f9]

63 **Grazi GL**, Ercolani G, Pierangeli F, Del Gaudio M, Cescon M, Cavallari A, Mazziotti A. Improved results of liver resection for hepatocellular carcinoma on cirrhosis give the procedure added value. *Ann Surg* 2001; **234**: 71-78 [PMID: 11420485]

64 **Jarnagin WR**, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, Corvera C, Weber S, Blumgart LH. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg* 2002; **236**: 397-406; discussion 406-407 [PMID: 12368667 DOI: 10.1097/01.sla.0000029003.66466.b3]

65 **Poon RT**, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Improving perioperative outcome expands the role of hepatectomy in management of benign and malignant hepatobiliary diseases: analysis of 1222 consecutive patients from a prospective database. *Ann Surg* 2004; **240**: 698-708; discussion 708-710 [PMID: 15383797]

66 **Sima CS**, Jarnagin WR, Fong Y, Elkin E, Fischer M, Wuest D, D' Angelica M, DeMatteo RP, Blumgart LH, Gönen M. Predicting the risk of perioperative transfusion for patients undergoing elective hepatectomy. *Ann Surg* 2009; **250**: 914-921 [PMID: 19953711]

67 **Aramaki O**, Takayama T, Higaki T, Nakayama H, Ohkubo T, Midorikawa Y, Moriguchi M, Matsuyama Y. Decreased blood loss reduces postoperative complications in resection for hepatocellular carcinoma. *J Hepatobiliary Pancreat Sci* 2014; **21**: 585-591 [PMID: 24638988 DOI: 10.1002/jhbp.101]

68 **Katz SC**, Shia J, Liau KH, Gonen M, Ruo L, Jarnagin WR, Fong Y, D'Angelica MI, Blumgart LH, Dematteo RP. Operative blood loss independently predicts recurrence and survival after resection of hepatocellular carcinoma. *Ann Surg* 2009; **249**: 617-623 [PMID: 19300227 DOI: 10.1097/SLA.0b013e31819ed22f]

69 **Ishizawa T**, Hasegawa K, Tsuno NH, Tanaka M, Mise Y, Aoki T, Imamura H, Beck Y, Sugawara Y, Makuuchi M, Takahashi K, Kokudo N. Predeposit autologous plasma donation in liver resection for hepatocellular carcinoma: toward allogenic blood-free operations. *J Am Coll Surg* 2009; **209**: 206-214 [PMID: 19632597 DOI: 10.1016/j.jamcollsurg.2009.03.004]

70 **Makuuchi M**, Takayama T, Gunvén P, Kosuge T, Yamazaki S, Hasegawa H. Restrictive versus liberal blood transfusion policy for hepatectomies in cirrhotic patients. *World J Surg* 1989; **13**: 644-648 [PMID: 2554598]

71 **Tomimaru Y**, Wada H, Marubashi S, Kobayashi S, Eguchi H, Takeda Y, Tanemura M, Noda T, Umeshita K, Doki Y, Mori M, Nagano H. Fresh frozen plasma transfusion does not affect outcomes following hepatic resection for hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 5603-5610 [PMID: 21105194]

72 **Yamazaki S**, Takayama T, Kimura Y, Moriguchi M, Higaki T, Nakayama H, Fujii M, Makuuchi M. Transfusion criteria for fresh frozen plasma in liver resection: a 3 + 3 cohort expansion study. *Arch Surg* 2011; **146**: 1293-1299 [PMID: 22106322 DOI: 10.1001/archsurg.2011.293]

73 **Ijichi M**, Takayama T, Toyoda H, Sano K, Kubota K, Makuuchi M. Randomized trial of the usefulness of a bile leakage test during hepatic resection. *Arch Surg* 2000; **135**: 1395-1400 [PMID: 11115338]

74 **Liu Z**, Jin H, Li Y, Gu Y, Zhai C. Randomized controlled trial of the intraoperative bile leakage test in preventing bile leakage after hepatic resection. *Dig Surg* 2012; **29**: 510-515 [PMID: 23392477 DOI: 10.1159/000346480]

75 **Mangram AJ**, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol* 1999; **20**: 250-278; quiz 279-280 [PMID: 10219875 DOI: 10.1086/501620]

76 **Liu CL**, Fan ST, Lo CM, Wong Y, Ng IO, Lam CM, Poon RT, Wong J. Abdominal drainage after hepatic resection is contraindicated in patients with chronic liver diseases. *Ann Surg* 2004; **239**: 194-201 [PMID: 14745327 DOI: 10.1097/01.sla.0000109153.71725.8c]

77 **Sun HC**, Qin LX, Lu L, Wang L, Ye QH, Ren N, Fan J, Tang ZY. Randomized clinical trial of the effects of abdominal drainage after elective hepatectomy using the crushing clamp method. *Br J Surg* 2006; **93**: 422-426 [PMID: 16491462 DOI: 10.1002/bjs.5260]

78 **Belghiti J**, Kabbej M, Sauvanet A, Vilgrain V, Panis Y, Fekete F. Drainage after elective hepatic resection. A randomized trial. *Ann Surg* 1993; **218**: 748-753 [PMID: 8257225]

79 **Franco D**, Karaa A, Meakins JL, Borgonovo G, Smadja C, Grange D. Hepatectomy without abdominal drainage. Results of a prospective study in 61 patients. *Ann Surg* 1989; **210**: 748-750 [PMID: 2556083]

80 **Burt BM**, Brown K, Jarnagin W, DeMatteo R, Blumgart LH, Fong Y. An audit of results of a no-drainage practice policy after hepatectomy. *Am J Surg* 2002; **184**: 441-445 [PMID: 12433610]

81 **Lu L**, Sun HC, Qin LX, Wang L, Ye QH, Ren N, Fan J, Tang ZY. Abdominal drainage was unnecessary after hepatectomy using the conventional clamp crushing technique. *J Gastrointest Surg* 2006; **10**: 302-308 [PMID: 16455466 DOI: 10.1016/j.gassur.2005.06.002]

82 **Yamazaki S**, Takayama T, Moriguchi M, Mitsuka Y, Okada S, Midorikawa Y, Nakayama H, Higaki T. Criteria for drain removal following liver resection. *Br J Surg* 2012; **99**: 1584-1590 [PMID: 23027077 DOI: 10.1002/bjs.8916]

83 **Nakayama H**, Takayama T, Okubo T, Higaki T, Midorikawa Y, Moriguchi M, Aramaki O, Yamazaki S. Subcutaneous drainage to prevent wound infection in liver resection: a randomized controlled

trial. *J Hepatobiliary Pancreat Sci* 2014; **21**: 509-517 [PMID: 24519844 DOI: 10.1002/jhbp.93]

84 **Hayashi Y**, Takayama T, Yamazaki S, Moriguchi M, Ohkubo T, Nakayama H, Higaki T. Validation of perioperative steroids administration in liver resection: a randomized controlled trial. *Ann Surg* 2011; **253**: 50-55 [PMID: 21233606 DOI: 10.1097/SLA.0b013e318204b6bb]

85 **Rahbari NN**, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R, Koch M, Makuuchi M, Dematteo RP, Christophi C, Banting S, Usatoff V, Nagino M, Maddern G, Hugh TJ, Vauthey JN, Greig P, Rees M, Yokoyama Y, Fan ST, Nimura Y, Figueras J, Capussotti L, Büchler MW, Weitz J. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**: 713-724 [PMID: 21236455 DOI: 10.1016/j.surg.2010.10.001]

86 **Balzan S**, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 824-828 [PMID: 16327492]

87 **Rahbari NN**, Reissfelder C, Koch M, Elbers H, Striebel F, Büchler MW, Weitz J. The predictive value of postoperative clinical risk scores for outcome after hepatic resection: a validation analysis in 807 patients. *Ann Surg Oncol* 2011; **18**: 3640-3649 [PMID: 21674269 DOI: 10.1245/s10434-011-1829-6]

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MINIREVIEWS

Immune response to hepatitis B vaccine among patients on hemodialysis

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Abstract

Infection with hepatitis B virus (HBV) poses a major health threat worldwide, where the magnitude and overburden of chronic carrier state approaches 150 million chronic carriers. The prevalence of HBV is greater among dialyzed patients compared to the general population owing to their increased vulnerability to blood and its products, along with hazards posed by contaminated hemodialysis tools and devices. An electronic systematic search of the published literature was carried and data on the immunological riposte to hepatitis B vaccination among hemodialysis patients was extracted from relevant studies. End stage renal disease patients on hemodialysis have a lower or an absolutely negative riposte to HBV vaccine. Several means have been tried to improve this response with some success, nevertheless none have been universally adopted. Genetic investigations are foreseen to make a break through concerning HBV vaccination.

Key words: Hemodialysis; Chronic kidney disease; Immune response; Vaccine; Adjuvant

Core tip: This article discussed the history of immunological riposte to various types of Hepatitis B vaccines among patients on hemodialysis based on published findings of an array of studies up to this year. Moreover, it tackled the possible causes for such a response and possible future ways out of this dilemma.

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INTRODUCTION

Infection with hepatitis B Virus (HBV) poses a major health threat worldwide, where the magnitude and overburden of chronic carrier state approaches 150 million chronic carriers. The prevalence of HBV is greater among dialyzed patients compared to the general population, this could be attributed to the

fact they are needy for the blood and its products and thus more vulnerable, along with the jeopardy posed by contaminated hemodialysis tools and devices[1]. Therefore HBV immunization is highly advised for patients suffering chronic kidney disease (CKD), whether pre-emptive or dialysis dependent, who are potential nominees for kidney transplant along with those on dialysis[2]. In spite of this, surges of the aforementioned infection among patients on hemodialysis are persistently encountered even in advanced countries[3-5].

The extent of renal failure has been described to determine the immunological riposte to hepatitis B immunization among this group of patients, where it has generally been reported to be suboptimal[6-8]. The seroconversion rates and antibody titers among chronic renal disease patients has been shown to be less than the general population along with a shorter duration of seroprotection[9]. The flawed effectiveness of HBV immunization among dialysis dependent patients can be justified by a group of determinants, noticeably the defective immunity owing to; azotemia, age[10], sex[11], body mass[12], nourishment of such patients[13], concomitant infection with HCV[14] or HIV[15], history of transfusion of blood or blood products[16] and having the major histocompatibility complex also to be associated with this response[17-19], along with failure to complete the full course of HBV vaccine[20].

In order to enhance the riposte degree to hepatitis B immunization in end-stage kidney disease a group of strategies have been embraced and these are; building up the vaccine dose[20], supplementary vaccine injections along with resorting to intradermal injections rather than the intramuscular in order to supplement the vaccination[21]. Hepatitis B vaccination among pre-dialysis chronic kidney disease patients results in higher seroprotection rates when compared to those patients on dialysis[22]. Adjuvants has been proposed to be of some help in raising the immunity, a good example for these adjuvants is levamisole, which is an antihelminthic with characteristics enabling it to stimulate suppressed T-cell action along with potentiation of B lymphocyte action[23-25]. The target of this review is to discuss inadequate immunological riposte to hepatitis B immunization, its determinants and the possible solutions.

MANAGEMENT

Search strategy and data extraction

An electronic systematic search of the published literature was carried and data on the immunological riposte to hepatitis B vaccination among hemodialysis patients was extracted from studies of relevance. The databases were searched with the words "Hepatitis B immunization", "dialysis", "immunological riposte", "retarded riposte", "non-responders" and "adjuvants" were used interchangeably in MEDLINE, Pubmed, MiPc library and Google.

Epidemiology of HBV among hemodialysis

Hepatitis B infection has been declining in the last two decades in artificial kidney facilities, a status that mirrors the outcomes of efforts made in providing efficient prophylaxis measures[26]. Variable prevalence of HBV infection among dialysis patients were reported from the different continents ranging between (6%), and (1.2%)[27-29]. In a large scale study including 8615 adult dialysis patients from different dialysis facilities in the Western world, hepatitis B prevalence rates ranged from 0% to 6.6%[5]. A principal determinant hindering the transmission of such an infection in artificial kidney facilities was the preservation of universal infection control measures. CDC guidelines advice segregation of patients who are antigen-positive, dedicating an independent nursing group and it further more prohibits sharing medicaments in artificial kidney facilities[30]. Undiminished vulnerability percentages to hepatitis B infection were particularly observed in renal facilities dealing with HB (S) Ag carriers. Such vulnerability could be controlled by the strict cohesion to the global precautions; however causal incidences can to an outbreak the whole facility[30,31]. History of immunization against hepatitis B targeting end stage renal disease sufferers started by the utilization of live attenuated virus derived from plasma, although it was initially reported to mount enough immunity, however, it was found later not to have induced a sufficient immunity. Currently supplied vaccines possess an outstanding safeness and immunogenicity account, providing protection rates falling just below 100% of the immunized group[32]. However, some population subgroups, including some people of normal health and immune-deficient individuals, riposte inadequately to immunization. Part of such sets, are chronic kidney disease patients, including pre-emptive and dialysis patients, whom are regarded to have vulnerability to contract HBV owing to transmission to those on dialysis through surrounding surfaces, expendables, or apparatuses during hemodialysis[33,34]. Hepatitis B vaccination, when combined with application of the other precautions, ended up in a definite and appreciable decline in new infections among hemodialysis patients and kidney facility personnel in Western countries[35,36]. Despite the fact that the frequency of HBV is absolutely squatty, a big proportion of vulnerable chronic kidney disease sufferers have to get the vaccine. Regulations meant with control of transmission of infections in renal facilities gives a feeling of protection to working personnel, nevertheless, chronic kidney disease sufferers' vaccination is yet regarded a subsidiary and pricy procedure, there for resulting in

a greater proportion of unimmunized chronic renal disease sufferers in some countries. Hepatitis B poses an intimidation for chronic renal sufferers on hemodialysis, regardless of precautions secured, let alone meanwhile they are subjected to hemodialysis in their local facilities, but again meanwhile accommodated by other units when considering superior chronic kidney disease sufferers' acclimation, vacation enjoyment. During such plots, the susceptible chronic kidney disease sufferers' acts as a probable HBV infection aim (undertaking dialysis in machines, units dedicated for infected patients) moreover they act as a probable harbor for the disease taking it back to their local facilities[37]. It should be noted that HBV can also be transmitted to dialyzed chronic kidney disease sufferers (as the rest of the general population) by other means known to transmit the disease (sexual route *etc.*). Those who caught the hepatitis infection can spread the disease in their home unit in turn, ahead of hepatitis B infection detection, unless active steps take place to protect patients from such an infection in terms of a fruitful immunization plan[38]. A great proportion of hemodialyzed chronic kidney disease sufferers acquiring HBV have a tendency to progress to chronic hepatic disease (unable to eliminate their virus). Such chronic kidney disease sufferers are rendered to have greater vulnerability on attempting kidney transplantation, further more they pose a potential harbor for the disease to both other chronic kidney disease sufferers and non-immune working personnel[39-41]. The aforementioned fact makes a comprehensively fruitful immunization plan imperative for chronic kidney disease sufferers and staff safeguard against this lifelong, enduring and possibly killing infection[42].

BOOSTING THE IMMUNOLOGICAL RESPONSE TO VACCINATION

Evolution of the vaccine

Comparative to numerous other infections, immunization, as a protective strategy, performs a crucial role in limitation of the HBV infection and its consequences[9]. Hitherto, there are triumvirate derivations of HBV vaccines. Saul Krugman's observation about immunogenicity of HBsAg and the immunizing properties of anti-HBS antibody facing HBV was a real breakthrough that resulted in producing the early vaccine derivation[26] incorporating an inactive HBsAg extracted out of the plasma of the HBV carrier persons. Merck and Pasteur institute simultaneously produced the early vaccine derivation making use of aforementioned observation. Then, Food and Drug Administration of the United States of America approved it during 1981[26]. The second derivation of HBV vaccine was engineered using recombinant DNA technology utilizing the yeast Saccharomyces cerevisiae resulting in the formulas; Engerix B along with Recombivax HB. Both vaccine formulas encompass HBsAg. Nevertheless, the third vaccines derivation mounts an appreciably greater protection if compared to HBsAg owing to the use of pre-S1 along with pre-S2 immune triggers; however, there availability is yet limited. Recombinant DNA technology has also been used to produce the third generation vaccines by mammalian cells[26,43]. Different American, European and Asian states adopted The WHO recommendation 1991 concerning large-scale HBV immunization by year 1997. Therefore there is an appreciable drop in pervasiveness of the HBV infection[43,44], and its complications including HCC and fulminating hepatitis[44].

Adjuvants

Several methods have been suggested to potentiate the outcome of HBV immunization and its riposte among chronic kidney disease sufferers on hemodialysis. Use of adjuvants was suggested to potentiate the riposte to immunization. Examples for such adjuvants are; high thymopentin doses[45]. Levamisole is another adjuvant that has been suggested to improve vaccination results among such patients. It is probable that it can also be efficient in boosting HBV vaccination riposte among HD dependent patients[46], however in terms of taking a rather mature decision it is prudent to conduct further research in this field in order to now the pros and cons of such agents. Fabrizi *et al*[47] found in their meta-analysis that a better immune response is mounted when GM-CSF is added as an adjuvant to HBV vaccine. Polymethylmethacrylate is another adjuvant that has been proposed to improve the immunity post vaccination[48]. HBV-AS04 encompassing the synergist 3-O-desacyl-40-monophosphoryl lipid A that is consistent with Engerix B customary, is a further adjuvant improving the immune response[26]. A Recent research conducted by Saade *et al*[49] has found that Advax (a polysaccharide adjuvant) induces a potent humoral and cellular induced immunity with minimal reactions in the preclinical phase. Yet most of the studies in this context have some methods limitation such as lack of randomization[50]. Thus a long term data about the sustainability of the effect of these adjuvants is to be verified.

Changing the route of administration and booster doses

Currently, HBV vaccinations, particularly the second derivation, are administered through the intramuscular (deltoid) route three times (on 0, 1 and 6 mo's period). Antibodies' to Anti-HBs titers above 10 IU/L are deemed effective. Considering revaccinating subjects or boosting a currently administered vaccine is required in situations where

the titers level falls beneath 10 IU/L such as time linked falls in titers, or those seen among high risk groups such as immunosuppressed, smokers, obese persons, kidney failure patients and those suffering hepatic disease[9,43].

Currently, administration of HBV immunization through either the intradermal and intramuscular injection routes is under evaluation in chronic kidney disease sufferers who undergo HD. Short term follow up, reflected that the former route of HBV immunization can mount a more potent immune riposte if compared to the later route[26]; nevertheless, such a believe has been refuted by long term follow up.

FUTURE PROSPECTS

Genetic investigations might help in the improvement of hepatitis B vaccines and there for it may lead to reduction in the proportion of vaccine failures[51]. Increased interferon (IFN)-gamma production was shown to be associated with positive response to vaccines[52,53]. As IL-18 is involved in IFN-gamma production[54-57], it was used as adjuvant to DNA vaccines against HBV[53,57,58]. Channarong *et al*[57] has devised a recombinant plasmid bearing a gene encoding HBsAg combined to DNA segment encoding full-length murine IL-18. All immunized mice showed a remarkable serum anti-HBsAg IgG response following two intramuscular injections of the vaccines on comparison to the level of mice vaccinated with the vaccine devoid of the DNA segment encoding IL-18. Recently Hu *et al*[59] found on animal experiments that adding calcineurin B subunit to Engerix mounted an higher hepatitis B antibodies both in dose and time dependent manner through promoting an inflammatory response where IFN-γ, IL-6, TNF-α are produced[59]. It is probable that in the near future all people throughout the world will be vaccinated on the mandatory basis.

CONCLUSION

Chronic kidney disease patients on hemodialysis tend to have no or at most a lower response to HBV vaccine. Several means have been tried to improve this response with some success, nevertheless, absolutely none have been universally adopted. Genetic investigations are foreseen to make a breakthrough concerning HBV immunization.

REFERENCES

1 **Martin P**, Friedman LS. Chronic viral hepatitis and the management of chronic renal failure. *Kidney Int* 1995; **47**: 1231-1241 [PMID: 7637252 DOI: 10.1038/ki.1995.177]

2 **Centers for Disease Control**. Hepatitis B Vaccination Recommendations for Adults. *MMWR* 2006; **55** (RR-16)

3 **Thompson ND**, Perz JF, Moorman AC, Holmberg SD. Nonhospital health care-associated hepatitis B and C virus transmission: United States, 1998-2008. *Ann Intern Med* 2009; **150**: 33-39 [PMID: 19124818 DOI: 10.7326/0003-4819-150-1-200901060-00007]

4 **Igaki N**, Nakaji M, Moriguchi R, Akiyama H, Tamada F, Oimomi M, Goto T. An outbreak of fulminant hepatitis B in immunocompromised hemodialysis patients. *J Gastroenterol* 2003; **38**: 968-976 [PMID: 14614604]

5 **Burdick RA**, Bragg-Gresham JL, Woods JD, Hedderwick SA, Kurokawa K, Combe C, Saito A, LaBrecque J, Port FK, Young EW. Patterns of hepatitis B prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney Int* 2003; **63**: 2222-2229 [PMID: 12753311]

6 Infection control requirements for dialysis facilities and clarification regarding guidance on parenteral medication vials. *MMWR Morb Mortal Wkly Rep* 2008; **57**: 875-876 [PMID: 18701878]

7 **Finelli L**, Miller JT, Tokars JI, Alter MJ, Arduino MJ. National surveillance of dialysis-associated diseases in the United States, 2002. *Semin Dial* 2005; **18**: 52-61 [PMID: 15663766]

8 **Bond TC**, Patel PR, Krisher J, Sauls L, Deane J, Strott K, Karp S, McClellan W. Association of standing-order policies with vaccination rates in dialysis clinics: a US-based cross-sectional study. *Am J Kidney Dis* 2009; **54**: 86-94 [PMID: 19346041 DOI: 10.1053/j.ajkd.2008.12.038]

9 **Grzegorzewska AE**. Hepatitis B vaccination in chronic kidney disease: review of evidence in non-dialyzed patients. *Hepat Mon* 2012; **12**: e7359 [PMID: 23326280 DOI: 10.5812/hepatmon.7359]

10 **Unger JK**, Peters H. Hepatitis B in chronic kidney disease: moving toward effective prevention. *Kidney Int* 2008; **73**: 799-801 [PMID: 18340351 DOI: 10.1038/ki.2008.57]

11 **Stevens CE**, Alter HJ, Taylor PE, Zang EA, Harley EJ, Szmuness W. Hepatitis B vaccine in patients receiving hemodialysis. Immunogenicity and efficacy. *N Engl J Med* 1984; **311**: 496-501 [PMID: 6235453]

12 **Vagelli G**, Calabrese G, Mazzotta A, Pratesi G, Gonella M. More about response to hepatitis B vaccine in hemodialysis patients. *Nephron* 1988; **49**: 171 [PMID: 2967921]

13 **Al Saran K**, Sabry A, Al Halawany Z, Ismail M. Factors affecting response to hepatitis B vaccine among hemodialysis patients in a large Saudi Hemodialysis Center. *Saudi J Kidney Dis Transpl* 2014; **25**: 185-191 [PMID: 24434410]

14 **Gandhi RT**, Wurcel A, Lee H, McGovern B, Shopis J, Geary M, Sivamurthy R, Sax PE, Ukomadu C. Response to hepatitis B vaccine in HIV-1-positive subjects who test positive for isolated antibody to hepatitis B core antigen: implications for hepatitis B vaccine strategies. *J Infect Dis* 2005; **191**: 1435-1441 [PMID: 15809901 DOI: 10.1086/429302]

15 **Ahuja TS**, Kumar S, Mansoury H, Rodriguez H, Kuo YF. Hepatitis B vaccination in human immunodeficiency virus-infected adults receiving hemodialysis. *Kidney Int* 2005; **67**: 1136-1141 [PMID: 15698455]

16 **Afsar B**. The relationship between erythropoietin resistance and antibody response to hepatitis B vaccine in hemodialysis patients. *Nephrourol Mon* 2013; **5**: 806-812 [PMID: 24282790]

17 **Pol S**, Legendre C, Mattlinger B, Berthelot P, Kreis H. Genetic basis of nonresponse to hepatitis B vaccine in hemodialyzed patients. *J Hepatol* 1990; **11**: 385-387 [PMID: 2290031]

18 **Lu Y**, Cheng Y, Yan W, Nardini C. Exploring the molecular causes of hepatitis B virus vaccination response: an approach with epigenomic and transcriptomic data. *BMC Med Genomics* 2014; **7**: 12 [PMID: 24612962]

19 **Davila S**, Froeling FE, Tan A, Bonnard C, Boland GJ, Snippe H, Hibberd ML, Seielstad M. New genetic associations detected in a host response study to hepatitis B vaccine. *Genes Immun* 2010; **11**: 232-238 [PMID: 20237496]

20 **Centers for Disease Control**. Epidemiology and Prevention of Vaccine-Preventable Diseases. The Pink Book: Course Textbook -12th ed, Second Printing. USA: Centers for Disease Control and Prevention, 2012

21 **Barraclough KA**, Wiggins KJ, Hawley CM, van Eps CL, Mudge DW, Johnson DW, Whitby M, Carpenter S, Playford EG. Intradermal versus intramuscular hepatitis B vaccination in hemodialysis patients: a prospective open-label randomized

controlled trial in nonresponders to primary vaccination. *Am J Kidney Dis* 2009; **54**: 95-103 [PMID: 19481320 DOI: 10.1053/j.ajkd.2009.03.010]
22 **DaRoza G**, Loewen A, Djurdjev O, Love J, Kempston C, Burnett S, Kiaii M, Taylor PA, Levin A. Stage of chronic kidney disease predicts seroconversion after hepatitis B immunization: earlier is better. *Am J Kidney Dis* 2003; **42**: 1184-1192 [PMID: 14655190]
23 **Chen LY**, Lin YL, Chiang BL. Levamisole enhances immune response by affecting the activation and maturation of human monocyte-derived dendritic cells. *Clin Exp Immunol* 2008; **151**: 174-181 [PMID: 18005262]
24 **Oladele OA**, Emikpe BO, Adeyefa CAO, Enibe F. Effects of levamisole hydrochloride on cellular immune response and flock performance of commercial broilers. *Rev Bras Cienc Avic* 2012; **14**: 259-265 [DOI: 10.1590/S1516-635X2012000400005]
25 **Božiæ1 F**, Banoviæ F, Šuran1 J, Crniæ AP. Adjuvant activity of levamisole for experimental F18ac+ *Escherichia coli* oral vaccine against porcine post-weaning colibacillosis. *Vet Arhiv* 2011; **81**: 199-209
26 **Edey M**, Barraclough K, Johnson DW. Review article: Hepatitis B and dialysis. *Nephrology* (Carlton) 2010; **15**: 137-145 [PMID: 20470270]
27 **Goodkin DA**, Young EW, Kurokawa K, Prütz KG, Levin NW. Mortality among hemodialysis patients in Europe, Japan, and the United States: case-mix effects. *Am J Kidney Dis* 2004; **44**: 16-21 [PMID: 15486869]
28 **Lioussfi Z**, Errami Z, Radoui A, Rhou H, Ezzaitouni F, Ouzeddoun N, Bayahia R, Benamar L. Viral hepatitis C and B among dialysis patients at the Rabat University Hospital: prevalence and risk factors. *Saudi J Kidney Dis Transpl* 2014; **25**: 672-679 [PMID: 24821177]
29 **Kalantari H**, Ebadi S, Yaran M, Maracy MR, Shahshahan Z. Prevalence and risk factors of hepatitis B and C viruses among hemodialysis patients in Isfahan, Iran. *Adv Biomed Res* 2014; **3**: 73 [PMID: 24627881]
30 **Centers for Disease Control.** Recommendations for preventing transmission of infections among chronic hemodialysis patients. *MMWR* 2001; **50** (RR-5): 1-43. Available from: URL: http://www.cdc.gov/mmwr/PDF/rr/rr5005.pdf
31 **Lanini S**, Puro V, Lauria FN, Fusco FM, Nisii C, Ippolito G. Patient to patient transmission of hepatitis B virus: a systematic review of reports on outbreaks between 1992 and 2007. *BMC Med* 2009; **7**: 15 [PMID: 19356228 DOI: 10.1186/1741-7015-7-15]
32 **Carniel EF**, Morcillo AM, Blotta MH, Da Silva MT, Mazzola TN, Antonio MA, Zanolli ML, Netto AA, Higashi HG, Raw I, Vilela MM. Immunogenicity and safety of combined intradermal recombinant Hepatitis B with BCG vaccines at birth. *Vaccine* 2008; **26**: 647-652 [PMID: 18155811 DOI: 10.1016/j.vaccine.2007.11.048]
33 **Alashek WA**, McIntyre CW, Taal MW. Hepatitis B and C infection in haemodialysis patients in Libya: prevalence, incidence and risk factors. *BMC Infect Dis* 2012; **12**: 265 [PMID: 23082935 DOI: 10.1186/1471-2334-12-265]
34 **Gasim GI**, Hamdan HZ, Hamdan SZ, Adam I. Epidemiology of hepatitis B and hepatitis C virus infections among hemodialysis patients in Khartoum, Sudan. *J Med Virol* 2012; **84**: 52-55 [PMID: 22052648 DOI: 10.1002/jmv.22256]
35 **Elamin S**, Abu-Aisha H. Prevention of hepatitis B virus and hepatitis C virus transmission in hemodialysis centers: review of current international recommendations. *Arab J Nephrol Transplant* 2011; **4**: 35-47 [PMID: 21469594]
36 **Gasim GI**. Hepatitis B virus in the Arab world: where do we stand? *Arab J Gastroenterol* 2013; **14**: 35-43 [PMID: 23820498 DOI: 10.1016/j.ajg.2013.04.002]
37 **Ayub MA**, Bacci MR, Fonseca FL, Chehter EZ. Hemodialysis and hepatitis B vaccination: a challenge to physicians. *Int J Gen Med* 2014; **7**: 109-114 [PMID: 24520201 DOI: 10.2147/IJGM.S57254]
38 **Eleftheriadis T**, Liakopoulos V, Leivaditis K, Antoniadi G, Stefanidis I. Infections in hemodialysis: a concise review. Part II: blood transmitted viral infections. *Hippokratia* 2011; **15**: 120-126 [PMID: 22110292]
39 **Urbánek P**. Viral hepatitis infections in chronic kidney disease patients and renal transplant recipients. *Kidney Blood Press Res* 2012; **35**: 454-467 [PMID: 22677941 DOI: 10.1159/000338309]
40 **Schroth RJ**, Hitchon CA, Uhanova J, Noreddin A, Taback SP, Moffatt ME, Zacharias JM. Hepatitis B vaccination for patients with chronic renal failure. *Cochrane Database Syst Rev* 2004; **3**: CD003775 [PMID: 15266500]
41 **Barraclough KA**, Playford EG. Hepatitis B virus infection in hemodialysis populations: progress toward prevention. *Kidney Int* 2010; **77**: 177-180 [PMID: 20075953 DOI: 10.1038/ki.2009.456]
42 **World Health Organization.** Media Centre: Hepatitis B. 2013. Available from: URL: http: //www.who.int/mediacentre/factsheets/fs204/en/
43 **Aspinall EJ**, Hawkins G, Fraser A, Hutchinson SJ, Goldberg D. Hepatitis B prevention, diagnosis, treatment and care: a review. *Occup Med* (Lond) 2011; **61**: 531-540 [PMID: 22114089 DOI: 10.1093/occmed/kqr136]
44 **Luo Z**, Li L, Ruan B. Impact of the implementation of a vaccination strategy on hepatitis B virus infections in China over a 20-year period. *Int J Infect Dis* 2012; **16**: e82-e88 [PMID: 22178658 DOI: 10.1016/j.ijid.2011.10.009]
45 **Fabrizi F**, Dixit V, Martin P. Meta-analysis: the adjuvant role of thymopentin on immunological response to hepatitis B virus vaccine in end-stage renal disease. *Aliment Pharmacol Ther* 2006; **23**: 1559-1566 [PMID: 16696803]
46 **Fabrizi F**, Dixit V, Messa P, Martin P. Meta-analysis: levamisole improves the immune response to hepatitis B vaccine in dialysis patients. *Aliment Pharmacol Ther* 2010; **32**: 756-762 [PMID: 20662784 DOI: 10.1111/j.1365-2036.2010.04410]
47 **Fabrizi F**, Ganeshan SV, Dixit V, Martin P. Meta-analysis: the adjuvant role of granulocyte macrophage-colony stimulating factor on immunological response to hepatitis B virus vaccine in end-stage renal disease. *Aliment Pharmacol Ther* 2006; **24**: 789-796 [PMID: 16918882]
48 **Duranti E**, Duranti D. Polymethylmethacrylate strengthens antibody response in hemodialysis patients not responding to hepatitis B vaccine: preliminary data. *Minerva Med* 2011; **102**: 469-474 [PMID: 22193378]
49 **Saade F**, Honda-Okubo Y, Trec S, Petrovsky N. A novel hepatitis B vaccine containing Advax™, a polysaccharide adjuvant derived from delta inulin, induces robust humoral and cellular immunity with minimal reactogenicity in preclinical testing. *Vaccine* 2013; **31**: 1999-2007 [PMID: 23306367 DOI: 10.1016/j.vaccine.2012.12.077]
50 **Moher D**, Pham B, Jones A, Cook DJ, Jadad AR, Moher M, Tugwell P, Klassen TP. Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? *Lancet* 1998; **352**: 609-613 [PMID: 9746022 DOI: 10.1016/S0140-6736(98)01085]
51 **Hennig BJ**, Fielding K, Broxholme J, Diatta M, Mendy M, Moore C, Pollard AJ, Rayco-Solon P, Sirugo G, van der Sande MA, Waight P, Whittle HC, Zaman SM, Hill AV, Hall AJ. Host genetic factors and vaccine-induced immunity to hepatitis B virus infection. *PLoS One* 2008; **3**: e1898 [PMID: 18365030 DOI: 10.1371/journal.pone.0001898]
52 **Petrescu L**, Stancu S, Tardei G, Santimbrean C, Penescu M, Mircescu G. Tuberculin skin test, interferon-gamma assay, and T cells subpopulations in hemodialysis patients. *J Ren Nutr* 2010; **20**: S109-S117 [PMID: 20797557 DOI: 10.1053/j.jrn.2010.06.011]
53 **Tang LL**, Liu KZ. Recent advances in DNA vaccine of hepatitis virus. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 228-231 [PMID: 14612273]
54 **Kim JJ**, Nottingham LK, Tsai A, Lee DJ, Maguire HC, Oh J, Dentchev T, Manson KH, Wyand MS, Agadjanyan MG, Ugen KE, Weiner DB. Antigen-specific humoral and cellular immune responses can be modulated in rhesus macaques through the use of IFN-gamma, IL-12, or IL-18 gene adjuvants. *J Med Primatol* 1999; **28**: 214-223 [PMID: 10593488]
55 **Tominaga K**, Yoshimoto T, Torigoe K, Kurimoto M, Matsui K, Hada T, Okamura H, Nakanishi K. IL-12 synergizes with IL-18

or IL-1beta for IFN-gamma production from human T cells. *Int Immunol* 2000; **12**: 151-160 [PMID: 10653850 DOI: 10.1093/intimm/12.2.151]

56 **Yoshimoto T**, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, Akira S, Nakanishi K. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. *J Immunol* 1998; **161**: 3400-3407 [PMID: 9759857]

57 **Channarong S**, Mitrevej A, Sinchaipanid N, Usuwantim K, Kulkeaw K, Chaicumpa W. Cloning, protein expression and immunogenicity of HBs-murine IL-18 fusion DNA vaccine. *Asian Pac J Allergy Immunol* 2007; **25**: 233-242 [PMID: 18402297]

58 **Chen JZ**, Zhu HH, Liu KZ, Chen Z. Enhancing cellular immune response to HBV M DNA vaccine in mice by codelivery of interleukin-18 recombinant. *J Zhejiang Univ Sci* 2004; **5**: 467-471 [PMID: 14994439]

59 **Hu M**, Su Z, Yin Y, Li J, Wei Q. Calcineurin B subunit triggers innate immunity and acts as a novel Engerix-B HBV vaccine adjuvant. *Vaccine* 2012; **30**: 4719-4727 [PMID: 22652401 DOI: 10.1016/j.vaccine.2012.05.040]

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ORIGINAL ARTICLE

Retrospective Study

Post-transplantation hepatocellular carcinoma recurrence: Patterns and relation between vascularity and differentiation degree

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Author contributions: Pecchi A, Besutti G, De Santis M and Torricelli P designed the study and reviewed imaging examinations; Nosseir S reviewed pathological reports; Del Giovane C carried out the statistical analysis; Tarantino G and Di Benedetto F provided all clinical information.
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Informed consent: All patients gave their verbal consent to the use of their data for scientific purposes at the moment of their admission to the Transplantation Department.
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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at anna_pecchi@yahoo.com.

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Abstract

AIM: To evaluate the relationship between hepatocellular carcinoma (HCC) vascularity and grade; to describe patterns and vascular/histopathological variations of post-transplantation recurrence.

METHODS: This retrospective study included 165 patients (143 men, 22 women; median age 56.8 years, range 28-70.4 years) transplanted for HCC who had a follow-up period longer than 2 mo. Pre-transplantation dynamic computed tomography or magnetic resonance examinations were retrospectively reviewed, classifying HCC imaging enhancement pattern into hypervascular and hypovascular based on presence of wash-in during arterial phase. All pathologic reports of the explanted livers were reviewed, collecting data about HCC differentiation degree. The association between imaging vascular pattern and pathological grade was estimated using the Fisher exact test. All follow-up clinical and imaging data were reviewed for evidence of recurrence. Recurrence rate was calculated and imaging features of recurrent tumor were collected, classifying early and late recurrences based on timing (< or $\geqslant$ 2 years after transplantation) and intrahepatic, extrahepatic and both intrahepatic and extrahepatic recurrences based on

location. All intrahepatic recurrences were classified as hypervascular or hypovascular and the differentiation degree was collected where available. The presence of variations in imaging enhancement pattern and pathological grade between the primary tumor and the intrahepatic recurrence was evaluated and the association between imaging and histopatholgical variations was estimated by using the χ^2 test.

RESULTS: Of the 163 patients with imaging evidence of viable tumor, 156 (95.7%) had hypervascular and 7 (4.3%) hypovascular HCC. Among the 125 patients with evidence of viable tumor in the explanted liver, 19 (15.2%) had grade 1, 56 (44.8%) grade 2, 40 (32%) grade 3 and 4 (3.2%) grade 4 HCC, while the differentiation degree was not assessable for 6 patients (4.8%). A significant association was found between imaging vascularity and pathological grade (P = 0.035). Post-transplantation recurrence rate was 14.55% (24/165). All recurrences occurred in patients who had a hypervascular primary tumor. Three patients (12.5%) experienced late recurrence; the location of the first recurrence was extrahepatic in 14 patients (58.3%), intrahepatic in 7 patients (29.2%) and both intrahepatic and extrahepatic in 3 patients (12.5%). Two patients had a variation in imaging characteristics between the primary HCC (hypervascular) and the intrahepatic recurrent HCC (hypovascular), while 1 patient had a variation of histopathological characteristics (from moderate to poor differentiation), however no association was found between imaging and histopathological variations.

CONCLUSION: A correlation was found between HCC grade and vascularity; some degree of variability may exist between the primary and the recurrence imaging/histopathological characteristics, apparently not correlated.

Key words: Contrast media; Hepatocellular carcinoma; Liver transplantation; Cell differentiation; Recurrence

Core tip: During hepatocarcinogenesis, besides the differentiation loss, blood supply changes occur. Recently, a correlation between higher histopathological grades and hypervascular dynamic-imaging enhancement pattern has been demonstrated. Hepatocellular carcinoma recurrence after transplantation is relatively common, however the issue of possible variations in imaging and histopathology between the primary and the recurrent tumor, and particularly the relationship between enhancement and grade changes, has never been investigated. We demonstrated a correlation between vascularity and pathological grade in a large population of transplanted patients, and some degree of variability between the primary and the recurrent tumor vascularity was found, though not associated with histopathological changes.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary hepatic malignancy[1], and it represents an important health issue due to its increasing incidence and poor survival[2]. HCC usually arises in the background of chronic liver diseases, in particular cirrhosis is the substrate of HCC in 80%-90% of cases[3]. Carcinogenesis in HCC is typically a multistep process, that comprehends low-grade and high grade dysplastic nodules possibly with neoplastic foci, early HCC, and overt HCC. Pathologically, HCC is graded based on differentiation degree into four degrees of cellular dysplasia and architectural tissue disarrangement (well-differentiated, moderately differentiated, poorly differentiated and undifferentiated, grade 1 to 4)[4]. During the hepatocarcinogenesis multistage process, besides the size growth and the loss of differentiation of the nodule, blood supply changes progressively occur, so that the hepatocellular nodule becomes more and more dependent on newly formed arteries and in parallel less dependent on the portal contribution[3].

Contrast-enhanced dynamic imaging, performed both with multidetector CT (MDCT) and magnetic resonance imaging (MRI), provides information about HCC vascularity. In particular, two different hemodynamic patterns have been featured: the typical, much more common, hypervascular pattern, and the less frequent hypovascular variant of HCC, which shows no arterial phase hypervascularity[5]. Recently, some studies have compared contrast enhanced dynamic imaging findings with histopathological differentiation degree, showing a correlation between higher pathological grades and hypervascular enhancement pattern[6-10], even though some investigators have reported a subsequent decrease in arterial blood supply in the late stage of HCC development (grade $\geqslant$ 3)[7-9].

Liver transplantation (LT) is the preferred treatment for selected patients with HCC. However, even after the introduction of selection criteria such as Milan Criteria[11], HCC recurrence rate after LT has been estimated to be 8%-17%[12-15]. Many investigators have reported on the spectrum of imaging findings of HCC recurrence after LT, distinguishing different patterns on the basis of recurrence location[12,13,16] or timing[14]. In particular, the majority of tumor burden in recurrent HCC is typically in extrahepatic locations[12,15,16] and the average time to recurrence

Table 1 Demographic and clinical characteristics of the study population

Demographic and clinical characteristics (n = 165)				
Sex	Male (%)			143 (86.7)
	Female (%)			22 (13.3)
Median age (range)				56.8 (28; 70.4)
HIV + (%)				15 (9.1)
Etiology of the underlying hepatic disease	Viral (%)	136 (82.4)	HBV-related	28 (17.0)
			HCV-related	92 (55.8)
			Mixed	16 (9.7)
	Not viral (%)	29 (17.6)	Alcoholic	16 (9.7)
			Cryptogenetic	6 (3.6)
			Other	7 (4.2)

ranges between 1 and 2 years after LT[14,16]. The hemodynamic imaging characteristics of recurred HCC have been scarcely reported, however, similarly to the primary HCC, the majority of recurred HCC appear as hypervascular lesions.

To our knowledge, the issue of possible variations in imaging and histopathological characteristics between the primary and the recurrent HCC, particularly referring to the relationship between enhancement pattern changes and differentiation degree changes, has never been investigated. The preliminary objective of this study was to evaluate the relationship between HCC contrast-enhanced dynamic imaging pattern and pathological differentiation degree in a population of patients transplanted for HCC. Additional aims were to describe the patterns and imaging features of HCC recurrence after LT and to evaluate the variations in imaging and histopathological characteristics between the primary HCC and the intrahepatic recurrence, particularly elucidating whether differentiation degree variations may justify contrast-enhanced imaging pattern changes.

MATERIALS AND METHODS

Patients

Between October 2004 and November 2011, a total of 172 consecutive patients with known HCC underwent LT at our hospital. During this period, another patient, transplanted without known HCC, had pathologic diagnosis of incidental HCC in the liver explant. Of these 173 patients, 8 patients were excluded because of a short follow up period (≤ 2 mo), as a result of perioperative mortality. The remaining 165 patients (143 men, 22 women; median age 56.8 years, range 28-70.4 years), who had a follow-up period longer than 2 mo, were included. Fifteen of them were HIV-infected patients. Clinical data about the etiology of the underlying liver disease, were collected. Table 1 summarizes demographic and clinical characteristics of the included patients.

Pre-transplantation imaging

All available MDCT and MRI dynamic hepatic examinations were reviewed, selecting those with evidence of viable tumor, and thus where vascularity was assessable. MDCT examinations were performed using a 64-slice CT scanner (Lightspeed VCT, GE Medical Systems, Milwaukee, Wisconsin, United States) with contrast enhancement and bolus-tracking technique to obtain a multiphase (arterial, portal and hepatic venous phases) examination after an unenhanced scan. Image reconstruction was obtained with a 2.5 mm slice thickness and a 2.5 mm interval. Dynamic MRI studies were conducted on a 1.5-T high field magnet (Philips Achieva, Philips Medical System, Best, The Netherlands) with a Phased Array coil. The protocol included axial T1- and T2-weighted sequences with and without fat suppression and axial dynamic three-dimensional T1-weighted fat-suppressed GRE sequences obtained before and after a bolus injection of gadopentetate dimeglumine (Gd-DOTA) in arterial, portal and hepatic venous phases.

Pre-transplantation imaging examinations were retrospectively reviewed by two experienced radiologists by consensus reading, both blinded to the results of the pathologic reports. From the last pre-LT examination with evidence of viable tumor, data about the HCC enhancement pattern were collected. In particular, based on the presence or absence of wash-in, which was defined as present when the lesion was hyperattenuating compared to the surrounding hepatic parenchyma during the arterial phase, lesions were classified into hypervascular and hypovascular.

Histopathology of the explanted liver

A pathologist experienced with liver pathologies reviewed all pathologic reports of the explanted livers, collecting data about the differentiation degree, scored according to the World Health Organization criteria[4] into well-differentiated (Grade 1), moderately differentiated (Grade 2), poorly differentiated (Grade 3), and undifferentiated (Grade 4) types. When different degrees were reported in the same explanted liver, the prevailing grade (the one demonstrated by the larger number of nodules) was considered.

Recurrence analysis: Imaging and histopathology

All available postoperative dynamic imaging examin-

Table 2 Pre-Transplantation imaging enhancement pattern and histopathological differentiation degree

Pre-LT imaging and transplant pathology		
Pre-LT imaging		
Enhancement pattern (n = 163)	Hypervascular (%)	156 (95.7)
	Hypovascular (%)	7 (4.3)
Histopathology		
Differentiation degree (n = 125)	Grade 1 (%)	19 (15.2)
	Grade 2 (%)	56 (44.8)
	Grade 3 (%)	40 (32)
	Grade 4 (%)	4 (3.2)
	Not assessable (%)	6 (4.8)

ations (MDCT or MRI) were retrospectively reviewed for evidence of recurrent HCC. Proof of recurrence was made on the basis of biopsy or growth of new lesions with appropriate radiologic features, combined with rising AFP levels or with negative work-up for another primary malignancy. Imaging features of recurrent HCC were collected. Based on recurrence timing, recurrences were divided into early (< 2 years after LT) and late (≥ 2 years after LT). With respect to tumor location at the moment of the first recurrence, three different patterns were distinguished: intrahepatic recurrence (allograft itself), extrahepatic recurrence and both intrahepatic and extrahepatic recurrence.

All follow-up dynamic imaging examinations were also reviewed to describe the enhancement pattern of all intrahepatic recurrences (those that occurred at the moment of the first recurrence and subsequent ones), in particular classifying lesions into hypervascular and hypovascular based on the presence of wash-in.

Intrahepatic recurrence histopathological differentiation degree was obtained by a review of the available pathological reports (in case of biopsy or resection of the recurred HCC).

Statistical analysis

The association between pretransplantation HCC enhancement pattern (hypervascular or hypovascular) and explanted liver HCC differentiation degree was evaluated by using the Fisher exact test. Recurrence rate was calculated. The presence of variations in imaging features (enhancement pattern) and histopathological characteristics (differentiation degree) between the primary and the intrahepatic recurred HCC was evaluated and the association between imaging and histopatholgical variations was estimated by using the χ^2 test. For all statistical analyses, a $P < 0.05$ was considered to indicate a statistically significant difference.

The statistical methods of this study were reviewed by Marta Di Nicola from Department of Experimental and Clinical Sciences, Laboratory of Biostatistics, University of Chieti, Italy.

RESULTS

Pre-transplantation imaging and histopathology of the explanted liver

Of the 165 patients who were included, 2 had no evidence of viable tumor in all dynamic imaging examinations performed within 6 mo to LT. Of the remaining 163 patients in whom evaluation of enhancement pattern was possible, 156 (95.7%) had evidence of hypervascular HCC (Table 2). Of these 163 patients, 125 patients had evidence of viable tumor in the explanted liver, while the remaining 38 patients had completely necrotic nodules as a result of pre-transplantation loco-regional therapies performed after imaging examinations. The distribution of the pathological differentiation degree of the primary HCC over the 125 patients who had viable tumor is summarized in Table 2. The differentiation degree was not assessable for 6 patients (4.8%). Different degrees were shown in the same explanted liver in 5 cases, and in such cases the prevailing grade was considered.

Both enhancement pattern and differentiation degree were available for 113 patients. Among them, a significant association was found between imaging enhancement pattern and histopathological differentiation degree (P = 0.035). As shown in Table 3, 50% (3/6) of the patients whit a hypovascular HCC had a well-differentiated tumor, *vs* 14% (15/107) of those with a hypervascular HCC. An explicative case of a well-differentiated HCC which was characterized by an atypical enhancement pattern in the pre-LT dynamic MDCT examination is depicted in Figure 1.

Recurrence analysis

Of the 165 patients included, 24 (14.55%) had evidence of HCC recurrence after the LT. The 1-, 3-, 5-years cumulative disease-free survival rates according to the Kaplan-Meier method were 92.96%, 83.9% and 82.84%, respectively. The mean duration of recurrence-free survival was 40.15 mo.

Time to development of the recurrence ranged from 1.55 to 41.85 mo after LT, with a median value of 12.36 mo. Three patients (12.5%) experienced late recurrence (≥ 2 years after LT), with a rate of 1.7% (3/175). All recurrences occurred in patients who had a hypervascular primary HCC, while none of the patients with hypovascular primary HCC had a recurrence after LT.

With respect to recurrence location at the moment of the first recurrence, 14 patients (58.3%) showed extrahepatic recurrence, 7 patients (29.2%) had intrahepatic recurrence and 3 patients (12.5%) showed both intrahepatic and extrahepatic recurrence. Eight patients had more than one recurrence site at the moment of the first recurrence. Only one of the 3 patients with late recurrence had intrahepatic recurrence. Recurrence patterns and most common imaging features of recurrence are further described

Table 3 Distribution of dynamic imaging enhancement patterns according to histopathological differentiation degrees

Dynamic imaging enhancement pattern and histopathological differentiation degree (n = 113)					
Enhancement pattern	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	***P* value**
Hypovascular	3	1	1	1	0.035
Hypervascular	15	52	37	3	

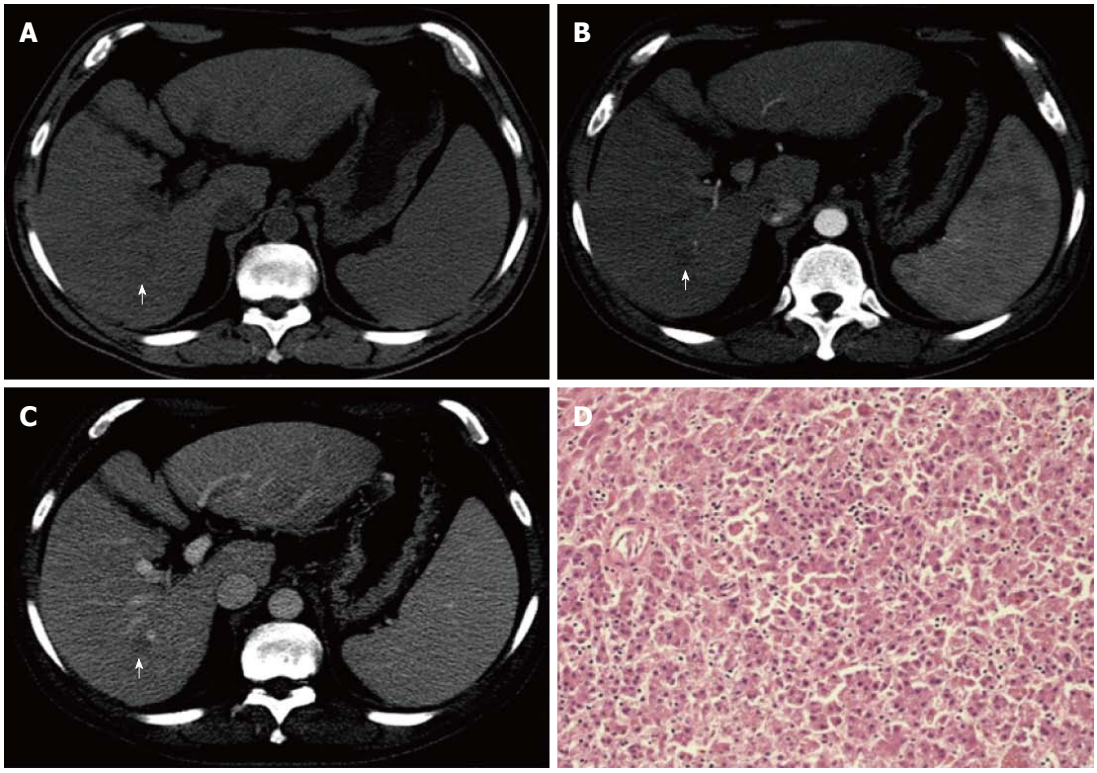

Figure 1 Pre-contrast (A), hepatic arterial (B) and hepatic portal venous (C) phases multidetector CT scans demonstrate a hypovascular hepatocellular carcinoma (arrows), with no hypervascularization during the arterial phase (wash-in). Pathologically (HE stain, × 100) (D), it was classified as well-differentiated (Grade 1).

in Table 4 and some recurrence cases illustrated in Figure 2. When extrahepatic recurrences had a solid component which was large enough to allow vascularity evaluation, they showed a contrast enhancement similar to the primary hypervascular HCC, hence usually already evident in the arterial phase of the examination. Frequently they also presented with some necrotic intralesional component.

Among the 14 patients who didn't show intrahepatic recurrence at the moment of the first recurrence, 4 had evidence of hepatic recurrence in further follow-up imaging examinations. On a total of 14 patients who firstly or subsequently developed an intrahepatic recurrent HCC, 12 had hypervascular and 2 had hypovascular recurrent HCCs, classified based on the presence of wash-in. Evidence of wash out was shown by all intrahepatic recurrences. Nine of them underwent liver biopsy with histological diagnosis of HCC, 4 scored as moderately and 5 scored as poorly-differentiated. Two patients (14.3%) showed a variation in imaging characteristics between the primary HCC (hypervascular) and the intrahepatic recurrent HCC (hypovascular). However, they didn't show any variation in histopathological characteristics (Figure 3). Only 1 patient, who had hypervascular enhancement pattern on pre-LT imaging as well as on recurrence imaging, had a variation of histopathological characteristics (from moderate to poor differentiation) (Figure 4). Therefore, no association was found between imaging and histopathological variations.

DISCUSSION

It is well known that during HCC development progressive changes in the vascular supply occur[3]: as dysplastic nodules undergo malignant transformation, abnormal neoplastic arterial supply increases while portal supply decreases. Classic HCC is exclusively supplied by the hepatic artery and lacks a portal venous supply, leading, on dynamic imaging examinations, to the typical hypervascular pattern

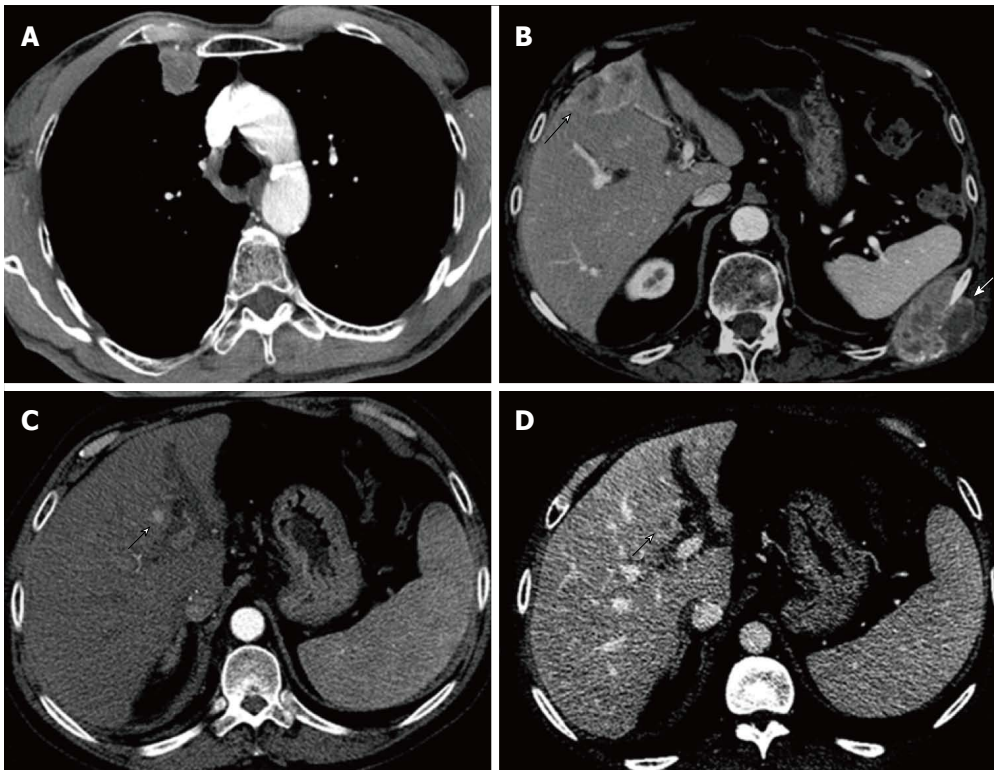

Figure 2 Representative multidetector CT images of different recurrence patterns based on the location at the moment of the first recurrence: (A) Extrahepatic recurrence presenting with a solitary pleural-based lung nodule; (B) Both intrahepatic and extrahepatic recurrence presenting with bone lesions and an intrahepatic lesion in segments IVb and V; (C and D) Intrahepatic recurrence presenting with a solitary hypervascular nodule in segment IV.

Table 4 Recurrence patterns based on the location at the moment of the first recurrence and most common imaging features of recurrence

Recurrence patterns (n = 24)		
Intrahepatic		7 (29.2%)
	Solitary nodule	3 (12.5%)
	Multifocal lesions	4 (16.7%)
Extrahepatic		14 (58.3%)
	Lung	9 (37.5%)
	Solitary nodule	4 (16.7%)
	Multiple nodules	5 (20.8%)
	Consolidation	/
	Bone	5 (20.8%)
	Osteolytic	5 (20.8%)
	Osteoblastic	/
	Lymph nodes	2 (8.3%)
	Brain	1 (4.2%)
	Spleen	1 (4.2%)
	Adrenal	1 (4.2%)
Intrahepatic and extrahepatic		3 (12.5%)

characterized by both wash-in in the arterial phase and wash-out in portal or delayed phases. Less frequently HCCs present as hypovascular lesions, enhancing less than the surrounding liver both on arterial and venous phase imaging, probably as a result of a dual blood supply, both arterial and portal[17,18].

Some investigators have recently reported a correlation between histopathologic grade and HCC blood supply in radiological and pathologic analyses. A tendency towards higher grades in tumors with hypervascular pattern was demonstrated[6-10], however there is some evidence that in the late stage of HCC development, the arterial blood supply decreases again[7-9].

In our population, comparatively with previous studies[9], the majority of HCCs were moderately or poorly differentiated, almost all of them being characterized by a typical hypervascular pattern. On the contrary, the few atypical hypovascular HCCs were predominantly distributed in the well-differentiated group, resulting in 50% of patients with hypovascular HCC *vs* only 15% of those with typical HCC having a grade 1 tumor. Therefore, despite the fact that in our population a very small number of patients had evidence of atypical HCC (4.3%), a correlation was found between differentiation degree and enhancement pattern (P = 0.035).

Recently, some authors have hypothesized the utility of imaging pattern as a prognostic factor for tumor outcome after locoregional treatment[19] or surgery[20,21]. In our study, the pre-LT imaging examinations of all the 24 patients who experienced HCC recurrence after LT showed hypervascular tumors, and all of them were graded as moderately or poorly differentiated on histopathology (n = 14 grade 2, n = 9 grade 3, n = 1 not assessable). No patient with hypovascular HCC at pre-LT imaging developed

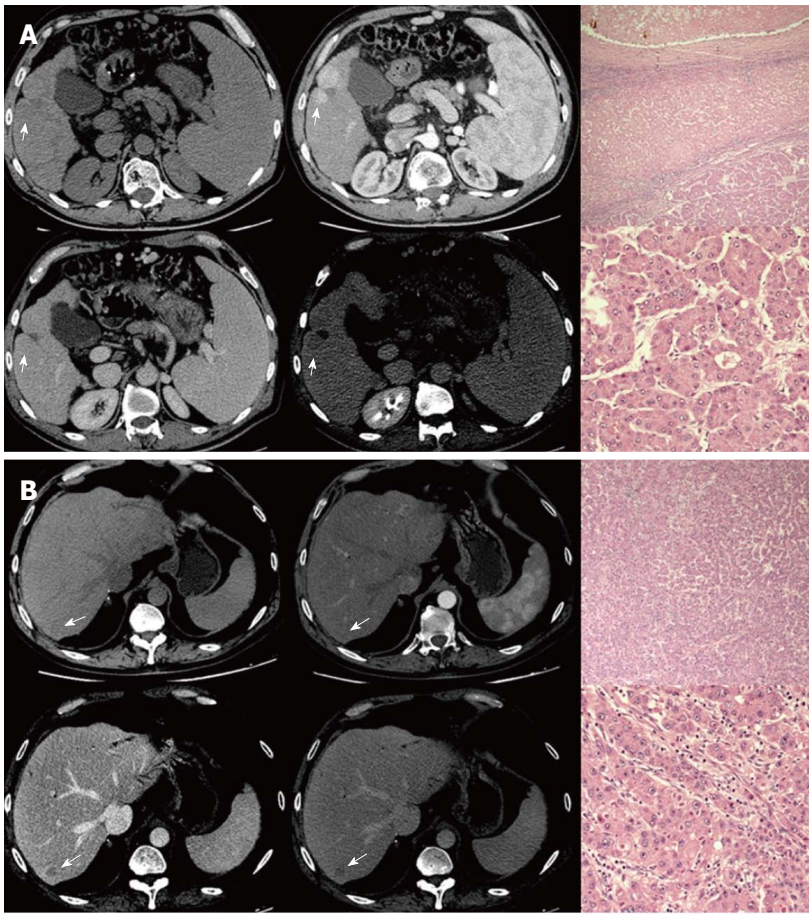

Figure 3 Multidetector CT scans demonstrating two hypervascular primary hepatocellular carcinoma nodules which were graded as moderately differentiated on histopathology (HE stain, magnification × 40 and × 200) (A); Multidetector CT scans of the same patient 2 years after LT demonstrating a hypovascular intrahepatic recurrent hepatocellular carcinoma, which was still graded as moderately differentiated on histopathology after resection (HE stain, magnification × 40 and × 200) (B).

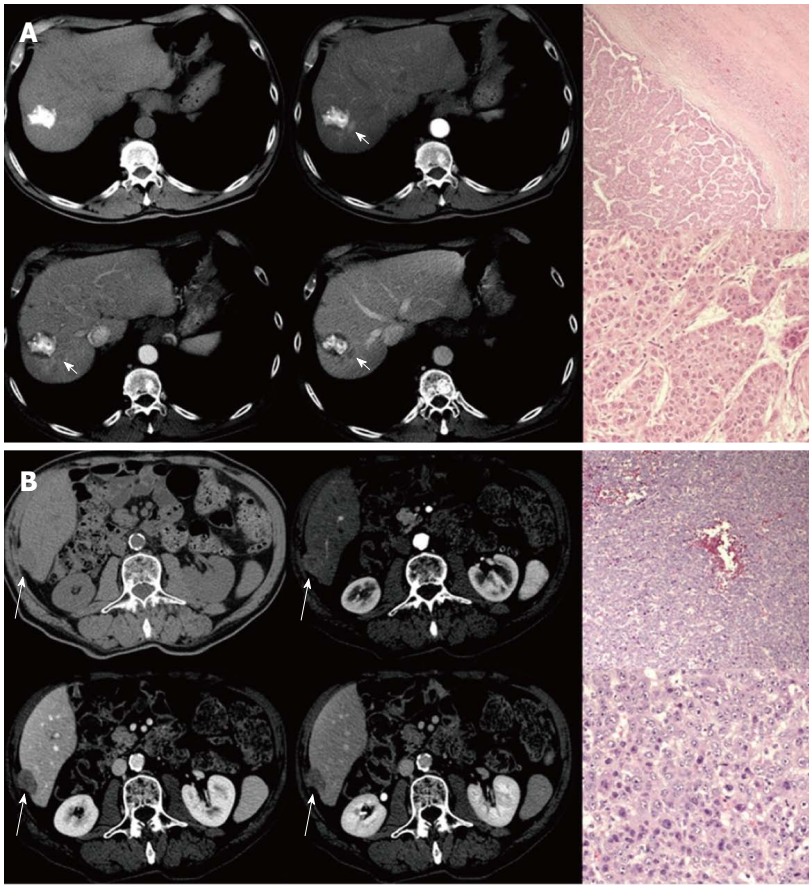

Figure 4 Multidetector CT scans demonstrating a hypervascular area representing viable hepatocellular carcinoma next to a previously lipiodolized nodule, graded as moderately differentiated on histopathology (HE stain, magnification × 40 and × 200) (A); Multidetector CT scans of the same patients 1 year after LT demonstrating a hypervascular intrahepatic recurrent hepatocellular carcinoma, which was graded as poorly differentiated on histopathology after resection (HE stain, magnification × 40 and × 200) (B).

post-LT recurrence, not even the few of them with a high histopathological degree. These findings suggest, albeit the number of cases is limited, a lower tendency towards recurrence and a longer recurrence-free survival in patients with hypovascular rather than hypervascular HCCs, underlying the potential prognostic role of vascular supply along with the pathological grade.

In our population the tumor recurrence rate after transplantation was 14.55%, which is similar to the rates observed in other studies with comparable follow-up period and in which Milan selection criteria were adopted[12,14,15]. Extrahepatic recurrence was the most common recurrence pattern with respect to tumor location, comparatively with other studies in which the focus was on the appearance of early recurrence[12,15,16]. Consistently with previous studies[14], the median time to recurrence was 12.36 mo and late recurrence was less common, representing only 12.5% of cases, with a rate of 1.7%.

Focusing on the variations in imaging and histopathological characteristics between the primary and the intrahepatic recurred HCC, among the 14 patients with intrahepatic recurrence, enhancement pattern changes (from hypervascular to hypovascular) were experienced by two patients and histopathological changes (from moderate to poor differentiation degree) were recorded in one patient, underlying that some degree of variability may exist between the primary and the recurred HCC. Even though the number of patients who had changes in imaging or pathological characteristics is small, it is to be noted that no correlation was found between enhancement and differentiation degree changes. In particular, the two patients who changed enhancement pattern did not show any variation in histopathology between the primary and the recurred HCC. On the other hand, in one patient a shift from moderately to poorly differentiated HCC was observed, while no change in the enhancement pattern was registered. This result agrees with the higher prevalence of hypervascular pattern among both grade 2 and 3 HCCs.

Some limitations of this study should be noted. First, it was designed retrospectively. Second, in the correlation analysis between imaging pattern and differentiation degree of the primary tumor, wash-out was not considered, while recently some studies have focused on hypervascular HCCs signal intensity during portal venous phase as an additional predictive factor for differentiation degree[22]. Finally, this study lacked a genomic and immunophenotypical analysis of both the primary and the recurrent HCC.

In conclusion, this study confirms the recently explored correlation between HCC differentiation degree and dynamic-imaging enhancement pattern. Moreover, our preliminary results show that some degree of variability may exist between the primary and the recurred HCC imaging characteristics. If tumoral histopathological characteristics do not seem to justify enhancement pattern variations, a possible explanation is perhaps to be found in changes which occur in liver parenchymal structure and vascularity following transplantation[23]. However, more studies with larger patient groups are needed to better explore the presence of and the reasons for enhancement pattern changes between the primary and the recurred HCC.

COMMENTS

Background

A correlation between histopathological grade and vascularity of hepatocellular carcinoma (HCC) has been recently demonstrated. More frequently HCC is hypervascular, an enhancement pattern that usually corresponds to moderate or poor differentiation degree. Liver transplantation is the optimal treatment for HCC, however recurrence rate still ranges from 8% to 17%. HCC recurrence is usually similar to the primary tumor, but some variability in enhancement pattern and histopathological characteristics may be expected.

Research frontiers

The relation between HCC pathological grade and vascularity introduces the possible role of imaging enhancement pattern as a prognostic factor for tumor outcome after transplantation. Moreover, in the field of HCC recurrence after transplantation, the reasons for variability between the primary tumor and the recurrence characteristics are still to be understood.

Innovations and breakthroughs

The recently introduced correlation between HCC vascularity and differentiation degree has been discussed in other studies, however the authors aimed to better explore this relationship by applying it to the issue of HCC recurrence after transplantation.

Applications

The most relevant future application of the study is the possible use of imaging enhancement pattern as a prognostic factor for tumor recurrence after transplantation; moreover the study of recurrence variability may allow to better understand the factors involved in post-transplantation recurrence.

Terminology

HCC is the most common type of liver tumor, arising from hepatocytes and typically originating in patients with chronic liver diseases; liver transplantation is the optimal, potentially curative treatment for HCC; recurrence of hepatocellular carcinoma after transplantation reflects hematogenous spread, lymphatic spread and peritoneal seeding of neoplastic cells.

Peer review

A very interesting topic, with a somewhat novel approach that justifies thee small number of patients included.

REFERENCES

1 **Ayuso C**, Rimola J, García-Criado A. Imaging of HCC. *Abdom Imaging* 2012; **37**: 215-230 [PMID: 21909721 DOI: 10.1007/s00261-011-9794-x]

2 **Shanbhogue AK**, Prasad SR, Takahashi N, Vikram R, Sahani DV. Recent advances in cytogenetics and molecular biology of adult hepatocellular tumors: implications for imaging and management. *Radiology* 2011; **258**: 673-693 [PMID: 21339346 DOI: 10.1148/radiol.10100376]

3 **Trevisani F**, Cantarini MC, Wands JR, Bernardi M. Recent advances in the natural history of hepatocellular carcinoma. *Carcinogenesis* 2008; **29**: 1299-1305 [PMID: 18515282 DOI: 10.1093/carcin/bgn113]

4 **Ishak KG**, Anthony PP, Sobin LH. Histological typing of tumors of the liver. World Health Organization, Geneva. Available from: URL: http: //whqlibdoc.who.int/publications/1994/3540581545_eng.pdf

5 **Bolondi L**, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S, Venturi AM, Piscaglia F. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 2005; **42**: 27-34 [PMID: 15954118]

6 **Tarhan NC**, Hatipoğlu T, Ercan E, Bener M, Keleş G, Başaran C,

Bilezikçi B. Correlation of dynamic multidetector CT findings with pathological grades of hepatocellular carcinoma. *Diagn Interv Radiol* 2011; **17**: 328-333 [PMID: 21328198 DOI: 10.4261/1305-3825.DIR.2682-09.3]

7 **Yoon SH**, Lee JM, So YH, Hong SH, Kim SJ, Han JK, Choi BI. Multiphasic MDCT enhancement pattern of hepatocellular carcinoma smaller than 3 cm in diameter: tumor size and cellular differentiation. *AJR Am J Roentgenol* 2009; **193**: W482-W489 [PMID: 19933622 DOI: 10.2214/AJR.08.1818]

8 **Asayama Y**, Yoshimitsu K, Nishihara Y, Irie H, Aishima S, Taketomi A, Honda H. Arterial blood supply of hepatocellular carcinoma and histologic grading: radiologic-pathologic correlation. *AJR Am J Roentgenol* 2008; **190**: W28-W34 [PMID: 18094269]

9 **Jang HJ**, Kim TK, Burns PN, Wilson SR. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. *Radiology* 2007; **244**: 898-906 [PMID: 17709836]

10 **Lee JH**, Lee JM, Kim SJ, Baek JH, Yun SH, Kim KW, Han JK, Choi BI. Enhancement patterns of hepatocellular carcinomas on multiphasicmultidetector row CT: comparison with pathological differentiation. *Br J Radiol* 2012; **85**: e573-e583 [PMID: 22919011 DOI: 10.1259/bjr/86767895]

11 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428]

12 **Kim YS**, Lim HK, Rhim H, Lee WJ, Joh JW, Park CK. Recurrence of hepatocellular carcinoma after liver transplantation: patterns and prognostic factors based on clinical and radiologic features. *AJR Am J Roentgenol* 2007; **189**: 352-358 [PMID: 17646461]

13 **Lee CH**, Brubaker LM, Gerber DA, Ku YM, Kim YH, Shin SS, Semelka RC. MRI findings of recurrent hepatocellular carcinoma after liver transplantation: preliminary results. *J Magn Reson Imaging* 2011; **33**: 1399-1405 [PMID: 21591009 DOI: 10.1002/jmri.22326]

14 **Chok KS**, Chan SC, Cheung TT, Chan AC, Fan ST, Lo CM. Late recurrence of hepatocellular carcinoma after liver transplantation. *World J Surg* 2011; **35**: 2058-2062 [PMID: 21597889 DOI: 10.1007/s00268-011-1146-z]

15 **Chan KM**, Chou HS, Wu TJ, Lee CF, Yu MC, Lee WC. Characterization of hepatocellular carcinoma recurrence after liver transplantation: perioperative prognostic factors, patterns, and outcome. *Asian J Surg* 2011; **34**: 128-134 [PMID: 22208688 DOI: 10.1016/j.asjsur.2011.08.005]

16 **Roayaie S**, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GE, Krieger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004; **10**: 534-540 [PMID: 15048797]

17 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051]

18 **Nakashima Y**, Nakashima O, Hsia CC, Kojiro M, Tabor E. Vascularization of small hepatocellular carcinomas: correlation with differentiation. *Liver* 1999; **19**: 12-18 [PMID: 9928760]

19 **Kawamura Y**, Ikeda K, Seko Y, Hosaka T, Kobayashi M, Saitoh S, Kumada H. Heterogeneous type 4 enhancement of hepatocellular carcinoma on dynamic CT is associated with tumor recurrence after radiofrequency ablation. *AJR Am J Roentgenol* 2011; **197**: W665-W673 [PMID: 21940538 DOI: 10.2214/AJR.11.6843]

20 **Choi JW**, Lee JM, Kim SJ, Yoon JH, Baek JH, Han JK, Choi BI. Hepatocellular carcinoma: imaging patterns on gadoxetic acid-enhanced MR Images and their value as an imaging biomarker. *Radiology* 2013; **267**: 776-786 [PMID: 23401584 DOI: 10.1148/radiol.13120775]

21 **Chung GE**, Lee JH, Yoon JH, Myung SJ, Lee K, Jang JJ, Lee JM, Kim SH, Suh KS, Kim YJ, Lee HS. Prognostic implications of tumor vascularity and its relationship to cytokeratin 19 expression in patients with hepatocellular carcinoma. *Abdom Imaging* 2012; **37**: 439-446 [PMID: 21584634 DOI: 10.1007/s00261-011-9756-3]

22 **Okamoto D**, Yoshimitsu K, Nishie A, Tajima T, Asayama Y, Ishigami K, Hirakawa M, Ushijima Y, Kakihara D, Nakayama T, Nishihara Y, Aishima S, Taketomi A, Kishimoto J, Honda H. Enhancement pattern analysis of hypervascular hepatocellular carcinoma on dynamic MR imaging with histopathological correlation: validity of portal phase imaging for predicting tumor grade. *Eur J Radiol* 2012; **81**: 1116-1121 [PMID: 21420813 DOI: 10.1016/j.ejrad.2011.02.056]

23 **Navasa M,** Feu F, García-Pagán JC, Jiménez W, Llach J, Rimola A, Bosch J, Rodés J. Hemodynamic and humoral changes after liver transplantation in patients with cirrhosis. *Hepatology* 1993; **17**: 355-360 [PMID: 8444409]

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CASE REPORT

Lesser celandine (pilewort) induced acute toxic liver injury: The first case report worldwide

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Abstract

Lesser celandine, also known as *Ranunculus ficaria*, is a herbaceous perennial plant that commonly utilizes piles and is taken either internally or used externally. The causality assessment of several reports provided evidence for the existence of Greater Celandine hepatotoxicity. However, there hasn't been any case report published thus far, about lesser celandine induced liver injury. Here, we present a case of 36-year-old woman admitted to the hospital with acute hepatitis and jaundice on her sclera with no history of drug abuse or alcohol consumption. However, the patient had a recent history of lesser celandine extract consumption for hemorrhoids, for about 10 d, prior to the admission. Viral hepatitis, autoimmune hepatitis, and drug induced toxic hepatitis were ruled out by further imaging studies and laboratory analysis. Using the Council for International Organizations of Medical Sciences scale, the type of liver injury was assumed as hepatocellular and was scored as 7 which shows probable causality. Immediate discontinuation of lesser celandine extract resulted in rapid decrease of the elevated enzymes. Herbs have been reported to cause liver injury and therefore should be suspected in the case of acute hepatitis with an unknown etiology. This case is important to be the first to explain hepatotoxicity caused by lesser celandine. Physicians should consider lesser celandine as a causative agent for hepatotoxicity.

Key words: Celandine; Acute liver toxicity; Hepatitis; Pilewort; Herb

Core tip: Herbs have been reported to cause liver injury and therefore should be suspected in the case of acute hepatitis with an unknown etiology. This case is the first to explain hepatotoxicity caused by lesser celandine. Physicians should consider lesser celandine as a causative agent for hepatotoxicity.

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INTRODUCTION

Numerous herbs can be hepatotoxic. This has been demonstrated by several case reports, case series and literature reviews[1-4]. However, the potential hepatotoxicity of a variety of chemicals in any herb and case presentations lacking diagnostic exclusion made it difficult to have a clear clinical assessment[5].

Greater Celandine (*Chelidonium majus L.*) has been used in traditional Chinese medicine as weel as in the Western world for its numerous biological activities[6,7]. Teschke *et al*[8,9] reviewed several reports from Europe regarding Greater Celandine (Chelidonium majus) hepatotoxicity. The causality assessment of these reports provided evidence for the existence of Greater Celandine hepatotoxicity[8,9].

Lesser celandine, also known as *Ranunculus ficaria*, is a herbaceous perennial plant. Lesser celandine is a herbal astringent that commonly utilizes piles and is taken either internally or used externally, and for this feature it is also known as pilewort[10]. A review of literature revealed that there hasn't been any case report published thus far, about lesser celandine induced liver injury. Here, we present the first case of toxic hepatitis associated with lesser celandine consumption.

CASE REPORT

A 36-year-old woman was admitted to the hospital for acute hepatitis. Her past medical history and family history did not reveal any significant disease. She did not have any history of alcohol consumption or drug abuse. However, the patient had a recent history of lesser celandine extract consumption for hemorrhoids, for about 10 d, prior to the admission. A detailed anamnesis showed that the patient consumed lesser celandine as tea, one cup per day for 3 d. Physical examination revealed jaundice on her sclera. Laboratory abnormalities included alanine aminotransferase (ALT 1830 IU/L; normal range: 0 to 45 U/L), aspartate aminotransferase (AST 1520 IU/L; normal range: 0 to 45 U/L), alkaline phosphatase (ALP 225 IU/L; normal range: 30 to 120 U/L), and total bilirubin (3.4 mg/dL; normal range: 0.174 to 1.04 mg/dL). Anti-HBs IgG was positive, anti-HCV, HCV PCR, Anti-HAV IgM, and anti-HEV IgM were negative. There was no serologic evidence for recent infections with herpes simplex virus (HSV), epstein-barr virus (EBV), cytomegalovirus (CMV), or varisella zoster virus (VZV). Autoimmune marker analysis included anti-nuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), anti-neutrophil cytoplasmic antibodies (ANCA), smooth muscle antibody (SMA), liver kidney microsomal antibody type 1 (LKM-1) and all markers were found to be negative. Abdominal ultrasonography was normal. Liver biopsy wasn't performed, because the patient did not give an informed consent for the procedure. Lesser celandine extract was immediately discontinued. Using the Council for International Organizations of Medical Sciences scale (CIOMS), the type of liver injury of our case was assumed as hepatocellular (ALT > 5N and R $\geqslant$ 5) and was scored as "7: probable" (Table 1)[11]. After discontinuation of lesser celandine, rapid recovery was observed in patient and liver enzyme levels returned to normal in 3 wk.

DISCUSSION

Hepatocellular, cholestatic, and mixed type (hepatocellular and cholestatic) injuries are the three types of herb induced liver injuries[12]. In the decision of different injury types, ratio R (ALT/ALP activity at the time liver injury is suspected, activity is measured by multiples of the highest point of the normal values) is used. If ALT > 2N or R $\geqslant$ 5, hepatocellular injury is assumed; if ALP > 2N or R $\leqslant$ 2, injury is cholestatic; if ALT > 2N and ALP is increased, with R > 2 and R < 5, mixed injury is assumed[11]. There are no diagnostic tests or specific criteria for herb induced hepatotoxicity. Careful history taking, insightful evaluation of laboratory findings and histopathology are essential for diagnosis. Liver biopsy might be helpful for the assessment of liver injury, however Teschke *et al*[13] reported that it is not essential for the diagnosis. The best way to determine causal agent is re-challenging. But this, for obvious reasons, is not ethically acceptable.

Lesser celandine can easily be confused with greater celandine. A case report from Germany provides an evidence for that by presenting a case with toxic hepatitis caused by greater celandine, however mentioning the name of the herb as lesser celandine in the abstract[14]. Herbs have been reported to cause liver injury and therefore should be suspected in the case of acute hepatitis with an unknown etiology. Our case suggests that physicians should consider lesser celandine as a causative agent for hepatotoxicity.

COMMENTS

Case characteristics

A 36-year-old woman was admitted to the hospital for fainting and a prior diagnosis of acute hepatitis from another hospital. She had a recent history of lesser celandine extract consumption for hemorrhoids, for about 10 d prior to the admission.

Clinical diagnosis

Physical examination revealed jaundice on her sclera.

Differential diagnosis

Viral hepatitis, autoimmune hepatitis and drug induced toxic hepatitis were

Table 1 Score of the presented patient according to Council for International Organizations of Medical Sciences Scale

Items for hepatocellular injury	Score	Result of the presented case[1]
1 Time to onset from the beginning of the drug/herb		
5-90 d (rechallenge: 1-15 d)	2	+
< 5 or > 90 d (rechallenge: > 15 d)	1	-
Alternative: Time to onset from cessation of the drug/herb		
≤ 15 d (except for slowly metabolized chemicals: > 15 d)	1	-
2 Course of ALT after cessation of the drug/herb		
Percentage difference between ALT peak and N		
Decrease ⩾ 50% within 8 d	3	+
Decrease ⩾ 50% within 30 d	2	-
No information or continued drug/herb use	0	-
Decrease ⩾ 50% after the 30th day	0	-
Decrease < 50% after the 30th day or recurrent increase	-2	-
3 Risk factors		
Alcohol use (drinks/d: > 2 for women, > 3 for men)	1	-
Alcohol use (drinks/d: ⩽ 2 for women, ⩽ 3 for men)	0	+
Age ⩾ 55 yr	1	-
Age < 55 yr	0	+
4 Concomitant drug(s) or herbs(s)		
None or no information	0	-
Concomitant drug or herb with incompatible time to onset	0	+
Concomitant drug or herb with compatible or suggestive time to onset	-1	-
Concomitant drug or herb known as hepatotoxin and with compatible or suggestive time to onset	-2	-
Concomitant drug or herb with evidence for its role in this case (positive rechallenge or validated test)	-3	-
5 Search for non drug/herb causes	"+" if negative	-
Group I (6 causes)		
Anti-HAV-IgM	+	-
HBsAg, anti-HBc-IgM, HBV-DNA	+	-
Anti-HCV, HCV-RNA	+	-
Hepatobiliary sonography/colour doppler sonography of liver vessels/endosonography/CT/MRC	+	-
Alcoholism (AST/ALT ⩾ 2)	+	-
Acute recent hypotension history (particularly if underlying heart disease)	+	-
Group II (6 causes)		
Complications of underlying disease(s) such as sepsis, autoimmune hepatitis, chronic hepatitis B or C, primary biliary cirrhosis or sclerosing cholangitis, genetic liver diseases	+	-
Infection suggested by PCR and titer change for CMV (anti-CMV-IgM, anti-CMV-IgG)	+	-
EBV (anti-EBV-IgM, anti-EBV-IgG)	+	-
HEV (anti-HEV-IgM, anti-HEV-IgG)	+	-
HSV (anti-HSV-IgM, anti-HSV-IgG)	+	-
VZV (anti-VZV-IgM, anti-VZV-IgG)	+	-
Evaluation of group I and II		
All causes-groups I and II - reasonably ruled out	2	+
The 6 causes of group I ruled out	1	-
5 or 4 causes of group I ruled out	0	-
Less than 4 causes of group I ruled out	-2	-
Non drug or herb cause highly probable	-3	-
6 Previous information on hepatotoxicity of the drug/herb		
Reaction labelled in the product characteristics	2	-
Reaction published but unlabelled	1	-
Reaction unknown	0	+
7 Response to unintentional readministration		
Doubling of ALT with the drug/herb alone, provided ALT below 5N before reexposure	3	-
Doubling of ALT with the drug(s) and herb(s) already given at the time of first reaction	1	-
Increase of ALT but less than N in the same conditions as for the first administration	-2	-
Other situations	0	+
Total Score		7

[1]The score of the patient for each 7 item for hepatocellular injury was indicated as "+" in the correspondent cell. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

ruled out.

Laboratory diagnosis

Alanine aminotransferase (ALT 1830 IU/L; normal range: 0 to 45 U/L), aspartate aminotransferase (AST 1520 IU/L; normal range: 0 to 45 U/L), alkaline phosphatase (ALP 225 IU/L; normal range: 30 to 120 U/L), and total bilirubin (3.4 mg/dL; normal range: 0.174 to 1.04 mg/dL) were assessed. Anti-HBs IgG was positive, anti-HCV, HCV PCR, Anti-HAV IgM, and anti-HEV IgM were negative. There was no serologic evidence for recent infections with herpes simplex virus, epstein-barr virus, cytomegalovirus, or varisella zoster virus. All autoimmune markers were negative.

Imaging diagnosis

Abdominal ultrasonography was normal.

Treatment
Lesser celandine extract was immediately discontinued.

Related reports
This is the first case of toxic hepatitis associated with lesser celandine consumption.

Term explanation
Greater Celandine (*Chelidonium majus L.*) is a perennial herb and is used in western phytotherapy and traditional Chinese medicine for its wide variety of biological activities. Lesser celandine, *Ranunculus ficaria* (syn. *Ficaria verna, F. ranunculoides* or *F. grandiflora*), also known as pilewort, is a herbaceous perennial plant.

Experiences and lessons
Herb induced liver injury is an important problem in clinical setting, because it can be an etiology of undiagnosed acute hepatitis. This case is important to be the first to explain hepatotoxicity caused by lesser celandine. Physicians should consider lesser celandine as a causative agent for hepatotoxicity.

Peer review
Lesser celandine (pilewort) induced acute toxic liver injury by Bulent Yilmez *et al* is a relatively good and interesting report.

REFERENCES

1 **Estes JD**, Stolpman D, Olyaei A, Corless CL, Ham JM, Schwartz JM, Orloff SL. High prevalence of potentially hepatotoxic herbal supplement use in patients with fulminant hepatic failure. *Arch Surg* 2003; **138**: 852-858 [PMID: 12912743]

2 **Seeff LB**. Herbal hepatotoxicity. *Clin Liver Dis* 2007; **11**: 577-596, vii [PMID: 17723921]

3 **Teschke R**, Schwarzenboeck A, Eickhoff A, Frenzel C, Wolff A, Schulze J. Clinical and causality assessment in herbal hepatotoxicity. *Expert Opin Drug Saf* 2013; **12**: 339-366 [PMID: 23458441 DOI: 10.1517/14740338.2013.774371]

4 **Teschke R**, Wolff A, Frenzel C, Schulze J, Eickhoff A. Herbal hepatotoxicity: a tabular compilation of reported cases. *Liver Int* 2012; **32**: 1543-1556 [PMID: 22928722 DOI: 10.1111/j.1478-3231.2012.02864.x]

5 **Teschke R**, Frenzel C, Glass X, Schulze J, Eickhoff A. Herbal hepatotoxicity: a critical review. *Br J Clin Pharmacol* 2013; **75**: 630-636 [PMID: 22831551 DOI: 10.1111/j.1365-2125.2012.04395.x]

6 **Colombo ML**, Bosisio E. Pharmacological activities of Chelidonium majus L. (Papaveraceae). *Pharmacol Res* 1996; **33**: 127-134 [PMID: 8870028]

7 **Gilca M**, Gaman L, Panait E, Stoian I, Atanasiu V. Chelidonium majus--an integrative review: traditional knowledge versus modern findings. *Forsch Komplementmed* 2010; **17**: 241-248 [PMID: 20980763 DOI: 10.1159/000321397]

8 **Teschke R**, Glass X, Schulze J. Herbal hepatotoxicity by Greater Celandine (Chelidonium majus): causality assessment of 22 spontaneous reports. *Regul Toxicol Pharmacol* 2011; **61**: 282-291 [PMID: 21893153 DOI: 10.1016/j.yrtph.2011.08.008]

9 **Teschke R**, Glass X, Schulze J, Eickhoff A. Suspected Greater Celandine hepatotoxicity: liver-specific causality evaluation of published case reports from Europe. *Eur J Gastroenterol Hepatol* 2012; **24**: 270-280 [PMID: 22189691 DOI: 10.1097/MEG.0b013e32834f993f]

10 **Chevallier A.** Encyclopedia of herbal medicine. Dk Pub, 2000: 258

11 **Teschke R**, Wolff A, Frenzel C, Schwarzenboeck A, Schulze J, Eickhoff A. Drug and herb induced liver injury: Council for International Organizations of Medical Sciences scale for causality assessment. *World J Hepatol* 2014; **6**: 17-32 [PMID: 24653791 DOI: 10.4254/wjh.v6.i1.17]

12 **Bunchorntavakul C**, Reddy KR. Review article: herbal and dietary supplement hepatotoxicity. *Aliment Pharmacol Ther* 2013; **37**: 3-17 [PMID: 23121117 DOI: 10.1111/apt.12109]

13 **Teschke R**, Frenzel C. Drug induced liver injury: do we still need a routine liver biopsy for diagnosis today? *Ann Hepatol* 2012; **13**: 121-126 [PMID: 24378275]

14 **Strahl S**, Ehret V, Dahm HH, Maier KP. [Necrotizing hepatitis after taking herbal remedies]. *Dtsch Med Wochenschr* 1998; **123**: 1410-1414 [PMID: 9856112]

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