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EDITORIAL

# Ethanol-induced hepatic autophagy: Friend or foe?

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## Abstract

Excessive alcohol intake may induce hepatic apoptosis, steatosis, fibrosis, cirrhosis and even cancer. Ethanol-induced activation of general or selective autophagy as mitophagy or lipophagy in hepatocytes is generally considered a prosurvival mechanism. On the other side of the coin, upregulation of autophagy in non-hepatocytes as stellate cells may stimulate fibrogenesis and subsequently induce detrimental effects on the liver. The autophagic response of other non-hepatocytes as macrophages and endothelial cells is unknown yet and needs to be investigated as these cells play important roles in ethanol-induced hepatic steatosis and damage. Selective pharmacological stimulation of autophagy in hepatocytes may be of therapeutic importance in alcoholic liver disease.

**Key words:** Macrophages; Autophagy; Hepatocytes; Lipophagy; Mitophagy; Stellate cells; Alcohol



**Core tip:** This short editorial discusses the impact of ethanol-induced upregulation of cytoprotective bulk and selective autophagy as mitophagy or lipohagy on various types of liver cells. While ethanol-induced activation of autophagy in hepatocytes is generally prosurvival mechanism, upregulation of autophagy in non-hepatocytes as stellate cells may stimulate fibrogenesis and subsequently induce detrimental effects on the liver as a whole. The autophagic response of other non-hepatocytes as macrophages and endothelial cells is unknown yet and needs to be investigated as these cells play important roles in ethanol-induced hepatic steatosis and damage.

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## TEXT

Excessive alcohol intake may induce hepatic apoptosis, steatosis, fibrosis, cirrhosis and even cancer. Although ethanol-induced autophagy in hepatocytes has been recently considered as antiapoptotic mechanism, activation of autophagy in non-hepatocytes as macrophages, endothelial cells and stellate cells is not clearly known yet. More importantly, whether ethanol-induced activation of autophagy in non-hepatocytes is protective or detrimental to the liver as a whole needs to be

explored.

Autophagy is a cytoprotective pathway for clearance of damaged proapoptotic cellular components following multiple forms of stress, including oxidative stress, endoplasmic reticulum stress, mitochondrial damage and excessive accumulation of lipid droplets (LDs). Morphologically, autophagy is characterized by the formation of isolation membranes, which engulf a region of the cell cytoplasm or selectively an organelle forming autophagosomes mediated by microtubule-associated protein light chain 3 (LC3). The autophagosomes then fuse with lysosomes *via* lysosomal-associated membrane protein 2 (LAMP-2), forming autolysosomes, where the contents of the cargo are digested by lysosomal cathepsins[1-4]. Chronic alcohol consumption may induce hepatic damage, ranging from early-stage steatosis to steatohepatitis, fibrosis, cirrhosis, and ultimately hepatic carcinoma.

Ethanol-induced hepatocyte steatosis is characterized by excessive accumulation of cytoplasmic LDs which may render hepatocytes more susceptible to toxic or stress factors (multi-hit mechanisms) resulting in the progression of alcoholic liver disease. Importantly, ethanol-induced hepatocytes steatosis is often associated with structural and functional mitochondrial damage resulting from ethanol metabolism and related oxidative stress[5,6]. Therefore and as we have reported recently, the selective autophagic clearance of damaged mitochondria (mitophagy) and excessive LDs (lipophagy) in hepatocytes of chronic ethanol-treated rats may be a prosurvival mechanism for prevention of hepatocytes apoptosis (*via* clearance of proapoptotic damaged mitochondria) and progression of hepatic steatosis. We have observed that in addition to the upregulation of general autophagy markers LC3-Ⅱ, LAMP-2 and lysosomal cathepsins in hepatocytes of ethanol-treated rats, there was also overexpression of PINK1 (a sensor of mitochondrial damage and specific marker of mitophagy) in mitochondria of hepatocytes in treated rats[4,6]. Recent studies supported this cytoprotective role of autophagy in response to chronic ethanol toxicity[7,8]. In an interesting study, Lin *et al*[7] observed that there was an enhancement of lipophagy and probably mitophagy in hepatocytes of acute and chronic ethanol-treated mice. Moreover, they found that pharmacological promotion of autophagy by carbamazepine or rapamycin enhanced the autophagic response to ethanol toxicity and subsequently alleviated steatosis and hepatocyte injury, while blocking autophagy elevated steatosis and hepatic injury[7].

On the other hand, a recent study demonstrated that activation of autophagy in hepatic stellate cells of chronic ethanol-treated mice increases hepatic fibrogenesis by providing the fuel necessary to support stellate cell activation; thus accelerating liver pathology[9]. Therefore, it seems that upregulation of autophagy in stellate cells by ethanol may be to some degree detrimental to the liver compared to activation of autophagy in hepatocytes. However, autophagic signaling in stellate cells could be relatively innocuous compared to those in hepatocytes, simply because the hepatocytes make up the bulk of the parenchyma and comprise the main functional element in the liver. Hence, the pro-survival signaling in hepatocytes predominates. Selective pharmacological stimulation of autophagy in hepatocytes may be of therapeutic importance in alcoholic liver disease.

What is unknown yet and needs to be explored: Does ethanol activate autophagy in hepatic macrophages? Is activation of autophagy in Kupffer cells (KCs) by ethanol exposure friend as in case of hepatocytes or foe as in stellate cells? To the best of our knowledge, no studies investigated the autophagic response of KCs to ethanol toxicity although these cells may play important role in hepatic damage under acute and chronic ethanol treatment[10]. An elegant study by Wan *et al*[11] demonstrated that KCs could be either proinflammatory (M1 type) or anti-inflammatory (M2 type) and the balance between the two types impact hepatic damage. They found that in acute and chronic ethanol-treated mice, there was an increase in KCs apoptosis. Further observation revealed that M2 KCs induced apoptosis in M1 counterparts. They suggest that promoting M2-induced M1 KC apoptosis may be cytoprotective for liver under ethanol exposure. Whether autophagy is activated in M2 KCs and switched off in M1 KCs needs to be investigated. Moreover, ethanol-mediated increases in RANTES/CCL5 by liver sinusoidal endothelial cells can promote the infiltration of immunocytes to the liver *via* sinusoids, which may accelerate liver injury. Therefore, there is a possibility of autophagy-mediated upregulation of RANTES/CCL5 in ethanol-exposed liver sinusoidal endothelial cells[12,13].

## REFERENCES


1 **Kroemer G**, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* 2008; **9**: 1004-1010 [PMID: 18971948 DOI: 10.1038/nrm2529]

2 **Kroemer G**, Mariño G, Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010; **40**: 280-293 [PMID: 20965422 DOI: 10.1016/j.molcel.2010.09.023]

3 **Singh R**, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. Autophagy regulates lipid metabolism. *Nature* 2009; **458**: 1131-1135 [PMID: 19339967 DOI: 10.1038/nature07976]

4 **Eid N**, Ito Y, Otsuki Y. The autophagic response to alcohol toxicity: the missing layer. *J Hepatol* 2013; **59**: 398 [PMID: 23624249 DOI: 10.1016/j.jhep.2013.03.038]

5 **Feldstein AE**, Bailey SM. Emerging role of redox dysregulation in alcoholic and nonalcoholic fatty liver disease. *Antioxid Redox Signal* 2011; **15**: 421-424 [PMID: 21254858 DOI: 10.1089/ars.2011.3897]

6 **Eid N**, Ito Y, Maemura K, Otsuki Y. Elevated autophagic sequestration of mitochondria and lipid droplets in steatotic hepatocytes of chronic ethanol-treated rats: an immunohistochemical and electron microscopic study. *J Mol Histol* 2013; **44**: 311-326 [PMID: 23371376 DOI: 10.1007/s10735-013-9483-x]

7 **Lin CW**, Zhang H, Li M, Xiong X, Chen X, Chen X, Dong XC, Yin XM. Pharmacological promotion of autophagy alleviates steatosis and injury in alcoholic and non-alcoholic fatty liver conditions in mice. *J Hepatol* 2013; **58**: 993-999 [PMID: 23339953 DOI: 10.1016/j.jhep.2013.01.011]

8 **King AL**, Swain TM, Mao Z, Udoh US, Oliva CR, Betancourt AM, Griguer CE, Crowe DR, Lesort M, Bailey SM. Involvement of the mitochondrial permeability transition pore in chronic

ethanol-mediated liver injury in mice. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G265-G277 [PMID: 24356880 DOI: 10.1152/ajpgi.00278.2013]

9 **Hernández-Gea V**, Ghiassi-Nejad Z, Rozenfeld R, Gordon R, Fiel MI, Yue Z, Czaja MJ, Friedman SL. Autophagy releases lipid that promotes fibrogenesis by activated hepatic stellate cells in mice and in human tissues. *Gastroenterology* 2012; **142**: 938-946 [PMID: 22240484 DOI: 10.1053/j.gastro.2011.12.044]

10 **Cohen JI**, Nagy LE. Pathogenesis of alcoholic liver disease: interactions between parenchymal and non-parenchymal cells. *J Dig Dis* 2011; **12**: 3-9 [PMID: 21091930 DOI: 10.1111/j.1751-2980.2010.00468.x]

11 **Wan J**, Benkdane M, Teixeira-Clerc F, Bonnafous S, Louvet A, Lafdil F, Pecker F, Tran A, Gual P, Mallat A, Lotersztajn S, Pavoine C. M2 Kupffer cells promote M1 Kupffer cell apoptosis: a protective mechanism against alcoholic and nonalcoholic fatty liver disease. *Hepatology* 2014; **59**: 130-142 [PMID: 23832548 DOI: 10.1002/hep.26607]

12 **van Golen RF**, van Gulik TM, Heger M. Mechanistic overview of reactive species-induced degradation of the endothelial glycocalyx during hepatic ischemia/reperfusion injury. *Free Radic Biol Med* 2012; **52**: 1382-1402 [PMID: 22326617 DOI: 10.1016/j.freeradbiomed.2012.01.013]

13 **Yeligar SM**, Machida K, Tsukamoto H, Kalra VK. Ethanol augments RANTES/CCL5 expression in rat liver sinusoidal endothelial cells and human endothelial cells via activation of NF-kappa B, HIF-1 alpha, and AP-1. *J Immunol* 2009; **183**: 5964-5976 [PMID: 19828633 DOI: 10.4049/jimmunol.0901564]

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REVIEW

# Hepatocellular carcinoma: Advances in diagnosis, management, and long term outcome

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## Abstract

Hepatocellular carcinoma (HCC) remains a common and lethal malignancy worldwide and arises in the setting of a host of diseases. The incidence continues to increase despite multiple vaccines and therapies for viruses such as the hepatitis B and C viruses. In addition, due to the growing incidence of obesity in Western society, there is anticipation that there will be a growing population with HCC due to non-alcoholic fatty liver disease. Due to the growing frequency of this disease, screening is recommended using ultrasound with further imaging using magnetic resonance imaging and multi-detector computed tomography used for further characterization of masses. Great advances have been made to help with the early diagnosis of small lesions leading to potential curative resection or transplantation. Resection and transplantation maybe used in a variety of patients that are carefully selected based on underlying liver disease. Using certain guidelines and clinical acumen patients may have good outcomes with either resection or transplantation however many patients are inoperable at time of presentation. Fortunately, the use of new locoregional therapies has made down staging patients a potential option making them potential surgical candidates. Despite a growing population with HCC, new advances in viral therapies, chemotherapeutics, and an expanding population of surgical and transplant candidates might all contribute to improved long-term survival of these patients.

**Key words:** Hepatocellular carcinoma; Transplantation; Survival; Locoregional therapy; Resection



**Core tip:** Hepatocellular carcinoma (HCC) is a growing malignancy with poor survival. New therapies for the hepatitis C virus may help prevent the development of this malignancy, however the growing obesity epidemic will continue to foster new cases of HCC. With the aid of advances in imaging patients might be diagnosed earlier making them candidates for curative resection or transplantation. In addition, with a growing population of patients undergoing surgery after being down-staged with locoregional therapy, we expect an improvement in long-term outcomes for HCC patients.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) remains a common malignancy despite the development of many new treatment modalities over the past two decades. Worldwide HCC represents the fifth most common cancer and the second most common cause of cancer related deaths. Primary liver malignancies account for approximately 7% of all cancers and 90% of those are HCC[1,2]. Unfortunately, this disease has been on the rise, in both developing and developed countries, and despite a multitude of new therapeutic modalities, patients with HCC still have poor long-term survival. In the United States between 1990-2004, there was a 40% increase in HCC related deaths while overall cancer mortality was significantly reduced[3]. As this disease continues to plague countries worldwide, the United States, despite all its resources, reports a 5-year survival of only 12%, which although quite low, has been improving over recent years[4].

Much of the pathophysiology of HCC has been attributed to the long-term inflammation associated with a variety of disease processes ultimately resulting in cirrhosis; however roughly 10% of tumors occur in non-cirrhotic patients[5]. Worldwide, the most common etiology is hepatitis B virus (HBV) that accounts for approximately 50% of all primary HCCs, despite the available therapeutic modalities used to treat and prevent this virus[6,7]. Chronically HBV infected patients have a 25% chance of developing HCC, however as vaccinations rates have increased worldwide the number of patients with HBV is declining[8]. In contrast to other primary liver diseases, underlying cirrhosis is not necessarily a requirement for the development of HCC in the setting of HBV. Patients infected with HBV who have high levels of viremia (> 2000 IU/mL), high aminotransferases, co-infection of hepatitis D virus and hepatitis C virus (HCV) are more prone to developing HCC. In addition, other risk factors include infection with HBV genotype C as compared to the other genotypes, advanced age, alcohol consumption, smoking, and family history[9]. The administration of nucleoside and nucleotide analogues helps with HBV suppression in patients infected with the virus and decreases the overall risk for developing HCC. In addition, great strides have been made in liver transplantation, as HBV prophylaxis in the form of lamivudine, a nucleoside analogue, and hepatitis B immunoglobulin (HBIG) have shown to increase HCC recurrence-free survival as compared with HBIG alone or no prophylaxis in patients undergoing transplantation[10].

In Western countries, HCV remains the most common cause of HCC, however this could be changing with promising new HCV therapies on the market[11]. Worldwide, HCV accounts for approximately 30% of HCC, but unlike HBV, underlying cirrhosis is usually present. Interestingly, HCC is the main cause of death in HCV cirrhosis and the first complication experienced in many of these patients as opposed to other complications such as ascites and gastrointestinal bleeding[12]. Much like HBV, patients with HCV have a higher risk of acquiring HCC with advanced age, alcohol consumption, smoking, infection with HBV and human immunodeficiency virus (HIV), genotype 1b, obesity, as well as diabetes. Patients treated effectively for HCV, not surprisingly, have a significantly decreased incidence of HCC[13]. In addition to the effect of the chronic processes, there has been implication that the core protein of HCV has the ability to modulate gene transcription, cell proliferation, and cell death associated in the development of HCC[14].

Therapy for HCV has been evolving over the years, and recently the introduction of new drugs, including polymerase inhibitors such as sofosbuvir, have made significant headway in controlling the virus. Sofosbuvir is a HCV NS5B polymerase inhibitor that acts against HCV, and is used in conjunction most often with ribavirin for genotype 2 and 3. In a randomized control trial ribavirin and sofosbuvir combination therapy showed a 93% and 85% sustained virologic response in genotype 2 and 3, respectively[15]. Randomized trials looking at double and triple therapy for genotype 1 showed that ledipasvir-sofosbuvir with or without ribavirin showed > 95% sustained virologic responses[16]. Thus, drugs such as sofosbuvir are changing the face of HCV therapy.

Alcohol is another common cause of cirrhosis globally, making it a major risk factor for the development of HCC. Alcohol consumption of 80 g/d or higher for more than ten years is associated with a five-fold increase in the development of HCC. In patients with HCV cirrhosis, the use of alcohol nearly doubles the incidence of HCC as compared to those who do not use alcohol[17]. Alcohol consumption is thought to increase oxidative stress due to metabolism of the ethanol and inflammation, which cause chronic changes in the liver leading to cirrhosis and subsequent HCC[18]. In addition the induction of CYP2E1, a P450 cytochrome, related to alcohol consumption generates reactive oxygen species leading to carcinogenic consequences[19].

In Western countries, much like alcohol, non-alcoholic fatty liver disease (NAFLD) is a significant cause of chronic liver disease. Unfortunately, obesity rates have been increasing worldwide, and this has become a problem as it increases all disease burdens associated with being overweight, both cancer and non-cancer related. NAFLD is the most common form of liver disease in adults in the United States and has been an increasing indication for liver transplantation as well. NAFLD can be simply mild steatosis or can progress to non-alcoholic steatohepatitis (NASH), which leads to cirrhosis[20]. It is concerning that NAFLD occurs in 90% of obese patients and 70% of patients with type 2 diabetes mellitus[21]. In patients with NASH cirrhosis, the reported

risk of developing HCC is as high as 12.8% over 3 years which is alarming given the growing obesity epidemic in both children and adults. The mechanism behind the carcinogenicity of NASH is oxidative stress, insulin resistance, adipocytokine disorder and hyperplasia[22]. Cytokines such as interleukin-6 and tumor necrosis factor and inflammation are also increased in NASH leading to activation of STAT3, which is has been shown to be an oncogenic transcription factor[23].

In addition to these more common etiologies, HCC has been associated with increased exposure to aflatoxins such as *Aspergillus flavus* and *Aspergillus parasiticus* more commonly seen in Africa and Asia. Aflatoxins contaminate corn, nuts, soybeans, and legumes. Studies have shows that frequent p53 mutation maybe seen in high aflatoxin exposure which may explain at least part of the tumorigenesis[19,24].

Other forms of chronic liver diseases have been associated with HCC such as hemochromatosis, hereditary tyrosinemia type Ⅰ, alpha-1 antitrypsin deficiency, as well as chronic Wilson's disease[25]. There has been contention regarding the implication of chronic Wilson's disease, however, without chelation therapy, there have been reports of patients developing HCC[26].

## DIAGNOSTICS

There are a multitude of diagnostic modalities available to physicians that may aid in the diagnosis of HCC. The technology has progressed over the years allowing the diagnosis of small HCC's that would otherwise have been missed using more conventional diagnostics. First, patients at risk for HCC, which include non-cirrhotic and cirrhotic HBV patients, chronic HCV, as well as other patients with chronic liver disease and cirrhosis, warrant screening. The American Association for the Study of Liver Disease (AASLD) has created guidelines for the screening of patients at risk for the development of HCC. These were generated in order to define a group of patients who would benefit from screening modalities in a cost-effective manner. These guidelines include patients who have a 1.5% chance of developing HCC or higher while infected with HCV, or a 0.2% chance with HBV infection. The HBV infected patients who meet these criteria include Asian men over the age of 40 years, Asian women over the age of 50 years, patients with HBV and cirrhosis, a family history of HCC, and Africans. In addition, patients who have HCV related cirrhosis, stage 4 primary biliary cirrhosis, hemochromatosis, and alpha-1-antitrypsin related cirrhosis should be screened as well[27].

In addition it should be noted that those infected with HIV along with either HCV or HBV should be closely monitored, as HCC tends to develop more readily and rapidly in this population. Although these are not included in any defined guidelines, this might become more prevalent as the HIV population has much improved outcomes and are living longer with the disease[28,29].

Ultrasound is universally the diagnostic choice for screening, as it remains cost-effective and benign to patients[30]. Guidelines state that patients at high risk for developing HCC as those mentioned above should undergo surveillance with non-contrast enhanced ultrasound every 6 mo[31]. In the past, contrast enhanced ultrasound was recommended however the increased number of false positives have led this modality being dropped recently from the diagnostic imaging recommendations for HCC. Lesions that are less than a centimeter should be followed up in 3 mo with repeat ultrasound. If the lesion remains stable it should be watched every 3 mo but if it enlarges, it should be worked up with further imaging. For lesions 1 cm or greater immediate follow up with multi-detector computed tomography (MDCT) or magnetic resonance imaging (MRI) is indicated. Arterial enhancement and delayed phase washout suggests the diagnosis of HCC[26].

Ultrasound alone has reported sensitivity of 58%-89% and specificity over 90% depending on the source[32,33]. Ultrasound itself does not subject the patients to any contrast, which may be a concern for many patients with underlying cirrhosis and concomitant renal disease. The accuracy of ultrasound maybe affected by underlying nodular cirrhosis which is present in most of these chronically diseased liver patients[29,34].

There have been a number of studies that suggest the use of combining alpha-fetoprotein (AFP) with ultrasound as the method of choice for screening the patients at high risk for developing HCC, however the AASLD has not included this in their recommendations due to the lack of sensitivity and specificity[35]. Sensitivity of AFP alone has been reported to range from 25% to 65% for detecting HCC as a screening tool, and continues to be debated in its combination use with ultrasound[36]. AFP has, however, been shown to be a poor prognostic factor when it comes to liver transplantation and disease recurrence. Values greater than 1000 mcg/L are associated with high degree of HCC recurrence after transplantation, and although may not be a contraindication, should be heavily weighted while considering for transplant[37,38].

Multi-detector computed tomography (CT) scanning remains a very useful tool in the diagnosis of HCC. Advances over the last 10 years have seen CT scanners become considerably faster while attempting to limit the radiation dose. The sensitivity of MDCT is reported at 81% as compared to 91% with MRI in a meta-analysis of 15 comparative studies between MRI and MDCT. The specificity of MDCT was 93% compared to 95% in the MRI group. CT scan does afford the ability to perform three-dimensional reconstructions that may help with operative planning which is an advantage over MRI[39]. Although a rare event, this mode of imaging does however place patients at risk for contrast induced nephropathy[40].

Although not included in standard diagnostic guidelines, modern advances show that perfusion CT scanning may offer more information regarding liver hemodynamics and blood flow directed toward tumors in the liver[41]. This may become more useful as transarterial

chemoembolization (TACE) is an evolving therapy for HCC. It also may aid in treatment monitoring. Current perfusion CT does, however, deliver a higher radiation dose as well as lower resolution[42].

MRI has been used extensively in the diagnosis of HCC and advances in imaging continue to improve its diagnostic capability. The contrast most commonly used for MRI is gadolinium-based; however newer contrasts are more hepatocyte specific. Gadoxetate dimeglumine is one of these newer agents used and has demonstrated improvement in distinguishing small HCCs including those less than 1 cm. It has also been shown to be effective in distinguishing HCC *vs* benign liver lesions as compared to other contrasts. Nearly half of this contrast is taken up by hepatocytes and subsequently excreted into the bile in comparison to roughly 5% by standard gadobenate dimeglumine, which supports the improved accuracy in diagnosing liver malignancies[43,44]. The use of gadolinium based contrast agents should however be used with caution in patients with renal failure given the risk of nephrogenic systemic fibrosis which is a rare disorder associated with fibrosis of the skin, joints, eyes, as well as internal viscera[45]. The other disadvantage to MRI is the relatively long time it takes to complete the study which maybe a challenge for critically ill transplant candidates who need more detailed imaging before listing for transplant.

Some newer non-imaging modalities for prognosticating HCC, once diagnosed, have been developed over the last ten years, which may pave the way for a tailored approach to treating this malignancy. They also may define populations that either may benefit from more aggressive therapies or a more conservative or palliative approach. More recently microRNA expression in HCC has become a possible prognosticating marker for outcomes in HCC. Looking at HCC and benign liver disease, Jiang *et al*[46], found that miR-199a, miR-21, and miR-301 all were expressed differentially in HCC tumors. They specifically looked at expression of microRNA's and found that patients could be potentially prognosticated based on specific microRNA expression[46,47].

Another study looking at gene expression in resected HCC specimens identified 5 genes that could be used for prognosticating HCC. These genes, *HN1, RAN*, *RAMP3*, *KRT19*, and *TAF9*, were chosen based on correlations with disease-specific survival (HR = 3.5; 95%CI: 1.9-6.6; $P < 0.0001$). This 5-gene score was found to be associated with disease-specific survival, which upon multivariate was independent of many of the tumor features[48].

In addition to gene-score and micro-RNA, Kamiyama *et al*[49] evaluated N-glycosylation of glycoproteins in regards to HCC. They analyzed 369 presumed curative hepatectomies for HCC, and found that the G2890 and G3560 N-glycans were associated with recurrence and prognosis. In fact these two glycans were found to correlate with tumor number, size, and vascular invasion. These biomarkers maybe useful in prognosticating resected patients in the future[49].

## THERAPY

Therapeutic options for HCC have grown considerably over the last few decades. Initially resection was the only option, but now transplantation has emerged as an effective intervention as well as the growing number of locoregional therapies which have been proven quite effective. Rahman performed a large meta-analysis looking at resection *vs* transplantation looking at comparable early cirrhotic patients. This study found that at 5 years there was a higher disease free survival in patients undergoing transplantation (OR = 0.39; 95%CI: 0.24-0.63; $P < 0.001$) although similar 5-year overall survival. However, at 10-years this study demonstrated a clear overall and disease-free survival for patients undergoing liver transplantation. They did however demonstrate a higher short-term mortality for transplant patients[50].

Resection remains the first line therapy in patients who have preserved liver function and can be completely resected. In patients with no underlying liver disease, roughly 70%-80% of the hepatic parenchyma can be resected safely due to the ability of the liver to regenerate. Ratio of remnant liver volume to body weight should be $\geqslant$ 0.8% according to most literature to avoid post-resection major complications including post-resection liver failure[51,52]. In cirrhotic patients it is thought that only 60% of the parenchyma can be resected leaving at a minimum 40% of functioning liver[53,54].

CT volumetrics are used to help in planning resection, however in cases where there is not enough predicted remnant liver, portal vein embolization (PVE), originally reported by Makuuchi *et al*[55] in 1990, may be utilized to increase the predicted hepatic reserve post-resection. Two-stage hepatectomies in which patients undergo PVE have been compared to one-stage hepatectomies by Schadde *et al*[56], and showed that they were comparable in outcomes. Two-stage hepatectomy was developed over 10 years ago to allow for more extensive R0 resections while allowing enough remnant liver. The groups were comparable and no significant differences were seen in complications with a relative risk of 0.9 ($P = 0.79$). There were also no significant differences in post-resection liver failure or mortality when comparing two-stage *vs* one-stage hepatectomy. This technique has expanded the ability to resect patients who would otherwise not be candidates for resection[56-58].

Unfortunately only 20%-30% of patients who present with HCC are candidates for resection due to either multifocal unresectable tumors or their underlying chronic liver disease. In Western countries only 5% of patients develop HCC without underlying liver disease as compared to that of 40% of Asian countries[30]. In well-selected candidates without chronic liver disease, survival rates at 5 years approach 70% or higher with surgical resection with margins greater than 1 cm and tumors less than 5 cm[59,60]. Furthermore, a randomized

control trial showed that 2 cm margins show decreased recurrence rate and improved survival when it comes to solitary tumors[61].

Selecting patients with chronic liver disease for resection remains a very difficult treatment decision when planning therapy for HCC. Operative mortality is increased in patients with cirrhosis as compared with non-cirrhotics. Determining who is an adequate candidate is difficult however. It is thought that Childs-Pugh A patients are suitable candidates, however these patients may also go into post resection failure, unexpectedly. Both the Childs-Pugh scoring system and model of end stage liver disease have been evaluated to aid in the selection criteria for resection candidates, however neither have been deemed reliable. When evaluating patients in the office a platelet count of 100000 or less, a history of esophageal varices and documented splenomegaly should be factored into the equation regarding liver resection as they all suggest significant portal hypertension. Furthermore, a hepatic venous pressure gradient of greater than 10 mmHg is also a poor prognostic factor for resection as it is a sign of significant liver disease although is very rarely available when initially working up a resection candidate. In patients with underlying liver disease a normal bilirubin, hepatic venous pressure gradient $\leqslant$ 10 mmHg, and a small isolated tumor ($\leqslant$ 3 cm) portends the best outcomes[3,62]. In patients who have preserved liver function without cirrhosis, anatomic resections should be performed if possible, as they have been associated with improved outcomes. This may not always be possible given certain locations of lesions as well as the patients overall liver function[63].

More recently laparoscopic and even robotic liver resections have become more common across the world. Not every HCC is amenable to minimally invasive approaches, but smaller, more peripheral lesions that have some distance from major hepatic vasculature can often be resected safely[64]. Major hepatectomies in 210 patients were performed either completely laparoscopic or hand-assisted and were described in one study that included six major hepatobiliary centers across the world. They reported both right and left formal hepatectomies and converted only 12% of cases to laparotomies. In addition to being able to be performed safely, laparoscopic liver resections have been shown to have less blood loss, in some studies less transfusion requirements, less overall intravenous narcotic usage, and decreased length of stay. In regards to HCC, patients who eventually underwent liver transplantation who had previous laparoscopic resection had shorter hepatectomy and operative times, less blood loss, and less blood transfusions as compared with those who underwent open resection prior to transplant[65]. In addition to laparoscopic procedures, some centers are performing robotic resections for a variety of cases from segmentectomies to major hepatectomies. Most of the comparative studies show similar blood loss in robotic *vs* laparoscopic resection, with slightly longer operative times in the robotic groups but data is mixed[66,67]. Although minimally invasive resections have been shown to be safe and have some benefit, these procedures should be done concomitantly with laparoscopic ultrasonography and should only be done by surgeons with vast laparoscopic and open experience.

Liver transplantation remains the mainstay therapy for patients with Childs-Pugh class B and C or moderate and severe cirrhosis with HCC, as well as those individuals who have unresectable tumors within Milan or UCSF criteria. The oncologic advantage to liver transplantation includes the ability to completely remove all previously identified tumors as well as any premalignant or non-radiologically present tumor. HCC is frequently a multifocal disease process, and often times patients are found to have numerous small HCC's upon explant of the liver during liver transplantation that were not otherwise seen on modern-day advanced imaging[68]. Initial results were poor as compared to patients transplanted for non-malignant liver disease, but in 1996 the Milan group defined a group of patients who could achieve excellent survival of 75% at four years. The group initially defined the Milan Criteria as single tumor < 5 cm, three lesions or less with none greater than 3 cm, with no distant metastasis, lymph node involvement, or lymphovascular invasion[69]. Since then, groups have expanded their criteria for transplant showing that good outcomes can be achieved. The UCSF criteria was based off their study in 2001 which showed a 75% survival at 5 years, and includes a single tumor $\leqslant$ 6.5 cm, three or fewer tumors all $\leqslant$ 4.5 cm with a total tumor diameter of $\leqslant$ 8 cm. Patients outside the UCSF criteria had less than a 30% 5 year survival rate[37].

In addition to the Milan and UCSF criteria, the Barcelona Clinic Liver Cancer Group created criteria including: 1 tumor < 7 cm, 3 tumors < 5 cm, 5 tumors < 3 cm, or down-staging to Milan criteria with pretransplant adjuvant therapies. They achieved excellent results with these expanded criteria with over 50% 5-year survival[70-72]. The Hangzhou group created a criteria as well consisting of total tumor diameter less $\leqslant$ 8 cm; total tumor diameter more than 8 cm, with histopathologic grade Ⅰ or Ⅱ and preoperative AFP level less than or equal to 400 ng/mL, simultaneously. With these criteria they achieved a 71% 5-year overall survival[73]. The European Metro Group created the Metroticket criteria, which consists of nodule size plus tumor number $\leqslant$ 7 and they also, achieved a 71% 5-year survival as well[74,75].

Liver transplantation and resection are both curative approaches to HCC, however in comparable patient populations, transplantation has been shown to increase recurrence-free survival as compared with liver resection. In one study, even outside of the Milan criteria there was a trend toward improved survival in liver transplantation although not statistically significant. This study had a 51.5% recurrence rate in liver resection as compared with only 29.5% in the transplant group ($P$ < 0.001). Of note patients who were in the resection group were

primarily Child's class A cirrhotics with only 13.1% being Child's class B. The rational behind transplantation being a superior treatment in these cirrhotic patients is that one has their tumor eradicated and cirrhosis is cured as well[76].

Although results have improved over the years making transplant an excellent option for those with HCC and significant liver disease, lifelong immunosuppression has its drawbacks, including infection, renal failure, diabetes, neurotoxicity, amongst many more. Over the history of liver transplantation for HCC, immunosuppression has improved significantly. The introduction of mTOR inhibitors for immunosuppression such as rapamycin, also known as sirolimus, which is thought to have anti-tumor properties related to its ability to decrease cell proliferation and angiogenesis. Some studies suggest better survival without major differences in complications in HCC patients who underwent liver transplantation, although further studies are necessary to further validate this treatment modality[77,78].

Tumor biology is an important part in the outcomes after liver transplantation, however often times is unavailable at time selection of transplant candidacy. There is a reported incidence of 3% of seeding biopsy tracts, making biopsy undesirable in many cases especially given the accuracy of present imaging modalities[79]. Poor differentiation and lymphovascular invasion are both poor predictive markers for outcomes following OLT, however these factors are not always available for transplant patient selection. As mentioned above, most criteria for HCC in liver transplantation are based on size and number of tumors, however there is evidence that poor differentiation predicts higher rate of recurrence than being outside the Milan criteria[80].

In addition to tumor biology AFP and protein-induced vitamin k absence or antagonist Ⅱ have been shown to be markers in the prognosis of HCC but results have been variable[81]. The combination of markers is associated with tumor recurrence and worsened survival after any treatments for HCC, and might be useful in monitoring for recurrence. This combination may also be used in some settings to predict treatment outcomes in certain groups of patients undergoing local therapies such as ablation or TACE[82].

It is also important to note that patients who are outside of criteria for liver transplant candidacy maybe down-staged. Resection in many of these cirrhotic patients is not an option, but locoregional therapy allows destruction of focal lesions without much damage to the uninvolved liver parenchyma. Many options are available to physicians including percutaneous ethanol injection (PEI) with 95% ethanol or 50% acetic acid (PAI), radiofrequency ablation (RFA), TACE, transarterial radioembolization (TARE). Many of these methods have been used in the attempted down staging of HCC for liver transplantation, and results have shown that response to these therapies may predict post-transplant outcomes. When successful, these therapies may induce complete tumor necrosis, and are associated with better recurrence free survival. In the more advanced tumor populations with stage Ⅲ/Ⅳ HCC, the small group of patients down-staged to within Milan had similar survival to those with lesser-advanced tumors[83].

The use of locoregional therapies is very effective in down staging, and disease control for liver transplantation, but also has a role outside transplantation. Given the multitude of therapies it is important to be educated to know what modality should be used in each specific situation. In patients who are not candidates for curative measures including liver transplantation and surgical resection, percutaneous ablation is the best therapeutic option in small tumors less than 3 cm. There are a host of ways to approach ablation, usually done under ultrasound guidance, including those mentioned above such as injection of alcohol, acetic acid, microwaves, laser, cryoablation, and the most commonly used radiofrequency[84].

Percutaneous ethanol injection has limited use, as it often does not perform well in setting of fibrosis and with larger tumors. This method is relatively low cost, however often times requires multiple treatment sessions with relatively poor outcomes in tumors greater than 2 cm. In small tumors less than 2 cm there are reports of complete tumor response. The approach of percutaneous RFA has been used however with more success in numerous studies, and the rational is that this method delivers a thermal energy insult to the tumor and a small area of non-tumor hepatic parenchyma which may induce necrosis of small satellite lesions not seen on imaging. A meta-analysis looking at the comparison of RFA and PEI, demonstrated statistically significant improved overall survival in the RFA group with OR of 2.32 as compared with 1.92 in the PEI group[85]. In addition, RFA was associated with a greater rate of complete tumor response, decreased number of treatments, and decreased local recurrence[86]. RFA is recommended for tumors $\leqslant$ 3 cm with up to three lesions treated simultaneously, a single lesion $\leqslant$ 5 cm who are not surgical candidates, and can be used in both Child-Pugh Class A and B liver disease relatively safely. These modalities must be monitored as recurrent or inadequately treated tumors maybe retreated, and recommendations are to re-image with MDCT or MRI within 1 mo[87].

Percutaneous ablation has become a frequently used and extremely effective modality for patients who either need to be down-staged or who are not candidates for transplant or resection; however, its use in larger tumors has been unsuccessful. TACE is the treatment of choice in larger and later staged tumors. The rational behind its use is that after the initial stage of HCC when the blood supply comes from the portal vein, the hepatic artery becomes the main feeder to the tumor. This procedure requires percutaneous access to the arterial system, and subsequent access to the hepatic artery and ultimately in the segmental branches to deliver treatment directly to the tumor limiting damage to surrounding normal hepatic parenchyma. The catheter

directed therapy delivers chemotherapeutics such as doxorubicin, mitomycin-C, cisplatin, amongst others and then uses vascular embolization material to cease blood flow and induce cellular injury to the tumor[88]. Unlike RFA, which has shown fairly consistent results, TACE has shown variable results with some studies showing limited benefit as compared to supportive therapies. This treatment modality has been shown to have higher morbidity from the ischemic insult, which places patients with more advanced liver disease at higher risk of post-procedure liver failure. The general recommendations for the use of TACE is for lesions < than 8 cm, > 3 lesions, with no evidence of extrahepatic extension or lymph node involvement, and with patients with relatively preserved liver function including Child-Pugh Class A and B liver disease. Mortality from this procedure is reported at less than 2% assuming appropriate candidacy of patients[1,89].

More recently the use of TACE with drug eluting beads (DEB) has been utilized with some improved side-effect profiles and perhaps a trend toward improved outcomes as compared to TACE alone in more advanced liver disease. The beads allow for the controlled release of the chemotherapeutic agents over a one-week period creating a longer tumor treatment period as well as also increasing local drug concentrations. Studies have demonstrated increased tumor concentration of the chemotherapeutics and decreased systemic concentrations, subsequently decreasing both liver toxicity and cardiac toxicity with the use TACE with DEB as compared to TACE alone[90,91]. The use of TACE with DEB maybe better suited in patients who have more advanced liver disease and are borderline candidates for TACE alone.

Unlike the therapies mentioned so far, external beam radiation has little role in the treatment of HCC due to its toxic effect on the diseased liver. There have, however, been major advances with the use of TARE with the use of microspheres coated with Yttrium-90 ($Y_{90}$). $Y_{90}$ is delivered to the tumor much like the chemotherapeutics distributed in TACE, allowing for localized radiation therapy limiting subsequent hepatic toxicity as compared to external beam radiation. Distribution of $Y_{90}$ is a form of brachytherapy that allows for internal radiation of the tumor alone, permitting for higher doses of radiation than standard external beam doses[85]. In addition, this modality has been shown to be safe in patients who have portal vein thrombosis (PVT) with no significant increases in post treatment liver failure, however there was a significantly decreased median survival for patients with PVT as compared to those without[89].

Although, no large randomized control trials are completed, there have been comparative studies looking at TARE *vs* TACE with a trend toward higher treatment response in the TARE group at 49% *vs* 35% ($P = 0.052$), and significant increase in time to disease progression at 13.3 mo compared with 8.4 mo, in the TARE and TACE groups, respectively. Much like other therapies, abdominal imaging with CT or MRI should be done to evaluate efficacy of treatment[85,92].

## CHEMOTHERAPY

HCC has been known as one of the most chemo-resistant tumors encountered by physicians all over the world. Many agents have been attempted yet with little tumor response and survival benefit. Currently sorafenib is the only drug recommended in the treatment of HCC. Sorafenib is a multi-tyrosine kinase inhibitor used in the treatment of a number of cancers but has time and time again shown improved outcomes in HCC. It functions to inhibit Raf-1 and B-Raf serine-threonine kinases, and receptors of tyrosine kinases of vascular endothelial growth factor receptors 1, 2, 3, and platelet derive growth factor receptor-β. The means by which this drug works is that *via* these pathways, it inhibits tumor-cell proliferation and angiogenesis, while increasing rate of apoptosis. The SHARP trial was a randomized double-blind, placebo controlled trial which showed a median time to radiologic progression of 5.5 mo in the sorafenib arm and 2.8 mo in the placebo arm. The median survival demonstrated a survival advantage in the sorafenib group as compared to the placebo group at 10.7 mo and 7.9 mo respectively ($P < 0.001$)[93].

Sorafenib has also been studied looking at adjuvant therapy in high-risk patients undergoing liver transplantation. A retrospective review looked at a small group of patients who underwent post-OLT sorafenib therapy suggesting its safety and potential benefit in regards to HCC recurrence and extending disease free and overall survival in high-risk transplant recipients[94]. Yoon *et al*[95] also looked at a small number of patients who were treated with sorafenib post-OLT and found similar results, although prospective data, which is ongoing, is required to determine the true potential benefit.

## CONCLUSION

The incidence of HCC has been increasing worldwide, and despite a multitude of diagnostics, established treatment modalities, and new innovative viral therapies and prophylaxis, it still remains an aggressive tumor and one of the more common causes of cancer related-death. However, with advanced surgical techniques including resection, liver transplantation, and percutaneous interventions, this malignancy can be cured in appropriately selected patients. The hope is that with new innovative therapies being developed for HCV, the incidence of HCV related HCC might decline, however we must educate western societies regarding weight reduction as the increasing degree of obesity and subsequent development of NASH will continue to increase HCC incidence. With the continuing advancement of newer imaging modalities, pathologic studies, surgical approaches, and improved patient selection, there is

optimism to improve outcomes for this deadly disease.

## REFERENCES


1 **European Association for Study of Liver; European Organisation for Research and Treatment of Cancer**. 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]

2 **World Health Organization.** Cancer Fact sheet N°297. [Cited 2014 Nov; updated 2015 Feb]. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/

3 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]

4 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]

5 **Simonetti RG**, Cammà C, Fiorello F, Politi F, D'Amico G, Pagliaro L. Hepatocellular carcinoma. A worldwide problem and the major risk factors. *Dig Dis Sci* 1991; **36**: 962-972 [PMID: 1649041]

6 **Altekruse SF**, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol* 2009; **27**: 1485-1491 [PMID: 19224838 DOI: 10.1200/JCO.2008.20.7753]

7 **Chang MH**, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, Liang DC, Shau WY, Chen DS. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997; **336**: 1855-1859 [PMID: 9197213 DOI: 10.1056/NEJM199706263362602]

8 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129 [PMID: 15014185 DOI: 10.1056/NEJMra031087]

9 **Yu MW**, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, Shih WL, Kao JH, Chen DS, Chen CJ. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; **97**: 265-272 [PMID: 15713961 DOI: 10.1093/jnci/dji043]

10 **Zimmerman MA**, Ghobrial RM, Tong MJ, Hiatt JR, Cameron AM, Busuttil RW. Antiviral prophylaxis and recurrence of hepatocellular carcinoma following liver transplantation in patients with hepatitis B. *Transplant Proc* 2007; **39**: 3276-3280 [PMID: 18089370 DOI: 10.1016/j.transproceed.2007.07.085]

11 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078]

12 **Sangiovanni A**, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, Del Ninno E, Morabito A, Colombo M. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. *Hepatology* 2006; **43**: 1303-1310 [PMID: 16729298 DOI: 10.1002/hep.21176]

13 **Lok AS**, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138-148 [PMID: 18848939 DOI: 10.1053/j.gastro.2008.09.014]

14 **Moriya K**, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 1998; **4**: 1065-1067 [PMID: 9734402 DOI: 10.1038/2053]

15 **Zeuzem S**, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, Illeperuma A, Svarovskaia E, Brainard DM, Symonds WT, Subramanian GM, McHutchison JG, Weiland O, Reesink HW, Ferenci P, Hézode C, Esteban R. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993-2001 [PMID: 24795201 DOI: 10.1056/NEJMoa1316145]

16 **Afdhal N**, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, Romero-Gomez M, Zarski JP, Agarwal K, Buggisch P, Foster GR, Bräu N, Buti M, Jacobson IM, Subramanian GM, Ding X, Mo H, Yang JC, Pang PS, Symonds WT, McHutchison JG, Muir AJ, Mangia A, Marcellin P. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889-1898 [PMID: 24725239 DOI: 10.1056/NEJMoa1402454]

17 **Morgan TR**, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S87-S96 [PMID: 15508108]

18 **Seitz HK**, Stickel F. Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol Chem* 2006; **387**: 349-360 [PMID: 16606331 DOI: 10.1515/BC.2006.047]

19 **Seitz HK**, Wang XD. The role of cytochrome P450 2E1 in ethanol-mediated carcinogenesis. *Subcell Biochem* 2013; **67**: 131-143 [PMID: 23400919 DOI: 10.1007/978-94-007-5881-0_3]

20 **Kirstein MM**, Vogel A. The pathogenesis of hepatocellular carcinoma. *Dig Dis* 2014; **32**: 545-553 [PMID: 25034287 DOI: 10.1159/000360499]

21 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219 [PMID: 12717402 DOI: 10.1053/jhep.2003.50193]

22 **Petta S**, Craxi A. Hepatocellular carcinoma and non-alcoholic fatty liver disease: from a clinical to a molecular association. *Curr Pharm Des* 2010; **16**: 741-752 [PMID: 20388084]

23 **Torres DM**, Harrison SA. Nonalcoholic steatohepatitis and noncirrhotic hepatocellular carcinoma: fertile soil. *Semin Liver Dis* 2012; **32**: 30-38 [PMID: 22418886 DOI: 10.1055/s-0032-1306424]

24 **Lunn RM**, Zhang YJ, Wang LY, Chen CJ, Lee PH, Lee CS, Tsai WY, Santella RM. p53 mutations, chronic hepatitis B virus infection, and aflatoxin exposure in hepatocellular carcinoma in Taiwan. *Cancer Res* 1997; **57**: 3471-3477 [PMID: 9270015]

25 **McGlynn KA**, London WT. Epidemiology and natural history of hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2005; **19**: 3-23 [PMID: 15757802 DOI: 10.1016/j.bpg.2004.10.004]

26 **Xu R**, Hajdu CH. Wilson disease and hepatocellular carcinoma. *Gastroenterol Hepatol* (N Y) 2008; **4**: 438-439 [PMID: 21904522]

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

28 **Giordano TP**, Kramer JR, Souchek J, Richardson P, El-Serag HB. Cirrhosis and hepatocellular carcinoma in HIV-infected veterans with and without the hepatitis C virus: a cohort study, 1992-2001. *Arch Intern Med* 2004; **164**: 2349-2354 [PMID: 15557414 DOI: 10.1001/archinte.164.21.2349]

29 **Montes Ramírez ML**, Miró JM, Quereda C, Jou A, von Wichmann MÁ, Berenguer J, González-García JJ, Hernando A, Ortega E, Sanz J, Arribas JR. Incidence of hepatocellular carcinoma in HIV-infected patients with cirrhosis: a prospective study. *J Acquir Immune Defic Syndr* 2014; **65**: 82-86 [PMID: 24419065 DOI: 10.1097/QAI.0b013e3182a685dc]

30 **Hennedige T**, Venkatesh SK. Imaging of hepatocellular carcinoma: diagnosis, staging and treatment monitoring. *Cancer Imaging* 2013; **12**: 530-547 [PMID: 23400006 DOI: 10.1102/1470-7330.2012.0044]

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

32 **Bolondi L**. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076-1084 [PMID: 14642630]

33 **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]

34 **Willatt JM**, Hussain HK, Adusumilli S, Marrero JA. MR Imaging of hepatocellular carcinoma in the cirrhotic liver: challenges and controversies. *Radiology* 2008; **247**: 311-330 [PMID: 18430871 DOI: 10.1148/radiol.2472061331]

35 **Thompson Coon J**, Rogers G, Hewson P, Wright D, Anderson R, Cramp M, Jackson S, Ryder S, Price A, Stein K. Surveillance

of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. *Health Technol Assess* 2007; **11**: 1-206 [PMID: 17767898]

36 **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]

37 **Figueras J**, Ibañez L, Ramos E, Jaurrieta E, Ortiz-de-Urbina J, Pardo F, Mir J, Loinaz C, Herrera L, López-Cillero P, Santoyo J. Selection criteria for liver transplantation in early-stage hepatocellular carcinoma with cirrhosis: results of a multicenter study. *Liver Transpl* 2001; **7**: 877-883 [PMID: 11679986 DOI: 10.1053/jlts.2001.27856]

38 **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]

39 **Chen L**, Zhang L, Bao J, Zhang J, Li C, Xia Y, Huang X, Wang J. Comparison of MRI with liver-specific contrast agents and multidetector row CT for the detection of hepatocellular carcinoma: a meta-analysis of 15 direct comparative studies. *Gut* 2013; **62**: 1520-1521 [PMID: 23929696 DOI: 10.1136/gutjnl-2013-305231]

40 **Davenport MS**, Khalatbari S, Cohan RH, Dillman JR, Myles JD, Ellis JH. Contrast material-induced nephrotoxicity and intravenous low-osmolality iodinated contrast material: risk stratification by using estimated glomerular filtration rate. *Radiology* 2013; **268**: 719-728 [PMID: 23579046 DOI: 10.1148/radiol.13122276]

41 **Ippolito D**, Sironi S, Pozzi M, Antolini L, Invernizzi F, Ratti L, Leone EB, Fazio F. Perfusion CT in cirrhotic patients with early stage hepatocellular carcinoma: assessment of tumor-related vascularization. *Eur J Radiol* 2010; **73**: 148-152 [PMID: 19054640 DOI: 10.1016/j.ejrad.2008.10.014]

42 **Okada M**, Kim T, Murakami T. Hepatocellular nodules in liver cirrhosis: state of the art CT evaluation (perfusion CT/volume helical shuttle scan/dual-energy CT, etc.). *Abdom Imaging* 2011; **36**: 273-281 [PMID: 21267563 DOI: 10.1007/s00261-011-9684-2]

43 **Lee JM**, Zech CJ, Bolondi L, Jonas E, Kim MJ, Matsui O, Merkle EM, Sakamoto M, Choi BI. Consensus report of the 4th International Forum for Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid Magnetic Resonance Imaging. *Korean J Radiol* 2011; **12**: 403-415 [PMID: 21852900 DOI: 10.3348/kjr.2011.12.4.403]

44 **Kogita S**, Imai Y, Okada M, Kim T, Onishi H, Takamura M, Fukuda K, Igura T, Sawai Y, Morimoto O, Hori M, Nagano H, Wakasa K, Hayashi N, Murakami T. Gd-EOB-DTPA-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading and portal blood flow. *Eur Radiol* 2010; **20**: 2405-2413 [PMID: 20490505 DOI: 10.1007/s00330-010-1812-9]

45 **Kaewlai R**, Abujudeh H. Nephrogenic systemic fibrosis. *AJR Am J Roentgenol* 2012; **199**: W17-W23 [PMID: 22733927 DOI: 10.2214/AJR.11.8144]

46 **Jiang J**, Gusev Y, Aderca I, Mettler TA, Nagorney DM, Brackett DJ, Roberts LR, Schmittgen TD. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clin Cancer Res* 2008; **14**: 419-427 [PMID: 18223217 DOI: 10.1158/1078-0432.CCR-07-0523]

47 **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]

48 **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]

49 **Kamiyama T**, Yokoo H, Furukawa J, Kurogochi M, Togashi T, Miura N, Nakanishi K, Kamachi H, Kakisaka T, Tsuruga Y, Fujiyoshi M, Taketomi A, Nishimura S, Todo S. Identification of novel serum biomarkers of hepatocellular carcinoma using glycomic analysis. *Hepatology* 2013; **57**: 2314-2325 [PMID: 23322672 DOI: 10.1002/hep.26262]

50 **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 DOI: 10.1007/s11605-012-1973-8]

51 **Chun YS**, Ribero D, Abdalla EK, Madoff DC, Mortenson MM, Wei SH, Vauthey JN. Comparison of two methods of future liver remnant volume measurement. *J Gastrointest Surg* 2008; **12**: 123-128 [PMID: 17924174 DOI: 10.1007/s11605-007-0323-8]

52 **Truant S**, Oberlin O, Sergent G, Lebuffe G, Gambiez L, Ernst O, Pruvot FR. Remnant liver volume to body weight ratio > or =0.5%: A new cut-off to estimate postoperative risks after extended resection in noncirrhotic liver. *J Am Coll Surg* 2007; **204**: 22-33 [PMID: 17189109 DOI: 10.1016/j.jamcollsurg.2006.09.007]

53 **Belghiti J**, Ogata S. Assessment of hepatic reserve for the indication of hepatic resection. *J Hepatobiliary Pancreat Surg* 2005; **12**: 1-3 [PMID: 15754091 DOI: 10.1007/s00534-004-0951-2]

54 **Abdalla EK**, Hicks ME, Vauthey JN. Portal vein embolization: rationale, technique and future prospects. *Br J Surg* 2001; **88**: 165-175 [PMID: 11167863 DOI: 10.1046/j.1365-2168.2001.01658.x]

55 **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]

56 **Schadde E**, Slankamenac K, Breitenstein S, Lesurtel M, De Oliveira M, Beck-Schimmer B, Dutkowski P, Clavien PA. Are two-stage hepatectomies associated with more complications than one-stage procedures? *HPB* (Oxford) 2013; **15**: 411-417 [PMID: 23458579 DOI: 10.1111/hpb.12001]

57 **Jaeck D**, Oussoultzoglou E, Rosso E, Greget M, Weber JC, Bachellier P. A two-stage hepatectomy procedure combined with portal vein embolization to achieve curative resection for initially unresectable multiple and bilobar colorectal liver metastases. *Ann Surg* 2004; **240**: 1037-149; discussion 1037-149; [PMID: 15570209]

58 **Clavien PA**, Petrowsky H, DeOliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. *N Engl J Med* 2007; **356**: 1545-1559 [PMID: 17429086 DOI: 10.1056/NEJMra065156]

59 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522 DOI: 10.1002/hep.510300629]

60 **Tang YH**, Wen TF, Chen X. Resection margin in hepatectomy for hepatocellular carcinoma: a systematic review. *Hepatogastroenterology* 2012; **59**: 1393-1397 [PMID: 22683956 DOI: 10.5754/hge10600]

61 **Shi M**, Guo RP, Lin XJ, Zhang YQ, Chen MS, Zhang CQ, Lau WY, Li JQ. Partial hepatectomy with wide versus narrow resection margin for solitary hepatocellular carcinoma: a prospective randomized trial. *Ann Surg* 2007; **245**: 36-43 [PMID: 17197963 DOI: 10.1097/01.sla.0000231758.07868.71]

62 **Llovet JM**, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 2005; **25**: 181-200 [PMID: 15918147 DOI: 10.1055/s-2005-871198]

63 **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]

64 **Reddy SK**, Tsung A, Geller DA. Laparoscopic liver resection. *World J Surg* 2011; **35**: 1478-1486 [PMID: 21181472 DOI: 10.1007/s00268-010-0906-5]

65 **Dagher I**, O'Rourke N, Geller DA, Cherqui D, Belli G, Gamblin TC, Lainas P, Laurent A, Nguyen KT, Marvin MR, Thomas M,

Ravindra K, Fielding G, Franco D, Buell JF. Laparoscopic major hepatectomy: an evolution in standard of care. *Ann Surg* 2009; **250**: 856-860 [PMID: 19806057 DOI: 10.1097/SLA.0b013e3181bcaf46]

66 **Berber E**, Akyildiz HY, Aucejo F, Gunasekaran G, Chalikonda S, Fung J. Robotic versus laparoscopic resection of liver tumours. *HPB* (Oxford) 2010; **12**: 583-586 [PMID: 20887327 DOI: 10.1111/j.1477-2574.2010.00234.x]

67 **Reggiani P**, Antonelli B, Rossi G. Robotic surgery of the liver: Italian experience and review of the literature. *Ecancermedicalscience* 2013; **7**: 358 [PMID: 24174991 DOI: 10.3332/ecancer.2013.358]

68 **Zarrinpar A**, Kaldas F, Busuttil RW. Liver transplantation for hepatocellular carcinoma: an update. *Hepatobiliary Pancreat Dis Int* 2011; **10**: 234-242 [PMID: 21669564]

69 **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]

70 **Llovet JM**, Bruix J, Fuster J, Castells A, Garcia-Valdecasas JC, Grande L, Franca A, Brú C, Navasa M, Ayuso MC, Solé M, Real MI, Vilana R, Rimola A, Visa J, Rodés J. Liver transplantation for small hepatocellular carcinoma: the tumor-node-metastasis classification does not have prognostic power. *Hepatology* 1998; **27**: 1572-1577 [PMID: 9620329 DOI: 10.1002/hep.510270616]

71 **Llovet JM**, Fuster J, Bruix J. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. *Liver Transpl* 2004; **10**: S115-S120 [PMID: 14762851 DOI: 10.1002/lt.20034]

72 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750 DOI: 10.1016/S0140-6736(03)14964-1]

73 **Zheng SS**, Xu X, Wu J, Chen J, Wang WL, Zhang M, Liang TB, Wu LM. Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008; **85**: 1726-1732 [PMID: 18580463 DOI: 10.1097/TP.0b013e31816b67e4]

74 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]

75 **Duffy JP**, Vardanian A, Benjamin E, Watson M, Farmer DG, Ghobrial RM, Lipshutz G, Yersiz H, Lu DS, Lassman C, Tong MJ, Hiatt JR, Busuttil RW. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg* 2007; **246**: 502-509; discussion 509-511 [PMID: 17717454 DOI: 10.1097/SLA.0b013e318148c704]

76 **Zheng Z**, Liang W, Milgrom DP, Zheng Z, Schroder PM, Kong NS, Yang C, Guo Z, He X. Liver transplantation versus liver resection in the treatment of hepatocellular carcinoma: a meta-analysis of observational studies. *Transplantation* 2014; **97**: 227-234 [PMID: 24142034 DOI: 10.1097/TP.0b013e3182a89383]

77 **Zimmerman MA**, Trotter JF, Wachs M, Bak T, Campsen J, Skibba A, Kam I. Sirolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2008; **14**: 633-638 [PMID: 18324656 DOI: 10.1002/lt.21420]

78 **Toso C**, Merani S, Bigam DL, Shapiro AM, Kneteman NM. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. *Hepatology* 2010; **51**: 1237-1243 [PMID: 20187107 DOI: 10.1002/hep.23437]

79 **Silva MA**, Hegab B, Hyde C, Guo B, Buckels JA, Mirza DF. Needle track seeding following biopsy of liver lesions in the diagnosis of hepatocellular cancer: a systematic review and meta-analysis. *Gut* 2008; **57**: 1592-1596 [PMID: 18669577 DOI: 10.1136/gut.2008.149062]

80 **DuBay D**, Sandroussi C, Sandhu L, Cleary S, Guba M, Cattral MS, McGilvray I, Ghanekar A, Selzner M, Greig PD, Grant DR. Liver transplantation for advanced hepatocellular carcinoma using poor tumor differentiation on biopsy as an exclusion criterion. *Ann Surg* 2011; **253**: 166-172 [PMID: 21294289]

81 **Nanashima A**, Sumida Y, Tobinaga S, Shibata K, Shindo H, Obatake M, Shibasaki S, Ide N, Nagayasu T. Postoperative changes in protein-induced vitamin K absence or antagonist II levels after hepatectomy in patients with hepatocellular carcinoma: relationship to prognosis. *HPB* (Oxford) 2006; **8**: 137-141 [PMID: 18333262 DOI: 10.1080/13651820500273475]

82 **Park H**, Park JY. Clinical significance of AFP and PIVKA-II responses for monitoring treatment outcomes and predicting prognosis in patients with hepatocellular carcinoma. *Biomed Res Int* 2013; **2013**: 310427 [PMID: 24455683 DOI: 10.1155/2013/310427]

83 **Chapman WC**, Majella Doyle MB, Stuart JE, Vachharajani N, Crippin JS, Anderson CD, Lowell JA, Shenoy S, Darcy MD, Brown DB. Outcomes of neoadjuvant transarterial chemoembolization to downstage hepatocellular carcinoma before liver transplantation. *Ann Surg* 2008; **248**: 617-625 [PMID: 18936575 DOI: 10.1097/SLA.0b013e31818a07d4]

84 **Lencioni R**. Loco-regional treatment of hepatocellular carcinoma. *Hepatology* 2010; **52**: 762-773 [PMID: 20564355 DOI: 10.1002/hep.23725]

85 **Bouza C**, López-Cuadrado T, Alcázar R, Saz-Parkinson Z, Amate JM. Meta-analysis of percutaneous radiofrequency ablation versus ethanol injection in hepatocellular carcinoma. *BMC Gastroenterol* 2009; **9**: 31 [PMID: 19432967 DOI: 10.1186/1471-230X-9-31]

86 **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]

87 **Meza-Junco J**, Montano-Loza AJ, Liu DM, Sawyer MB, Bain VG, Ma M, Owen R. Locoregional radiological treatment for hepatocellular carcinoma; Which, when and how? *Cancer Treat Rev* 2012; **38**: 54-62 [PMID: 21726960 DOI: 10.1016/j.ctrv.2011.05.002]

88 **Brown DB**, Gould JE, Gervais DA, Goldberg SN, Murthy R, Millward SF, Rilling WS, Geschwind JF, Salem R, Vedantham S, Cardella JF, Soulen MC. Transcatheter therapy for hepatic malignancy: standardization of terminology and reporting criteria. *J Vasc Interv Radiol* 2009; **20**: S425-S434 [PMID: 19560030 DOI: 10.1016/j.jvir.2009.04.021]

89 **Bruix J**, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S179-S188 [PMID: 15508083]

90 **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]

91 **Kulik LM**, Carr BI, Mulcahy MF, Lewandowski RJ, Atassi B, Ryu RK, Sato KT, Benson A, Nemcek AA, Gates VL, Abecassis M, Omary RA, Salem R. Safety and efficacy of 90Y radiotherapy for hepatocellular carcinoma with and without portal vein thrombosis. *Hepatology* 2008; **47**: 71-81 [PMID: 18027884 DOI: 10.1002/hep.21980]

92 **Salem R**, Lewandowski RJ, Kulik L, Wang E, Riaz A, Ryu RK, Sato KT, Gupta R, Nikolaidis P, Miller FH, Yaghmai V, Ibrahim SM, Senthilnathan S, Baker T, Gates VL, Atassi B, Newman S, Memon K, Chen R, Vogelzang RL, Nemcek AA, Resnick SA, Chrisman HB, Carr J, Omary RA, Abecassis M, Benson AB, Mulcahy MF. Radioembolization results in longer time-to-progression and reduced toxicity compared with chemoembolization in patients with hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 497-507.e2 [PMID: 21044630 DOI: 10.1053/j.gastro.2010.10.049]

93 **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]

94 **Saab S**, McTigue M, Finn RS, Busuttil RW. Sorafenib as adjuvant

therapy for high-risk hepatocellular carcinoma in liver transplant recipients: feasibility and efficacy. *Exp Clin Transplant* 2010; **8**: 307-313 [PMID: 21143097]

95 **Yoon DH**, Ryoo BY, Ryu MH, Lee SG, Hwang S, Suh DJ, Lee HC, Kim TW, Ahn CS, Kim KH, Moon DB, Kang YK. Sorafenib for recurrent hepatocellular carcinoma after liver transplantation. *Jpn J Clin Oncol* 2010; **40**: 768-773 [PMID: 20494947 DOI: 10.1093/jjco/hyq055]

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REVIEW

# Hepatocellular carcinoma: Surgeon's view on latest findings and future perspectives

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## Abstract

Hepatocellular carcinoma (HCC) is the most common liver-derived malignancy with a high fatality rate. Risk factors for the development of HCC have been identified and are clearly described. However, due to the lack of tumor-specific symptoms, HCC are diagnosed at progressed tumor stages in most patients, and thus curative therapeutic options are limited. The focus of this review is on surgical therapeutic options which can be offered to patients with HCC with special regard to recent findings, not exclusively focused on surgical therapy, but also to other treatment modalities. Further, potential promising future perspectives for the treatment of HCC are discussed.

**Key words:** Hepatocellular carcinoma; Surgical therapy; Interventional therapy; Multimodal treatment; Extended indications



**Core tip:** This review presents an overview on most important knowledge on hepatocellular carcinoma (HCC) for surgeons and describes the common surgical and non-surgical therapeutic options for the treatment of HCC. Further, a perspective on novel aspect and future decision aids is given.

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## EPIDEMIOLOGY

Hepatocellular carcinoma (HCC) is a very common malignant disease with more than 700000 new patients diagnosed per year. Interestingly, the incidence of HCC varies relevantly throughout the world. Whereas HCC is a very common malignant disease in sub-Saharan Africa, and central and south-east Asia with incidence rates of 20-47/100000 habitants, the incidence of

HCC is comparably low in developed western countries (incidence rate 2-6/100000 habitants)[1,2]. However, diagnosis of HCC is continuously increasing in the developed countries throughout the past decades[3]. There are data from the United States showing a tripling of HCC incidence during the recent 30 years[4]. Reasons for this increase are multifactorial as follows. Besides an increasing incidence of chronic hepatitis C in developed countries, improved treatment of liver cirrhosis and cirrhosis-associated complications causes a longer survival of these patients with consequently a longer possible time period to develop HCC in the cirrhotic liver. Furthermore, screening programs for patients at risk increase the rate of newly diagnosed HCC.

When analyzing the incidence-to-mortality ratio, HCC is the second most common cause of cancer-related death. In 2012, 782000 new cases and 746000 deaths due to HCC are reported worldwide[3,5]. Risk factors for the development of HCC are well known and clearly described. The major risk factor for development of HCC is liver cirrhosis: 80%-90% of patients autopsied for HCC display signs of cirrhosis[6]. Most common causes for cirrhosis and subsequent HCC are chronic infections with hepatitis B virus (HBV) and HCV[6], chronic alcoholic liver disease, and increasingly non-alcoholic fatty liver disease[7]. According to Parkin *et al*[8], more than 50% of HCC are associated to HBV infection worldwide. Interestingly, in case of HBV infection, development of cirrhosis is not a prerequisite for the development of HCC, as there are up to 29% of cases of spontaneous HCC in non-cirrhotic HBV-infected livers[7,9]. Furthermore, in patients with HCV infection, HCC is not exclusively based on HCV-associated cirrhosis, as up to 54% of patients can develop HCC without having cirrhosis[9]. The risk to develop HCC in alcoholic liver disease has also been clearly demonstrated to be relevantly elevated for daily ingestion of more than 60 g alcohol[10]. Hereditary liver diseases, such as Wilson's disease, hemochromatosis, alpha-1-antitrypsin deficiency, or autoimmune hepatitis play a minor role in the development of HCC. Interestingly, the geographic distribution of underlying diseases and risk factors for development of HCC varies, and also gender, and ethnic group display differences in the distribution of risk factors[7].

## DIAGNOSTICS

As classical tumor-associated symptoms are lacking, patients at risk with known chronic viral hepatitis benefit from screening and surveillance programs as recommended by the American Association for the Study of Liver Diseases and European Association for the Study of the Liver (EASL)-European Organization for Research and Treatment of Cancer (EORTC) practice guidelines[11,12]. The aims of surveillance programs are to detect HCC at early stages, enable the patient to obtain curative treatment, and thus reduce HCC-associated mortality. Actual EASL-EORTC Clinical Practice Guidelines recommend abdominal ultrasound every 6 mo in patients at risk[12]. Despite the advantage of cost-effectiveness and non-invasiveness, ultrasound has the disadvantage to be investigator-dependent, which compromises sensitivity. Thus, for dubious findings, additional diagnostics, such as contrast-enhanced ultrasound, computed tomography (CT) and magnetic resonance imaging offer examination tools investigating contrast agent dynamics in suspected nodules. HCC are classically characterized by an arterial hypervascularisation, thus showing typical hyperintense contrast agent accumulation in early arterial imaging phase and a washout phenomenon in portal venous imaging phase[3]. The significance for contrast enhanced ultrasound is uncertain according to the clinical practice guidelines, and nuclear imaging (PET-CT) in not appropriate for early diagnosis of HCC. Diagnosis of HCC is accepted if at least two complementary imaging techniques show classical features of HCC or HCC is proven by biopsy. This consensus is reflected by the Eurotransplant criteria for exceptional MELD application for HCC. Tumor markers are not recommended for screening routine due to the lack of sensitivity and the fact that especially early HCC do not express alpha-fetoprotein (AFP) in up to 40% of the cases[13,14]. Sensitivity and specificity of AFP are dependent on AFP serum levels[15]. According to the current EASL-EORTC and European Society for Medical Oncology clinical practice guidelines, AFP levels > 400 ng/mL are accepted to prove HCC[12,16]. Furthermore, AFP can be used as a progression parameter in case of AFP-expressing HCC after treatment, although serum AFP levels do not correlate with tumor size or tumor stage. Thus, the extent of AFP level does not allow any conclusions on the presence of metastases or vascular invasion, which might be helpful for the surgeon[17].

## PROGNOSIS

Prognosis for patients with HCC basically depends on the tumor stage at the time point of diagnosis, as defined by the barcelona clinic liver cancer (BCLC) classification system, as well as the fact whether the tumor is treated or not. The BCLC classification system stratifies HCC according to patients performance status, tumor size and number of nodules, tumor Okuda stage, and the presence or absence of liver function impairment and portal hypertension, and degree of cirrhosis as stratified by Child-Pugh score[18]. Cabibbo *et al*[19] recently reported outcome data from 320 patients with untreated HCC at different BCLC stages. Median survival in the entire cohort was 6.8 mo, whereas median survival rates ranged from 1.8 (BCLC D) to 33 mo (BCLC A). These data underline impressively the necessity for screening programs for patients at risk, since late diagnosis of HCC is associated with a very poor prognosis for these patients.

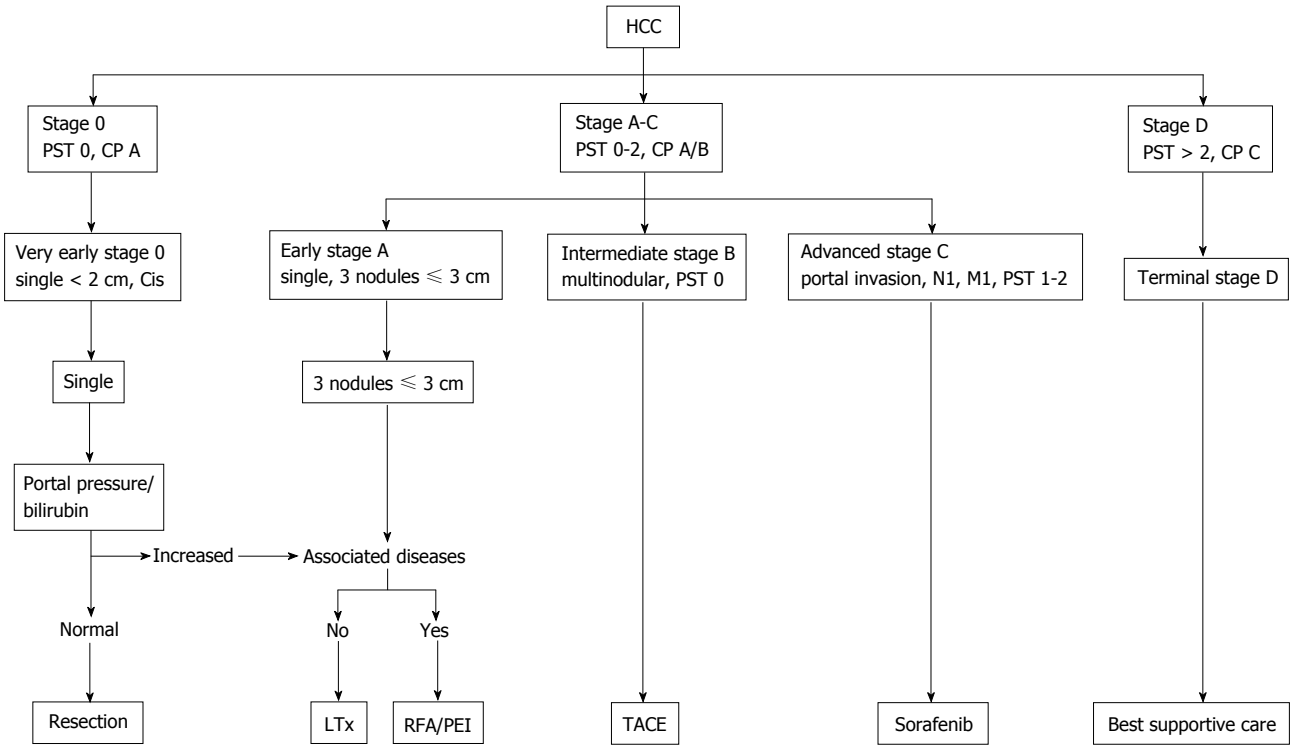


**Figure 1 Flow chart displaying the recommended treatment according to the barcelona clinic liver cancer tumor stage (adapted from European Association for the Study of the Liver-European Organization for Research and Treatment of Cancer Clinical Practice Guidelines).** CP: Child-Pugh; HCC: Hepatocellular carcinoma; PST: Performance status; Cis: Carcinoma *in situ*; LTx: Liver transplantation; RFA: Radiofrequency ablation; PEI: Percutaneous ethanol injection; TACE: Transarterial chemoembolization.

## TREATMENT MODALITIES

In general, treatment of patients with HCC is a multidisciplinary therapy approach. There are manifold treatment options which can be offered to our patients with HCC. The EASL-EORTC guidelines present a treatment algorithm which therapy is recommended to which patient, taking into account patient's performance status, Child-Pugh stage, as well as tumor diameter and number of nodules (Figure 1) as given by the BCLC status[12,18]. According to this recommendation, surgical approach for HCC is restricted to very early stages of HCC, *i.e.*, singular tumors with a diameter < 2 cm, and early stage HCC, *i.e.*, either a single tumor < 5 cm or 3 nodules each < 3 cm (Milan criteria[20]). For patients with contraindications for liver surgery or transplantation, thus considered not suitable for a surgical approach, should be treated with non-surgical procedures, *i.e.,* local ablative therapies, intravascular embolizing approaches, or palliative chemotherapy. Also, according to the guidelines, patients with advanced stages of HCC are considered not to profit from surgical resection of their respective tumor. The decision for the most suitable and success-promising approach for the respective patient must be defined in multidisciplinary tumor boards in which representatives of all specialist departments involved in HCC therapy including experienced hepatobiliary surgeons must be present. However, the restriction of surgery to very early and early stages of HCC is increasingly challenged with increasing evidence for broadening the indication for surgery.

### *Best supportive care*

As previously stated, expected survival for patients with advanced and terminal stage of HCC is very short, so that some patients actively decide not to undergo any palliative therapy due to their very limited life expectancy. For these patients, best supportive care and help through an ambulatory palliative care institution is reasonable, since patients lose relevant life expectancy under a palliative setting without any HCC-directed treatment[21,22]. Unfortunately, there are no data available on the benefit of best supportive care for these patients who decline any palliative treatment option. However, it is known that quality of life (QoL) is an independent predictor for survival in HCC patients[23], and thus it is conceivable that QoL improvement for these patients might be an effective strategy to optimize life expectancy without any tumor-directed intervention.

### *Systemic chemotherapy*

So far, there have been many efforts to develop effective drug treatment for HCC, either in the adjuvant setting after surgical tumor removal by liver resection or transplantation, or in the palliative setting. Unfortunately, there are no really seminal pharmacological approaches available, yet. The sole drug which has found the way into clinical practice is the multi-kinase inhibitor sorafenib[24,25]. In the setting of advanced or unresectable HCC, sorafenib

has been demonstrated to prolong median survival from 7.9 to 10.7 mo and median time to progression from 2.8 to 5.5 mo, respectively. Unfortunately, sorafenib treatment is recommended to patients with Child-Pugh A and stable B cirrhosis only[26], and is associated with distinct side effects. In particular gastrointestinal side effects, hemorrhages, exanthema, hand-foot-syndrome, and cardio-vascular symptoms, which often lead to treatment discontinuation or dose reduction are common[27]. For patients with advanced cirrhosis (Child-Pugh C), sorafenib therapy is not recommended due to the limited life expectancy caused by the cirrhosis stage, a lack of evidence concerning efficacy of sorafenib treatment in this patient subgroup, and to avoid severe side effects following impaired hepatic drug metabolism. In case of sorafenib treatment failure, oxaliplatin-based treatment regimens have been demonstrated to be a suitable second-line chemotherapy achieving progression free survival and overall survival of 4.2 and 9.3 mo, respectively, with an acceptable rate of side effects[28,29]. Furthermore, numerous clinical trials have investigated the use of a variety of tyrosine kinase inhibitors, mTOR inhibitors, VEGF receptor-, FGF receptor-, and PDGF-receptor-blocking multikinase tyrosine kinase inhibitors, as well as classical chemotherapeutic drugs, such as, *e.g.*, doxorubicin. Unfortunately, none of these trials has demonstrated a striking effect in a palliative or adjuvant treatment setting[30,31]. Despite the proven effect in the palliative setting, adjuvant systemic chemotherapy with sorafenib after liver resection for HCC showed no beneficial effects on recurrence-free survival in the current randomized, double-blind, placebo-controlled STORM-trial (NCT0069277)[32]. However, there are some promising results that interferon might improve overall and recurrence-free survival in the adjuvant setting[33,34]. But interferon therapy is also accompanied by a variety of side effects such as flu-like symptoms, fever, fatigue, myalgia, and cephalgia, which might limit the suitability of this treatment option in many patients. In conclusion, there is actually no drug for adjuvant treatment after surgical tumor resection.

### *Percutaneous interventions (radio frequency ablation, laser-induced thermo therapy, cryo, percutaneous ethanol injection, microwave ablation)*

According to the BCLC treatment algorithm, percutaneous tumor destruction by radio frequency ablation (RFA), or percutaneous ethanol injection (PEI) is indicated for early stages of HCC in patients which are not suitable for liver resection or liver transplantation[12]. Other percutaneous ablative techniques, such as laser-induced thermo therapy (LITT), cryotherapy, and microwave ablation (MWA) are actually not recommended in the EASL-EORTC clinical practice guidelines. This fact is due to the novelty of some of these techniques, with a consequent lack of evidence for the use and comparability of these techniques to the established and recommended techniques. However, these techniques will be presented briefly in the followings.

**RFA**: RFA is the most frequently used approach to destroy intrahepatic tumor masses with a diameter up to 5 cm by application of thermal energy into the tumor to induce thermal tumor necrosis. It is recommended by the EASL-EORTC guidelines for early-stages of HCC in patients who are not eligible for surgery or transplantation. According to an actual systematic Cochrane Database review[35], there is moderate evidence, that RFA is superior to percutaneous ethanol injection[36], but inferior to hepatic resection of HCC[37,38] with regard to recurrence-free and overall survival. In contrast, RFA is superior to hepatic resection with regard to procedure-related complications due to the less invasive character of the procedure[39]. Besides definite treatment of HCC in patients who are not eligible for surgery or liver transplantation, RFA can be performed both percutaneously and with a laparoscopic approach. RFA is a major bridging therapy option for patients on the waiting list for liver transplantation. Due to the very limited approach during RFA, this procedure can be performed repeatedly without causing severe intraabdominal adhesions, and thus RFA does not complicate subsequent liver transplantation[40]. Additionally, Huang *et al*[41] could demonstrate in a non-randomized prospective parallel cohort study comparing RFA and liver resection for small HCC < 2 cm, that RFA is well tolerated by patients, and impairs health-related quality of life significantly less than liver resection.

**LITT:** LITT plays obviously only a minor role, since there is only one actual large report on LITT experience in 113 HCC patients reporting both an excellent tumor response in small lesions < 2 cm after a single LITT treatment, and also in larger tumors up to 5 cm after repeated LITT sessions, with favorable 5-year survival rates of 30%[42]. Besides this report, there are only few publications with small patient cohorts using LITT for treatment of HCC. Randomized studies comparing different ablation techniques are missing in the literature. Thus, LITT seems to play a minor role among the percutaneous intervention options when compared to RFA or PEI.

**Cryotherapy**: The evidence for the use of cryotherapy for treatment of HCC is limited. There is only one systematic Cochrane Database review by Awad *et al*[43], who finish their analysis with a quite ambiguous conclusion, in that there is not enough evidence so far in favor or against cryotherapy[43]. However, there are some single center reports in the literature reporting excellent, size-dependent tumor ablation rates and even 10-year survival rates of approximately 9% in patients with cirrhosis-based HCC[44] with acceptably low procedure-related complication rates[44,45]. Interestingly, tumor response to cryotherapy as assessed by the

modified Response Evaluation Criteria in Solid Tumors criteria[46] has been demonstrated to be an independent predictor of overall survival for HCC[47]. However, in contrast to the use of cryotherapy for the treatment of colorectal liver metastases[48], cryotherapy is not used routinely for the treatment of unresectable HCC. The main reason for this is certainly the advanced and expensive technique with multiple possible pitfalls due to the use of liquid nitrogen. To the author's knowledge, there is also no ongoing trial comparing cryotherapy to the established interventional procedures. Thus, evidence in favor or against the use of cryotherapy will be owing, and some authors even expect an end of the use of cryotherapy[49]. In contrast, as stated by Awad *et al*[43], evidence is insufficient "to recommend or refute cryotherapy" to patients with HCC[43], and indication for cryotherapy is finally based on the experience and expertise of the respective treating physician.

**PEI**: PEI is an alternative very low-priced method to apply pure ethanol directly to the targeted tumor. However, the efficacy of this method is limited by the fact that ethanol spread within the tumor tissue might be altered by septa or a tumor capsule and equable ethanol distribution within the tumor is not warranted. In two recent studies from eastern Asia, PEI has been demonstrated to achieve inferior results compared to RFA[36,50], which might be attributable to an inferior rate of tumor response (or vice versa increased treatment failure) using PEI and thus resulting in decreased survival rates[36]. Accordingly, in the EASL-EORTC guidelines, PEI is recommended for small HCCs (BCLC 0 or A), and especially in cases of larger tumors up to 5 cm when RFA is not feasible due to technical reasons, as, *e.g.*, subcapsular tumor localization or adjacent to the gallbladder, to large vessels or the hepatic hilum[12,51].

**MWA**: According to the actual EASL-EORTC guidelines, microwave ablation is not generally recommended and remains to be evaluated. The advantage of MWA compared to RFA is that treatment efficacy is affected in a lesser degree by the cooling effect of large blood vessels located in the proximity of the ablation area. Recently, Zhang *et al*[52] demonstrated comparable results for MWA and RFA with regard to overall survival, local progression, and the degree of local tumor ablation for HCC < 3 cm[52]. Additionally, MWA shows the same frequency of post-ablation syndrome, *i.e.*, occurrence of low-grade fever, nausea, vomiting, malaise, and post-interventional pain[53] as RFA[54]. In an actual multicenter study, Groeschl *et al*[55] demonstrated excellent complete tumor ablation rates of approximately 94% as assessed by histology after resection of the respective pre-treated tumors, with a reported median recurrence-free survival of 25 mo. Taking into account, that MWA is by far more cost-effective compared to RFA[56], and is associated with a low complication rate of approximately 11%[57], MWA might be a promising alternative ablative technique, which even might replace RFA as standard treatment for unresectable HCC. From the surgical point of view, MWA seems not to have relevant influence on complication rate after resection of the MWA pre-treated HCC[55], but so far there are too few data to state whether neo-adjuvant MWA prior to HCC resection is really a suitable approach and will be of benefit for the patients. Equally, there is no evidence yet, whether MWA or RFA is superior in a neo-adjuvant setting with regard to overall and recurrence-free survival, as well as resection-related morbidity. This remains to be elucidated in prospective, randomized trials.

### *Intravascular approaches (transarterial chemoembolization, selective internal radiotherapy)*

According to the EASL-EORTC guidelines, transarterial chemoembolization (TACE) is recommended for intermediate stages of HCC[12]. In contrast, selective internal radiotherapy (SIRT; synonymous: radioembolization) is a comparatively novel approach for intravascular tumor-directed therapy, which can also be used for intravascular treatment of HCC. SIRT is not recommended in the EASL-EORTC-guidelines, due to the lack of data from randomized clinical trials comparing SIRT and TACE in intermediate stages of HCC.

**TACE**: The rationale for TACE is, that intrahepatic malignancies and especially HCC are almost exclusively nourished *via* the hepato-arterial vasculature. Consequently, a catheter device is placed *via* femoral artery into the tumor-supplying branch of the proper hepatic artery, and a combination of high-dose chemotherapy and vessel occluding agents are applied selectively to the tumor area, whereas non-tumorous liver areas remain basically unaffected. As reviewed by Marelli *et al*[58], a huge variety of chemotherapeutic drugs is used, whereas doxorubicin is the most common agent. This chemotherapeutic drug is usually applied in combination with lipiodol as a carrier which offers the beneficial effect that is can be visualized by X-rays, and persists for several weeks in the liver after administration[59]. Also for vessel occlusion, a variety of embolization agents is available, whereas gelatin sponge particles, and polyvinyl alcohol particles are the most commonly used embolizing agents[58].

Besides its recommendation in the EASL-EORTC guidelines for treatment of intermediate stage HCC, TACE is also widely used as a palliative treatment approach. Interestingly, according to a recent Cochrane Database review, there is no evidence for the use of TACE for treatment of unresectable HCC[60,61]. However, due to overall heterogenous data from retrospective, and also prospective studies and the previously demonstrated capacity of TACE to prolong survival for patients with unresectable HCC in two randomized clinical trials[62,63] as well as some older reviews[64,65], TACE has been included in the EASL-EORTC guidelines and still represents the golden treatment standard for patients with unresectable intermediate stages of HCC according to the BCLC staging algorithm[12]. The

recently published Canadian clinical recommendations also state that TACE probably provides benefit and thus also recommend TACE for patients with intermediate stages of HCC[66].

Furthermore, TACE is used regularly as a bridging therapy for patients with HCC on the waiting list for liver transplantation. However, there is no distinct evidence for the effectiveness of TACE to prevent patient drop-out from waiting list during the waiting time for transplantation[67] and to improve post-transplant overall and recurrence-free survival[68,69]. There is also evidence, that response of HCC to pre-transplant TACE can be an indicator for favorable outcome after liver transplantation with regard to delayed tumor recurrence[70]. In a study by Sotiropoulos *et al*[71], TACE even failed to reproducibly down-stage multifocal HCC or to induce entire tumor necrosis, and provided at best an "acceptable" tumor control[71]. However, the same group demonstrated, that response to TACE and TACE-induced complete tumor necrosis at the time point of liver transplantation is associated with a very low recurrence rate and improved survival after liver transplantation[68,72] thus establishing an indication for pre-transplant TACE. At least, TACE can be safely performed in patients with cirrhosis and hyperbilirubinemia who are on the waiting list for liver transplantation[73] without increasing the risk for complications during transplantation.

TACE has also been investigated as a neoadjuvant treatment option prior to liver resection for resectable HCC. There is one actual meta-analysis that demonstrates that preoperative TACE does not effectively influence postoperative overall and recurrence-free survival respectively. Furthermore, preoperative TACE has no effect on intra- or extrahepatic tumor recurrence, and is therefore not recommended as a preoperative strategy for resectable HCC[74]. Interestingly, there are some reports that adjuvant TACE, *i.e.*, after curative resection of HCC might offer benefits with regard to disease-free and overall survival[75].

**SIRT**: In contrast to TACE, SIRT uses Yttrium 90 ($^{90}$Y)-loaded glass microspheres which are applied *via* a trans-arterial catheter system into the tumor supplying arterial vasculature. $^{90}$Y decays to zirconium ($^{90}$Zr) with a physical half-life of approximately 65 h. During this decay process, an average energy of approximately 0.94MeV is emitted. $^{90}$Y is a pure β radiation emitter. The corresponding β radiation penetrates into the surrounding tissue with a depth of a maximum of 11 mm, leading to tissue destruction and fibrosis[76].

So far, SIRT has not been widely used for the treatment of resectable or unresectable HCC. There are only very few studies investigating the potential beneficial effect of this treatment modality. There is a very recent review article by Sangro *et al*[77], who compared published outcome data from patients after SIRT for unresectable HCC. When comparing treatment efficacy of SIRT to TACE or systemic sorafenib therapy, median overall survival rates are comparably between SIRT and the other treatment modality, respectively, especially for intermediate or advanced stages of HCC[78]. SIRT has been reported to be a safe treatment option with a frequency of the so-called post-radioembolization syndrome (fatigue, nausea, vomiting, anorexia, fever, abdominal pain) of 20%-55%[79]. However, large prospective trials comparing SIRT *vs* TACE are lacking, yet, and also cost-effectiveness analyses for $^{90}$Y radioembolization are lacking[79]. Besides its use as a definite treatment option for patients with otherwise non-resectable HCC, SIRT has also been used as a bridging therapy prior to liver transplantation[69,80]. The emerging role of SIRT, both as a definite treatment modality as well as a curative or a bridging therapy option has been elaborated in detail in a review article by Lau *et al*[81]. The authors also admit, that evidence for the use of SIRT in the respective intention is quite low, due to the short time of investigation so far and the scarcity of studies investigating a potential role of SIRT in concurrence to established treatment modalities for HCC patients. However, in a recent study by El Fouly *et al*[82], SIRT has shown equivalent survival probabilities, less hospitalizations, less treatment sessions and a lower complication rate in patients with intermediate stage B of HCC when compared to TACE[82].

### *Radiotherapy*

Whereas radiotherapy for treatment HCC has been considered inappropriate for a long time due to severe radiation-associated complications and liver failure, more than 600 articles have been published within the recent 5 years on this topic. This enthusiasm can be attributed to the development of novel radiotherapy technologies during the recent decade, which now allow precise application of high-dose radiation to the tumor tissue while sparing the rest of the liver and adjacent organs.

This has led to the more widespread use of radiotherapy for the treatment of HCC, especially in patients with unresectable tumors. In 2008, Tse *et al*[83] published their results on stereotactic body radiotherapy for patients with unresectable HCC proving the safety of this treatment option. Finally, due to the possibility to effectively use radiotherapy in advanced stages of liver cirrhosis (Child-Pugh B/C) the use of radiotherapy has been implemented in HCC treatment guidelines from the Korean Liver Cancer Study Group and the National Comprehensive Cancer Network. But despite the proven efficacy and safety, radiotherapy has not found its way into the EASL-EORTC guidelines, yet. However, Jihye *et al*[84] proposed a possible way of integration of radiotherapy in the BCLC guidelines. Another advantage of radiotherapy is the possibility of combination therapy with established treatment options, such as TACE[85,86] or systemic chemotherapy[87] although side effects and hepatic toxicity are critical limitations for this treatment approach. Perspectively, there are some promising experimental data on the possibility to induce radiosensitization of HCC cells using the aurora kinase

inhibitor VE-465 potently suppressing tumor growth and enhancing tumor-responsiveness to radiotherapy[88]. Thus, radiotherapy opens new therapeutic options but its use is currently limited to a scarce comprehensive availability of this therapy option.

### *Surgical treatment options*

**Liver resection:** The surgical approach to HCC represents the only treatment option which allows entire and reliable removal of the tumor from the patient and therefore the potential of cure. Principally, there are two surgical concepts for the treatment of HCC: liver resection and liver transplantation. Latter offers the additional benefit that underlying liver disease which nourishes development of further malignancies in sense of precancerosis, is also removed. The disadvantage of liver transplantation is - despite all progresses and technical improvements of this procedure - the higher mortality risk when compared to liver resection as well as the necessity for life-long immunosuppression with all associated side effects.

When dealing with liver resection, two principle questions have to be discussed: whether to remove the HCC by anatomic or non-anatomic/atypical resection, and to perform this procedure open or laparoscopically.

The surgical approach is limited by the mandatory need to maintain sufficient functional liver remnant. In non-cirrhotic patients, maximum extent of resection can be calculated by the remnant liver volume - body weight ratio (RLV-BWR). Several reports have demonstrated that patients with a RLV-BWR $\leqslant$ 0.5%-0.8% are at high risk for postoperative hepatic dysfunction and increased mortality[89-91]. Another approach to indicate limit for liver resection is the future liver remnant (FLR), referred to the total liver volume. The safe limit is considered to be at a FLR of > 20% in patients with healthy livers[92,93]. In cirrhotic patients, parenchymal functional and regenerative capacities are relevantly reduced. Consequently, a RLV-BWR $\geqslant$ 1.4% or a FLR of at least 30%-40% are considered as critical threshold for development of postoperative complications[92,94].

Thus, from the surgical point of view, contraindications against liver surgery for HCC might be considered only to be the insufficient future liver remnant after resection. Worse prognostic factors for the outcome are vascular invasion[95], infiltration of adjacent organs[96], and presence of lymph node metastases[97] at the time of diagnosis, but represent no contraindications against surgery *per se*. Resection of HCC with these degrees of tumor extent can be safely resected (when respecting resection limits), and survival after resection is for sure improved when compared to best supportive therapy or palliative chemotherapy approaches.

### *Anatomic vs atypical resection*

There has been an intense debate whether anatomic or atypical resection for HCC should be preferred. Rationale for atypical resection was the idea of parenchymal-sparing surgery with an as marginal as possible loss of - in most cases - functionally altered liver parenchyma. This idea of parenchymal-preserving liver surgery is based on the limited possibilities to assess functional liver reserve after liver resection, and the fear to induce postoperative liver failure due to a too aggressive resection extent[98]. In contrast, anatomic liver resection is rationale since it is known that HCC spread along the nourishing portal venous branch distributing satellite nodules within the same anatomical segment. Thus, anatomic resection allows removal of the known tumor, as well as of potential undetectable satellite metastases[99]. Meanwhile, there is strong evidence that anatomic resection is superior to non-anatomic, *i.e.*, atypical resection for HCC. As evaluated by several meta-analyses, anatomic resection is associated with improved survival rates, and delayed intrahepatic and systemic disease-recurrence with no differences regarding perioperative morbidity or mortality[100-102]. Interestingly, one recently published meta-analysis by Tang *et al*[103] could not demonstrate superiority of anatomic resection, but this seems to be explainable by the different trial selection compared to previous analyses. Thus, a general recommendation for anatomical or non-anatomical resection cannot be given. Decision on the extent of liver resection is based on the tumor location within an anatomical segment. Based on the available data, anatomic resection should be performed in non-cirrhotic livers. In patients with cirrhotic livers, potential oncologic disadvantage of a non-anatomic resection has to be accepted with regard to the necessity for maintenance of sufficient functional liver remnant volume and function.

### *Open vs laparoscopic*

Since the first description of laparoscopic liver resection in 1992 by Gagner *et al*[104] performing a non-anatomic liver resection for focal nodular hyperplasia, there has been an enormous increase of laparoscopic liver resections with increasing extent of resections up to right hemihepatectomies[105], and more and more complex procedures, such as tumor resection in the postero-superior segments (Ⅶ, Ⅷ, Ⅳa)[106,107]. Also, in the treatment of HCC, laparoscopic approaches have been established. But there are so far only few specialized centers worldwide reporting on laparoscopic liver resections for the treatment of HCC.

According to several meta-analyses comparing open *vs* laparoscopic liver resections for HCC, laparoscopic liver resections are a safe procedure with comparable overall and recurrence-free survival rates[108]. Laparoscopic liver resections are associated with reduced intraoperative blood loss and subsequent requirement for packed red blood cells. Furthermore, laparoscopic liver resections are effective to provide negative resection margins, and are associated with shorter hospitalization and less postoperative complications[108,109]. As the findings could also be observed for patients with cirrhosis, laparoscopic liver resection can be safely applied to patient with resectable HCC. However, extensive experience is

necessary especially for more complex procedures, and thus, this procedure is reserved to specialized centers. With increasing experience, the extent of liver resection and localization of HCC within the liver will become more and more secondary, and only assessment of liver functional reserve as estimated predominantly by liver volumetry in western centers, and by indocyanine green excretion dynamics in eastern centers will determine the limits for laparoscopic liver resection, as it is also the case for open liver resections[110,111]. A recent review article analyzing major laparoscopic hepatectomies independent from HCC, could include a total of 29 studies from 1998 to 2011 with a total of more than 2600 patients, underlining the fast expansion of laparoscopy for liver resection[112]. However, learning curve for laparoscopic liver surgery is very flat, as demonstrated by Vigano *et al*[113] or Dagher *et al*[114], showing that at least 60 to 90 laparoscopic liver resections are necessary to perform for a surgeon, before a state of experience and standard is achieved[115]. Since laparoscopic liver resection for HCC has predominantly to be performed in cirrhotic livers, learning curve is expectedly even flatter than in non-cirrhotic livers[116]. However, meanwhile development of surgical technique is progressing, and the first reports of robotic liver resections for HCC are published. The largest study including 41 patients reports excellent morbidity and mortality rates of 7% and 0%, respectively, as well as 2-year overall and disease-free survival rates of 94% and 74%, respectively[117]. Thus, technological progress also finds its way into liver surgery with promising first results and experiences[118], providing more possibilities for further development and improvement of treatment options and thus the prognosis for our patients with HCC.

**Liver transplantation:** First human liver transplantation was performed at the University of Colorado in 1963 by Starzl *et al*[119]. The first successful liver transplantation was performed 4 years later again by Starzl *et al*[120] in a girl suffering from HCC. Since that time, this former experimental treatment option has developed to a widespread, highly standardized and successful therapy. Whereas the girl died 13 mo after transplantation due to tumor recurrence, patients undergoing liver transplantation for HCC have nowadays an excellent survival prognosis with 10-year survival rates of 50%[121]. These excellent outcome results have been achieved after implementation of the so-called Milan criteria described by Mazzaferro *et al*[20] in 1996. The Milan criteria indicate a benefit for patients undergoing liver transplantation for HCC under definite circumstances: one single nodule with a size up to 5 cm, or two or three nodules each up to 3 cm without signs of lymph node metastases and vascular invasion. Currently, these criteria which have been validated prospectively several times[122] are the selection basis for our patients for liver transplantation. However, these criteria are challenged repeatedly by attempts to expand the selection criteria for liver transplantation. For example, patients selected to the so called University of California, San Francisco (UCSF) criteria which contain larger size limits for the respective tumor nodules (single tumor < 6.5 cm, maximum of 3 total tumors with none > 4.5 cm, and cumulative tumor size < 8 cm)[123] show comparable outcome results to patients who were selected according to the Milan criteria[124]. Mazzaferro *et al*[125] themselves challenged their own "traditional" Milan criteria by the new Milan criteria, also called up-to-seven criteria. These criteria comprise patients with HCC in which the sum of diameters (in cm) and of the number of all HCC nodules is equal or less than seven. They showed that patients with tumor dimensions within these up-to-seven criteria had similar 5-year overall survival rates as patients with HCC within the "traditional" Milan criteria[125]. However, application of the up-to-seven criteria requires a careful patient selection. Besides these two probably most famous extended criteria of eligibility for liver transplantation for HCC, there is a multitude of further criteria with different limits for maximum tumor size of number of tumor nodules. Recently, these criteria have been reviewed, and Chan *et al*[126] demonstrate in their review article that these several stratification criteria yield quite similar overall survival rates[126] leading to postulations to extend criteria for liver allocation for HCC patients on the waiting list. However, the attempts to extend eligibility criteria are counteracted by the constricted availability of donor organs, as also stated by Mazzaferro *et al*[122] himself.

To overcome the lack of post-mortal donor organs, transplantation of partial liver grafts has been developed and advanced. The technique was first described by Pichlmayr *et al*[127] in 1989, and the first series of successful split liver transplantations has been published by Broelsch *et al*[128] in 1990. Approximately at the same time, transplantation of grafts from living donors has been developed[129,130]. However, one could assume, that implantation of a partial liver graft with subsequent liver regeneration to the extent of the recipient's demand might represent a massive systemic proliferative stimulus[131], which might - in combination with immunosuppression-induced attenuated tumor-defense[132,133] - enhance tumor cell proliferation and promote early disease recurrence. Interestingly, a recent meta-analysis showed that there is no difference in recurrence-free and overall survival for patients after liver transplantation using living-donated partial liver grafts compared to deceased donor grafts[134]. Thus, living donor liver transplantation represents a safe method for HCC treatment, especially with the advantage, that allocation is not performed by the central allocation authorities (*e.g.*, Eurotransplant), and not limited by tumor size criteria (*e.g.*, Milan criteria).

### *Transplantation vs liver resection*

According to the EASL-EORTC guidelines, there is a clear separation between the indications for liver resection and liver transplantation for HCC, respectively. However, it is a legitimate question, whether resection of HCC

which should be treated with transplantation is equally effective, and *vice versa* if prognosis of patients treated by resection might be improved by transplantation. This is relevant in two ways. First, resection of HCC in patients which should be treated by transplantation would preserve the scarce resource donor organ, and second, transplantation instead of resection abolishes not only the main tumor, but also possible undetected additional small tumors, and removes the diseased liver which is the nutrient medium for further HCC development.

In 2013, there was a Cochrane analysis by Taefi *et al*[135], which concluded without any clear results due to a lack of appropriate studies. However, there are four additional meta-analyses investigating the superiority of any of these two treatment options. In mutual agreement, all publications demonstrate superiority for liver transplantation for the treatment of HCC at the early BCLC stage A[136-138] or AJCC stage Ⅰ and Ⅱ HCC[139]. In contrast, liver resection has been demonstrated to yield similar outcome results compared to liver transplantation in patients with incomplete cirrhosis (Ishak score 0-4), thus for these patients, liver transplantation should be avoided in this subgroup of patients[139]. Unfortunately, there are nor studies comparing liver resection and liver transplantation for patients with Child B or Child C cirrhosis. However, one might speculate that these patients might benefit from liver transplantation due to the fact that transplantation reliefs both tumor and life-limiting diseased liver.

### *Surgical approach vs interventional tumor therapy*

As stated above, the EASL-EORTC guidelines represent a recommendation for HCC treatment and assign to each tumor stage a recommended - and thus assumingly best - treatment modality. In the decision algorithm, surgery is only recommended for very early and early stages of HCC (Figure 1). However, there are comparative studies investigating the value of surgical approaches for patients with more advanced tumor stages.

There is a recent meta-analysis with approximately 21000 patients which clearly demonstrates superiority of surgical resection over RFA and PEI in early stages of HCC[38], which is according to the guidelines a domain of transplantation or RFA. These findings are confirmed by a meta-analysis by Xu *et al*[140] also showing a significantly improved survival benefit for patients with early stages of HCC undergoing surgery instead of RFA.

When trying to further expand indication for hepatic surgery towards HCC stage B (intermediate stage), one has to compare the outcome results from surgery and TACE. So far, there are no meta-analyses available comparing this issue. There are several very recent publications investigating the role of surgery for HCC stage B. There is one prospective randomized controlled trial comparing TACE and surgery. The authors can clearly demonstrate a survival benefit for patients undergoing liver resection independent of the performance status[141]. These findings are underlined by data from two retrospective analyses enrolling together approximately 1300 patients[142,143] demonstrating a long-term survival benefit for patients undergoing hepatic resection for HCC stage B.

### *Perspectives*

**Systemic chemotherapy?** So far, advance for systemic chemotherapy for HCC have been very disappointing. Even the gain in survival for patients treated with sorafenib, the only agent which has made its way into clinical use in palliative situations, is not groundbreaking[24,25]. In the adjuvant setting, sorafenib has failed to provide beneficial effects in a randomized, double-blind, placebo-controlled phase Ⅲ study (STORM trial). As very recently reviewed in detail by Germano *et al*[144], a multitude of approaches for systemic HCC therapy has failed to show efficacy in the adjuvant or palliative situations, respectively. However, there are many other promising agents, and with the increased understanding of HCC cancerogenesis and cancer-related signaling pathways, molecular targeted therapy is the hope for some breakthroughs in the near future. For example, there are two recent reports for the safety and efficacy of the MET receptor tyrosine kinase inhibitor tivantinib which show promising results[145,146]. The reader might be referred to the excellent article by Germano *et al*[144] for further detailed information.

**Expanding criteria for liver transplantation?** So far, liver transplantation is accepted as a curative treatment strategy for patients with a very early stage (0) of HCC who are not suitable for liver resection due to impaired excretory liver function or portal hypertension, and for patients with early stage (A) HCC without concomitant diseases. Since the groundbreaking work by Mazzaferro *et al*[20] in 1996 defining tumor character limits associated with excellent patient outcome after liver transplantation for HCC, these size limits have been integrated in most guidelines (Eurotransplant, German Bundesärztekammer…). Since other tumor size criteria (*e.g.*, UCSF, up-to-seven) have been demonstrated to show comparable overall and recurrence-free survival rates as the Milan criteria, there is an ongoing intense discussion on the extension of the very strict and limiting Milan criteria towards expanded criteria. However, this will be a relevant matter of debate since expansion of the recipient criteria might lead to a still increasing demand and consumption of the "scarce resource donor organ" and a continuative withdrawal of urgently needed donor organs to patients with other indications for liver transplantation.

**Expanding indications for surgery beyond the BCLC criteria?** Up to now, resection as most favorable treatment option is only recommended for the very early stage (0) of HCC in patients with normal portal venous pressure and normal serum bilirubin. However, as reviewed in detail by Guglielmi *et al*[147], the rigid

EASL-EORTC limits for liver resection can be expanded to more advanced tumor stages. Tumor size criteria from EASL-EORTC guidelines are not considered as limits for hepatic surgery for example in eastern countries, and especially portal hypertension is not an obstacle against surgery in well selected patients. Thus, acceptable morbidity and mortality rates after hepatic resection in patients with cirrhosis and portal hypertension have been reported which are comparable to those of patients without portal hypertension[148-150].

When expanding the criteria for surgical resection, outcome results of hepatic surgery have to be compared to those of the respective treatment modality recommended by the EASL-EORTC guidelines.

When comparing liver resection *vs* RFA (for patients with stage A HCC who are not suitable for transplantation), there are several meta-analyses showing that hepatic resection is superior to RFA (or PEI) for HCC with regard to recurrence-free and overall survival[38,140,151,152]. Whereas this superiority of surgery is accepted especially for tumors with diameters > 3 cm, there are some contradictory results for small HCC with a diameter < 3 cm. Interestingly, a cost-effectiveness analysis by Cucchetti *et al*[153] demonstrated that liver resection for a HCC of 3-5 cm is more cost effective than RFA, whereas in patients with two or three tumor nodules each < 3 cm RFA is superior with regard to cost-effectiveness. Based on these insights, indication for liver resection might be extended to early stages of HCC.

For the intermediate stage of HCC (BCLC B), guidelines recommend TACE as local therapy for tumor control with an expected median overall survival of 20 mo. There are only few data available for this subgroup of patients. But there are data from two retrospective analyses[143,154] and one prospective non-randomized study[155] which clearly demonstrate that in patients with stage B of HCC with preserved liver function liver resection provides an improvement of overall survival rate when compared to TACE. However, the evidence for this is still limited, since there are no meta-analyses available, yet.

When expanding indication for surgery by another step, outcome of patients with advanced stages (stage C) of HCC with portal invasion, lymph node metastases or distant metastases, have to be compared between surgery and palliative systemic chemotherapy. The critical point is for sure the presence or absence of portal infiltration, since macrovascular invasion is known to be one of the most reliable predictors of poor prognosis. However, there are some data showing even for patients with macrovascular invasion a survival benefit after surgery compared to palliative systemic therapy[156]. However, this approach requires patient selection, and achievement of high 5-year survival rates[157] can certainly not generalized in a situation of advanced stages of HCC. But in contrast, macrovascular invasion per se has been demonstrated to allow surgical approach and thus offering a chance of improved survival to patients with otherwise severely limited prognosis[156,158].

Thus, based on the findings cited above, the rigid limits of the EASL-EORTC guidelines will be expanded slowly but constantly. In East Asian treatment algorithms, surgery is of paramount importance even for tumor stages beyond BCLC 0 and A[159]. The non-inferiority or even superiority of surgery compared to the recommended treatment modalities is most probably due to the rapid development of surgical techniques, a more profound understanding of liver anatomy and physiology, improved methods for preoperative risk assessment and liver functional remnant estimation, as well as improved intensive care regimes which overall helped surgery to a striking progress and improvement of patient safety and outcome. Ongoing research and further development in this field of research will still promote the advance of surgery, which will most probably be the most exciting and promising perspective for future HCC therapy.

**Significance of HCC-causing underlying disease?**

There are numerous studies investigating chromosomal aberrations in HCC[160]. When comparing the patterns of chromosomal aberrations in HCC on the basis of different underlying diseases, remarkable differences in the frequency of aberrations have been demonstrated[161]. Thus, one might speculate that different underlying hepatic diseases leading to development of HCC might be associated with different degrees of chromosomal instability in the diseased liver parenchyma. Consequently, underlying disease might also be associated with the pattern of chromosomal aberrations in the respective HCCs and thus might also determine the prognosis of the patient by determining the dynamics of tumor recurrence. Indeed, a recently published large Japanese study including approximately 11950 patients after curative resection of HCC showed that patients with viral hepatitis B or C as underlying disease had a significantly worse overall and recurrence-free survival when compared to patients with non-viral underlying hepatic diseases[162]. This might be of increasing interest in the near future, since patients with different underlying diseases might require a differently close-mesh aftercare.

## CONCLUSION

Since striking medical breakthroughs for the effective curative non-surgical treatment of HCCs are lacking, surgery will play a pivotal role in the multidisciplinary management of patients with HCC in the future. Up to now, surgical treatment is the only therapeutic option that can offer cure to the patient. Even for patients with cirrhosis as a kind of "precancerosis", liver transplantation offers - within the respective given legal framework - the opportunity to relieve the patient both from the tumor burden and the tumor-favoring disease. Whether a patient can be subjected to liver surgery for HCC and will profit from surgery has to be evaluated

carefully in a multidisciplinary context, to offer both best benefit to the patient, minimal risk for complications and procedure-related mortality, as well as best quality of life.

From a surgeon's point of view, surgery is the central treatment option with regard to tumor treatment, and is thus the most effective therapy to allow the best overall survival and recurrence free survival to patients. Surgery will further gain importance since there are emerging insights that indications for liver resection for HCC can be expanded to tumor staged beyond the actual recommendations. Additional treatment options are both valuable and continuously improving tools for patients who are unsuitable for surgery, and might also further gain importance in multimodal settings with possible perioperative use of percutaneous, intravascular, or pharmacological approaches.

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## REFERENCES


1 **Bolondi L**. Screening for hepatocellular carcinoma in cirrhosis. *J Hepatol* 2003; **39**: 1076-1084 [PMID: 14642630]
2 **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]
3 **Dhanasekaran R**, Limaye A, Cabrera R. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis, and therapeutics. *Hepat Med* 2012; **4**: 19-37 [PMID: 24367230 DOI: 10.2147/HMER.S16316]
4 **National Cancer Institute**. Surveillance, Epidemiology, and End Results Program. Available from: URL: http://www.seer.cancer.gov/faststats
5 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
6 **Simonetti RG**, Cammà C, Fiorello F, Politi F, D'Amico G, Pagliaro L. Hepatocellular carcinoma. A worldwide problem and the major risk factors. *Dig Dis Sci* 1991; **36**: 962-972 [PMID: 1649041]
7 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124]
8 **Parkin DM**. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006; **118**: 3030-3044 [PMID: 16404738]
9 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101]
10 **Donato F**, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, Decarli A, Trevisi P, Ribero ML, Martelli C, Porru S, Nardi G. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol* 2002; **155**: 323-331 [PMID: 11836196]
11 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051]
12 **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]
13 **Sturgeon CM**, Duffy MJ, Hofmann BR, Lamerz R, Fritsche HA, Gaarenstroom K, Bonfrer J, Ecke TH, Grossman HB, Hayes P, Hoffmann RT, Lerner SP, Löhe F, Louhimo J, Sawczuk I, Taketa K, Diamandis EP. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in liver, bladder, cervical, and gastric cancers. *Clin Chem* 2010; **56**: e1-48 [PMID: 20207771 DOI: 10.1373/clinchem.2009.133124]
14 **Chen DS**, Sung JL, Sheu JC, Lai MY, How SW, Hsu HC, Lee CS, Wei TC. Serum alpha-fetoprotein in the early stage of human hepatocellular carcinoma. *Gastroenterology* 1984; **86**: 1404-1409 [PMID: 6201411]
15 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575 [PMID: 11394657]
16 **Jelic S**, Sotiropoulos GC; ESMO Guidelines Working Group. Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; **21** Suppl 5: v59-v64 [PMID: 20555104 DOI: 10.1093/annonc/mdq166]
17 **Behne T**, Copur MS. Biomarkers for hepatocellular carcinoma. *Int J Hepatol* 2012; **2012**: 859076 [PMID: 22655201 DOI: 10.1155/2012/859076]
18 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312]
19 **Cabibbo G**, Maida M, Genco C, Parisi P, Peralta M, Antonucci M, Brancatelli G, Cammà C, Craxì A, Di Marco V. Natural history of untreatable hepatocellular carcinoma: A retrospective cohort study. *World J Hepatol* 2012; **4**: 256-261 [PMID: 23060970 DOI: 10.4254/wjh.v4.i9.256]
20 **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]
21 **Eun HS**, Kim MJ, Kim HJ, Ko KH, Moon HS, Lee ES, Kim SH, Lee HY, Lee BS. The retrospective cohort study for survival rate in patients with advanced hepatocellular carcinoma receiving radiotherapy or palliative care. *Korean J Hepatol* 2011; **17**: 189-198 [PMID: 22102385 DOI: 10.3350/kjhep.2011.17.3.189]
22 **Davila JA**, Duan Z, McGlynn KA, El-Serag HB. Utilization and outcomes of palliative therapy for hepatocellular carcinoma: a population-based study in the United States. *J Clin Gastroenterol* 2012; **46**: 71-77 [PMID: 22157221 DOI: 10.1097/MCG.0b013e318224d669]
23 **Yeo W**, Mo FK, Koh J, Chan AT, Leung T, Hui P, Chan L, Tang A, Lee JJ, Mok TS, Lai PB, Johnson PJ, Zee B. Quality of life is predictive of survival in patients with unresectable hepatocellular carcinoma. *Ann Oncol* 2006; **17**: 1083-1089 [PMID: 16600982]
24 **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]
25 **Kane RC**, Farrell AT, Madabushi R, Booth B, Chattopadhyay S, Sridhara R, Justice R, Pazdur R. Sorafenib for the treatment of unresectable hepatocellular carcinoma. *Oncologist* 2009; **14**: 95-100 [PMID: 19144678 DOI: 10.1634/theoncologist.2008-0185]
26 **Pinter M**, Sieghart W, Graziadei I, Vogel W, Maieron A, Königsberg R, Weissmann A, Kornek G, Plank C, Peck-Radosavljevic M. Sorafenib in unresectable hepatocellular carcinoma from mild to advanced stage liver cirrhosis. *Oncologist* 2009; **14**: 70-76 [PMID: 19144684 DOI: 10.1634/theoncologist.2008-0191]
27 **Lencioni R**, Kudo M, Ye SL, Bronowicki JP, Chen XP, Dagher L, Furuse J, Geschwind JF, de Guevara LL, Papandreou C, Takayama T, Yoon SK, Nakajima K, Lehr R, Heldner S, Sanyal AJ. GIDEON (Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib): second interim analysis. *Int J Clin Pract* 2014; **68**: 609-617 [PMID: 24283303 DOI: 10.1111/ijcp.12352]

28 **Petrelli F**, Coinu A, Borgonovo K, Cabiddu M, Ghilardi M, Lonati V, Barni S. Oxaliplatin-based chemotherapy: a new option in advanced hepatocellular carcinoma. a systematic review and pooled analysis. *Clin Oncol* (R Coll Radiol) 2014; **26**: 488-496 [PMID: 24856442 DOI: 10.1016/j.clon.2014.04.031]

29 **Patrikidou A**, Sinapi I, Regnault H, Fayard F, Bouattour M, Fartoux L, Faivre S, Malka D, Ducreux M, Boige V. Gemcitabine and oxaliplatin chemotherapy for advanced hepatocellular carcinoma after failure of anti-angiogenic therapies. *Invest New Drugs* 2014; **32**: 1028-1035 [PMID: 24748335 DOI: 10.1007/s10637-014-0100-y]

30 **Peck-Radosavljevic M**. Drug therapy for advanced-stage liver cancer. *Liver Cancer* 2014; **3**: 125-131 [PMID: 24945003 DOI: 10.1159/000343868]

31 **Liccioni A**, Reig M, Bruix J. FOLFOX-4 vs. doxorubicin for hepatocellular carcinoma: could a negative result be accepted as positive? *J Hepatol* 2014; **61**: 164-165 [PMID: 24681343 DOI: 10.1016/j.jhep.2014.03.024]

32 **Bruix J**, Takayama T, Mazzaferro V, Chau GY, Yang J, Kudo M, Cai J, Poon RTP, Han KH, Tak WY, Lee HC, Song T, Roayaie S, Bolondi L, Lee KS, Makuuchi M, Souza F, Le Berre MA, Meinhardt G, Llovet JM. Oral Abstract Session, Gastrointestinal (Noncolorectal) Cancer. *J Clin Oncol* 2014; **32**: 5s

33 **Wang J**, He XD, Yao N, Liang WJ, Zhang YC. A meta-analysis of adjuvant therapy after potentially curative treatment for hepatocellular carcinoma. *Can J Gastroenterol* 2013; **27**: 351-363 [PMID: 23781519]

34 **Zhuang L**, Zeng X, Yang Z, Meng Z. Effect and safety of interferon for hepatocellular carcinoma: a systematic review and meta-analysis. *PLoS One* 2013; **8**: e61361 [PMID: 24069133 DOI: 10.1371/journal.pone.0061361]

35 **Weis S**, Franke A, Mössner J, Jakobsen JC, Schoppmeyer K. Radiofrequency (thermal) ablation versus no intervention or other interventions for hepatocellular carcinoma. *Cochrane Database Syst Rev* 2013; **12**: CD003046 [PMID: 24357457 DOI: 10.1002/14651858.CD003046.pub3]

36 **Lin ZZ**, Shau WY, Hsu C, Shao YY, Yeh YC, Kuo RN, Hsu CH, Yang JC, Cheng AL, Lai MS. Radiofrequency ablation is superior to ethanol injection in early-stage hepatocellular carcinoma irrespective of tumor size. *PLoS One* 2013; **8**: e80276 [PMID: 24244668 DOI: 10.1371/journal.pone.0080276]

37 **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]

38 **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]

39 **Feng Q**, Chi Y, Liu Y, Zhang L, Liu Q. Efficacy and safety of percutaneous radiofrequency ablation versus surgical resection for small hepatocellular carcinoma: a meta-analysis of 23 studies. *J Cancer Res Clin Oncol* 2015; **141**: 1-9 [PMID: 24889505]

40 **Feng K**, Ma KS. Value of radiofrequency ablation in the treatment of hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 5987-5998 [PMID: 24876721 DOI: 10.3748/wjg.v20.i20.5987]

41 **Huang G**, Chen X, Lau WY, Shen F, Wang RY, Yuan SX, Geng WX, Zhou WP. Quality of life after surgical resection compared with radiofrequency ablation for small hepatocellular carcinomas. *Br J Surg* 2014; **101**: 1006-1015 [PMID: 24863168 DOI: 10.1002/bjs.9539]

42 **Eichler K**, Zangos S, Gruber-Rouh T, Vogl TJ, Mack MG. Magnetic resonance-guided laser-induced thermotherapy in patients with oligonodular hepatocellular carcinoma: long-term results over a 15-year period. *J Clin Gastroenterol* 2012; **46**: 796-801 [PMID: 22955262 DOI: 10.1097/MCG.0b013e3182641806]

43 **Awad T**, Thorlund K, Gluud C. Cryotherapy for hepatocellular carcinoma. *Cochrane Database Syst Rev* 2009; (**4**): CD007611 [PMID: 19821432 DOI: 10.1002/14651858.CD007611.pub2]

44 **Rong G**, Bai W, Dong Z, Wang C, Lu Y, Zeng Z, Qu J, Lou M, Wang H, Gao X, Chang X, An L, Chen Y, Yang Y. Cryotherapy for cirrhosis-based hepatocellular carcinoma: a single center experience from 1595 treated cases. *Front Med* 2015; **9**: 63-71 [PMID: 25001101]

45 **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]

46 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]

47 **Li H**, Guo Z, Si T, Wang H. EASL and mRECIST responses are independent predictors of survival in hepatocellular carcinoma patients treated with cryoablation. *Eur J Gastroenterol Hepatol* 2013; **25**: 620-627 [PMID: 23325276 DOI: 10.1097/MEG.0b013e32835ced13]

48 **Schuld J**, Richter S, Kollmar O. The role of cryosurgery in the treatment of colorectal liver metastases: a matched-pair analysis of cryotherapy vs. liver resection. *Hepatogastroenterology* 2014; **61**: 192-196 [PMID: 24895819]

49 **Sheen AJ**, Siriwardena AK. The end of cryotherapy for the treatment of nonresectable hepatic tumors? *Ann Surg Oncol* 2005; **12**: 202-204 [PMID: 15827810]

50 **Osaki Y**, Nishikawa H. Treatment for hepatocellular carcinoma in Japan over the last three decades: Our experience and published work review. *Hepatol Res* 2015; **45**: 59-74 [PMID: 24965914 DOI: 10.1111/hepr.12378]

51 **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]

52 **Zhang L**, Wang N, Shen Q, Cheng W, Qian GJ. Therapeutic efficacy of percutaneous radiofrequency ablation versus microwave ablation for hepatocellular carcinoma. *PLoS One* 2013; **8**: e76119 [PMID: 24146824 DOI: 10.1371/journal.pone.0076119]

53 **Carrafiello G**, Laganà D, Ianniello A, Dionigi G, Novario R, Recaldini C, Mangini M, Cuffari S, Fugazzola C. Post-radiofrequency ablation syndrome after percutaneous radiofrequency of abdominal tumours: one centre experience and review of published works. *Australas Radiol* 2007; **51**: 550-554 [PMID: 17958690]

54 **Andreano A**, Galimberti S, Franza E, Knavel EM, Sironi S, Lee FT, Meloni MF. Percutaneous microwave ablation of hepatic tumors: prospective evaluation of postablation syndrome and postprocedural pain. *J Vasc Interv Radiol* 2014; **25**: 97-105.e1-2 [PMID: 24286938 DOI: 10.1016/j.jvir.2013.09.005]

55 **Groeschl RT**, Pilgrim CH, Hanna EM, Simo KA, Swan RZ, Sindram D, Martinie JB, Iannitti DA, Bloomston M, Schmidt C, Khabiri H, Shirley LA, Martin RC, Tsai S, Turaga KK, Christians KK, Rilling WS, Gamblin TC. Microwave ablation for hepatic malignancies: a multiinstitutional analysis. *Ann Surg* 2014; **259**: 1195-1200 [PMID: 24096760 DOI: 10.1097/SLA.0000000000000234]

56 **Zhang XG**, Zhang ZL, Hu SY, Wang YL. Ultrasound-guided ablative therapy for hepatic malignancies : a comparison of the therapeutic effects of microwave and radiofrequency ablation. *Acta Chir Belg* 2014; **114**: 40-45 [PMID: 24720137]

57 **Abdelaziz A**, Elbaz T, Shousha HI, Mahmoud S, Ibrahim M, Abdelmaksoud A, Nabeel M. Efficacy and survival analysis of percutaneous radiofrequency versus microwave ablation for hepatocellular carcinoma: an Egyptian multidisciplinary clinic experience. *Surg Endosc* 2014; **28**: 3429-3434 [PMID: 24935203]

58 **Marelli L**, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, Tibballs J, Meyer T, Patch DW, Burroughs AK. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. *Cardiovasc Intervent Radiol* 2007; **30**: 6-25 [PMID: 17103105]

59 **Petersen J**, Henninger B, Glodny B, Jaschke W. [Transarterial chemoembolisation in hepatocellular carcinoma]. *Wien Med Wochenschr* 2013; **163**: 123-127 [PMID: 23413009 DOI: 10.1007/s10354-013-0180-x]

60 **Oliveri RS**, Wetterslev J, Gluud C. Transarterial (chemo)embolisation

for unresectable hepatocellular carcinoma. *Cochrane Database Syst Rev* 2011; (**3**): CD004787 [PMID: 21412886 DOI: 10.1002/14651858.CD004787.pub2]
61 **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]
62 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J; Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862]
63 **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]
64 **Cammà C**, Schepis F, Orlando A, Albanese M, Shahied L, Trevisani F, Andreone P, Craxì A, Cottone M. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. *Radiology* 2002; **224**: 47-54 [PMID: 12091661]
65 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794]
66 **Boily G**, Villeneuve JP, Lacoursière L, Chaudhury P, Couture F, Ouellet JF, Lapointe R, Goulet S, Gervais N; Comité de l'évolution des pratiques en oncologie. Transarterial embolization therapies for the treatment of hepatocellular carcinoma: CEPO review and clinical recommendations. *HPB* (Oxford) 2015; **17**: 52-65 [PMID: 24961288 DOI: 10.1111/hpb.12273]
67 **Belghiti J**, Carr BI, Greig PD, Lencioni R, Poon RT. Treatment before liver transplantation for HCC. *Ann Surg Oncol* 2008; **15**: 993-1000 [PMID: 18236111 DOI: 10.1245/s10434-007-9787-8]
68 **Seehofer D**, Nebrig M, Denecke T, Kroencke T, Weichert W, Stockmann M, Somasundaram R, Schott E, Puhl G, Neuhaus P. Impact of neoadjuvant transarterial chemoembolization on tumor recurrence and patient survival after liver transplantation for hepatocellular carcinoma: a retrospective analysis. *Clin Transplant* 2012; **26**: 764-774 [PMID: 22432589 DOI: 10.1111/j.1399-0012.2012.01609.x]
69 **Heckman JT**, Devera MB, Marsh JW, Fontes P, Amesur NB, Holloway SE, Nalesnik M, Geller DA, Steel JL, Gamblin TC. Bridging locoregional therapy for hepatocellular carcinoma prior to liver transplantation. *Ann Surg Oncol* 2008; **15**: 3169-3177 [PMID: 18696158 DOI: 10.1245/s10434-008-0071-3]
70 **Otto G**, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, Hoppe-Lotichius M, Schuchmann M, Victor A, Pitton M. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl* 2006; **12**: 1260-1267 [PMID: 16826556]
71 **Sotiropoulos GC**, Malagó M, Molmenti E, Paul A, Nadalin S, Brokalaki EI, Verhagen R, Dirsch O, Gerken G, Lang H, Broelsch CE. Efficacy of transarterial chemoembolization prior to liver transplantation for hepatocellular carcinoma as found in pathology. *Hepatogastroenterology* 2005; **52**: 329-332 [PMID: 15816428]
72 **Sotiropoulos GC**, Malago M, Molmenti EP, Radtke A, Brokalaki EI, Nadalin S, Lang H, Frilling A, Baba HA, Kühl H, Verhagen R, Broelsch CE. Disease course after liver transplantation for hepatocellular carcinoma in patients with complete tumor necrosis in liver explants after performance of bridging treatments. *Eur J Med Res* 2005; **10**: 539-542 [PMID: 16356871]
73 **Thorat A**, Lee CF, Wu TH, Chan KM, Chou HS, Lee WC. Safety of transarterial chemoembolization as bridging therapy in HCC patients with hyperbilirubinemia on the waiting list for liver transplantation: a centre experience. *Hepatogastroenterology* 2013; **60**: 2076-2079 [PMID: 24719950]
74 **Zhou Y**, Zhang X, Wu L, Ye F, Su X, Shi L, Li B. Meta-analysis: preoperative transcatheter arterial chemoembolization does not improve prognosis of patients with resectable hepatocellular carcinoma. *BMC Gastroenterol* 2013; **13**: 51 [PMID: 23509884 DOI: 10.1186/1471-230X-13-51]
75 **Cheng X**, Sun P, Hu QG, Song ZF, Xiong J, Zheng QC. Transarterial (chemo)embolization for curative resection of hepatocellular carcinoma: a systematic review and meta-analyses. *J Cancer Res Clin Oncol* 2014; **140**: 1159-1170 [PMID: 24752339]
76 **Murthy R**, Habbu A, Salem R. Trans-arterial hepatic radioembolisation of yttrium-90 microspheres. *Biomed Imaging Interv J* 2006; **2**: e43 [PMID: 21614250 DOI: 10.2349/biij.2.3.e43]
77 **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]
78 **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* 2015; **35**: 1036-1047 [PMID: 24750853 DOI: 10.1111/liv.12574]
79 **Kim YH**, Kim do Y. Yttrium-90 radioembolization for hepatocellular carcinoma: what we know and what we need to know. *Oncology* 2013; **84** Suppl 1: 34-39 [PMID: 23428856 DOI: 10.1159/000345887]
80 **Marin HL**, Furth EE, Olthoff K, Shaked A, Soulen MC. Histopathologic outcome of neoadjuvant image-guided therapy of hepatocellular carcinoma. *J Gastrointestin Liver Dis* 2009; **18**: 169-176 [PMID: 19565046]
81 **Lau WY**, Lai EC, Leung TW. Current role of selective internal irradiation with yttrium-90 microspheres in the management of hepatocellular carcinoma: a systematic review. *Int J Radiat Oncol Biol Phys* 2011; **81**: 460-467 [PMID: 20888138 DOI: 10.1016/j.ijrobp.2010.06.010]
82 **El Fouly A**, Ertle J, El Dorry A, Shaker MK, Dechêne A, Abdella H, Mueller S, Barakat E, Lauenstein T, Bockisch A, Gerken G, Schlaak JF. In intermediate stage hepatocellular carcinoma: radioembolization with yttrium 90 or chemoembolization? *Liver Int* 2015; **35**: 627-635 [PMID: 25040497 DOI: 10.1111/liv.12637]
83 **Tse RV**, Hawkins M, Lockwood G, Kim JJ, Cummings B, Knox J, Sherman M, Dawson LA. Phase I study of individualized stereotactic body radiotherapy for hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Clin Oncol* 2008; **26**: 657-664 [PMID: 18172187]
84 **Jihye C**, Jinsil S. Application of Radiotherapeutic Strategies in the BCLC-Defined Stages of Hepatocellular Carcinoma. *Liver Cancer* 2012; **1**: 216-225 [PMID: 24159586 DOI: 10.1159/000343836]
85 **Kim SW**, Oh D, Park HC, Lim do H, Shin SW, Cho SK, Gwak GY, Choi MS, Paik YH, Paik SW. Transcatheter arterial chemoembolization and radiation therapy for treatment-naïve patients with locally advanced hepatocellular carcinoma. *Radiat Oncol J* 2014; **32**: 14-22 [PMID: 24724047 DOI: 10.3857/roj.2014.32.1.14]
86 **Chen WJ**, Yuan SF, Zhu LJ, Sun XN, Zheng W. Three-dimensional conformal radiotherapy in combination with transcatheter arterial chemoembolization in the treatment of hepatocellular carcinoma. *J BUON* 2014; **19**: 692-697 [PMID: 25261654]
87 **Chen SW**, Lin LC, Kuo YC, Liang JA, Kuo CC, Chiou JF. Phase 2 study of combined sorafenib and radiation therapy in patients with advanced hepatocellular carcinoma. *Int J Radiat Oncol Biol Phys* 2014; **88**: 1041-1047 [PMID: 24661657 DOI: 10.1016/j.ijrobp.2014.01.017]
88 **Lin ZZ**, Chou CH, Cheng AL, Liu WL, Chia-Hsien Cheng J. Radiosensitization by combining an aurora kinase inhibitor with radiotherapy in hepatocellular carcinoma through cell cycle interruption. *Int J Cancer* 2014; **135**: 492-501 [PMID: 24375034 DOI: 10.1002/ijc.28682]
89 **Truant S**, Oberlin O, Sergent G, Lebuffe G, Gambiez L, Ernst O, Pruvot FR. Remnant liver volume to body weight ratio > or =0.5%: A new cut-off to estimate postoperative risks after extended resection in noncirrhotic liver. *J Am Coll Surg* 2007; **204**: 22-33 [PMID: 17189109]
90 **Breitenstein S**, Apestegui C, Petrowsky H, Clavien PA. "State of the art" in liver resection and living donor liver transplantation: a worldwide survey of 100 liver centers. *World J Surg* 2009; **33**:

797-803 [PMID: 19172348 DOI: 10.1007/s00268-008-9878-0]
91 **Yaprak O**, Guler N, Altaca G, Dayangac M, Demirbas T, Akyildiz M, Ulusoy L, Tokat Y, Yuzer Y. Ratio of remnant to total liver volume or remnant to body weight: which one is more predictive on donor outcomes? *HPB* (Oxford) 2012; **14**: 476-482 [PMID: 22672550 DOI: 10.1111/j.1477-2574.2012.00483.x]
92 **Guglielmi A**, Ruzzenente A, Conci S, Valdegamberi A, Iacono C. How much remnant is enough in liver resection? *Dig Surg* 2012; **29**: 6-17 [PMID: 22441614 DOI: 10.1159/000335713]
93 **Abdalla EK**, Adam R, Bilchik AJ, Jaeck D, Vauthey JN, Mahvi D. Improving resectability of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1271-1280 [PMID: 16955381]
94 **Lin XJ**, Yang J, Chen XB, Zhang M, Xu MQ. The critical value of remnant liver volume-to-body weight ratio to estimate posthepatectomy liver failure in cirrhotic patients. *J Surg Res* 2014; **188**: 489-495 [PMID: 24569034 DOI: 10.1016/j.jss.2014.01.023]
95 **Nanashima A**, Tobinaga S, Kunizaki M, Miuma S, Taura N, Takeshita H, Hidaka S, Sawai T, Nakao K, Nagayasu T. Strategy of treatment for hepatocellular carcinomas with vascular infiltration in patients undergoing hepatectomy. *J Surg Oncol* 2010; **101**: 557-563 [PMID: 20213729 DOI: 10.1002/jso.21534]
96 **Zhou YM**, Sui CJ, Li B, Xu F, Kan T, Yang JM. Results of en bloc resection for hepatocellular carcinoma extending to adjacent organs. *Can J Surg* 2012; **55**: 222-226 [PMID: 22617536 DOI: 10.1503/cjs.028410]
97 **Xiaohong S**, Huikai L, Feng W, Ti Z, Yunlong C, Qiang L. Clinical significance of lymph node metastasis in patients undergoing partial hepatectomy for hepatocellular carcinoma. *World J Surg* 2010; **34**: 1028-1033 [PMID: 20174806 DOI: 10.1007/s00268-010-0400-0]
98 **Cucchetti A**, Cescon M, Trevisani F, Pinna AD. Current concepts in hepatic resection for hepatocellular carcinoma in cirrhotic patients. *World J Gastroenterol* 2012; **18**: 6398-6408 [PMID: 23197885 DOI: 10.3748/wjg.v18.i44.6398]
99 **Hasegawa K**, Kokudo N. Surgical treatment of hepatocellular carcinoma. *Surg Today* 2009; **39**: 833-843 [PMID: 19784720 DOI: 10.1007/s00595-008-4024-z]
100 **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]
101 **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]
102 **Ye JZ**, Miao ZG, Wu FX, Zhao YN, Ye HH, Li LQ. Recurrence after anatomic resection versus nonanatomic resection for hepatocellular carcinoma: a meta-analysis. *Asian Pac J Cancer Prev* 2012; **13**: 1771-1777 [PMID: 22901120]
103 **Tang YH**, Wen TF, Chen X. Anatomic versus non-anatomic liver resection for hepatocellular carcinoma: a systematic review. *Hepatogastroenterology* 2013; **60**: 2019-2025 [PMID: 24719944]
104 **Gagner M**, Rheault M, Dubuc J. Laparoscopic partial hepatectomy for liver tumor (abstract). *Surg Endosc* 1992; **6**: 99
105 **Takahashi M**, Wakabayashi G, Nitta H, Takeda D, Hasegawa Y, Takahara T, Ito N. Pure laparoscopic right hepatectomy by anterior approach with hanging maneuver for large intrahepatic cholangiocarcinoma. *Surg Endosc* 2013; **27**: 4732-4733 [PMID: 24170066 DOI: 10.1007/s00464-013-3202-2]
106 **Yoon YS**, Han HS, Cho JY, Ahn KS. Total laparoscopic liver resection for hepatocellular carcinoma located in all segments of the liver. *Surg Endosc* 2010; **24**: 1630-1637 [PMID: 20035349 DOI: 10.1007/s00464-009-0823-6]
107 **Lee W**, Han HS, Yoon YS, Cho JY, Choi Y, Shin HK. Role of intercostal trocars on laparoscopic liver resection for tumors in segments 7 and 8. *J Hepatobiliary Pancreat Sci* 2014; **21**: E65-E68 [PMID: 24841194 DOI: 10.1002/jhbp.123]
108 **Twaij A**, Pucher PH, Sodergren MH, Gall T, Darzi A, Jiao LR. Laparoscopic vs open approach to resection of hepatocellular carcinoma in patients with known cirrhosis: systematic review and meta-analysis. *World J Gastroenterol* 2014; **20**: 8274-8281 [PMID: 25009403 DOI: 10.3748/wjg.v20.i25.8274]
109 **Xiong JJ**, Altaf K, Javed MA, Huang W, Mukherjee R, Mai G, Sutton R, Liu XB, Hu WM. Meta-analysis of laparoscopic vs open liver resection for hepatocellular carcinoma. *World J Gastroenterol* 2012; **18**: 6657-6668 [PMID: 23236242 DOI: 10.3748/wjg.v18.i45.6657]
110 **Herman P**, Coelho FF. Laparoscopic resection for hepatocellular carcinoma: eastern and western experiences. *Chin J Cancer Res* 2014; **26**: 234-236 [PMID: 25035647 DOI: 10.3978/j.issn.1000-9604.2014.06.19]
111 **Kim HJ**, Kim MK. Laparoscopic resection for hepatocellular carcinoma: comparison between Middle Eastern and Western experience. *Chin J Cancer Res* 2014; **26**: 245-246 [PMID: 25035650 DOI: 10.3978/j.issn.1000-9604.2014.06.20]
112 **Lin NC**, Nitta H, Wakabayashi G. Laparoscopic major hepatectomy: a systematic literature review and comparison of 3 techniques. *Ann Surg* 2013; **257**: 205-213 [PMID: 23263192 DOI: 10.1097/SLA.0b013e31827da7fe]
113 **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]
114 **Dagher I**, O'Rourke N, Geller DA, Cherqui D, Belli G, Gamblin TC, Lainas P, Laurent A, Nguyen KT, Marvin MR, Thomas M, Ravindra K, Fielding G, Franco D, Buell JF. Laparoscopic major hepatectomy: an evolution in standard of care. *Ann Surg* 2009; **250**: 856-860 [PMID: 19806057 DOI: 10.1097/SLA.0b013e3181bcaf46]
115 **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 DOI: 10.1007/s00534-012-0571-1]
116 **Chan FK**, Cheng KC, Yeung YP. Laparoscopic liver resection: lessons learnt after 100 cases. *Hong Kong Med J* 2014; **20**: 386-392 [PMID: 24722724 DOI: 10.12809/hkmj134066]
117 **Lai EC**, Yang GP, Tang CN. Robot-assisted laparoscopic liver resection for hepatocellular carcinoma: short-term outcome. *Am J Surg* 2013; **205**: 697-702 [PMID: 23561638 DOI: 10.1016/j.amjsurg.2012.08.015]
118 **Pelletier JS**, Gill RS, Shi X, Birch DW, Karmali S. Robotic-assisted hepatic resection: a systematic review. *Int J Med Robot* 2013; **9**: 262-267 [PMID: 23749316 DOI: 10.1002/rcs.1500]
119 **Starzl TE**, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR. Homotransplantation of the liver in humans. *Surg Gynecol Obstet* 1963; **117**: 659-676 [PMID: 14100514]
120 **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]
121 **Herrero JI**, Sangro B, Pardo F, Quiroga J, Iñarrairaegui M, Rotellar F, Montiel C, Alegre F, Prieto J. Liver transplantation in patients with hepatocellular carcinoma across Milan criteria. *Liver Transpl* 2008; **14**: 272-278 [PMID: 18306328 DOI: 10.1002/lt.21368]
122 **Mazzaferro V**, Bhoori S, Sposito C, Bongini M, Langer M, Miceli R, Mariani L. Milan criteria in liver transplantation for hepatocellular carcinoma: an evidence-based analysis of 15 years of experience. *Liver Transpl* 2011; **17** Suppl 2: S44-S57 [PMID: 21695773 DOI: 10.1002/lt.22365]
123 **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]
124 **Lai Q**, Nudo F, Mennini G, Spoletini G, Morabito V, Levi SG, Melandro F, Guglielmo N, Berloco PB, Rossi M. Expanded criteria for hepatocellular carcinoma after liver transplantation: a 20-year evolution. *Hepatogastroenterology* 2013; **60**: 2039-2041 [PMID: 24719947]
125 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M,

Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]

126 **Chan SC**, Fan ST. Selection of patients of hepatocellular carcinoma beyond the Milan criteria for liver transplantation. *Hepatobiliary Surg Nutr* 2013; **2**: 84-88 [PMID: 24570921 DOI: 10.3978/j.issn.2304-3881.2012.12.04]

127 **Pichlmayr R**, Ringe B, Gubernatis G, Hauss J, Bunzendahl H. [Transplantation of a donor liver to 2 recipients (splitting transplantation)--a new method in the further development of segmental liver transplantation]. *Langenbecks Arch Chir* 1988; **373**: 127-130 [PMID: 3287073]

128 **Broelsch CE**, Emond JC, Whitington PF, Thistlethwaite JR, Baker AL, Lichtor JL. Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. *Ann Surg* 1990; **212**: 368-375; discussion 375-377 [PMID: 2396888]

129 **Raia S**, Nery JR, Mies S. Liver transplantation from live donors. *Lancet* 1989; **2**: 497 [PMID: 2570198]

130 **Strong R**, Ong TH, Pillay P, Wall D, Balderson G, Lynch S. A new method of segmental orthotopic liver transplantation in children. *Surgery* 1988; **104**: 104-107 [PMID: 3291166]

131 **Rupertus K**, Kollmar O, Scheuer C, Junker B, Menger MD, Schilling MK. Major but not minor hepatectomy accelerates engraftment of extrahepatic tumor cells. *Clin Exp Metastasis* 2007; **24**: 39-48 [PMID: 17260102]

132 **Rodríguez-Perálvarez M**, De la Mata M, Burroughs AK. Liver transplantation: immunosuppression and oncology. *Curr Opin Organ Transplant* 2014; **19:** 253-260

133 **Chak E**, Saab S. Risk factors and incidence of de novo malignancy in liver transplant recipients: a systematic review. *Liver Int* 2010; **30**: 1247-1258 [PMID: 20602682 DOI: 10.1111/j.1478-3231.2010.02303.x]

134 **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

135 **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 DOI: 10.1002/14651858.CD006935.pub2]

136 **Zheng Z**, Liang W, Milgrom DP, Zheng Z, Schroder PM, Kong NS, Yang C, Guo Z, He X. Liver transplantation versus liver resection in the treatment of hepatocellular carcinoma: a meta-analysis of observational studies. *Transplantation* 2014; **97**: 227-234 [PMID: 24142034 DOI: 10.1097/TP.0b013e3182a89383]

137 **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]

138 **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]

139 **Seshadri RM**, Besur S, Niemeyer DJ, Templin M, McKillop IH, Swan RZ, Martinie JB, Russo MW, Iannitti DA. Survival analysis of patients with stage I and II hepatocellular carcinoma after a liver transplantation or liver resection. *HPB* (Oxford) 2014; **16**: 1102-1109 [PMID: 24964271 DOI: 10.1111/hpb.12300]

140 **Xu G**, Qi FZ, Zhang JH, Cheng GF, Cai Y, Miao Y. Meta-analysis of surgical resection and radiofrequency ablation for early hepatocellular carcinoma. *World J Surg Oncol* 2012; **10**: 163 [PMID: 22897815 DOI: 10.1186/1477-7819-10-163]

141 **Yin L**, Li H, Li AJ, Lau WY, Pan ZY, Lai EC, Wu MC, Zhou WP. Partial hepatectomy vs. transcatheter arterial chemoembolization for resectable multiple hepatocellular carcinoma beyond Milan Criteria: a RCT. *J Hepatol* 2014; **61**: 82-88 [PMID: 24650695 DOI: 10.1016/j.jhep.2014.03.012]

142 **Liu PH**, Lee YH, Hsu CY, Hsia CY, Huang YH, Chiou YY, Lin HC, Huo TI. Surgical resection is better than transarterial chemoembolization for hepatocellular carcinoma beyond Milan criteria independent of performance status. *J Gastrointest Surg* 2014; **18**: 1623-1631 [PMID: 24871082 DOI: 10.1007/s11605-014-2546-9]

143 **Zhong JH**, Xiang BD, Gong WF, Ke Y, Mo QG, Ma L, Liu X, Li LQ. Comparison of long-term survival of patients with BCLC stage B hepatocellular carcinoma after liver resection or transarterial chemoembolization. *PLoS One* 2013; **8**: e68193 [PMID: 23874536 DOI: 10.1371/journal.pone.0068193]

144 **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]

145 **Santoro A**, Simonelli M, Rodriguez-Lope C, Zucali P, Camacho LH, Granito A, Senzer N, Rimassa L, Abbadessa G, Schwartz B, Lamar M, Savage RE, Bruix J. A Phase-1b study of tivantinib (ARQ 197) in adult patients with hepatocellular carcinoma and cirrhosis. *Br J Cancer* 2013; **108**: 21-24 [PMID: 23287988 DOI: 10.1038/bjc.2012.556]

146 **Santoro A**, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, Van Vlierberghe H, Trojan J, Kolligs FT, Weiss A, Miles S, Gasbarrini A, Lencioni M, Cicalese L, Sherman M, Gridelli C, Buggisch P, Gerken G, Schmid RM, Boni C, Personeni N, Hassoun Z, Abbadessa G, Schwartz B, Von Roemeling R, Lamar ME, Chen Y, Porta C. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol* 2013; **14**: 55-63 [PMID: 23182627 DOI: 10.1016/S1470-2045(12)70490-4]

147 **Guglielmi A**, Ruzzenente A, Conci S, Valdegamberi A, Vitali M, Bertuzzo F, De Angelis M, Mantovani G, Iacono C. Hepatocellular carcinoma: surgical perspectives beyond the barcelona clinic liver cancer recommendations. *World J Gastroenterol* 2014; **20**: 7525-7533 [PMID: 24976693 DOI: 10.3748/wjg.v20.i24.7525]

148 **Ishizawa T**, Mise Y, Aoki T, Hasegawa K, Beck Y, Sugawara Y, Kokudo N. Surgical technique: new advances for expanding indications and increasing safety in liver resection for HCC: the Eastern perspective. *J Hepatobiliary Pancreat Sci* 2010; **17**: 389-393 [PMID: 19924372 DOI: 10.1007/s00534-009-0231-2]

149 **Giannini EG**, Savarino V, Farinati F, Ciccarese F, Rapaccini G, Marco MD, Benvegnù L, Zoli M, Borzio F, Caturelli E, Chiaramonte M, Trevisani F; Italian Liver Cancer (ITA.LI.CA) group. Influence of clinically significant portal hypertension on survival after hepatic resection for hepatocellular carcinoma in cirrhotic patients. *Liver Int* 2013; **33**: 1594-1600 [PMID: 23654354 DOI: 10.1111/liv.12199]

150 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, Ramacciato G, Grazi GL, Pinna AD. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009; **250**: 922-928 [PMID: 19855258 DOI: 10.1097/SLA.0b013e3181b977a5]

151 **Li L**, Zhang J, Liu X, Li X, Jiao B, Kang T. Clinical outcomes of radiofrequency ablation and surgical resection for small hepatocellular carcinoma: a meta-analysis. *J Gastroenterol Hepatol* 2012; **27**: 51-58 [PMID: 22004366 DOI: 10.1111/j.1440-1746.2011.06947.x]

152 **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]

153 **Cucchetti A**, Piscaglia F, Cescon M, Colecchia A, Ercolani G, Bolondi L, Pinna AD. Cost-effectiveness of hepatic resection versus percutaneous radiofrequency ablation for early hepatocellular carcinoma. *J Hepatol* 2013; **59**: 300-307 [PMID: 23603669 DOI: 10.1016/j.jhep.2013.04.009]

154 **Ho MC**, Huang GT, Tsang YM, Lee PH, Chen DS, Sheu JC, Chen CH. Liver resection improves the survival of patients with multiple

hepatocellular carcinomas. *Ann Surg Oncol* 2009; **16**: 848-855 [PMID: 19159983 DOI: 10.1245/s10434-008-0282-7]

155 **Luo J**, Peng ZW, Guo RP, Zhang YQ, Li JQ, Chen MS, Shi M. Hepatic resection versus transarterial lipiodol chemoembolization as the initial treatment for large, multiple, and resectable hepatocellular carcinomas: a prospective nonrandomized analysis. *Radiology* 2011; **259**: 286-295 [PMID: 21330557 DOI: 10.1148/radiol.10101072]

156 **Ruzzenente A**, Capra F, Pachera S, Iacono C, Piccirillo G, Lunardi M, Pistoso S, Valdegamberi A, D'Onofrio M, Guglielmi A. Is liver resection justified in advanced hepatocellular carcinoma? Results of an observational study in 464 patients. *J Gastrointest Surg* 2009; **13**: 1313-1320 [PMID: 19418103 DOI: 10.1007/s11605-009-0903-x]

157 **Ban D**, Shimada K, Yamamoto Y, Nara S, Esaki M, Sakamoto Y, Kosuge T. Efficacy of a hepatectomy and a tumor thrombectomy for hepatocellular carcinoma with tumor thrombus extending to the main portal vein. *J Gastrointest Surg* 2009; **13**: 1921-1928 [PMID: 19727969 DOI: 10.1007/s11605-009-0998-0]

158 **Chok KS**, Cheung TT, Chan SC, Poon RT, Fan ST, Lo CM. Surgical outcomes in hepatocellular carcinoma patients with portal vein tumor thrombosis. *World J Surg* 2014; **38**: 490-496 [PMID: 24132826 DOI: 10.1007/s00268-013-2290-4]

159 **Han KH**, Kudo M, Ye SL, Choi JY, Poon RT, Seong J, Park JW, Ichida T, Chung JW, Chow P, Cheng AL. Asian consensus workshop report: expert consensus guideline for the management of intermediate and advanced hepatocellular carcinoma in Asia. *Oncology* 2011; **81** Suppl 1: 158-164 [PMID: 22212951 DOI: 10.1159/000333280]

160 **Homayounfar K**, Gunawan B, Cameron S, Haller F, Baumhoer D, Uecker S, Sander B, Ramadori G, Lorf T, Füzesi L. Pattern of chromosomal aberrations in primary liver cancers identified by comparative genomic hybridization. *Hum Pathol* 2009; **40**: 834-842 [PMID: 19200581 DOI: 10.1016/j.humpath.2008.11.005]

161 **Homayounfar K**, Schwarz A, Enders C, Cameron S, Baumhoer D, Ramadori G, Lorf T, Gunawan B, Sander B. Etiologic influence on chromosomal aberrations in European hepatocellular carcinoma identified by CGH. *Pathol Res Pract* 2013; **209**: 380-387 [PMID: 23706943 DOI: 10.1016/j.prp.2013.04.004]

162 **Utsunomiya T**, Shimada M, Kudo M, Ichida T, Matsui O, Izumi N, Matsuyama Y, Sakamoto M, Nakashima O, Ku Y, Takayama T, Kokudo N. A comparison of the surgical outcomes among patients with HBV-positive, HCV-positive, and non-B non-C hepatocellular carcinoma: a nationwide study of 11,950 patients. *Ann Surg* 2015; **261**: 513-520 [PMID: 25072437]

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REVIEW

# Intermediate hepatocellular carcinoma: How to choose the best treatment modality?

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## Abstract

Intermediate stage, or stage B according to Barcelona Clinic Liver Cancer classification, of hepatocellular carcinoma (HCC) comprises a heterogeneous population with different tumor burden and liver function. This heterogeneity is confirmed by the large variability of treatment choice and disease-relate survival. The aim of this review was to highlight the existing evidences regarding this specific topic. In a multidisciplinary evaluation, patients with large (> 5 cm) solitary HCC should be firstly considered for liver resection (LR). When LR is unfeasible, locoregional treatments are evaluable therapeutic options, being transarterial chemoembolization (TACE), the most used procedure. Percutaneous ablation can be an evaluable treatment for large HCC. However, the efficacy of all ablative procedures decrease as tumor size increases over 3 cm. In clinical practice, a combination treatment strategy [TACE or transarterial radioembolization (TARE)-plus percutaneous ablation] is "a priori" preferred in a relevant percentage of these patients. On the other hands, sorafenib is the treatment of choice in patients who are unsuitable to surgery and/or with a contraindication to locoregional treatments. In multifocal HCC, TACE is the first-line treatment. The role of TARE is still undefined. Surgery may have also a role in the treatment of multifocal HCC in selected cases (patients with up to three nodules, multifocal HCC involving 2-3 adjacent liver segments). In some patients with bilobar disease the combination of LR and ablative treatment may be a valuable option. The choice of the best treatment in the patient with intermediate stage HCC should be "patient-tailored" and made by a multidisciplinary team.

**Key words:** Hepatocellular carcinoma; Percutaneous ablation; Hepatectomy; Chemoembolization; Liver transplantation; Combination therapy



**Core tip:** Intermediate stage, or stage B according to Barcelona Clinic Liver Cancer classification, of hepatocellular carcinoma (HCC) comprises a heterogeneous population with different tumor burden and liver function. This heterogeneity is confirmed by the large variability in treatment and survival, the choice of the best treatment in the patient with intermediate stage HCC is a difficult task. A multisciplinary evaluation of each intermediate stage HCC patient is recommended

for planning the best therapeutic strategy and this review was aimed to discuss about the existing evidences regarding this topic. Due to the heterogeneity of intermediate HCC, the use of different therapies (combination treatment) is likely the best choice in most of the cases offering the opportunity of a treatment tailored to the single patient.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide and the leading cause of death among cirrhotic patients[1,2]. The management of this cancer represents a challenge for physicians being complicated by the coexistence in the same patient of two severe diseases, HCC and cirrhosis. Therefore, in the last two decades several staging and prognostic systems have been proposed to better define the prognosis and the treatment strategy[3-9]. The Barcelona Clinic Liver Cancer (BCLC) classification was first published in 1999 by Llovet *et al*[6] and is actually the most widely used staging system. The BCLC classification takes into account cancer characteristics (number and size of nodules, macrovascular invasion, and extrahepatic metastasis), cirrhosis related variables (liver function and portal hypertension), and general health status of the patients (performance status). Using these parameters, five distinct HCC stages each associated with different prognosis and specific treatment recommendations are identified. In Western countries, between 20% and 30% of the HCC population at their first observation falls into the stage B and many patients progress to this stage during follow-up. Intermediate stage or stage B, according to BCLC, of HCC includes all Child-Pugh A or B patients, with a performance status 0, and with a single nodule > 5 cm, or multiple nodules > 3 in number or at least one of these > 3 cm, without macrovascular invasion and extrahepatic metastases. According to these criteria, the intermediate stage comprises a heterogeneous population with different tumor burden and liver function. This heterogeneity is confirmed by the large variability in survival among control patients of randomized controlled trials on transarterial chemoembolization (TACE), with a 1-year survival rate ranging from 3% and 75% (median 49.6%-test for heterogeneity, $P < 0.0001$)[10]. The unique treatment recommended by BCLC group for stage B patients is TACE with a wide range of expected survival, from 14 to 45 mo[11]. Therefore TACE is effective only in a proportion of intermediate patients, the others might likely benefit from other treatments. Due to this heterogeneity, diverse therapies, single or combined, are offered to intermediate patients in field practice. Unfortunately, guidelines to define the best therapeutic approach in the single patient are lacking. The main problem is to distinguish between stage B patients with expected better survival who could have the largest benefit from an aggressive therapeutic approach, and those with poor prognosis in whom treatment should be modulated to offer the best quality and duration of life to the patient. In the attempt to solve this issue, a panel of European experts in 2012 discussed about unresolved questions in the management of stage B patients and proposed a sub-classification into four stages to facilitate treatment decisions[12]. This sub-classification was based on Child-Pugh score, up-to-seven criteria, ECOG (Eastern Cooperative Oncology Group PS performance status), and portal vein thrombosis. The need for a sub-classification of intermediate patients has been claimed also by Asian experts[13] who recently proposed a modification of the European sub-classification using alpha-feto protein to re-classify patients into three modified stages[14]. Further studies are needed before these sub-classifications can be implemented in field practice.

Actually, a multisciplinary evaluation of each intermediate stage HCC patient is recommended for planning the best therapeutic strategy[15,16] and the aim of this review is to discuss about the existing evidences regarding this topic.

## THERAPEUTIC PROCEDURES

### *Liver resection*

According to the BCLC classification, patients with intermediate HCC are unsuitable for liver resection (LR). However, in the last decades advances in surgical technique, preoperative preparation, and postoperative care have expanded LR indications. Nowadays, peri-operative mortality after LR has decreased from 15% to less than 5% in referral centers. To prevent the occurrence of postoperative liver failure, two selection protocols have been proposed based on extimated resection volume and: (1) bilirubin serum level and indocyanine green retention rate at 15 min[17]; and (2) MELD score and serum sodium level[18]. Laparoscopic video-assisted LR is increasingly used as an alternative to the classical open procedure for reducing the risk of postoperative liver deterioration[19]. However, this technique is performed only in few centers and in a restricted proportion of patients due to the stringent selection criteria. In patients with huge cancer masses and poor remnant liver volume after LR, pre-operative percutaneous transhepatic portal vein embolization has been used to increase the size of non-tumorous liver[20,21]. In cirrhotic patients, this procedure may cause severe complications in up to 20% of cases and its use should be carefully evaluated[22].

**Table 1 Main transarterial chemoembolization contraindications in Barcelona Clinic Liver Cancer stage B hepatocellular carcinoma**

| |
|---|
| Liver failure |
| Refractory ascites |
| Encephalopathy |
| Bilirubin level > 3 mg/dL |
| Renal failure |
| Creatinine > 2 mg/dL or creatinine clearance < 30 mL/min |
| Coagulopathy |
| Platelet count < $50 \times 10^9$/L |
| Prothrombin time < 50% or prolonged > 4 s |
| Portal hypertension |
| Variceal bleeding within past 3 mo |
| Varices at high risk of bleeding |
| Circulatory impairment |
| Main portal venous thrombosis |
| Severely reduced portal flow or hepatofugal blood flow |
| Untreatable arteriovenous fistula |
| Hepatic artery thrombosis |
| Severe atheromatosis |

### *Transarterial treatments*

According to European and American guidelines, TACE is the first line treatment for BCLC B stage patients, but a large variability exists in the protocols, schedule, and indications among centers[23,24]. TACE can be performed with chemotherapeutic agents emulsified with lipiodol followed by embolic agents (conventional transarterial chemoembolization or c-TACE) or with embolic microspheres preloaded with chemotherapeutic agents [Drug Eluting-Beds-TACE (DEB-TACE)]. Main contraindications to TACE are shown in the Table 1. TACE can be scheduled at fixed intervals or "on demands". Prospective comparative studies between the two schedules are lacking, but this last option is likely more effective reducing the exposure of patients to the toxic effects of the treatment and increasing the compliance. When c-TACE is used, radiological assessment of tumor response must be done with magnetic resonance imaging because computed tomography evaluation is hindered by artifacts caused by lipiodol retention. It is not established how many times TACE can be repeated, but the treatment should be shifted from TACE to sorafenib (stage migration strategy)[11] in patients who have not experienced at least a partial response (according to mRECIST criteria)[25] after two TACE cycles. Furthermore, TACE should be discontinued when a deterioration of the performance status or of the liver function occurr.

Transarterial radioembolization (TARE) is a novel treatment using hepatic intra-arterial infusion of radioactive substances such as β-emitting yttrium-90 integral to the glass matrix of microspheres or Iodine-131-labeled lipiodol. Published series showed comparable median survival and toxic effects among patients treated with TACE and TARE, and therefore no defined selection criteria to choose between these techniques have been established so far[11,26-30]. Further studies are needed to evaluate the utility of TARE and its role in the treatment strategy of BCLC B stage patients. However, a widespread use of TARE is limited by its high costs.

### *Percutaneous treatments*

Thermal ablation using radiofrequency (RFA), microwave (MWA), or laser (LA), is the most widely employed locoregional treatment for HCC. It achieves a complete ablation rate > 90% in nodules ⩽ 3 cm[31-33]. Due to the improvement in devices and techniques, percutaneous ablation has been demonstrated effective also for the treatment of large HCC[34-37]. In these cases, overlapping ablative technique with multiple electrode insertions or simultaneous use of multiple applicators are required to ablate the tumor[38]. This last technique may be more effective because the simultaneous activation of multiple electrodes has a synergistic effect increasing the ablation volume and reducing the procedural time.

### *Combination of locoregional treatments*

The combination treatment strategy, using both transarterial and percutaneous procedures, offers the opportunity of a treatment tailored to the single patient. The occlusion of the hepatic arterial flow supplying the tumor with TACE would theoretically increase the ablation volume after RFA/MWA/LA by reducing the heat loss due to blood flow[39]. Furthermore, the alternate use of intravascular and percutaneous approach allows to increase the time interval between TACE procedures reducing the risk of liver failure caused by cumulating toxic effects. Several studies have evaluated the efficacy of combined locoregional treatments[40-49]. Metanalysis of observational and randomized controlled studies comparing single and combined locoregional treatments showed significant better survival in patients who underwent to combined treatment[50-55].

The combination of TACE and sorafenib has been evaluated in some studies[56-61]. The rationale of sorafenib use is to block vascular endothelial growth factor (VEGF) receptors for counterbalancing the increase in VEGF induced by post-TACE ischemia which facilitates tumor growth and metastasis[62]. It is still unclear if sorafenib potentiate the therapeutic effects of TACE[63]. However, a recent metanalysis including both randomized and retrospective trials showed that TACE-sorafenib combination increased the risk of adverse reactions, but was associated with better overall survival and longer time-to-progression[64].

## TUMOR BURDEN AND TREATMENT STRATEGY

### *Monofocal HCC*

In the setting of a multidisciplinary evaluation, patients with large (> 5 cm) solitary HCC should be firstly considered for LR[65-68]. Radical LR can be considered a valuable option in patients with: (1) peripherically located HCC, < 30% of tissue destroyed as evaluated

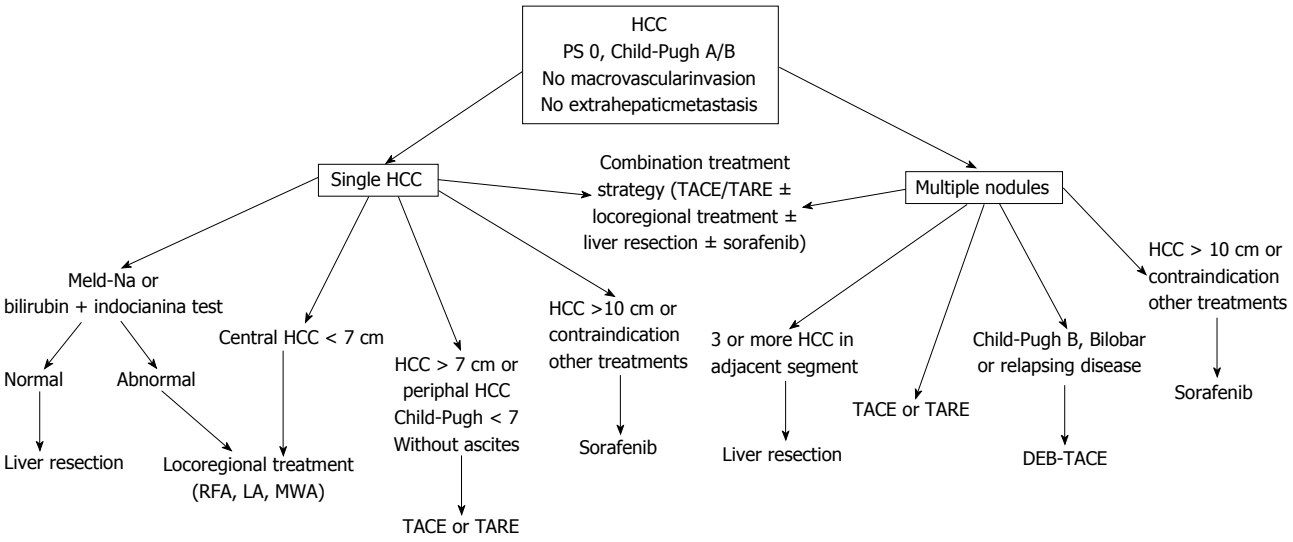


**Figure 1 Treatment strategy for monofocal and multifocal hepatocellular carcinoma.** HCC: Hepatocellular carcinoma; RFA: Radiofrequency; MWA: Micro-wave; LA: Laser; DEB-TACE: Drug Eluting-Beds-TACE; TACE: Transarterial chemoembolization; TARE: Transarterial radioembolization.

at imaging, or > 50% compensatory hepatic hypertrophy[65]; (2) no or mild portal hypertension; and (3) no history of liver decompensation.

When LR is unfeasible, locoregional treatments are evaluable therapeutic options and the most used is TACE. Best candidates to TACE are patients with well preserved liver function (Child-Pugh score $\leqslant$ 7) and without ascites. Complete HCC necrosis after TACE is seldom observed and local recurrence rates within one year are as high as 60%[69]. Up to now, there are no studies designed to define the maximum tumor size that can be treated. In the two RCTs showing survival benefit of TACE compared to best supportive care, the mean size of HCC was 5-7 cm (range 4-14 cm)[70,71]. In patients with large solitary tumor, TARE may be preferred because there are some evidences of a higher rate of response after TARE as compared to TACE[28].

Percutaneous ablation can be an evaluable treatment for large HCC. However, the efficacy of all ablative procedures decrease as size increases over 3 cm and the probability of obtaining the complete ablation of nodules larger than 7 cm is very low[35,72]. Candidates for percutaneous local ablation are patients with centrally located HCC having a diameter no more than 7 cm, in whom a complete response rate > 80% has been reported[72,73]. In patients with residual peripherical cancer tissue after ablation, the use of TACE increases the rate of complete tumor response[74]. In practice field, a combination treatment strategy (combination of TACE or TARE with percutaneous ablation) is "a priori" preferred in a relevant percentage of these patients.

In patients who are unsuitable to surgery and with contraindication to locoregional treatments or with huge HCC masses (> 10 cm) sorafenib is the treatment of choice. A subanalysis of the SHARP trial has shown that in BCLC B patients sorafenib as compared to placebo increased the median overall survival (14.5 mo *vs* 11.4 mo, HR = 0.72) and the time-to-progression (6.9 mo *vs* 4.4 mo, HR = 0.47)[75] (Figure 1).

### *Multifocal HCC*

Most of BCLC B stage patients are affected by multifocal HCC. In these cases, TACE is the first-line treatment. Best candidates are patients with few nodules having a small size: no more than 5 nodules with a size up to 5 cm is likely a good proposal[12]. According to a multicenter European trial, DEB-TACE is more effective than c-TACE in patients classified as Child-Pugh B, and with bi-lobar or relapsing disease, but differences in survival between patients treated with these techniques have not been demonstrated up to now[76-79]. The role of TARE in the management of BCLC B stage patients with multifocal disease is still undefined. However, in some case TARE might be teoretically more safe than TACE as in patients with portal thrombosis because of only minimal embolic effect of microspheres[80]. In field practice, the combined use of transarterial and percutaneous treatment for multifocal HCC is used by many centers and in the position paper of the AISF (Associazione Italiana per lo Studio del Fegato) this approach is recommended as "particularly evaluable"[66]. The use in the same patient of combined locoregional treatments and sorafenib might be theoretically useful, but due to high costs it should be evaluated by a multidisciplinary team.

Surgery may have also a role in the treatment of multifocal HCC in well selected cases[81]. In fact, LR may be a valuable treatment in patients with up to three nodules and multifocal HCC involving 2-3 adjacent liver segments. In some patients with bilobar disease the combination of LR and ablative treatment may be a valuable option. TACE before surgical resection should

not be recommended because this strategy offers no benefit[82] (Figure 1).

## LIVER TRANSPLANTATION AND DOWN-STAGING STRATEGY

In selected BCLC B stage patients treatment can be performed with the aim of reducing the tumor burden within Milan criteria. This is the downstaging strategy and patients who have been successfully treated can undergo to liver transplantation with good results[83-85]. The most used treatment for downstaging is TACE[86]. After downstaging treatment, a waiting period of at least 3-6 mo before performing liver transplantation is recommended[87]. During this time, patients should be carefully monitored for tumor response with imaging. The rationale of this strategy is to evaluate tumor biology and risk of recurrence after transplant. In fact, about a third of these patients can be affected by HCC with aggressive biology that can progress during the waiting period and they are not good candidates for transplantation due to the high risk of recurrence[88,89]. Other factors that can indicate a high risk of post-transplant recurrence are AFP serum level above a threshold of 400-1000 ng/mL[80,81,90,91] and poor HCC differentiation at histology[92]. The use in combination with locoregional treatments of systemic targeted therapy with sorafenib may theoretically further increase the rate of tumor control and reduce the recurrences, but appropriately designed studies are needed to confirm it[93].

## CONCLUSION

The choice of the best treatment in the patient with intermediate stage HCC is a difficult task. It should be made by a multidisciplinary team. Due to the heterogeneity of intermediate HCCs, the use of different therapies (combination treatment) is likely the best choice in most of the cases offering the opportunity of a treatment tailored to the single patient.

## REFERENCES


1 **World Health Organization**. International Agency for Research in Cancer. Globocan 2008: Cancer incidence and mortality worldwide. Available from: URL: http://globocan.iarc.fr

2 **Sangiovanni A**, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, Del Ninno E, Morabito A, Colombo M. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. *Hepatology* 2006; **43**: 1303-1310 [PMID: 16729298 DOI: 10.1002/hep.21176]

3 **Okuda K**, Obata H, Nakajima Y, Ohtsuki T, Okazaki N, Ohnishi K. Prognosis of primary hepatocellular carcinoma. *Hepatology* 1984; **4**: 3S-6S [PMID: 6319264 DOI: 10.1002/hep.1840040703]

4 **Chevret S**, Trinchet JC, Mathieu D, Rached AA, Beaugrand M, Chastang C. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. *J Hepatol* 1999; **31**: 133-141 [PMID: 10424293 DOI: 10.1016/S0168-8278(99)80173-1]

5 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]

6 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338 [PMID: 10518312 DOI: 10.1055/s-2007-1007122]

7 **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]

8 **Leung TW**, Tang AM, Zee B, Yu SC, Lai PB, Lau WY, Johnson PJ. Factors predicting response and survival in 149 patients with unresectable hepatocellular carcinoma treated by combination cisplatin, interferon-alpha, doxorubicin and 5-fluorouracil chemotherapy. *Cancer* 2002; **94**: 421-427 [PMID: 11905412 DOI: 10.1002/cncr.10236]

9 **Kudo M**, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J Gastroenterol* 2003; **38**: 207-215 [PMID: 12673442 DOI: 10.1007/s005350300038]

10 **Cabibbo G**, Enea M, Attanasio M, Bruix J, Craxì A, Cammà C. A meta-analysis of survival rates of untreated patients in randomized clinical trials of hepatocellular carcinoma. *Hepatology* 2010; **51**: 1274-1283 [PMID: 20112254 DOI: 10.1002/hep.23485]

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 **Bolondi L**, Burroughs A, Dufour JF, Galle PR, Mazzaferro V, Piscaglia F, Raoul JL, Sangro B. Heterogeneity of patients with intermediate (BCLC B) Hepatocellular Carcinoma: proposal for a subclassification to facilitate treatment decisions. *Semin Liver Dis* 2012; **32**: 348-359 [PMID: 23397536 DOI: 10.1055/s-0032-1329906]

13 **Park JW**, Amarapurkar D, Chao Y, Chen PJ, Geschwind JF, Goh KL, Han KH, Kudo M, Lee HC, Lee RC, Lesmana LA, Lim HY, Paik SW, Poon RT, Tan CK, Tanwandee T, Teng G, Cheng AL. Consensus recommendations and review by an International Expert Panel on Interventions in Hepatocellular Carcinoma (EPOIHCC). *Liver Int* 2013; **33**: 327-337 [PMID: 23331661 DOI: 10.1111/liv.12083]

14 **Wang JH**, Kee KM, Lin CY, Hung CH, Chen CH, Lee CM, Lu SN. Validation and modification of a proposed substaging system for patients with intermediate hepatocellular carcinoma. *J Gastroenterol Hepatol* 2015; **30**: 358-363 [PMID: 25088668 DOI: 10.1111/jgh.12686]

15 **Livraghi T**, Brambilla G, Carnaghi C, Tommasini MA, Torzilli G. Is it time to reconsider the BCLC/AASLD therapeutic flow-chart? *J Surg Oncol* 2010; **102**: 868-876 [PMID: 20886553 DOI: 10.1002/jso.21733]

16 **Piscaglia F**, Bolondi L. The intermediate hepatocellular carcinoma stage: Should treatment be expanded? *Dig Liver Dis* 2010; **42** Suppl 3: S258-S263 [PMID: 20547312 DOI: 10.1016/S1590-8658(10)60514-2]

17 **Imamura H**, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. *J Hepatobiliary Pancreat Surg* 2005; **12**: 16-22 [PMID: 15754094]

18 **Cescon M**, Cucchetti A, Grazi GL, Ferrero A, Viganò L, Ercolani G, Zanello M, Ravaioli M, Capussotti L, Pinna AD. Indication of the extent of hepatectomy for hepatocellular carcinoma on cirrhosis by a simple algorithm based on preoperative variables. *Arch Surg* 2009; **144**: 57-63; discussion 63 [PMID: 19153326 DOI: 10.1001/archsurg.2008.522]

19 **Croome KP**, Yamashita MH. Laparoscopic vs open hepatic resection for benign and malignant tumors: An updated meta-analysis. *Arch Surg* 2010; **145**: 1109-1118 [PMID: 21079101 DOI: 10.1001/archsurg.2010.227]

20 **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]

21 **Haghighi KS**, Glenn D, Gruenberger T, Morris DL. Extending the limits for curative liver resections by portal vein embolization. *Int Surg* 2009; **94**: 43-47 [PMID: 20099426]

22 **Ribero D**, Curley SA, Imamura H, Madoff DC, Nagorney DM, Ng KK, Donadon M, Vilgrain V, Torzilli G, Roh M, Vauthey JN. Selection for resection of hepatocellular carcinoma and surgical strategy: indications for resection, evaluation of liver function, portal vein embolization, and resection. *Ann Surg Oncol* 2008; **15**: 986-992 [PMID: 18236112 DOI: 10.1245/s10434-007-9731-y]

23 **Marelli L**, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, Tibballs J, Meyer T, Patch DW, Burroughs AK. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. *Cardiovasc Intervent Radiol* 2007; **30**: 6-25 [PMID: 17103105]

24 **Bargellini I**, Florio F, Golfieri R, Grosso M, Lauretti DL, Cioni R. Trends in utilization of transarterial treatments for hepatocellular carcinoma: results of a survey by the Italian Society of Interventional Radiology. *Cardiovasc Intervent Radiol* 2014; **37**: 438-444 [PMID: 23719667 DOI: 10.1007/s00270-013-0656-5]

25 **Lencioni R**, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52-60 [PMID: 20175033 DOI: 10.1055/s-0030-1247132]

26 **Carr BI**, Kondragunta V, Buch SC, Branch RA. Therapeutic equivalence in survival for hepatic arterial chemoembolization and yttrium 90 microsphere treatments in unresectable hepatocellular carcinoma: a two-cohort study. *Cancer* 2010; **116**: 1305-1314 [PMID: 20066715 DOI: 10.1002/cncr.24884]

27 **El Fouly A**, Ertle J, El Dorry A, Shaker MK, Dechêne A, Abdella H, Mueller S, Barakat E, Lauenstein T, Bockisch A, Gerken G, Schlaak JF. In intermediate stage hepatocellular carcinoma: radioembolization with yttrium 90 or chemoembolization? *Liver Int* 2015; **35**: 627-635 [PMID: 25040497 DOI: 10.1111/liv.12637]

28 **Salem R**, Lewandowski RJ, Kulik L, Wang E, Riaz A, Ryu RK, Sato KT, Gupta R, Nikolaidis P, Miller FH, Yaghmai V, Ibrahim SM, Senthilnathan S, Baker T, Gates VL, Atassi B, Newman S, Memon K, Chen R, Vogelzang RL, Nemcek AA, Resnick SA, Chrisman HB, Carr J, Omary RA, Abecassis M, Benson AB, Mulcahy MF. Radioembolization results in longer time-to-progression and reduced toxicity compared with chemoembolization in patients with hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 497-507.e2 [PMID: 21044630 DOI: 10.1053/j.gastro.2010.10.049]

29 **Hilgard P**, Hamami M, Fouly AE, Scherag A, Müller S, Ertle J, Heusner T, Cicinnati VR, Paul A, Bockisch A, Gerken G, Antoch G. Radioembolization with yttrium-90 glass microspheres in hepatocellular carcinoma: European experience on safety and long-term survival. *Hepatology* 2010; **52**: 1741-1749 [PMID: 21038413 DOI: 10.1002/hep.23944]

30 **Sangro B**, Carpanese L, Cianni R, Golfieri R, Gasparini D, Ezziddin S, Paprottka PM, Fiore F, Van Buskirk M, Bilbao JI, Ettorre GM, Salvatori R, Giampalma E, Geatti O, Wilhelm K, Hoffmann RT, Izzo F, Iñarrairaegui M, Maini CL, Urigo C, Cappelli A, Vit A, Ahmadzadehfar H, Jakobs TF, Lastoria S. Survival after yttrium-90 resin microsphere radioembolization of hepatocellular carcinoma across Barcelona clinic liver cancer stages: a European evaluation. *Hepatology* 2011; **54**: 868-878 [PMID: 21618574 DOI: 10.1002/hep.24451]

31 **Shiina S**, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122-130 [PMID: 16012942 DOI: 10.1053/j.gastro.2005.04.009]

32 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma < or =4 cm. *Gastroenterology* 2004; **127**: 1714-1723 [PMID: 15578509 DOI: 10.1053/j.gastro.2004.09.003]

33 **Seki T**, Wakabayashi M, Nakagawa T, Imamura M, Tamai T, Nishimura A, Yamashiki N, Okamura A, Inoue K. Percutaneous microwave coagulation therapy for patients with small hepatocellular carcinoma: comparison with percutaneous ethanol injection therapy. *Cancer* 1999; **85**: 1694-1702 [PMID: 10223562]

34 **Goldberg SN**, Solbiati L, Hahn PF, Cosman E, Conrad JE, Fogle R, Gazelle GS. Large-volume tissue ablation with radio frequency by using a clustered, internally cooled electrode technique: laboratory and clinical experience in liver metastases. *Radiology* 1998; **209**: 371-379 [PMID: 9807561 DOI: 10.1148/radiology.209.2.9807561]

35 **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]

36 **Strickland AD**, Clegg PJ, Cronin NJ, Swift B, Festing M, West KP, Robertson GS, Lloyd DM. Experimental study of large-volume microwave ablation in the liver. *Br J Surg* 2002; **89**: 1003-1007 [PMID: 12153625 DOI: 10.1046/j.1365-2168.2002.02155.x]

37 **Brace CL**, Sampson LA, Hinshaw JL, Sandhu N, Lee FT. Radiofrequency ablation: simultaneous application of multiple electrodes via switching creates larger, more confluent ablations than sequential application in a large animal model. *J Vasc Interv Radiol* 2009; **20**: 118-124 [PMID: 19019701 DOI: 10.1016/j.jvir.2008.09.021]

38 **Dodd GD**, Frank MS, Aribandi M, Chopra S, Chintapalli KN. Radiofrequency thermal ablation: computer analysis of the size of the thermal injury created by overlapping ablations. *AJR Am J Roentgenol* 2001; **177**: 777-782 [PMID: 11566672]

39 **Tsochatzis EA**, Fatourou EM, Triantos CK, Burroughs AK. Transarterial therapies for hepatocellular carcinoma. *Recent Results Cancer Res* 2013; **190**: 195-206 [PMID: 22941022 DOI: 10.1007/978-3-642-16037-0_13]

40 **Tanaka K**, Okazaki H, Nakamura S, Endo O, Inoue S, Takamura Y, Sugiyama M, Ohaki Y. Hepatocellular carcinoma: treatment with a combination therapy of transcatheter arterial embolization and percutaneous ethanol injection. *Radiology* 1991; **179**: 713-717 [PMID: 1851313 DOI: 10.1148/radiology.179.3.1851313]

41 **Bartolozzi C**, Lencioni R, Caramella D, Vignali C, Cioni R, Mazzeo S, Carrai M, Maltinti G, Capria A, Conte PF. Treatment of large HCC: transcatheter arterial chemoembolization combined with percutaneous ethanol injection versus repeated transcatheter arterial chemoembolization. *Radiology* 1995; **197**: 812-818 [PMID: 7480761 DOI: 10.1148/radiology.197.3.7480761]

42 **Koda M**, Murawaki Y, Mitsuda A, Oyama K, Okamoto K, Idobe Y, Suou T, Kawasaki H. Combination therapy with transcatheter arterial chemoembolization and percutaneous ethanol injection compared with percutaneous ethanol injection alone for patients with small hepatocellular carcinoma: a randomized control study. *Cancer* 2001; **92**: 1516-1524 [PMID: 11745230]

43 **Akamatsu M**, Yoshida H, Obi S, Sato S, Koike Y, Fujishima T, Tateishi R, Imamura M, Hamamura K, Teratani T, Shiina S, Ishikawa T, Omata M. Evaluation of transcatheter arterial embolization prior to percutaneous tumor ablation in patients with hepatocellular carcinoma: a randomized controlled trial. *Liver Int* 2004; **24**: 625-629 [PMID: 15566514 DOI: 10.1111/j.1478-3231.2004.0963.x]

44 **Becker G**, Soezgen T, Olschewski M, Laubenberger J, Blum HE, Allgaier HP. Combined TACE and PEI for palliative treatment of unresectable hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6104-6109 [PMID: 16273634]

45 **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]

46 **Kamada K**, Kitamoto M, Aikata H, Kawakami Y, Kono H, Imamura M, Nakanishi T, Chayama K. Combination of transcatheter arterial chemoembolization using cisplatin-lipiodol suspension and percutaneous ethanol injection for treatment of advanced small hepatocellular carcinoma. *Am J Surg* 2002; **184**: 284-290 [PMID: 12354601 DOI: 10.1016/S0002-9610(02)00933-9]

47 **Cheng BQ**, Jia CQ, Liu CT, Fan W, Wang QL, Zhang ZL, Yi CH. Chemoembolization combined with radiofrequency ablation

for patients with hepatocellular carcinoma larger than 3 cm: a randomized controlled trial. *JAMA* 2008; **299**: 1669-1677 [PMID: 18398079 DOI: 10.1001/jama.299.14.1669]

48 **Tanaka K**, Nakamura S, Numata K, Kondo M, Morita K, Kitamura T, Saito S, Kiba T, Okazaki H, Sekihara H. The long term efficacy of combined transcatheter arterial embolization and percutaneous ethanol injection in the treatment of patients with large hepatocellular carcinoma and cirrhosis. *Cancer* 1998; **82**: 78-85 [PMID: 9428482]

49 **Dettmer A**, Kirchhoff TD, Gebel M, Zender L, Malek NP, Panning B, Chavan A, Rosenthal H, Kubicka S, Krusche S, Merkesdal S, Galanski M, Manns MP, Bleck JS. Combination of repeated single-session percutaneous ethanol injection and transarterial chemoembolisation compared to repeated single-session percutaneous ethanol injection in patients with non-resectable hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 3707-3715 [PMID: 16773687]

50 **Marelli L**, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, Yu D, Meyer T, Patch DW, Burroughs AK. Treatment outcomes for hepatocellular carcinoma using chemoembolization in combination with other therapies. *Cancer Treat Rev* 2006; **32**: 594-606 [PMID: 17045407]

51 **Yan S**, Xu D, Sun B. Combination of radiofrequency ablation with transarterial chemoembolization for hepatocellular carcinoma: a meta-analysis. *Dig Dis Sci* 2012; **57**: 3026-3031 [PMID: 22585384 DOI: 10.1007/s10620-012-2212-6]

52 **Lu Z**, Wen F, Guo Q, Liang H, Mao X, Sun H. Radiofrequency ablation plus chemoembolization versus radiofrequency ablation alone for hepatocellular carcinoma: a meta-analysis of randomized-controlled trials. *Eur J Gastroenterol Hepatol* 2013; **25**: 187-194 [PMID: 23134976 DOI: 10.1097/MEG.0b013e32835a0a07]

53 **Wang N**, Guan Q, Wang K, Zhu B, Yuan W, Zhao P, Wang X, Zhao Y. TACE combined with PEI versus TACE alone in the treatment of HCC: a meta-analysis. *Med Oncol* 2011; **28**: 1038-1043 [PMID: 20632218 DOI: 10.1007/s12032-010-9620-2]

54 **Wang W**, Shi J, Xie WF. Transarterial chemoembolization in combination with percutaneous ablation therapy in unresectable hepatocellular carcinoma: a meta-analysis. *Liver Int* 2010; **30**: 741-749 [PMID: 20331507 DOI: 10.1111/j.1478-3231.2010.02221.x]

55 **Ni JY**, Liu SS, Xu LF, Sun HL, Chen YT. Meta-analysis of radiofrequency ablation in combination with transarterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 2013; **19**: 3872-3882 [PMID: 23840128 DOI: 10.3748/wjg.v19.i24.3872]

56 **Bai W**, Wang YJ, Zhao Y, Qi XS, Yin ZX, He CY, Li RJ, Wu KC, Xia JL, Fan DM, Han GH. Sorafenib in combination with transarterial chemoembolization improves the survival of patients with unresectable hepatocellular carcinoma: a propensity score matching study. *J Dig Dis* 2013; **14**: 181-190 [PMID: 23324079 DOI: 10.1111/1751-2980.12038]

57 **Kudo M**, Imanaka K, Chida N, Nakachi K, Tak WY, Takayama T, Yoon JH, Hori T, Kumada H, Hayashi N, Kaneko S, Tsubouchi H, Suh DJ, Furuse J, Okusaka T, Tanaka K, Matsui O, Wada M, Yamaguchi I, Ohya T, Meinhardt G, Okita K. Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma. *Eur J Cancer* 2011; **47**: 2117-2127 [PMID: 21664811 DOI: 10.1016/j.ejca.2011.05.007]

58 **Sansonno D**, Lauletta G, Russi S, Conteduca V, Sansonno L, Dammacco F. Transarterial chemoembolization plus sorafenib: a sequential therapeutic scheme for HCV-related intermediate-stage hepatocellular carcinoma: a randomized clinical trial. *Oncologist* 2012; **17**: 359-366 [PMID: 22334456 DOI: 10.1634/theoncologist.2011-0313]

59 **Muhammad A**, Dhamija M, Vidyarthi G, Amodeo D, Boyd W, Miladinovic B, Kumar A. Comparative effectiveness of traditional chemoembolization with or without sorafenib for hepatocellular carcinoma. *World J Hepatol* 2013; **5**: 364-371 [PMID: 23898369 DOI: 10.4254/wjh.v5.i7.364]

60 **Choi GH**, Shim JH, Kim MJ, Ryu MH, Ryoo BY, Kang YK, Shin YM, Kim KM, Lim YS, Lee HC. Sorafenib alone versus sorafenib combined with transarterial chemoembolization for advanced-stage hepatocellular carcinoma: results of propensity score analyses. *Radiology* 2013; **269**: 603-611 [PMID: 23864102 DOI: 10.1148/radiol.13130150]

61 **Qu XD**, Chen CS, Wang JH, Yan ZP, Chen JM, Gong GQ, Liu QX, Luo JJ, Liu LX, Liu R, Qian S. The efficacy of TACE combined sorafenib in advanced stages hepatocellullar carcinoma. *BMC Cancer* 2012; **12**: 263 [PMID: 22721173 DOI: 10.1186/1471-2407-12-263]

62 **Sergio A**, Cristofori C, Cardin R, Pivetta G, Ragazzi R, Baldan A, Girardi L, Cillo U, Burra P, Giacomin A, Farinati F. Transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. *Am J Gastroenterol* 2008; **103**: 914-921 [PMID: 18177453 DOI: 10.1111/j.1572-0241.2007.01712.x]

63 **Weintraub JL**, Salem R. Treatment of hepatocellular carcinoma combining sorafenib and transarterial locoregional therapy: state of the science. *J Vasc Interv Radiol* 2013; **24**: 1123-1134 [PMID: 23562168 DOI: 10.1016/j.jvir.2013.01.494]

64 **Zhang L**, Hu P, Chen X, Bie P. Transarterial chemoembolization (TACE) plus sorafenib versus TACE for intermediate or advanced stage hepatocellular carcinoma: a meta-analysis. *PLoS One* 2014; **9**: e100305 [PMID: 24945380 DOI: 10.1371/journal.pone.0100305]

65 **Zhang ZM**, Guo JX, Zhang ZC, Jiang N, Zhang ZY, Pan LJ. Therapeutic options for intermediate-advanced hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 1685-1689 [PMID: 21483627 DOI: 10.3748/wjg.v17.i13.1685]

66 **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]

67 **Régimbeau JM**, Farges O, Shen BY, Sauvanet A, Belghiti J. Is surgery for large hepatocellular carcinoma justified? *J Hepatol* 1999; **31**: 1062-1068 [PMID: 10604580 DOI: 10.1016/S0168-8278(99)80319-5]

68 **Poon RT**, Fan ST, Wong J. Selection criteria for hepatic resection in patients with large hepatocellular carcinoma larger than 10 cm in diameter. *J Am Coll Surg* 2002; **194**: 592-602 [PMID: 12022599 DOI: 10.1016/S1072-7515(02)01163-8]

69 **Terzi E**, Golfieri R, Piscaglia F, Galassi M, Dazzi A, Leoni S, Giampalma E, Renzulli M, Bolondi L. Response rate and clinical outcome of HCC after first and repeated cTACE performed "on demand". *J Hepatol* 2012; **57**: 1258-1267 [PMID: 22871502 DOI: 10.1016/j.jhep.2012.07.025]

70 **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]

71 **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]

72 **Yin XY**, Xie XY, Lu MD, Xu HX, Xu ZF, Kuang M, Liu GJ, Liang JY, Lau WY. Percutaneous thermal ablation of medium and large hepatocellular carcinoma: long-term outcome and prognostic factors. *Cancer* 2009; **115**: 1914-1923 [PMID: 19241423 DOI: 10.1002/cncr.24196]

73 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-577; quiz 578 [PMID: 22158026 DOI: 10.1038/ajg.2011.425]

74 **Pacella CM**, Bizzarri G, Cecconi P, Caspani B, Magnolfi F,

Bianchini A, Anelli V, Pacella S, Rossi Z. Hepatocellular carcinoma: long-term results of combined treatment with laser thermal ablation and transcatheter arterial chemoembolization. *Radiology* 2001; **219**: 669-678 [PMID: 11376253]

75 **Bruix J**, Raoul JL, Sherman M, Mazzaferro V, Bolondi L, Craxi A, Galle PR, Santoro A, Beaugrand M, Sangiovanni A, Porta C, Gerken G, Marrero JA, Nadel A, Shan M, Moscovici M, Voliotis D, Llovet JM. Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: subanalyses of a phase III trial. *J Hepatol* 2012; **57**: 821-829 [PMID: 22727733 DOI: 10.1016/j.jhep.2012.06.014]

76 **Xie F**, Zang J, Guo X, Xu F, Shen R, Yan L, Yang J, He J. Comparison of transcatheter arterial chemoembolization and microsphere embolization for treatment of unresectable hepatocellular carcinoma: a meta-analysis. *J Cancer Res Clin Oncol* 2012; **138**: 455-462 [PMID: 22179199]

77 **Kooby DA**, Egnatashvili V, Srinivasan S, Chamsuddin A, Delman KA, Kauh J, Staley CA, Kim HS. Comparison of yttrium-90 radioembolization and transcatheter arterial chemoembolization for the treatment of unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2010; **21**: 224-230 [PMID: 20022765 DOI: 10.1016/j.jvir.2009.10.013]

78 **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]

79 **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]

80 **Kulik LM**, Carr BI, Mulcahy MF, Lewandowski RJ, Atassi B, Ryu RK, Sato KT, Benson A, Nemcek AA, Gates VL, Abecassis M, Omary RA, Salem R. Safety and efficacy of 90Y radiotherapy for hepatocellular carcinoma with and without portal vein thrombosis. *Hepatology* 2008; **47**: 71-81 [PMID: 18027884]

81 **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]

82 **Yamasaki S**, Hasegawa H, Kinoshita H, Furukawa M, Imaoka S, Takasaki K, Kakumoto Y, Saitsu H, Yamada R, Oosaki Y, Arii S, Okamoto E, Monden M, Ryu M, Kusano S, Kanematsu T, Ikeda K, Yamamoto M, Saoshiro T, Tsuzuki T. A prospective randomized trial of the preventive effect of pre-operative transcatheter arterial embolization against recurrence of hepatocellular carcinoma. *Jpn J Cancer Res* 1996; **87**: 206-211 [PMID: 8609071]

83 **Ravaioli M**, Grazi GL, Piscaglia F, Trevisani F, Cescon M, Ercolani G, Vivarelli M, Golfieri R, D'Errico Grigioni A, Panzini I, Morelli C, Bernardi M, Bolondi L, Pinna AD. Liver transplantation for hepatocellular carcinoma: results of down-staging in patients initially outside the Milan selection criteria. *Am J Transplant* 2008; **8**: 2547-2557 [PMID: 19032223 DOI: 10.1111/j.1600-6143.2008.02409.x]

84 **Yao FY**, Kerlan RK, Hirose R, Davern TJ, Bass NM, Feng S, Peters M, Terrault N, Freise CE, Ascher NL, Roberts JP. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. *Hepatology* 2008; **48**: 819-827 [PMID: 18688876 DOI: 10.1002/hep.22412]

85 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]

86 **Chapman WC**, Majella Doyle MB, Stuart JE, Vachharajani N, Crippin JS, Anderson CD, Lowell JA, Shenoy S, Darcy MD, Brown DB. Outcomes of neoadjuvant transarterial chemoembolization to downstage hepatocellular carcinoma before liver transplantation. *Ann Surg* 2008; **248**: 617-625 [PMID: 18936575 DOI: 10.1097/SLA.0b013e31818a07d4]

87 **Pomfret EA**, Washburn K, Wald C, Nalesnik MA, Douglas D, Russo M, Roberts J, Reich DJ, Schwartz ME, Mieles L, Lee FT, Florman S, Yao F, Harper A, Edwards E, Freeman R, Lake J. Report of a national conference on liver allocation in patients with hepatocellular carcinoma in the United States. *Liver Transpl* 2010; **16**: 262-278 [PMID: 20209641 DOI: 10.1002/lt.21999]

88 **Millonig G**, Graziadei IW, Freund MC, Jaschke W, Stadlmann S, Ladurner R, Margreiter R, Vogel W. Response to preoperative chemoembolization correlates with outcome after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2007; **13**: 272-279 [PMID: 17256758 DOI: 10.1002/lt.21033]

89 **Otto G**, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, Hoppe-Lotichius M, Schuchmann M, Victor A, Pitton M. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl* 2006; **12**: 1260-1267 [PMID: 16826556 DOI: 10.1002/lt.20837]

90 **Vibert E**, Azoulay D, Hoti E, Iacopinelli S, Samuel D, Salloum C, Lemoine A, Bismuth H, Castaing D, Adam R. Progression of alphafetoprotein before liver transplantation for hepatocellular carcinoma in cirrhotic patients: a critical factor. *Am J Transplant* 2010; **10**: 129-137 [PMID: 20070666 DOI: 10.1111/j.1600-6143.2009.02750.x]

91 **Toso C**, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology* 2009; **49**: 832-838 [PMID: 19152426 DOI: 10.1002/hep.22693]

92 **Decaens T**, Roudot-Thoraval F, Badran H, Wolf P, Durand F, Adam R, Boillot O, Vanlemmens C, Gugenheim J, Dharancy S, Bernard PH, Boudjema K, Calmus Y, Hardwigsen J, Ducerf C, Pageaux GP, Hilleret MN, Chazouillères O, Cherqui D, Mallat A, Duvoux C. Impact of tumour differentiation to select patients before liver transplantation for hepatocellular carcinoma. *Liver Int* 2011; **31**: 792-801 [PMID: 21645209 DOI: 10.1111/j.1478-3231.2010.02425.x]

93 **Gutierrez JA**, Gish RG. Efficacy of combination treatment modalities for intermediate and advanced hepatocellular carcinoma: intra-arterial therapies, sorafenib and novel small molecules. *Transl Cancer Res* 2013; **2**: 460-471

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REVIEW

# Composite prognostic models across the non-alcoholic fatty liver disease spectrum: Clinical application in developing countries

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## Abstract

Heterogeneity in clinical presentation, histological severity, prognosis and therapeutic outcomes characteristic of non-alcoholic fatty liver disease (NAFLD) necessitates the development of scientifically sound classification schemes to assist clinicians in stratifying patients into meaningful prognostic subgroups. The need for replacement of invasive liver biopsies as the standard method whereby NAFLD is diagnosed, graded and staged with biomarkers of histological severity injury led to the development of composite prognostic models as potentially viable surrogate alternatives. In the present article, we review existing scoring systems used to (1) confirm the presence of undiagnosed hepatosteatosis; (2) distinguish between simple steatosis and NASH; and (3) predict advanced hepatic fibrosis, with particular emphasis on the role of NAFLD as an independent cardio-metabolic risk factor. In addition, the incorporation of functional genomic markers and application of emerging imaging technologies are discussed as a means to improve the diagnostic accuracy and predictive performance of promising composite models found to be most appropriate for widespread clinical adoption.

**Key words:** Liver biopsy; Genomics; Steatohepatitis; Non-invasive biomarkers; Histological severity; Non-alcoholic fatty liver disease



**Core tip:** Non-alcoholic fatty liver disease (NAFLD) remains largely underdiagnosed and undertreated in general practice. In view of the limitations inherent to liver biopsy and peripheral surrogate biomarkers used in the diagnosis and assessment of histological severity in NAFLD, a number of composite prognostic models

have entered the clinical domain as potentially viable alternatives. Lifestyle-based intervention remains the cornerstone of treatment in patients with NAFLD. The widespread clinical adoption of composite diagnostic and predictive models could however prove useful in informing clinical and therapeutic decision making with the goal of adding value to patient care across the NAFLD spectrum.

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## INTRODUCTION

In coming decades, the developing world is expected to bear an increasingly disproportionate share of the overall health and financial burden attributable to chronic non-communicable diseases (NCDs) once considered merely as reflections of affluence[1,2]. Economic prosperity is inexorably linked to a globalization of modernity which fuels an ongoing reversal of the social obesity gradient in non-industrialized nations[3,4]. This epidemiological trend is mirrored in the emergence of non-alcoholic fatty liver disease (NAFLD) as a major health concern in non-occidental countries, affecting individuals across boundaries for age, sex and ancestral background[5]. Despite growing evidence as to its magnitude and preventability, this "hepatic pandemic" remains largely underdiagnosed and undertreated in routine medical practice[6].

NAFLD encompasses a broad spectrum of hepatic abnormalities and is typified by marked inter-individual heterogeneity in clinical presentation, histological severity, prognosis and therapeutic outcomes. While the majority of uncomplicated steatosis is non-progressive, approximately 20%-30% of patients will develop non-alcoholic steatohepatitis (NASH), a more aggressive necro-inflammatory phenotype associated with increased risk for advanced fibrosis predisposing towards cirrhosis, portal hypertension, decompensated liver failure and hepatocellular carcinoma[7,8]. The pathological evaluation and classification of biopsied liver tissue remains the definitive standard investigation whereby a suspected diagnosis of NAFLD is confirmed and histological severity quantified to assist prognostication and the selection of appropriate therapeutic intervention[9]. This approach is to an extent advantageous as it allows for the concurrent assessment of multiple histological parameters and may help identify unexpected hepatic pathology or comorbidities. Liver biopsy is however limited insofar as it is an invasive, expensive and time-consuming procedure which poses significant physical risk, including a 0.1% risk of mortality[9]. Moreover, it is subject to sampling error and intra- as well as inter-observer variability in interpretation, and does not adequately reflect dynamic changes in disease severity over time. Given its restricted availability in resource-limited settings, compounded by the high overall prevalence of NAFLD, liver biopsy may not always be logistically feasible in the developing world[10].

An appreciation for the abovementioned shortcomings creates an incentive to develop and validate robust and cost-effective risk classification tools as potentially viable alternatives. Growing insight into the molecular and genetic mechanisms underlying the development and pathogenic progression of NAFLD has led to the identification of novel peripheral biomarkers (Table 1) allowing for the non-invasive assessment of underlying hepatic injury. The recognition that individual risk markers have insufficient discriminatory power and limited clinical utility in stratifying patients into meaningful prognostic subgroups has led to the development and validation of composite diagnostic and predictive models as potentially viable alternatives[17-20].

A growing number of complex and often patented biomarker panels and risk classification schemes have recently entered the clinical domain. Their use is however not always applicable in resource-limited settings. In the present article, we provide an overview of non-invasive composite models used to (1) confirm the presence of undiagnosed hepatosteatosis; (2) differentiate between simple fatty liver and NASH; and (3) predict advanced hepatic fibrosis, with particular emphasis on the relationship between histological severity and cardio-metabolic risk. The advantages and shortcomings of these models are discussed in relation to the potential added value of emerging genomic applications as a means of improving their performance, weighed against the reality of its implementation. In conclusion, suggestions are provided as to how ongoing research may confirm their clinical utility as robust and cost-effective population-based screening tools used to facilitate prognostication, assist in the selection of appropriate treatment and intervention strategies, predict adverse clinical outcomes, and ultimately allow for the more goal-directed use of liver biopsy in developing nations.

## COMPOSITE DIAGNOSTIC MODELS FOR HEPATIC STEATOSIS

Intra-hepatocyte accumulation of neutral triglycerides in excess of 5% of liver mass is the defining pathological feature of NAFLD. Once considered a "first hit" mechanism underlying its etiology, this is now rather thought to protect against oxidative stress driven by increased intra-hepatic free fatty acid (FFA) flux arising in the context of visceral adiposity and insulin resistance (IR) as components of the metabolic syndrome[21]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are commonly elevated in

**Table 1 Peripheral biomarkers used for the assessment of necro-inflammation and fibrotic injury in non-alcoholic fatty liver disease**

| Pathogenic mechanism | Biomarker | | NASH | Advanced fibrosis | Ref. |
|---|---|---|---|---|---|
| Hepatocyte apoptosis | Apoptotic markers | CK-18 ↑ | X | | El Bassat *et al*[11] |
| | | sFas ↑ | X | | |
| Oxidative stress | Lipid peroxidation products | Ox-LDL ↑ | X | | Kawanaka *et al*[12] |
| | | TBARS ↑ | X | | Machado *et al*[13] |
| | | MDA ↑ | X | | |
| | | HNE ↑ | X | | |
| | Indicators of altered redox potential | GSH ↑ | X | | |
| | | SOD ↓ | X | | |
| | | TRX ↑ | X | | |
| Chronic inflammation | Acute phase reactants | CRP | X | | Jarrar *et al*[14] |
| | | Ferritin | X | | Braunersreuther *et al*[15] |
| | | PTX3 | X | | |
| | Adipocytokines | Leptin | X | | |
| | | Adiponectin | | X | |
| | | TNF-α | X | | |
| | | IL-1β | X | | |
| | | IL-6 | | X | |
| | | TGF-α/β1 | X | | |
| Increased hepatocellular turnover | Extracellular matrix components | HA | | X | Baranova *et al*[16] |
| | | Laminin | | X | |
| | | Type Ⅳ collagen 7S | | X | |
| | | PⅢNP | | X | |
| | | MMP-2/9 | | X | |
| | | TIMP-1 | | X | |

CK-18: Cytokeratin-18; sFAS: Soluble Fas; Ox-LDL: Oxidized low-density lipoprotein cholesterol; TBARS: Thiobarbituric acid reactive substances; MDA: Malondialdehyde; HNE: Hydroxynonenal; GSH: Reduced glutathione; SOD: Superoxide dismutase; TRX: Thioredoxin; CRP: C-reactive protein; PTX3: Pentraxin 3; TNF-α: Tumor necrosis factor α; IL: Interleukin; TGF: Transforming growth factor; HA: Hyaluronic acid; NASH: Non-alcoholic steatohepatitis; MMP: Matrix metalloproteinase; PⅢNP; Procollagen 3 n-terminal propeptide; TIMP: Tissue inhibitor of metalloproteinase.

patients with this condition, and increased ALT reliably predicts the development of adverse cardiac events and cardiovascular mortality[22,23]. Although used to determine eligibility for further diagnostic work-up and imaging, liver enzymes may however remain normal in up to 80% of NAFLD cases[24]. In addition, while elevated gamma glutamyl-transferase (GGT) has greater specificity for hepatosteatosis and is a sensitive marker for early IR, it is clear that no single biochemical test is considered ideal for confirming a suspected diagnosis of NAFLD.

Imaging modalities such as ultrasonography further have limited diagnostic utility in moderate steatosis, while the application of more complex technologies in resource-limited environments is still largely restricted by their expense. Whether routine screening for hepatic steatosis in high-risk asymptomatic patients is practical or cost-effective remains subject to debate, as the vast majority are likely to present with uncomplicated and non-progressive disease. Its identification is however of significant clinical relevance, since even uncomplicated fatty liver, once regarded as relatively benign and showing a favorable prognosis, is associated with increased risk for overall and liver-related mortality[25,26].

Emerging evidence suggests that hepatic steatosis independently predicts the development of new-onset ischemic heart disease as well as adverse cardiac events irrespective of traditional cardio-metabolic traits[27,28]. The importance of hepatosteatosis as predictor of adverse clinical outcomes and mortality in patients with established cardiovascular disease (CVD) however remains uncertain[29]. Results from prior investigation into the value of existing clinical stratification tools in estimating cardio-metabolic risk and predicting new-onset CVD in patients with the metabolic syndrome and/or NAFLD are conflicting[30]. Multiple studies have further reported positive correlations between the severity of hepatic steatosis and proportional derangements in several early markers of subclinical atherosclerotic burden and cardiovascular risk[31-33]. Composite scoring systems validated against measures of steatosis severity may therefore prove useful as non-invasive tools in the diagnosis of NAFLD.

Several diagnostic models for hepatic steatosis have been developed to date. Anthropometric correlates for central obesity, fasting triglycerides and GGT levels are utilized in the fatty liver index commonly used in epidemiological studies, which along with its sex-specific derivative, the lipid accumulation product, accurately predicts the presence of hepatic steatosis, and could help clinicians identify patients at increased cardio-metabolic risk eligible for further diagnostic evaluation and for whom suitable lifestyle-based interventions may be indicated[34-37]. Subsequently developed models such as the NAFLD liver fat score[38] substitute GGT for the AST/ALT ratio (AAR) in addition to incorporating clinical or biochemical markers of IR. A particular advantage of recent panels is the ability to not only identify hepatic steatosis using a high cut-off value, but also reliably exclude it using a low cut-off value[39,40]. The widespread

**Table 2 Composite diagnostic models for non-alcoholic steatohepatitis**

| Biomarker panel | Components | Formula | AUC | Ref. |
|---|---|---|---|---|
| HAIR score | Hypertension<br>Elevated ALT (> 40 IU/L)<br>Insulin Resistance (index > 5) | Weighted sum (0-4) | 0.9 | Dixon *et al*[52] |
| Gholam's model | DM Ⅱ<br>AST level | Algorithm | 0.9 | Gholam *et al*[53] |
| NASH clinical scoring system for morbid obesity | Hypertension<br>DM Ⅱ<br>Elevated AST (> 27 IU/L)<br>Elevated ALT (> 27 IU/L)<br>OSA<br>non-black race | Weighted sum (0-7) | Not reported | Campos *et al*[54] |
| Ulitsky's score | DM Ⅱ<br>Elevated ALT (> 40 IU/L)<br>Elevated triglycerides (> 150 mg/dL)<br>OSA | Weighted sum (0-5) | Not reported | Ulitsky *et al*[55] |
| NASH predictive index | Female sex<br>Body mass index<br>HOMA-IR<br>AST levels<br>ALT levels | Algorithm | 0.78 | Zein *et al*[56] |

AUC: Area under curve; ALT: Alanine aminotransferase; DM Ⅱ: Diabetes mellitus type Ⅱ; AST: Aspartate aminotransferase; OSA: Obstructive sleep apnoea; HOMA-IR: Homeostatic Measurement Assessment of Insulin Resistance; NASH: Non-alcoholic steatohepatitis.

clinical use of these models is currently restricted by their limited utility in quantifying steatosis severity in obese patients in addition to not accurately predicting new-onset ischemic heart disease or cardiovascular mortality[41-43].

## COMPOSITE DIAGNOSTIC MODELS FOR NASH

Liver-specific therapeutic interventions such as thiazolidinedione pharmacotherapy could pose specific benefit in non-cirrhotic NASH[44]. There is still however insufficient evidence to justify the routine clinical use of any specific targeted treatments at this time. Numerous studies have shown that a approximately 10% reduction in body weight improves both metabolic abnormalities and histological changes in patients with NAFLD/NASH and as such, a multidisciplinary lifestyle-based therapeutic approach incorporating a tailored low-calorie dietary regimen and moderate physical exercise remains the cornerstone of treatment for this condition[45-47]. To ensure the successful implementation of population-based lifestyle intervention programs aimed at preventing the onset or progression of NAFLD in resource-limited healthcare settings, it is imperative to foster collaboration between clinicians and the public sector in accordance with standardized guidelines and assisted by the necessary ethico-legal and governmental frameworks[48].

It can be argued that the abovementioned findings call into question the validity of diagnostic confirmation for NASH. Delineating between uncomplicated steatosis and this more aggressive phenotype however has important prognostic significance, since compared to patients with simple fatty liver, those with steatohepatitis are at greater risk for cardiovascular as well as liver-related mortality[49]. The association between NASH and increased cardio-metabolic risk is further evidenced by significant correlations reported between the extent of necro-inflammatory injury and the degree of endothelial as well as diastolic dysfunction[50,51]. A number of simple composite diagnostic models for NASH have been developed to date, largely incorporating readily available clinical data in addition to several routinely performed biochemical tests (Table 2).

### *Socio-demographic characteristics*

Epidemiological studies have reported marked gender-specific and population-related disparity in the prevalence and severity of NAFLD, with ethnicity and sex considered co-dependent risk modifiers further influenced by age as well as environmental exposures acting on a genetic background[57,58]. Such evidence emphasizes the importance of a population-based approach to chronic disease risk screening integrating socio-demographic variables in developing composite risk models for application as screening tools. The incidence of NASH is higher in females particularly of older age, while risk for progression to this inflammatory phenotype is inversely related to African ancestry[59]. These observations are reflected in the inclusion of non-black ethnicity and female sex in the NASH clinical scoring system for morbid obesity[54] and NASH predictive index[56], respectively.

### *Cardio-metabolic risk traits*

The near-universal incorporation of cardiovascular risk traits defined by the metabolic syndrome as a component of diagnostic models for NASH accords with the well-

evidenced association between these entities[60]. Direct biochemical confirmation or clinical approximation of IR is of particular relevance in this regard, as insulin-mediated intra-hepatic FFA flux potentiates cardiomyocyte ischemia contributing towards diastolic dysfunction, adaptive remodelling and subsequent cardiac injury. IR is therefore considered the primary mechanism underlying the emergence of a distinct "dysfunctional cardiovascular phenotype" of NAFLD, particularly associated with increased cardio-metabolic risk[61].

### *Liver enzyme levels*

Separately, an elevation in either AST or ALT is not considered a reliable indicator of necro-inflammatory hepatic injury, and as such, normal levels do not confidently exclude a diagnosis of NASH[62]. Increased GGT however independently predicts not only progression to NASH[63] but also new-onset CVD in addition to cardiac mortality[64].

### *Obstructive sleep apnoea*

The association between obstructive sleep apnoea and central obesity as well as the metabolic syndrome validates its incorporation into several diagnostic models for NASH. Severe chronic hypoxemia is positively correlated with steatosis grade and indirectly promotes hepatic necro-inflammation[65] although its association with other determinants of histological injury remains unclear[66,67]. Intermittent hypoxia induces intra-hepatic FFA flux which promotes progression to NASH *via*: (1) activation of nuclear factor kappa beta and increased production of pro-inflammatory adipocytokines; as well as (2) up-regulation of reactive oxygen species (ROS) synthesis mediated by the NADPH oxidase complex[68].

### *Oxidative and inflammatory biomarkers*

Chronic inflammation and oxidative stress are considered important "second-hit" pathogenic mechanisms underlying progression to NASH. The relationship between the metabolic syndrome and high-sensitivity C-reactive protein is well-recognized[69] and a preponderance of evidence now supports its utility as a reliable predictive marker for new-onset CVD and subclinical atherosclerosis[70-73]. There is however general concern regarding the putative value of adipocytokines, acute phase reactants and oxidative biomarkers in the diagnosis of NASH due to their general lack of specificity[74]. While plasma caspase-generated cytokeratin-18 (CK-18) reflecting hepatocyte apoptosis is considered a highly accurate and potentially useful non-invasive diagnostic marker for NASH[75], the low sensitivity of CK-18 limits its potential viability as screening tool in the clinical setting[76]. It also remains unclear to what extent the incorporation of CK-18 into composite diagnostic models is deterministic of their accuracy.

### *Iron parameters*

Emerging evidence suggests that ferritin is not only independently associated with diagnostic features of the metabolic syndrome, but reliably predicts its presentation as composite entity as well as the onset of full-blown type Ⅱ diabetes mellitus (DM Ⅱ) and adverse cardiac events[77-79]. It has been proposed that the well-evidenced pathogenic relationship between hyperferritinemia and the metabolic syndrome is mediated by undiagnosed hepatosteatosis[80] which in turn exacerbates the association between this condition and increased risk for DM Ⅱ and atherosclerotic disease[81]. Up to 30% of NAFLD patients present with baseline hyperferritinemia[82] considered a reliable predictive marker for NASH, validating its incorporation into the NAFIC score shown to outperform both the HAIR score and Gholam's model[83]. The utility of ferritin in the non-invasive prediction of advanced hepatic fibrosis and increased histological severity however remains contested[84-87].

In the absence of a corresponding elevation in transferrin saturation (TS), inflammation-mediated hyperferritinemia accompanied by decreased serum iron constitutes a clinico-biochemical profile consistent with the "anaemia of chronic disease"[88,89] and compatible with a diagnosis of hepatosteatosis and/or the dysmetabolic iron overload syndrome (DIOS)[82]. Mendler *et al*[90] first described the presence of unexplained mild-to-moderate sinusoidal hepatic siderosis invariably associated with decreased insulin sensitivity and termed this condition insulin resistance-associated hepatic iron overload (IR-HIO). In less than two decades, DIOS has emerged as an important differential diagnosis for type Ⅰ hereditary hemochromatosis (HH) in patients at increased cardio-metabolic risk presenting with deranged iron profiles and a persistent elevation in liver transaminases[91]. Due to their striking similarities, it has been proposed that a superficial distinction between the "iron phenotypes of obesity" which characterize the metabolic syndrome, NAFLD and DIOS in fact belies their common pathological basis[82].

Standardized selection criteria for mutation screening of the hemochromatosis (*HFE*) gene to confirm a suspected diagnosis of HH are already in place based on well-established diagnostic algorithms. However, there remains a pressing need to develop and validate cost-effective non-invasive pre-screen diagnostic algorithms to assist clinicians in differentiating between type Ⅰ HH and DIOS as common causes of hepatic siderosis with the goal of informing clinical and therapeutic decision making. Riva *et al*[91] re-evaluated the diagnostic criteria for DIOS and showed that the presence of two or more metabolic syndrome features and a normal TS percentage in patients with confirmed hepatosteatosis corresponded with mild to moderate hepatic siderosis, showing a predominantly sinusoidal distribution typical of "classic" DIOS. By comparison, a significant peripheral elevation in TS dissociated from cardio-metabolic risk traits corresponded to more severe hepatocellular iron accumulation consistent with the histological presentation of HH. In this context, the routine implementation of a similar validated diagnostic tool could assist clinicians

**Table 3 Composite predictive models for advanced hepatic fibrosis in chronic liver disease**

| Biomarker panel | Components | Formula | Validated in adult NAFLD | Ref. |
|---|---|---|---|---|
| APRI index | AST, platelet count | Ratio | √ | Kruger *et al*[96] |
| BAAT score | Age, BMI, ALT, triglycerides | Weighted sum | √ | Ratziu *et al*[97] |
| BARD score | BMI, AST, ALT, DM Ⅱ | Weighted sum | √ | Harrison *et al*[98] |
| Cirrhosis discrimination score | AST, ALT, platelet count, INR | Weighted sum | × | Udell *et al*[99] |
| FIB-4 index | Age, AST, ALT, platelet count | Algorithm | √ | Shah *et al*[100] |
| Fibrosis probability index | Age, AST, previous alcohol use, HOMA-IR, cholesterol | Algorithm | × | Sud *et al*[101] |
| Forns index | Age, platelet count, GGT, cholesterol | Algorithm | √ | Forns *et al*[102] |
| Gholam's score | ALT, HbA1C | Weighted score | √ | Gholam *et al*[53] |
| Hui model | BMI, bilirubin, albumin, platelet count | Algorithm | × | Hui *et al*[103] |
| King score | Age, AST, platelet count, INR | Algorithm | × | Cross *et al*[104] |
| Lok index | AST, ALT, platelet count, INR | Algorithm | × | Lok *et al*[105] |
| NAFLD fibrosis score | Age, BMI, platelet count, albumin, AAR, IFG/DM Ⅱ | Algorithm | √ | Angulo *et al*[106] |

NAFLD: Non-alcoholic fatty liver disease; APRI: Aspartate aminotransferase to platelet ratio index; AST: Aspartate aminotransferase; BMI: Body mass index; ALT: Alanine aminotransferase; DM Ⅱ: Diabetes mellitus type Ⅱ; HOMA-IR: Homeostatic Measurement Assessment of Insulin Resistance; IFG: Impaired fasting glucose; GGT: Gamma glutamyl-transferase; INR: International normalized ratio; AAR: AST/ALT ratio.

in stratifying obese patients into meaningful subgroups based on the need for extended follow-up evaluation. This could confirm a suspected diagnosis of hepatosteatosis and/or DIOS, in addition to identifying a subgroup of patients set to derive optimal benefit from the timely implementation of suitable lifestyle-based intervention strategies and targeted therapeutic modalities aimed at decreasing cumulative cardio-metabolic risk early on in the disease process.

## COMPOSITE PREDICTIVE MODELS FOR ADVANCED HEPATIC FIBROSIS

Establishing the extent and severity of fibrotic injury in patients with NAFLD is of significant clinical relevance for reliable prediction of overall and liver-related mortality[92]. In addition to its prognostic importance, confirmation of advanced hepatic fibrosis may identify patients eligible for enrolment in screening programs aimed at monitoring risk for progression to cirrhosis and its associated complications[93]. Fibrosis severity is moreover correlated with greater carotid intima media thickness measurements, decreased coronary blood flow reserves as well as microvascular dysfunction, suggesting that patients with advanced fibrotic injury should be considered at high-risk for CVD, warranting more aggressive and sustained intervention *via* lifestyle-based risk reduction methods[94,95]. Several scoring panels used for the prediction of advanced hepatic fibrosis are outlined in Table 3.

### *Socio-demographic characteristics*

Risk for pathogenic progression and greater histological severity increases dramatically with age[107]. Older age is therefore considered a predictor variable in multiple composite models for advanced fibrosis.

### *Cardio-metabolic risk traits and liver enzyme levels*

The metabolic syndrome as composite entity, in addition to its individual components, are accurate predictors of histological severity in NASH[108]. The AAR is typically < 1 in patients with uncomplicated NAFLD. However, ALT levels decrease with resolution of necro-inflammation and as fibrotic injury progresses, which causes decreased clearance of AST from the sinusoidal space resulting in gradual reversal of the AAR[62]. An AAR of > 1 is considered a reliable indicator of cirrhosis, while a cut-off value of 0.8 can be used to predict advanced hepatic fibrosis[109].

The abovementioned variables constitute the basis for most of the composite predictive models for advanced hepatic fibrosis developed to date. Despite the high specificity (100%) of the BAAT score, its low sensitivity largely restricts more widespread use in clinical practice. The NAFLD fibrosis score (NFS) has proven useful in accurately predicting as well as excluding advanced hepatic fibrosis using high and low cut-off values, respectively. Although the NFS has been extensively investigated and validated in different population groups, it has limited utility in intermediate stages of non-severe fibrosis[110-112]. The BARD score compares favourably to the NFS, and is both easier to calculate and does not produce intermediary results of indeterminate significance. However, its utility is hampered by the significant proportion of patients who, despite mild disease severity, are allocated high total scores due to obesity[113,114]. Although the NFS and BARD score accurately predict the onset of cirrhotic complications and liver-related mortality, their clinical application towards these goals is undermined by the inclusion of DM Ⅱ as independent predictor of adverse clinical outcome[115,116].

### *Markers of impaired hepatic functioning*

Progression from severe hepatic fibrosis to cirrhosis ultimately leads to decompensated liver failure and portal hypertension, biochemically reflected as thrombocytopenia, increased prothrombin time, hypoalbuminemia and hyperbilirubinemia. Platelet count is an

ideal biomarker for the prediction of advanced fibrosis in many chronic liver diseases[117] and features in multiple composite models currently available to the clinician. The clinical relevance of the AST to platelet ratio index (APRI) as a simple screening tool to predict advanced fibrosis has been demonstrated in the resource-limited South African setting, comparing favourably to the NFS with superior accuracy to the AAR[96]. While the APRI could serve to decrease the need for liver biopsy in this context, its utility in the evaluation of intermediary stages of fibrosis severity remains unclear[118,119].

Addition of age to the APRI yields the FIB-4 index, providing a model which, while still easily and affordably calculable, consistently outperforms other non-invasive tools in comparative studies for the identification as well as exclusion of advanced hepatic fibrosis[120,121]. The international normalized ratio (INR) is not only a significant independent predictor for advanced hepatic fibrosis in NAFLD, but improves the positive predictive value of the BARD score when incorporated as an additional variable, without compromising its negative predictive value[122]. Age, platelet count and/or INR are also included in several other predictive models which have not yet been validated in adult patients with NAFLD. These scoring panels incorporate a number of components known to independently predict fibrosis severity in NAFLD, including bilirubin[123], GGT[124] and albumin[106].

### *Markers of hepatocellular turnover*

Markers which reflect increased deposition or decreased degradation of extracellular matrix (ECM) components are obvious candidates for the evaluation of fibrosis severity in chronic liver disease. The accuracy of direct fibrotic biomarkers may exceed that for simple biomarker panels incorporating indirect markers of hepatic dysfunction[125]. ECM components are included in a number of complex patented biomarker panels, including the Original European Liver Fibrosis (OELF) score, simplified European Liver Fibrosis (ELF) score and NASH Diagnostics panel. One of the most widely investigated direct biomarkers is the high molecular weight polysaccharide hyaluronic acid (HA), with increased levels, resulting from accelerated collagen synthesis and decreased hepatic sinusoidal clearance, shown to accurately predict advanced fibrosis in NAFLD[126,127]. However, a significant drawback to the use of ECM markers such as HA is their lack of liver-specificity, as levels are affected by diverse factors such as renal failure, extra-hepatic fibrogenesis and dietary habits[128].

## LIMITATIONS OF COMPOSITE PROGNOSTIC MODELS

Individual biomarkers used for the peripheral evaluation of hepatic injury in NAFLD invariably fall short of the hypothetical ideal. Composite diagnostic and predictive models show greater discriminatory power compared to single-variable analysis and there is considerable interest in their potential value as non-invasive risk assessment tools. A number of important limitations however currently impede their routine use in clinical practice, as outlined below.

### *Diagnostic and predictive models are defined by variability*

Different models which assess the same outcome may differ markedly in their composition as well as methods employed for risk calculation, and many were initially developed for use in other chronic liver diseases such as viral hepatitis. Existing models were constructed against histological end-points defined by variable classification schemes and evaluated using liver biopsy as an imperfect diagnostic standard. Selection bias poses a major general concern, as initial studies often utilize heterogeneous and highly selected patient cohorts as well as different reference populations. Despite promising findings supporting their clinical value, confirming the reproducibility and robustness of existing composite prognostic models will depend on their external validation in large-scale prospective studies, considered a prerequisite for extrapolation to the general population.

### *Lack of consensus regarding clinically meaningful thresholds for histological severity*

Predictive models for the non-invasive assessment of the extent and severity of fibrotic injury should ideally reflect a dimensional pathogenic spectrum raging from ECM deposition though initial scarring, bridging as well as advanced fibrosis, and ultimately different stages of compensated and decompensated cirrhosis. While existing predictive models have proven useful in excluding advanced fibrosis, many require further validation in cases of intermediate severity. Their positive predictive value is also modest at best, and likely inferior to that of more complex scoring panels[129], which are again limited as the direct biomarkers they employ lack standardization as well as liver-specificity. A number of suggestions have been proposed in an attempt to address these shortcomings, including the concurrent use of multiple prognostic models towards the same goal[130]. The development of progressively more complex risk assessment schemes is intuitively a plausible solution, and while improving the performance for cirrhosis, this approach does not greatly increase the predictive accuracy of these models for NASH or non-severe fibrosis[131].

### *Limited value in predicting complications*

Composite non-invasive models have limited utility in the prediction of cirrhotic complications such as variceal bleeding[132]. There is still a disproportionate focus on liver-related as opposed to cardiovascular events, which is particularly relevant as CVD is the primary cause of mortality in NAFLD patients. Establishing

the utility of composite models in predicting cardio-metabolic complications, adverse clinical outcomes and mortality risk is therefore an important research focus for future prospective studies. Moreover, an important research objective in this regard would be to focus on determining to what extent the addition of a genomics component to prognostic models allows for the accurate prediction of long-term clinical outcomes.

## IMPROVING CLINICAL RISK PREDICTION ACROSS THE NAFLD SPECTRUM

There is ongoing research interest in determining to what extent the addition of biochemical and functional genomic markers and/or the application of emerging imaging technologies can assist in overcoming the abovementioned limitations restricting the more widespread clinical implementation of existing composite prognostic models applicable across the NAFLD spectrum by improving their diagnostic accuracy and predictive performance.

### *Incorporation of personalized genomic testing to existing composite prognostic models*

Epidemiological evidence concerning the extent towards which susceptibility for NAFLD involves a substantial heritability component remains conflicted[133,134]. This has led to ongoing research interest in elucidating the genetic mechanisms that may underlie marked population-based variation in disease prevalence and inter-patient heterogeneity in histological severity characteristic of this complex disease trait[135,136].

Two seminal population-based genome-wide association studies (GWAS) conducted in 2008[137,138] identified a common non-synonymous single-nucleotide polymorphism in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene (rs738409 C > G; I148M), which encodes the multifunctional lipolytic enzyme adiponutrin (ADPN)[139] as a major determinant of inter-individual variation in hepatic fat content and plasma liver enzyme levels. The PNPLA3 rs738409 SNP has subsequently been reproducibly associated with increased susceptibility towards the onset and progression of NAFLD across boundaries for age, sex and ethnic background[139-144]. Findings from at least two recent meta-analyses have also confirmed the clinical relevance of PNPLA3 rs738409 as a potent genetic risk factor for NASH, severe hepatic fibrosis and hepatocellular carcinoma[145,146].

Existing evidence concerning the exact molecular mechanisms which underlie the association between PNPLA3 rs738409 and increased susceptibility towards the onset and progression of hepatosteatosis however remains both inconclusive and conflicting[147]. ADPN possesses both triacylglycerol (TAG) lipase and acylglycerol O-acyltransferase activity with the known relationship between PNPLA3 rs738409 and decreased TAG hydrolysis[148,149] supporting a loss-of-function mechanism as underlying the promotion of macrovesicular hepatosteatosis due to increased hepatic lipid accumulation and/or export associated with impaired lipidation of very low-density lipoprotein particles[150,151]. PNPLA3 knockout *in vivo* does not however lead to increased intra-hepatic TAG accumulation[152] with increased lysophosphatidic acid acyltransferase activity and TAG synthesis noted for G-allele carriers, but rather supports a gain-of-function mechanism contributing towards the development of hepatosteatosis[153]. Li *et al*[154] however suggested that increased susceptibility towards hepatosteatosis associated with PNPLA3 rs738409 could be explained by a combination of increased TAG synthesis as well as decreased hydrolysis in the context of excess intra-hepatic FFA accumulation.

While PNPLA3 rs738409 confers susceptibility towards hepatosteatosis independent of extra-hepatic metabolic phenotypes, visceral adiposity mediates the severity of hepatic lipid infiltration in risk-allele carriers as well as the association of this genetic variant with related cardio-metabolic risk traits[155,156]. The rs738409 variant similarly confers risk for increased histological severity dissociated from its effects on central obesity and IR[157,158]. Its pathogenic role in impaired lipid homeostasis as evidenced by a peripheral decrease in serum triglyceride, total and high-density lipoprotein cholesterol[159,160] is furthermore unmasked in the presence of increased visceral adiposity[161] and impaired glucose tolerance[162], in addition to being modulated by lifestyle and dietary habits[161,163]. It has further been proposed that lipotoxicity and inflammatory stress resulting from impaired intra-hepatic lipid metabolism and free cholesterol deposition associated with PNPLA3 rs738409 activates dormant hepatic stellate cells (HSCs), leading to increased fibrogenesis. Severe hepatic fibrosis may occur in the absence of pathologically evident NASH, supporting the hypothesis that a direct stimulating effect on HSCs also contributes towards hepatic fibrogenesis independent of hepatic necro-inflammation[164].

Collectively, the abovementioned findings provide a useful framework for ongoing research aimed at establishing to what extent the incorporation of PNPLA3 genotyping as a component of existing composite prognostic models used in NAFLD/NASH could improve their performance. The addition of PNPLA3 genotyping to the NAFLD liver fat score fails to significantly increase its diagnostic accuracy[38], suggesting limited utility as a diagnostic marker for hepatosteatosis. However, the combined assessment of PNPLA3 rs378409 genotype along with fasting insulin and ALT levels has been shown to reliably predict the presence of undiagnosed NASH[165]. The observation that the genotype distribution and allele frequencies for PNPLA3 rs738409 closely mirror population-based prevalence rates for hepatosteatosis further supports the notion that a degree of the known variation in disease susceptibility observed between different population groups evident for NAFLD has a partly genetic basis[134]. This emphasizes the need for

further research aimed at clarifying the role of ethnicity as a putative modulator of genetic risk conferred by PNPLA3 rs738409. Evidence of gene-diet interactions that may be relevant to a specific population supports the need for development of lifestyle-based intervention strategies aimed at decreasing cumulative risk for development of NAFLD and progression to NASH[166].

GWAS have identified a number of other genetic variants as potential risk modifiers for progressive necroinflammatory injury and advanced hepatic fibrosis in NAFLD. For example, the PiS/PiZ variant of the α-1 antitrypsin (AAT) gene implicated in endoplasmic reticulum stress has been shown to predict hyperferritinemia and parenchymal iron overload independent of TS percentage despite its dissociation from risk for increased histological severity[167]. In a similar study, Valenti *et al*[168] investigated the utility of four polymorphic variants in the HFE, AAT, ferroportin-1 and beta-globin genes functioning in known iron metabolism pathways as putative predictive markers for parenchymal iron overload and increased histological severity. In this study[168], the authors demonstrated that that hepatocellular iron overload could be explained by risk-variant carriage in 63% of cases, with the beta-thalassemia trait showing the highest predictive accuracy for moderate-to-severe hepatic fibrosis in patients with NAFLD and/or DIOS.

The role of causative mutations in the HFE gene implicated in type Ⅰ HH as non-deterministic modifiers of susceptibility towards the onset and pathogenic progression of NAFLD remains incompletely elucidated. In an Italian study, Valenti *et al*[88] showed that heterozygosity for the deleterious C282Y mutation confers susceptibility towards hepatosteatosis even in the absence of overt cardio-metabolic risk. The C282Y mutation has been associated with NASH[169] as well as hepatocellular iron accumulation which could promote the progression of hepatic fibrogenesis, thereby conferring risk for increased histological severity secondary to iron-mediated oxidative stress and lipotoxicity[170-172]. Findings from conflicting studies[173,174] as well as a systematic review and meta-analysis[175] however fail to support the notion that HFE genotype is a significant genetic determinant of risk for the onset or progression of NAFLD and/or DIOS. In patients with a known diagnosis of NAFLD, confirmation that HFE genotype allows for the non-invasive prediction of parenchymal as opposed to sinusoidal iron overload could also provide evidence supporting the relevance of personalized genomic testing in the non-invasive differentiation between hepatosteatosis/DIOS and type Ⅰ HH. This issue was addressed in a study conducted by Valenti *et al*[176] who showed that, despite the association between C282Y mutation carriage and parenchymal siderosis typical of type Ⅰ HH, only approximately 33% of NAFLD patients exhibited this pattern of iron distribution, with HFE genotype explaining less than 50% of phenotypic variance for this trait. These findings suggest that HFE genotyping as a stand-alone genomic test most likely has limited utility as a reliable predictive marker for hepatocellular iron overload.

There is growing recognition that genetic variants implicated in the aberrant regulation of the ferroportin-hepcidin axis play a major role in the etiopathogenesis of iron-related disorders[177]. In accordance with this notion, a polymorphic variant (rs855791) in the matriptase-2 (*TMPRSS6*) gene has been associated with dysfunctional down-regulation of hepcidin expression implicated in the pathogenesis of a genetic subtype of iron-deficiency anaemia. It remains unclear however to what extent the association between TMPRSS6 rs855791 and iron deficiency is either mediated by or independent of hepcidin status, with existing evidence indicating that the pleiotropic effects this genetic variant exerts on serum iron profiles are likely context-dependent[178-180]. The putative role of TMPRSS6 rs855791 in the etiology of NAFLD has recently started to garner increasing research attention. Emerging evidence suggests that TMPRSS6 rs855791 is associated with hypoferritinemia and decreased hepatic iron stores independent of serum ferritin and HFE genotype, and may exert a protective effect against the development of hepatic siderosis and DM Ⅱ in patients with NAFLD[181,182]. Ongoing investigative effort is required not only to further elucidate the clinical significance of TMPRSS6 rs855791 as a potential genetic risk modifier for NASH and/or advanced hepatic siderosis but also to replicate its apparent dissociation from susceptibility towards DIOS considered in the context of known environmental and epistatic modulators of phenotypic expression in different population groups.

### *Incorporation of emerging imaging methodologies as a component of existing composite prognostic models*

Findings from multiple studies and meta-analyses further support the utility of transient elastography (TE) in diagnosing or excluding advanced hepatic fibrosis and cirrhosis with excellent accuracy, providing a reliable non-invasive tool used to assess and monitor the progression of fibrogenic activity in patients with NAFLD/NASH. To what extent extra-hepatic metabolic risk phenotypes as well as underlying pathology contribute towards variation in liver stiffness measurement (LSM) and influences the efficacy of TE however remains incompletely understood[183-187]. Emerging evidence further suggests that the concurrent assessment of LSM and PNPLA3 genotype alongside existing composite prognostic models used to predict advanced hepatic fibrosis in NAFLD could improve their performance with the goal of decreasing the need for invasive liver biopsy[188,189]. Future studies should ideally aim to validate these preliminary findings in large-scale population-based prospective studies.

## CONCLUSION

The routine clinical adoption of composite prognostic models as viable non-invasive risk stratification tools offers distinct advantages over the use of individual peripheral biomarkers, which have limited utility

compared to liver biopsy as the current standard for the diagnosis, grading and staging of NAFLD. In future, improved non-invasive diagnostic and predictive models incorporating genomics could allow for the timely implementation of tailored therapeutic intervention, aimed at preventing disease onset as well as decreasing risk for cardiovascular- and liver-related mortality in patients with NAFLD[190]. A number of important limitations however continue to impede more widespread clinical application as part of routine patient management, including the need for external validation in large-scale prospective studies to confirm their reproducibility and robustness as well as applicability in the general population. In response, ongoing investigative effort is required in order to assess new combinations of readily available biomarkers as novel prognostic models which could serve as cost-effective screening tools in a specific target population. In resource-limited settings the establishment and ongoing development of a database resource for health outcome studies may provide a viable alternative to prospective clinical trials for validation of eligibility criteria and treatment algorithms guided partly from the genetic background.

Given the decreasing cost and growing availability of genetic testing in the clinical domain, the routine use of personalized genomic risk screening in developing nations soon become a reality. This provides motivation for future research aimed at clarifying to what extent the incorporation of functional genomic markers and/or the application of emerging imaging technologies including TE could assist in overcoming the abovementioned limitations evident for existing composite prognostic models by improving their diagnostic accuracy and predictive performance. In particular, considering the emerging role of DIOS as an important secondary cause of hepatic siderosis distinct from type Ⅰ HH known to pose independent risk for new-onset CVD, subclinical atherosclerosis and decompensated liver failure, there is a pressing need to develop and validate non-invasive pre-clinical diagnostic algorithms to differentiate between DIOS and type Ⅰ HH in patients with the metabolic syndrome and/or NAFLD. The clinical implementation of a validated diagnostic model for DIOS could potentially allow for a more comprehensive approach to cardiovascular risk screening as well, allowing clinicians to identify an obese patient subgroup set to derive optimal benefit from the timely implementation of more aggressive and sustained lifestyle-based intervention strategies as well as tailored therapeutic adjuncts aimed at decreasing cumulative cardio-metabolic risk for early on in the disease process.

In addition to allowing for a more goal-directed use of liver biopsy in resource-limited settings, clinically validated composite models may in future be used to determine eligibility for genetic testing as part of the emerging arsenal of available tools used to guide patient management and improve the standard of patient care in NAFLD. Towards this goal, a pathology-supported genetic testing approach could prove useful in developing standardized pre-screen diagnostic algorithms to select non-responders to standard therapies and genetically uncharacterized NAFLD patients set to derive additional benefit from extended whole genome or exome sequencing[191,192]. Next-generation sequencing could be used to validate common susceptibility variants implicated in the etiopathogenesis of NAFLD, thereby supporting the development and validation of a genomics-based screening panel to provide greater insight into the value of personalized medicine applications[193,194]. In this context, a transdisciplinary "omics" approach has been proposed as a means of developing novel prognostic signatures incorporating relevant biochemical and genomic markers to optimize clinical diagnosis and improve risk management in patients with NAFLD[195]. Extension of genomics-based risk profiling beyond the limited scope of single-gene testing assisted by next-generation sequencing has the potential to facilitate the detection of both known and novel genetic variations aimed at the prevention of cumulative cardio-metabolic risk across the NAFLD spectrum.

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## REFERENCES

1 **Murray CJ**, Lopez AD. Measuring the global burden of disease. *N Engl J Med* 2013; **369**: 448-457 [PMID: 23902484 DOI: 10.1056/NEJMra1201534]
2 **Boutayeb A**, Boutayeb S. The burden of non communicable diseases in developing countries. *Int J Equity Health* 2005; **4**: 2 [PMID: 15651987 DOI: 10.1007/978-0-387-78665-0_31]
3 **Prentice AM**. The emerging epidemic of obesity in developing countries. *Int J Epidemiol* 2006; **35**: 93-99 [PMID: 16326822 DOI: 10.1093/ije/dyi272]
4 **Egger G**. Obesity, chronic disease, and economic growth: a case for "big picture" prevention. *Adv Prev Med* 2011; **2011**: 149158 [PMID: 21991431 DOI: 10.4061/2011/149158]
5 **Chang CY**. Understanding the relationship between PNPLA3, NAFLD and insulin resistance: do ethnic differences bring more questions or more answers? *Liver Int* 2011; **31**: 1246-1249 [PMID: 22093451 DOI: 10.1111/j.1478-3231.2011.02612.x]
6 **Bergqvist CJ**, Skoien R, Horsfall L, Clouston AD, Jonsson JR, Powell EE. Awareness and opinions of non-alcoholic fatty liver disease by hospital specialists. *Intern Med J* 2013; **43**: 247-253 [PMID: 22646061 DOI: 10.1111/j.1445-5994.2012.02848.x]
7 **Serfaty L**, Lemoine M. Definition and natural history of metabolic steatosis: clinical aspects of NAFLD, NASH and cirrhosis. *Diabetes Metab* 2008; **34**: 634-637 [PMID: 19195623 DOI: 10.1016/S1262-3636(08)74597-X]
8 **Basaranoglu M**, Basaranoglu G, Sentürk H. From fatty liver to fibrosis: a tale of "second hit". *World J Gastroenterol* 2013; **19**: 1158-1165 [PMID: 23483818 DOI: 10.3748/wjg.v19.i8.1158]
9 **Brunt EM**, Tiniakos DG. Histopathology of nonalcoholic fatty

liver disease. *World J Gastroenterol* 2010; **16**: 5286-5296 [PMID: 21072891 DOI: 10.3748/wjg.v16.i42.5286]
10 **Nalbantoglu IL**, Brunt EM. Role of liver biopsy in nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 9026-9037 [PMID: 25083076 DOI: 10.3748/wjg.v20.i27.9026]
11 **El Bassat H**, Ziada DH, Hasby EA, Nagy H, Abo Ryia MH. Apoptotic and anti-apoptotic seromarkers for assessment of disease severity of non-alcoholic steatohepatitis. *Arab J Gastroenterol* 2014; **15**: 6-11 [PMID: 24630506 DOI: 10.1016/j.ajg.2014.01.009]
12 **Kawanaka M**, Mahmood S, Niiyama G, Izumi A, Kamei A, Ikeda H, Suehiro M, Togawa K, Sasagawa T, Okita M, Nakamura H, Yodoi J, Yamada G. Control of oxidative stress and reduction in biochemical markers by Vitamin E treatment in patients with nonalcoholic steatohepatitis: a pilot study. *Hepatol Res* 2004; **29**: 39-41 [PMID: 15135345]
13 **Machado MV**, Ravasco P, Jesus L, Marques-Vidal P, Oliveira CR, Proença T, Baldeiras I, Camilo ME, Cortez-Pinto H. Blood oxidative stress markers in non-alcoholic steatohepatitis and how it correlates with diet. *Scand J Gastroenterol* 2008; **43**: 95-102 [PMID: 18938777]
14 **Jarrar MH**, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, Elariny H, Goodman Z, Chandhoke V, Younossi ZM. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **27**: 412-421 [PMID: 18081738 DOI: 10.1111/j.1365-2036.2007.03586.x]
15 **Braunersreuther V**, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 727-735 [PMID: 22371632 DOI: 10.3748/wjg.v18.i8.727]
16 **Baranova A**, Lal P, Birerdinc A, Younossi ZM. Non-invasive markers for hepatic fibrosis. *BMC Gastroenterol* 2011; **11**: 91 [PMID: 21849046 DOI: 10.1186/1471-230X-11-91]
17 **Alkhouri N**, McCullough AJ. Noninvasive Diagnosis of NASH and Liver Fibrosis Within the Spectrum of NAFLD. *Gastroenterol Hepatol* (N Y) 2012; **8**: 661-668 [PMID: 24683373 DOI: 10.1016/s0016-5085(10)62097-5]
18 **Festi D**, Schiumerini R, Marzi L, Di Biase AR, Mandolesi D, Montrone L, Scaioli E, Bonato G, Marchesini-Reggiani G, Colecchia A. Review article: the diagnosis of non-alcoholic fatty liver disease -- availability and accuracy of non-invasive methods. *Aliment Pharmacol Ther* 2013; **37**: 392-400 [PMID: 23278163 DOI: 10.1111/apt.12186]
19 **Castera L**, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 666-675 [PMID: 24061203 DOI: 10.1038/nrgastro.2013.175]
20 **Machado MV**, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. *J Hepatol* 2013; **58**: 1007-1019 [PMID: 23183525 DOI: 10.1016/j.jhep.2012.11.021]
21 **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]
22 **Schindhelm RK**, Diamant M, Dekker JM, Tushuizen ME, Teerlink T, Heine RJ. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab Res Rev* 2006; **22**: 437-443 [PMID: 16832839 DOI: 10.1002/dmrr.666]
23 **Yun KE**, Shin CY, Yoon YS, Park HS. Elevated alanine aminotransferase levels predict mortality from cardiovascular disease and diabetes in Koreans. *Atherosclerosis* 2009; **205**: 533-537 [PMID: 19159884 DOI: 10.1016/j.atherosclerosis.2008.12.012]
24 **Esteghamati A**, Jamali A, Khalilzadeh O, Noshad S, Khalili M, Zandieh A, Morteza A, Nakhjavani M. Metabolic syndrome is linked to a mild elevation in liver aminotransferases in diabetic patients with undetectable non-alcoholic fatty liver disease by ultrasound. *Diabetol Metab Syndr* 2010; **2**: 65 [PMID: 21047423 DOI: 10.1186/1758-5996-2-65]
25 **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]
26 **Ong JP**, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* 2008; **49**: 608-612 [PMID: 18682312 DOI: 10.1016/j.jhep.2008.06.018]
27 **Misra VL**, Khashab M, Chalasani N. Nonalcoholic fatty liver disease and cardiovascular risk. *Curr Gastroenterol Rep* 2009; **11**: 50-55 [PMID: 19166659 DOI: 10.1007/s11894-009-0008-4]
28 **Ahmed MH**, Barakat S, Almobarak AO. Nonalcoholic fatty liver disease and cardiovascular disease: has the time come for cardiologists to be hepatologists? *J Obes* 2012; **2012**: 483135 [PMID: 23320150 DOI: 10.1155/2012/483135]
29 **Wong VW**, Wong GL, Yip GW, Lo AO, Limquiaco J, Chu WC, Chim AM, Yu CM, Yu J, Chan FK, Sung JJ, Chan HL. Coronary artery disease and cardiovascular outcomes in patients with non-alcoholic fatty liver disease. *Gut* 2011; **60**: 1721-1727 [PMID: 21602530 DOI: 10.1136/gut.2011.242016]
30 **Dekker JM**, Girman C, Rhodes T, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ. Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study. *Circulation* 2005; **112**: 666-673 [PMID: 16061755 DOI: 10.1161/circulationaha.104.516948]
31 **Fracanzani AL**, Burdick L, Raselli S, Pedotti P, Grigore L, Santorelli G, Valenti L, Maraschi A, Catapano A, Fargion S. Carotid artery intima-media thickness in nonalcoholic fatty liver disease. *Am J Med* 2008; **121**: 72-78 [PMID: 18187076 DOI: 10.1016/j.amjmed.2007.08.041]
32 **Iacobellis G**, Barbarini G, Letizia C, Barbaro G. Epicardial fat thickness and nonalcoholic fatty liver disease in obese subjects. *Obesity* (Silver Spring) 2014; **22**: 332-336 [PMID: 24115757 DOI: 10.1002/oby.20624]
33 **Tarantino G**, Costantini S, Finelli C, Capone F, Guerriero E, La Sala N, Gioia S, Castello G. Carotid intima-media thickness is predicted by combined eotaxin levels and severity of hepatic steatosis at ultrasonography in obese patients with Nonalcoholic Fatty Liver Disease. *PLoS One* 2014; **9**: e105610 [PMID: 25268946 DOI: 10.1371/journal.pone.0105610]
34 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293 DOI: 10.1186/1471-230X-6-33]
35 **Bedogni G**, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. *BMC Gastroenterol* 2010; **10**: 98 [PMID: 20738844 DOI: 10.1186/1471-230X-10-98]
36 **Jung CH**, Lee WJ, Hwang JY, Yu JH, Shin MS, Lee MJ, Jang JE, Leem J, Park JY, Kim HK. Assessment of the fatty liver index as an indicator of hepatic steatosis for predicting incident diabetes independently of insulin resistance in a Korean population. *Diabet Med* 2013; **30**: 428-435 [PMID: 23278318 DOI: 10.1111/dme.12104]
37 **Zelber-Sagi S**, Webb M, Assy N, Blendis L, Yeshua H, Leshno M, Ratziu V, Halpern Z, Oren R, Santo E. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. *World J Gastroenterol* 2013; **19**: 57-64 [PMID: 23326163 DOI: 10.3748/wjg.v19.i1.57]
38 **Kotronen A**, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, Orho-Melander M, Yki-Järvinen H. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology* 2009; **137**: 865-872 [PMID: 19524579 DOI: 10.1053/j.gastro.2009.06.005]
39 **Lee JH**, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis* 2010; **42**: 503-508 [PMID: 19766548 DOI: 10.1016/j.dld.2009.08.002]
40 **Fuyan S**, Jing L, Wenjun C, Zhijun T, Weijing M, Suzhen W,

Yongyong X. Fatty liver disease index: a simple screening tool to facilitate diagnosis of nonalcoholic fatty liver disease in the Chinese population. *Dig Dis Sci* 2013; **58**: 3326-3334 [PMID: 23900558 DOI: 10.1007/s10620-013-2774-y]

41 **Calori G**, Lattuada G, Ragogna F, Garancini MP, Crosignani P, Villa M, Bosi E, Ruotolo G, Piemonti L, Perseghin G. Fatty liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology* 2011; **54**: 145-152 [PMID: 21488080 DOI: 10.1002/hep.24356]

42 **Borman MA**, Ladak F, Crotty P, Pollett A, Kirsch R, Pomier-Layrargues G, Beaton M, Duarte-Rojo A, Elkashab M, Myers RP. The Fatty Liver Index has limited utility for the detection and quantification of hepatic steatosis in obese patients. *Hepatol Int* 2013; **7** Suppl 2: 592-599 [DOI: 10.1111/jgh.12557]

43 **Jiang ZY**, Xu CY, Chang XX, Li WW, Sun LY, Yang XB, Yu LF. Fatty liver index correlates with non-alcoholic fatty liver disease, but not with newly diagnosed coronary artery atherosclerotic disease in Chinese patients. *BMC Gastroenterol* 2013; **13**: 110 [PMID: 23834773 DOI: 10.1186/1471-230X-13-110]

44 **Dyson J**, Day C. Treatment of non-alcoholic fatty liver disease. *Dig Dis* 2014; **32**: 597-604 [PMID: 25034293 DOI: 10.1159/000360511]

45 **Harrison SA**, Day CP. Benefits of lifestyle modification in NAFLD. *Gut* 2007; **56**: 1760-1769 [PMID: 17911352 DOI: 10.1136/gut.2006.112094]

46 **Rafiq N**, Younossi ZM. Effects of weight loss on nonalcoholic fatty liver disease. *Semin Liver Dis* 2008; **28**: 427-433 [PMID: 18956298 DOI: 10.1055/s-0028-1091986]

47 **Scaglioni F**, Marino M, Ciccia S, Procaccini A, Busacchi M, Loria P, Lonardo A, Malavolti M, Battistini NC, Pellegrini M, Carubbi F, Bellentani S. Short-term multidisciplinary non-pharmacological intervention is effective in reducing liver fat content assessed non-invasively in patients with nonalcoholic fatty liver disease (NAFLD). *Clin Res Hepatol Gastroenterol* 2013; **37**: 353-358 [PMID: 23273500 DOI: 10.1016/j.clinre.2012.10.009]

48 **Centis E**, Marzocchi R, Suppini A, Dalle Grave R, Villanova N, Hickman IJ, Marchesini G. The role of lifestyle change in the prevention and treatment of NAFLD. *Curr Pharm Des* 2013; **19**: 5270-5279 [PMID: 23394095 DOI: 10.2174/13816128113192900008]

49 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

50 **Villanova N**, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, Marchesini G. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 473-480 [PMID: 15981216 DOI: 10.1002/hep.20781]

51 **Goland S**, Shimoni S, Zornitzki T, Knobler H, Azoulai O, Lutaty G, Melzer E, Orr A, Caspi A, Malnick S. Cardiac abnormalities as a new manifestation of nonalcoholic fatty liver disease: echocardiographic and tissue Doppler imaging assessment. *J Clin Gastroenterol* 2006; **40**: 949-955 [PMID: 17063117 DOI: 10.1097/01.mcg.0000225668.53673.e6]

52 **Dixon JB**, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; **121**: 91-100 [PMID: 11438497 DOI: 10.1053/gast.2001.25540]

53 **Gholam PM**, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. *Am J Gastroenterol* 2007; **102**: 399-408 [PMID: 17311652 DOI: 10.1111/j.1572-0241.2006.01041.x]

54 **Campos GM**, Bambha K, Vittinghoff E, Rabl C, Posselt AM, Ciovica R, Tiwari U, Ferrel L, Pabst M, Bass NM, Merriman RB. A clinical scoring system for predicting nonalcoholic steatohepatitis in morbidly obese patients. *Hepatology* 2008; **47**: 1916-1923 [PMID: 18433022 DOI: 10.1002/hep.22241]

55 **Ulitsky A**, Ananthakrishnan AN, Komorowski R, Wallace J, Surapaneni SN, Franco J, Saeian K, Gawrieh S. A noninvasive clinical scoring model predicts risk of nonalcoholic steatohepatitis in morbidly obese patients. *Obes Surg* 2010; **20**: 685-691 [PMID: 20336392 DOI: 10.1007/s11695-010-0118-y]

56 **Zein CO**, Edmison JM, Schluchter M, Feldstein AE, Zein NN, McCullough A. A NASH predictive index (NPI) for use in patients with nonalcoholic fatty liver disease. *Hepatol* 2007; **46**: 747A

57 **Schwimmer JB**, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics* 2005; **115**: e561-e565 [PMID: 15867021 DOI: 10.1542/peds.2004-1832]

58 **Pan JJ**, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol* 2014; **6**: 274-283 [PMID: 24868321 DOI: 10.4254/wjh.v6.i5.274]

59 **Hashimoto E**, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J Gastroenterol* 2011; **46** Suppl 1: 63-69 [PMID: 20844903 DOI: 10.1007/s00535-010-0311-8]

60 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]

61 **Bhatia LS**, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? *Eur Heart J* 2012; **33**: 1190-1200 [PMID: 22408036 DOI: 10.1093/eurheartj/ehr453]

62 **Verma S**, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int* 2013; **33**: 1398-1405 [PMID: 23763360 DOI: 10.1111/liv.12226]

63 **Alam S**, Noor-E-Alam SM, Chowdhury ZR, Alam M, Kabir J. Nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients of Bangladesh. *World J Hepatol* 2013; **5**: 281-287 [PMID: 23717739 DOI: 10.4254/wjh.v5.i5.281]

64 **Lee DS**, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, Wang TJ, Benjamin EJ, D'Agostino RB, Vasan RS. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol* 2007; **27**: 127-133 [PMID: 17095717 DOI: 10.1161/01.ATV.0000251993.20372.40]

65 **Tanné F**, Gagnadoux F, Chazouillères O, Fleury B, Wendum D, Lasnier E, Lebeau B, Poupon R, Serfaty L. Chronic liver injury during obstructive sleep apnea. *Hepatology* 2005; **41**: 1290-1296 [PMID: 15915459 DOI: 10.1002/hep.20725]

66 **Weingarten TN**, Mantilla CB, Swain JM, Kendrick ML, Oberhansley JM, Burcham RJ, Ribeiro TC, Watt KD, Schroeder DR, Narr BJ, Sprung J. Nonalcoholic steatohepatitis in bariatric patients with a diagnosis of obstructive sleep apnea. *Obes Facts* 2012; **5**: 587-596 [PMID: 22986647 DOI: 10.1159/000342677]

67 **Tahiri M**, Drighil A, Jalal Y, Ghellab D, Hliwa W, Fouad H, Badre W, Bellabah A, Habbal R, Alaoui R. Chronic permanent hypoxemia predisposes to mild elevation of liver stiffness. *World J Gastroenterol* 2014; **20**: 10564-10569 [PMID: 25132776 DOI: 10.3748/wjg.v20.i30.10564]

68 **Mirrakhimov AE**, Polotsky VY. Obstructive sleep apnea and non-alcoholic Fatty liver disease: is the liver another target? *Front Neurol* 2012; **3**: 149 [PMID: 23087670 DOI: 10.3389/fneur.2012.00149]

69 **Hirsso PK**, Timonen MJ, Jokelainen JJ, Hiltunen LA, Laakso MA, Hedberg PS, Ruokonen AO, Koskela P, Härkönen PK, Keinänen-Kiukaanniemi SM, Rajala UM. Association between high-sensitive measurement of C-reactive protein and metabolic syndrome as defined by International Diabetes Federation, National Cholesterol Education Program, and World Health Organization criteria in a population-based cohort of 55-year-old Finnish individuals. *Diabetes Care* 2006; **29**: 2177-2178 [PMID: 16936176 DOI: 10.2337/dc06-0951]

70 **Pfützner A**, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. *Diabetes Technol Ther* 2006; **8**: 28-36 [PMID: 16472048 DOI: 10.1089/dia.2006.8.28]

71 **Ridker PM**. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory

hypothesis toward consensus. *J Am Coll Cardiol* 2007; **49**: 2129-2138 [PMID: 17531663 DOI: 10.1016/j.jacc.2007.02.052]
72 **Targher G**. High-sensitivity C-reactive protein, obesity, and subclinical atherosclerosis: implications of JUPITER from the MESA study. *Arterioscler Thromb Vasc Biol* 2011; **31**: 1251-1252 [PMID: 21593456 DOI: 10.1161/ATVBAHA.111.228320]
73 **van der Velde M**, Bello AK, Brantsma AH, El Nahas M, Bakker SJ, de Jong PE, Gansevoort RT. Do albuminuria and hs-CRP add to the International Diabetes Federation definition of the metabolic syndrome in predicting outcome? *Nephrol Dial Transplant* 2012; **27**: 2275-2283 [PMID: 22231032 DOI: 10.1093/ndt/gfr634]
74 **Pearce SG**, Thosani NC, Pan JJ. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. *Biomark Res* 2013; **1**: 7 [PMID: 24252302 DOI: 10.1186/2050-7771-1-7]
75 **Feldstein AE**, Alkhouri N, De Vito R, Alisi A, Lopez R, Nobili V. Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. *Am J Gastroenterol* 2013; **108**: 1526-1531 [PMID: 23752877 DOI: 10.1038/ajg.2013.168]
76 **Cusi K**, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, Ortiz-Lopez C, Hecht J, Feldstein AE, Webb A, Louden C, Goros M, Tio F. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **60**: 167-174 [PMID: 23973932 DOI: 10.1016/j.jhep.2013.07.042]
77 **Sharifi F**, Nasab NM, Zadeh HJ. Elevated serum ferritin concentrations in prediabetic subjects. *Diab Vasc Dis Res* 2008; **5**: 15-18 [PMID: 18398807 DOI: 10.3132/dvdr.2008.003]
78 **Park SK**, Ryoo JH, Kim MG, Shin JY. Association of serum ferritin and the development of metabolic syndrome in middle-aged Korean men: a 5-year follow-up study. *Diabetes Care* 2012; **35**: 2521-2526 [PMID: 22933431 DOI: 10.2337/dc12-0543]
79 **Abril-Ulloa V**, Flores-Mateo G, Solà-Alberich R, Manuel-y-Keenoy B, Arija V. Ferritin levels and risk of metabolic syndrome: meta-analysis of observational studies. *BMC Public Health* 2014; **14**: 483 [PMID: 24884526 DOI: 10.1186/1471-2458-14-483]
80 **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 DOI: 10.1016/j.jhep.2006.09.018]
81 **Juárez-Rojas JG**, Medina-Urrutia AX, Jorge-Galarza E, González-Salazar C, Kimura-Hayama E, Cardoso-Saldaña G, Posadas-Sánchez R, Martínez-Alvarado R, Posadas-Romero C. Fatty liver increases the association of metabolic syndrome with diabetes and atherosclerosis. *Diabetes Care* 2013; **36**: 1726-1728 [PMID: 23250798 DOI: 10.2337/dc12-1276]
82 **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]
83 **Sumida Y**, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, Eguchi Y, Suzuki Y, Imai S, Kanemasa K, Fujita K, Chayama K, Yasui K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Okanoue T. A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 257-268 [PMID: 20842510 DOI: 10.1007/s00535-010-0305-6]
84 **Manousou P**, Kalambokis G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, Leandro G, Arvaniti V, Germani G, Patch D, Calvaruso V, Mikhailidis DP, Dhillon AP, Burroughs AK. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. *Liver Int* 2011; **31**: 730-739 [PMID: 21457446 DOI: 10.1111/j.1478-3231.2011.02488.x]
85 **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]
86 **Chandok N**, Minuk G, Wengiel M, Uhanova J. Serum ferritin levels do not predict the stage of underlying non-alcoholic fatty liver disease. *J Gastrointestin Liver Dis* 2012; **21**: 53-58 [PMID: 22457860]
87 **Yoneda M**, Thomas E, Sumida Y, Imajo K, Eguchi Y, Hyogo H, Fujii H, Ono M, Kawaguchi T, Schiff ER. Clinical usage of serum ferritin to assess liver fibrosis in patients with non-alcoholic fatty liver disease: Proceed with caution. *Hepatol Res* 2014; **44**: E499-E502 [PMID: 24628717 DOI: 10.1111/hepr.12327]
88 **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]
89 **Ausk KJ**, Ioannou GN. Is obesity associated with anemia of chronic disease? A population-based study. *Obesity (Silver Spring)* 2008; **16**: 2356-2361 [PMID: 18719644 DOI: 10.1038/oby.2008.353]
90 **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]
91 **Riva A**, Trombini P, Mariani R, Salvioni A, Coletti S, Bonfadini S, Paolini V, Pozzi M, Facchetti R, Bovo G, Piperno A. Revaluation of clinical and histological criteria for diagnosis of dysmetabolic iron overload syndrome. *World J Gastroenterol* 2008; **14**: 4745-4752 [PMID: 18720534 DOI: 10.3748/wjg.14.4745]
92 **Angulo P**. Diagnosing steatohepatitis and predicting liver-related mortality in patients with NAFLD: two distinct concepts. *Hepatology* 2011; **53**: 1792-1794 [PMID: 21557278 DOI: 10.1002/hep.24403]
93 **Poynard T**, Munteanu M, Deckmyn O, Ngo Y, Drane F, Castille JM, Housset C, Ratziu V, Imbert-Bismut F. Validation of liver fibrosis biomarker (FibroTest) for assessing liver fibrosis progression: proof of concept and first application in a large population. *J Hepatol* 2012; **57**: 541-548 [PMID: 22612998 DOI: 10.1016/j.jhep.2012.04.025]
94 **Targher G**, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia* 2008; **51**: 1947-1953 [PMID: 18762907 DOI: 10.1007/s00125-008-1135-4]
95 **Yilmaz Y**, Kurt R, Yonal O, Polat N, Celikel CA, Gurdal A, Oflaz H, Ozdogan O, Imeryuz N, Kalayci C, Avsar E. Coronary flow reserve is impaired in patients with nonalcoholic fatty liver disease: association with liver fibrosis. *Atherosclerosis* 2010; **211**: 182-186 [PMID: 20181335 DOI: 10.1016/j.atherosclerosis.2010.01.049]
96 **Kruger FC**, Daniels CR, Kidd M, Swart G, Brundyn K, van Rensburg C, Kotze M. APRI: a simple bedside marker for advanced fibrosis that can avoid liver biopsy in patients with NAFLD/NASH. *S Afr Med J* 2011; **101**: 477-480 [PMID: 21920102]
97 **Ratziu V**, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123 [PMID: 10833486 DOI: 10.1016/s0016-5085(00)70364-7]
98 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]
99 **Udell JA**, Wang CS, Tinmouth J, FitzGerald JM, Ayas NT, Simel DL, Schulzer M, Mak E, Yoshida EM. Does this patient with liver disease have cirrhosis? *JAMA* 2012; **307**: 832-842 [PMID: 22357834 DOI: 10.1001/jama.2012.186]
100 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112 [PMID: 19523535 DOI: 10.1016/j.cgh.2009.05.033]
101 **Sud A**, Hui JM, Farrell GC, Bandara P, Kench JG, Fung C, Lin R, Samarasinghe D, Liddle C, McCaughan GW, George J. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004; **39**: 1239-1247 [PMID: 15122752 DOI: 10.1002/hep.20207]
102 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-

Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992 [PMID: 12297848 DOI: 10.1016/s0270-9139(02)00107-6]

103 **Hui AY**, Chan HL, Wong VW, Liew CT, Chim AM, Chan FK, Sung JJ. Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. *Am J Gastroenterol* 2005; **100**: 616-623 [PMID: 15743360 DOI: 10.1111/j.1572-0241.2005.41289.x]

104 **Cross TJ**, Rizzi P, Berry PA, Bruce M, Portmann B, Harrison PM. King's Score: an accurate marker of cirrhosis in chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2009; **21**: 730-738 [PMID: 19430302 DOI: 10.1097/MEG.0b013e32830dfcb]

105 **Lok AS**, Ghany MG, Goodman ZD, Wright EC, Everson GT, Sterling RK, Everhart JE, Lindsay KL, Bonkovsky HL, Di Bisceglie AM, Lee WM, Morgan TR, Dienstag JL, Morishima C. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005; **42**: 282-292 [PMID: 15986415 DOI: 10.1002/hep.20772]

106 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]

107 **Gan L**, Chitturi S, Farrell GC. Mechanisms and implications of age-related changes in the liver: nonalcoholic Fatty liver disease in the elderly. *Curr Gerontol Geriatr Res* 2011; **2011**: 831536 [PMID: 21918648 DOI: 10.1155/2011/831536]

108 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362 [PMID: 10573511 DOI: 10.1002/hep.510300604]

109 **Sorbi D**, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol* 1999; **94**: 1018-1022 [PMID: 10201476 DOI: 10.1016/s0002-9270(99)00061-1]

110 **Qureshi K**, Clements RH, Abrams GA. The utility of the "NAFLD fibrosis score" in morbidly obese subjects with NAFLD. *Obes Surg* 2008; **18**: 264-270 [PMID: 18214632 DOI: 10.1007/s11695-007-9295-8]

111 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

112 **Treeprasertsuk S**, Björnsson E, Enders F, Suwanwalaikorn S, Lindor KD. NAFLD fibrosis score: a prognostic predictor for mortality and liver complications among NAFLD patients. *World J Gastroenterol* 2013; **19**: 1219-1229 [PMID: 23482703 DOI: 10.3748/wjg.v19.i8.1219]

113 **Ruffillo G**, Fassio E, Alvarez E, Landeira G, Longo C, Domínguez N, Gualano G. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. *J Hepatol* 2011; **54**: 160-163 [PMID: 20934232 DOI: 10.1016/j.jhep.2010.06.028]

114 **Cichoż-Lach H**, Celiński K, Prozorow-Król B, Swatek J, Słomka M, Lach T. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. *Med Sci Monit* 2012; **18**: CR735-CR740 [PMID: 23197236 DOI: 10.12659/msm.883601]

115 **Angulo P**, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Barrera F, Haflidadottir S, Day CP, George J. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2013; **145**: 782-789.e4 [PMID: 23860502 DOI: 10.1053/j.gastro.2013.06.057]

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

117 **Yoneda M**, Fujii H, Sumida Y, Hyogo H, Itoh Y, Ono M, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Imajo K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T. Platelet count for predicting fibrosis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 1300-1306 [PMID: 21750883 DOI: 10.1007/s00535-011-0436-4]

118 **Yilmaz Y**, Yonal O, Kurt R, Bayrak M, Aktas B, Ozdogan O. Noninvasive assessment of liver fibrosis with the aspartate transaminase to platelet ratio index (APRI): Usefulness in patients with chronic liver disease: APRI in chronic liver disease. *Hepat Mon* 2011; **11**: 103-106 [PMID: 22087126]

119 **Agilli M**, Aydin FN, Cayci T, Kurt YG. Is aspartate aminotransferase-to-platelet ratio index a biomarker in the evaluation of advanced fibrosis in non-alcoholic fatty liver disease? *Gastroenterol Rep* (Oxf) 2014; **2**: 323-324 [PMID: 25371481 DOI: 10.1093/gastro/gou063]

120 **McPherson S**, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 1265-1269 [PMID: 20801772 DOI: 10.1136/gut.2010.216077]

121 **McPherson S**, Anstee QM, Henderson E, Day CP, Burt AD. Are simple noninvasive scoring systems for fibrosis reliable in patients with NAFLD and normal ALT levels? *Eur J Gastroenterol Hepatol* 2013; **25**: 652-658 [PMID: 23325287 DOI: 10.1097/MEG.0b013e32835d72cf]

122 **Lee TH**, Han SH, Yang JD, Kim D, Ahmed M. Prediction of Advanced Fibrosis in Nonalcoholic Fatty Liver Disease: An Enhanced Model of BARD Score. *Gut Liver* 2013; **7**: 323-328 [PMID: 23710314 DOI: 10.5009/gnl.2013.7.3.323]

123 **Demir M**, Lang S, Schlattjan M, Drebber U, Wedemeyer I, Nierhoff D, Kaul I, Sowa J, Canbay A, Töx U, Steffen HM. NIKEI: a new inexpensive and non-invasive scoring system to exclude advanced fibrosis in patients with NAFLD. *PLoS One* 2013; **8**: e58360 [PMID: 23555578 DOI: 10.1371/journal.pone.0058360]

124 **Franzini M**, Fornaciari I, Fierabracci V, Elawadi HA, Bolognesi V, Maltinti S, Ricchiuti A, De Bortoli N, Marchi S, Pompella A, Passino C, Emdin M, Paolicchi A. Accuracy of b-GGT fraction for the diagnosis of non-alcoholic fatty liver disease. *Liver Int* 2012; **32**: 629-634 [PMID: 22098947 DOI: 10.1111/j.1478-3231.2011.02673.x]

125 **Tanwar S**, Trembling PM, Ellis E, Parkes L, Herold C, Schuppan D, Rosenberg WM. Direct non-invasive serum markers of liver fibrosis predict fibrosis evolution in chronic hepatitis C but are increased by interferon-based therapy. *Gut* 2012; **61**: A143-A144 [DOI: 10.1136/gutjnl-2012-302514b.172]

126 **Sakugawa H**, Nakayoshi T, Kobashigawa K, Yamashiro T, Maeshiro T, Miyagi S, Shiroma J, Toyama A, Nakayoshi T, Kinjo F, Saito A. Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2005; **11**: 255-259 [PMID: 15633226 DOI: 10.3748/wjg.v11.i2.255]

127 **Kaneda H**, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K. Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2006; **21**: 1459-1465 [PMID: 16911693 DOI: 10.1111/j.1440-1746.2006.04447.x]

128 **Adams LA**. Biomarkers of liver fibrosis. *J Gastroenterol Hepatol* 2011; **26**: 802-809 [PMID: 21198831 DOI: 10.1111/j.1440-1746.2010.06612.x]

129 **Adams LA**, George J, Bugianesi E, Rossi E, De Boer WB, van der Poorten D, Ching HL, Bulsara M, Jeffrey GP. Complex non-invasive fibrosis models are more accurate than simple models in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2011; **26**: 1536-1543 [PMID: 21950746 DOI: 10.1111/j.1440-1746.2011.06774.x]

130 **Wong L**, Roberts M, McCorry R. Identifying NAFLD in patients attending a lipid clinic - do non-invasive scoring systems for fibrosis have a role? *Gut* 2014; **63**: 89-90 [DOI: 10.1136/gutjnl-2014-307263.189]

131 **Neuschwander-Tetri BA**, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, Zein CO, Brunt EM, Kleiner DE, McCullough AJ, Sanyal AJ, Diehl AM, Lavine JE, Chalasani N, Kowdley KV. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 913-924 [PMID: 20648476 DOI: 10.1002/hep.23784]

132 **Naveau S**, Gaudé G, Asnacios A, Agostini H, Abella A, Barri-Ova N, Dauvois B, Prévot S, Ngo Y, Munteanu M, Balian A, Njiké-Nakseu M, Perlemuter G, Poynard T. Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. *Hepatology* 2009; **49**: 97-105 [PMID: 19053048 DOI: 10.1002/hep.22576]

133 **Schwimmer JB**, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1585-1592 [PMID: 19208353 DOI: 10.1053/j.gastro.2009.01.050]

134 **Tarnoki AD**, Tarnoki DL, Bata P, Littvay L, Osztovits J, Jermendy G, Karlinger K, Lannert A, Preda I, Kiss RG, Molnar AA, Garami Z, Baffy G, Berczi V. Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. *Liver Int* 2012; **32**: 1287-1293 [PMID: 22651705 DOI: 10.1111/j.1478-3231.2012.02823.x]

135 **Anstee QM**, Daly AK, Day CP. Genetic modifiers of non-alcoholic fatty liver disease progression. *Biochim Biophys Acta* 2011; **1812**: 1557-1566 [PMID: 21840395 DOI: 10.1016/j.bbadis.2011.07.017]

136 **Dongiovanni P**, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. *Curr Pharm Des* 2013; **19**: 5219-5238 [PMID: 23394097 DOI: 10.2174/1381612811319990381]

137 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]

138 **Yuan X**, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, Zhang W, Vollenweider P, Stirnadel H, Johnson T, Bergmann S, Beckmann ND, Li Y, Ferrucci L, Melzer D, Hernandez D, Singleton A, Scott J, Elliott P, Waeber G, Cardon L, Frayling TM, Kooner JS, Mooser V. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet* 2008; **83**: 520-528 [PMID: 18940312 DOI: 10.1016/j.ajhg.2008.09.012]

139 **He S**, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, Cohen JC, Hobbs HH. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010; **285**: 6706-6715 [PMID: 20034933 DOI: 10.1074/jbc.M109.064501]

140 **Hotta K**, Yoneda M, Hyogo H, Ochi H, Mizusawa S, Ueno T, Chayama K, Nakajima A, Nakao K, Sekine A. Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease. *BMC Med Genet* 2010; **11**: 172 [PMID: 21176169 DOI: 10.1186/1471-2350-11-172]

141 **Valenti L**, Alisi A, Galmozzi E, Bartuli A, Del Menico B, Alterio A, Dongiovanni P, Fargion S, Nobili V. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 1274-1280 [PMID: 20648474 DOI: 10.1002/hep.23823]

142 **Wagenknecht LE**, Palmer ND, Bowden DW, Rotter JI, Norris JM, Ziegler J, Chen YD, Haffner S, Scherzinger A, Langefeld CD. Association of PNPLA3 with non-alcoholic fatty liver disease in a minority cohort: the Insulin Resistance Atherosclerosis Family Study. *Liver Int* 2011; **31**: 412-416 [PMID: 21281435 DOI: 10.1111/j.1478-3231.2010.02444.x]

143 **Peng XE**, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X. Genetic variants in PNPLA3 and risk of non-alcoholic fatty liver disease in a Han Chinese population. *PLoS One* 2012; **7**: e50256 [PMID: 23226254 DOI: 10.1371/journal.pone.0050256]

144 **Dongiovanni P**, Donati B, Fares R, Lombardi R, Mancina RM, Romeo S, Valenti L. PNPLA3 I148M polymorphism and progressive liver disease. *World J Gastroenterol* 2013; **19**: 6969-6978 [PMID: 24222941 DOI: 10.3748/wjg.v19.i41.6969]

145 **Sookoian S**, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 1883-1894 [PMID: 21381068 DOI: 10.1002/hep.24283]

146 **Singal AG**, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, Waljee AK. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014; **109**: 325-334 [PMID: 24445574 DOI: 10.1038/ajg.2013.476]

147 **Sookoian S**, Pirola CJ. PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 6018-6026 [PMID: 23155331 DOI: 10.3748/wjg.v18.i42.6018]

148 **Huang Y**, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, Hobbs HH. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci USA* 2010; **107**: 7892-7897 [PMID: 20385813 DOI: 10.1073/pnas.1003585107]

149 **Pingitore P**, Pirazzi C, Mancina RM, Motta BM, Indiveri C, Pujia A, Montalcini T, Hedfalk K, Romeo S. Recombinant PNPLA3 protein shows triglyceride hydrolase activity and its I148M mutation results in loss of function. *Biochim Biophys Acta* 2014; **1841**: 574-580 [PMID: 24369119 DOI: 10.1016/j.bbalip.2013.12.006]

150 **Perttilä J**, Huaman-Samanez C, Caron S, Tanhuanpää K, Staels B, Yki-Järvinen H, Olkkonen VM. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. *Am J Physiol Endocrinol Metab* 2012; **302**: E1063-E1069 [PMID: 22338072 DOI: 10.1152/ajpendo.00125.2011]

151 **Pirazzi C**, Adiels M, Burza MA, Mancina RM, Levin M, Ståhlman M, Taskinen MR, Orho-Melander M, Perman J, Pujia A, Andersson L, Maglio C, Montalcini T, Wiklund O, Borén J, Romeo S. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. *J Hepatol* 2012; **57**: 1276-1282 [PMID: 22878467 DOI: 10.1016/j.jhep.2012.07.030]

152 **Basantani MK**, Sitnick MT, Cai L, Brenner DS, Gardner NP, Li JZ, Schoiswohl G, Yang K, Kumari M, Gross RW, Zechner R, Kershaw EE. Pnpla3/Adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. *J Lipid Res* 2011; **52**: 318-329 [PMID: 21068004 DOI: 10.1194/jlr.M01120]

153 **Kumari M**, Schoiswohl G, Chitraju C, Paar M, Cornaciu I, Rangrez AY, Wongsiriroj N, Nagy HM, Ivanova PT, Scott SA, Knittelfelder O, Rechberger GN, Birner-Gruenberger R, Eder S, Brown HA, Haemmerle G, Oberer M, Lass A, Kershaw EE, Zimmermann R, Zechner R. Adiponutrin functions as a nutritionally regulated lysophosphatidic acid acyltransferase. *Cell Metab* 2012; **15**: 691-702 [PMID: 22560221 DOI: 10.1016/j.cmet.2012.04.008]

154 **Li JZ**, Huang Y, Karaman R, Ivanova PT, Brown HA, Roddy T, Castro-Perez J, Cohen JC, Hobbs HH. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. *J Clin Invest* 2012; **122**: 4130-4144 [PMID: 23023705 DOI: 10.1172/JCI65179]

155 **Graff M**, North KE, Franceschini N, Reiner AP, Feitosa M, Carr JJ, Gordon-Larsen P, Wojczynski MK, Borecki IB. PNPLA3 gene-by-visceral adipose tissue volume interaction and the pathogenesis of fatty liver disease: the NHLBI family heart study. *Int J Obes* (Lond) 2013; **37**: 432-438 [PMID: 22546774 DOI: 10.1038/ijo.2012.65]

156 **Guichelaar MM**, Gawrieh S, Olivier M, Viker K, Krishnan A, Sanderson S, Malinchoc M, Watt KD, Swain JM, Sarr M, Charlton MR. Interactions of allelic variance of PNPLA3 with nongenetic factors in predicting nonalcoholic steatohepatitis and nonhepatic complications of severe obesity. *Obesity* (Silver Spring) 2013; **21**: 1935-1941 [PMID: 23418085 DOI: 10.1002/oby.20327]

157 **Kantartzis K**, Peter A, Machicao F, Machann J, Wagner S, Königsrainer I, Königsrainer A, Schick F, Fritsche A, Häring HU, Stefan N. Dissociation between fatty liver and insulin resistance in

humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes* 2009; **58**: 2616-2623 [PMID: 19651814 DOI: 10.2337/db09-0279]

158 **Speliotes EK**, Butler JL, Palmer CD, Voight BF, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology* 2010; **52**: 904-912 [PMID: 20648472 DOI: 10.1002/hep.23768]

159 **Goran MI**, Walker R, Le KA, Mahurkar S, Vikman S, Davis JN, Spruijt-Metz D, Weigensberg MJ, Allayee H. Effects of PNPLA3 on liver fat and metabolic profile in Hispanic children and adolescents. *Diabetes* 2010; **59**: 3127-3130 [PMID: 20852027 DOI: 10.2337/db10-0554]

160 **Zain SM**, Mohamed R, Mahadeva S, Cheah PL, Rampal S, Basu RC, Mohamed Z. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. *Hum Genet* 2012; **131**: 1145-1152 [PMID: 22258181 DOI: 10.1007/s00439-012-1141-y]

161 **Palmer CN**, Maglio C, Pirazzi C, Burza MA, Adiels M, Burch L, Donnelly LA, Colhoun H, Doney AS, Dillon JF, Pearson ER, McCarthy M, Hattersley AT, Frayling T, Morris AD, Peltonen M, Svensson PA, Jacobson P, Borén J, Sjöström L, Carlsson LM, Romeo S. Paradoxical lower serum triglyceride levels and higher type 2 diabetes mellitus susceptibility in obese individuals with the PNPLA3 148M variant. *PLoS One* 2012; **7**: e39362 [PMID: 22724004 DOI: 10.1371/journal.pone.0039362]

162 **Krarup NT**, Grarup N, Banasik K, Friedrichsen M, Færch K, Sandholt CH, Jørgensen T, Poulsen P, Witte DR, Vaag A, Sørensen T, Pedersen O, Hansen T. The PNPLA3 rs738409 G-allele associates with reduced fasting serum triglyceride and serum cholesterol in Danes with impaired glucose regulation. *PLoS One* 2012; **7**: e40376 [PMID: 22792295 DOI: 10.1371/journal.pone.0040376]

163 **Stojkovic IA**, Ericson U, Rukh G, Riddestråle M, Romeo S, Orho-Melander M. The PNPLA3 Ile148Met interacts with overweight and dietary intakes on fasting triglyceride levels. *Genes Nutr* 2014; **9**: 388 [PMID: 24563329 DOI: 10.1007/s12263-014-0388-4]

164 **Tomita K**, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, Okada Y, Kurihara C, Irie R, Yokoyama H, Shimamura K, Usui S, Ebinuma H, Saito H, Watanabe C, Komoto S, Kawaguchi A, Nagao S, Sugiyama K, Hokari R, Kanai T, Miura S, Hibi T. Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. *Hepatology* 2014; **59**: 154-169 [PMID: 23832448 DOI: 10.1002/hep.26604]

165 **Hyysalo J**, Männistö VT, Zhou Y, Arola J, Kärjä V, Leivonen M, Juuti A, Jaser N, Lallukka S, Käkelä P, Venesmaa S, Simonen M, Saltevo J, Moilanen L, Korpi-Hyövalti E, Keinänen-Kiukaanniemi S, Oksa H, Orho-Melander M, Valenti L, Fargion S, Pihlajamäki J, Peltonen M, Yki-Järvinen H. A population-based study on the prevalence of NASH using scores validated against liver histology. *J Hepatol* 2014; **60**: 839-846 [PMID: 24333862 DOI: 10.1016/j.jhep.2013.12.009]

166 **Maglio C**, Pirazzi C, Pujia A, Valenti L, Romeo S. The PNPLA3 I148M variant and chronic liver disease: When a genetic mutation meets nutrients. *Food Research International* 2014; **63** (Suppl B): 239-243

167 **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]

168 **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]

169 **Bonkovsky HL**, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht RW, Banner BF. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 421-429 [PMID: 10488699 DOI: 10.1016/s0168-8278(99)80032-4]

170 **Nelson JE**, Bhattacharya R, Lindor KD, Chalasani N, Raaka S, Heathcote EJ, Miskovsky E, Shaffer E, Rulyak SJ, Kowdley KV. HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. *Hepatology* 2007; **46**: 723-729 [PMID: 17680648 DOI: 10.1002/hep.21742]

171 **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 DOI: 10.1002/hep.22059]

172 **Nelson JE**, Klintworth H, Kowdley KV. Iron metabolism in Nonalcoholic Fatty Liver Disease. *Curr Gastroenterol Rep* 2012; **14**: 8-16 [PMID: 22124850 DOI: 10.1007/s11894-011-0234-4]

173 **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 DOI: 10.1053/jhep.2002.33892]

174 **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 DOI: 10.1002/hep.20023]

175 **Hernaez R**, Yeung E, Clark JM, Kowdley KV, Brancati FL, Kao WH. Hemochromatosis gene and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 2011; **55**: 1079-1085 [PMID: 21354231 DOI: 10.1016/j.jhep.2011.02.013]

176 **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]

177 **Ganz T**, Nemeth E. The hepcidin-ferroportin system as a therapeutic target in anemias and iron overload disorders. *Hematology Am Soc Hematol Educ Program* 2011; **2011**: 538-542 [PMID: 22160086]

178 **Nai A**, Pagani A, Silvestri L, Campostrini N, Corbella M, Girelli D, Traglia M, Toniolo D, Camaschella C. TMPRSS6 rs855791 modulates hepcidin transcription in vitro and serum hepcidin levels in normal individuals. *Blood* 2011; **118**: 4459-4462 [PMID: 21873547 DOI: 10.1182/blood-2011-06-364034]

179 **Traglia M**, Girelli D, Biino G, Campostrini N, Corbella M, Sala C, Masciullo C, Viganò F, Buetti I, Pistis G, Cocca M, Camaschella C, Toniolo D. Association of HFE and TMPRSS6 genetic variants with iron and erythrocyte parameters is only in part dependent on serum hepcidin concentrations. *J Med Genet* 2011; **48**: 629-634 [PMID: 21785125 DOI: 10.1136/jmedgenet-2011-100061]

180 **Galesloot TE**, Geurts-Moespot AJ, den Heijer M, Sweep FC, Fleming RE, Kiemeney LA, Vermeulen SH, Swinkels DW. Associations of common variants in HFE and TMPRSS6 with iron parameters are independent of serum hepcidin in a general population: a replication study. *J Med Genet* 2013; **50**: 593-598 [PMID: 23794717 DOI: 10.1136/jmedgenet-2013-101673]

181 **Gan W**, Guan Y, Wu Q, An P, Zhu J, Lu L, Jing L, Yu Y, Ruan S, Xie D, Makrides M, Gibson RA, Anderson GJ, Li H, Lin X, Wang F. Association of TMPRSS6 polymorphisms with ferritin, hemoglobin, and type 2 diabetes risk in a Chinese Han population. *Am J Clin Nutr* 2012; **95**: 626-632 [PMID: 22301935 DOI: 10.3945/ajcn.111.025684]

182 **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]

183 **Yoneda M**, Yoneda M, Fujita K, Inamori M, Tamano M, Hiriishi H, Nakajima A. Transient elastography in patients with non-alcoholic

fatty liver disease (NAFLD). *Gut* 2007; **56**: 1330-1331 [PMID: 17470477 DOI: 10.1136/gut.2007.126417]

184 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]

185 **Mahadeva S**, Mahfudz AS, Vijayanathan A, Goh KL, Kulenthran A, Cheah PL. Performance of transient elastography (TE) and factors associated with discordance in non-alcoholic fatty liver disease. *J Dig Dis* 2013; **14**: 604-610 [PMID: 23859493 DOI: 10.1111/1751-2980.12088]

186 **Suzuki K**, Yoneda M, Imajo K, Kirikoshi H, Nakajima A, Maeda S, Saito S. Transient elastography for monitoring the fibrosis of non-alcoholic fatty liver disease for 4 years. *Hepatol Res* 2013; **43**: 979-983 [PMID: 23294411 DOI: 10.1111/hepr.12039]

187 **Yoshioka K**, Hashimoto S, Kawabe N. Measurement of liver stiffness as a non-invasive method for diagnosis of non-alcoholic fatty liver disease. *Hepatol Res* 2015; **45**: 142-151 [PMID: 25040931 DOI: 10.1111/hepr.12388]

188 **Burlone ME**, Rossini A, Momo E, Colletta C, Leutner M, Minisini R, Pirisi M. A composite score including BMI liver stiffness and rs738409 PNPLA3 genotype might spare liver biopsies to most NAFLD patients maintaining 95% diagnostic accuracy. [Updated 2012 Nov 12]. Available from: URL: http://www.aspe.vb.it/it/downloads/Studio NASH.pdf

189 **Petta S**, Vanni E, Bugianesi E, Di Marco V, Cammà C, Cabibi D, Mezzabotta L, Craxì A. The combination of liver stiffness measurement and NAFLD fibrosis score improves the noninvasive diagnostic accuracy for severe liver fibrosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2015; **35**: 1566-1573 [PMID: 24798049 DOI: 10.1111/liv.12584]

190 **Sumida Y**, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2014; **20**: 475-485 [PMID: 24574716 DOI: 10.3748/wjg.v20.i2.475]

191 **Kotze MJ**, Lückhoff HK, Peeters AV, Baatjes K, Schoeman M, van der Merwe L, Grant KA, Fisher LR, van der Merwe N, Pretorius J, van Velden DP, Myburgh EJ, Pienaar FM, van Rensburg SJ, Yako YY, September AV, Moremi KE, Cronje FJ, Tiffin N, Bouwens CS, Bezui. Genomic medicine and risk prediction across the disease spectrum. *Crit Rev Clin Lab Sci* 2015: 1-18 [PMID: 25597499]

192 **Fisher LR**, Pretorius J, Kruger FC, Daniels C, Kidd M, Kotze MJ. Development of a pathology supported genetic testing strategy for improved clinical management of patients with non-alcoholic fatty liver disease (NAFLD). *Histopathol* 2012; **61** Suppl 1

193 **Lim JW**, Dillon J, Miller M. Proteomic and genomic studies of non-alcoholic fatty liver disease--clues in the pathogenesis. *World J Gastroenterol* 2014; **20**: 8325-8340 [PMID: 25024592 DOI: 10.3748/wjg.v20.i26.8325]

194 **Gerhard GS**, Chu X, Wood GC, Gerhard GM, Benotti P, Petrick AT, Gabrielsen J, Strodel WE, Still CD, Argyropoulos G. Next-generation sequence analysis of genes associated with obesity and nonalcoholic fatty liver disease-related cirrhosis in extreme obesity. *Hum Hered* 2013; **75**: 144-151 [PMID: 24081230 DOI: 10.1159/000351719]

195 **Gorden DL**, Myers DS, Ivanova PT, Fahy E, Maurya MR, Gupta S, Min J, Spann NJ, McDonald JG, Kelly SL, Duan J, Sullards MC, Leiker TJ, Barkley RM, Quehenberger O, Armando AM, Milne SB, Mathews TP, Armstrong MD, Li C, Melvin WV, Clements RH, Washington MK, Mendonsa AM, Witztum JL, Guan Z, Glass CK, Murphy RC, Dennis EA, Merrill AH, Russell DW, Subramaniam S, Brown HA. Biomarkers of NAFLD progression: a lipidomics approach to an epidemic. *J Lipid Res* 2015; **56**: 722-736 [PMID: 25598080 DOI: 10.1194/jlr.P056002]

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REVIEW

# Traditional Chinese medicine for prevention and treatment of hepatocarcinoma: From bench to bedside

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## Abstract

Traditional Chinese medicine (TCM) has played a positive role in the management of hepatocarcinoma. Hepatocarcinoma patients may present Qi-stagnation, damp-heat, blood stasis, Qi-deficiency, Yin-deficiency and other TCM syndromes (Zheng). Modern treatments such as surgery, transarterial chemoembolization (TACE) and high intensity focus ultrasound treatment would influence the manifestation of TCM syndromes. Herbs with traditional efficacy of tonifying Qi, blood and Yin, soothing liver-Qi stagnation, clearing heat and detoxifying and dissolving stasis, have been demonstrated to be potent to prevent hepatocarcinogenesis. TCM has been widely used in all aspects of integrative therapy in hepatocarcinoma, including surgical resection, liver transplantation, TACE, local ablative therapies and even as monotherapy for middle-advanced stage hepatocarcinoma. Clinical practices have confirmed that TCM is effective to alleviate clinical symptoms, improve quality of life and immune function, prevent recurrence and metastasis, delay tumor progression, and prolong survival time in hepatocarcinoma patients. The effective mechanism of TCM against hepatocarcinoma is related to inducing apoptosis, autophagy, anoikis and cell senescence, arresting cell cycle, regulating immune function, inhibiting metastasis and angiogenesis, reversing drug resistance and enhancing effects of chemotherapy. Along with the progress of research in this field, TCM will contribute more to the prevention and treatment of hepatocarcinoma.

**Key words:** Hepatocarcinoma; Traditional Chinese medicine; Prevention; Treatment; Traditional Chinese medicine syndrome (Zheng); Therapeutic principle; Chinese herbal formula; Chinese herb



**Core tip:** Hepatocarcinoma may present different Traditional Chinese medicine (TCM) syndromes (Zheng). Syndromes are associated with hepatocarcinoma progression and prognosis to a certain degree. Modern technologies have been exploited to elucidate the relation between syndromes and biomedical sciences. Chinese herbs or herbal components have been demonstrated

to be effective to prevent and treat hepatocarcinoma. Contemporary TCM physicians have established some effective herbal formulas and Chinese patent herbal drugs for hepatocarcinoma prevention and treatment.

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## INTRODUCTION

Hepatocarcinoma is the sixth most common malignancy and the third principal cause of cancer deaths worldwide[1]. The incidence of hepatocarcinoma is increasing by cause of hepatitis virus infection and other factors[2-4]. Due to latent onset and rapid progression, only a minority of patients with early-stage disease are suitable for potentially curative therapy, *i.e.*, surgical resection or liver transplantation. Treatments for unresectable disease, such as local ablative therapies, transarterial chemoembolization (TACE) and systemic therapy with sorafenib, are essentially palliative[5]. Cancer recurrence is another obstacle to successful treatment, and there is a shortage of preventive means for recurrence. It is important to develop novel approaches for hepatocarcinoma prevention and treatment.

Traditional Chinese medicine (TCM) has played a positive role in the management of hepatocarcinoma[6,7]. TCM has been widely used in all aspects of integrative therapy in hepatocarcinoma, including surgical resection, liver transplantation, chemoembolization, targeted therapy, and even as monotherapy for middle-advanced stage hepatocarcinoma[6-8]. TCM treatment mainly includes multiple herbal therapy and Chinese patent herbal drug therapy. Clinical practices have confirmed that TCM is effective to alleviate clinical symptoms, improve quality of life (QOL), palliate myelosuppression, improve immune function, prevent recurrence and metastasis, delay tumor progression, and prolong survival time[6-9].

TCM therapy is a syndrome differentiation based treatment. Based on TCM pathogenesis, TCM syndrome patterns and different disease stage, TCM physicians employ different therapeutic methods, and prescribe multiple herbs for hepatocarcinoma treatment[10]. The pathogenesis of hepatocarcinoma is related to weakened body defense or deficiency in liver and kidney, liver stagnation, dampness-heat, and blood stasis. Since these pathological factors are also seen in other diseases, contemporary TCM has developed the concept of cancerous toxicity (Ai-Du) to discriminate liver cancer from other common diseases, and underscore the application of anti-cancer therapy to improve overall therapeutic efficacy in hepatocarcinoma[11,12].

During past decades, TCM has been extensively explored to prevent and treat hepatocarcinoma. In this paper, we comprehensively review the experimental and clinical efficacy of TCM against hepatocarcinogenesis and hepatocarcinoma, TCM syndromes in hepatocarcinoma patients and the effective mechanism of TCM in the treatment of hepatocarcinoma, to provide new insights into hepatocarcinoma management.

## HEPATOCARCINOMA PREVENTION BY TCM

Disease prevention has long been practiced in TCM. Taking preventive measures against disease or treating before sick had been established as a basic principle for disease control since *Inner Canon of Emperor Huang* (Huang-Di-Nei-Jing) (400 B.C.), Dr. Zhong-Jing Zhang, one of the most prominent Chinese physicians in history, had developed another principle for liver disease prevention in *Synopsis of Golden Cabinet* (Jin-Gui-Yao-Lüe) (A.D 200 - 210). Dr. Zhang observed that liver diseases tend to spread to the spleen, and proposed that the spleen-Qi should be reinforced before it is affected. Hepatocarcinoma prevention by Chinese herbs or related products has been extensively studied in recent decades.

### *Classical herbal formulas*

Chinese herbal formula or Chinese herbal compound prescription is the most representative application form of Chinese herbs. It has been shown that some classical herbal formulas have preventive effects against hepatocarcingenesis. Ren-shen-bie-jia-jian, a formula for nourishing Qi and dissolving stasis, is effective in inhibiting hepatocarcinogenesis and down-regulation of transforming growth factor-β type Ⅱ receptor (TGF-β ⅡR)[13]. Xiao-chai-hu-tang, a formula used for soothing liver-Qi stagnation, may inhibit N-nitrosomorpholine (NNM) induced hepatocarcinogenesis and increase the proportion of helper T lymphocytes in rats[14]. Shi-quan-da-bu-tang, a classical formula for tonifying Qi-blood, inhibits NNM induced hepatocarcinogenesis and increases interleukin-2 (IL-2) receptor-positive lymphocytes in rats[15]. However, the more exploited and studied are modern formulas.

### *Liver and/or kidney-tonifying herbal formulas*

According to TCM theory, the liver and kidney share the same origin, and thus tonifying liver and/or kidney is frequently used to prevent hepatocarcinoma. Nourishing Yin Decoction, a formula for tonifying liver Yin, may inhibit aflatoxin B1 (AFB1) induced hepatocarcinogenesis[16]. A-L tonic capsule, a tonifying liver and kidney Yin and nourishing Qi based Chinese patent drug for improving the immune system in cancer patients, may reduce DNA content and improve its distribution in rat hepatocarcinogenesis induced by diethylnitrosamine (DEN)[17]. Bu Shen Prescription, a formula composed of eight herbs for tonifying kidney, inhibits DEN induced hepato-

carcinogenesis in rats[18].

Liu *et al*[19] established Fu-zheng-hua-yu formula for tonifying kidney and dissolving stasis, and demonstrated that it may inhibit hepatocarcinogenesis and induce cell cycle S phase arrest in DEN treated rat models. Fu-zheng-hua-yu formula has been developed as a Chinese patent drug for liver fibrosis treatment. Huqi San, a formula for tonifying kidney and nourishing Qi, inhibits the over-expression of c-jun, c-fos and c-myc oncogenes and liver preneoplastic lesions induced by DEN[20,21].

### *Spleen-strengthening herbal formulas*

Strengthening spleen is an important principle for hepatocarcinoma prevention. Ganfujian, a formula composed of dietary and medicinal Chinese herbs for strengthening spleen, has showed effects in inhibiting hepatocarcinogenesis, and down-regulation of CDK4 (cyclin-dependent kinase 4), cyclin D1 and PCNA (proliferating cell nuclear antigen) in DEN induced hepatic carcinogenesis in rats[22]. Qiu *et al*[23] have established an herbal recipe for strengthening spleen, regulating Qi, removing heat, and softening hard lumps and resolving phlegm (SRRS). SRRS recipe has been demonstrated to have preventive effects against DEN induced hepatocacinogenesis[23]. Jianpi Jiedu Recipe, an herbal formula for strengthening spleen and detoxification, has been showed to be effective in inhibiting DEN induced hepatocarcinogenesis, down-regulating miR-199a and Phosphatidylinositol-4,5-bisphosphate 3-kinaseV-akt murine thymoma viral oncogene homolog (PI3K/AKT), and up-regulating p70s6k[24,25].

### *Detoxifying herbal formulas*

In addition to tonifying treatments, eliminating pathogenic factors is another important principle for hepatocarcinoma prevention. Removing Toxic Heat Decoction, a formula based on detoxifying and tonifying liver Yin, may inhibit AFB1 induced hepatocarcinogenesis[16]. Gao *et al*[26] evaluated Gan-Zheng oral solution, a formula for detoxification and dissolving stasis, in a DEN induced rat model and demonstrated that it is effective in inhibiting hepatocarcinogenesis and down-regulating intercellular adhesion molecule 1 (ICAM-1)[26]. Zao-Lian mixture, a formula for detoxification, dissolving stasis, strengthening spleen, and soothing liver, may inhibit hepatocarcinogenesis and protect liver function[27]. Sb/Bs remedy, containing *Scutellaria baicalensis* Georgi (Sb) and *Bupleurum scorzonerifolfium* Willd (Bs), suppressed N-nitrosobis(2-oxopropyl)amine (BOP) induced liver tumours, increased serum tumor necrosis factor (TNF)-alpha and TGF-beta1, decreased 8-OHdG expression, and increased caspase-3 and apoptosis[28].

### *Stasis-dissolving and other herbal formulas*

Professor Fang's group has investigated different TCM treatments in DEN mediated hepatocainogenesis, and found that the more efficient treatments are, in order, promoting circulation and removing stasis, clearing heat and detoxifying, and strengthening spleen. All those treatments up-regulated GTPase-activating protein expression and down-regulated Ras expression. Expression of growth factor receptor-bound protein 2 and Raf 1, and son of sevenless was inhibited by those treatments, respectively[29-31]. Herbal compound 861, a formula composed of herbs with dissolving stasis and nourishing Qi efficacy, prevents 2-acetylaminofluorene (2-AAF) induced hepatocarcinogenesis in rats[32]. A new anti-tumor formula with tonifying Qi, soothing liver and dissolving stasis effects inhibits hepatocarcinogenesis induced by HBV and AFB1 accompanied by superoxide dismutase (SOD) and glutathione S-transferase (GST) activation and decreased malondialdehyde (MDA)[33].

### *Tonic herbs*

Ginseng, one of the most frequently used herbs for tonifying Qi, has been reported to be effective in inhibiting DEN induced hepatocellular carcinoma in rats[34]. Dang-Gui (*Angelica sinensis*), a common used blood tonic, is potent to inhibit AFB1 induced mutagenicity in Ames test and hepatocarcinogenesis induced by AFB1[35,36]. Ying-tonifying herbs such as Nü-Zhen-Zi (*Ligustrum lucidum* Ait.), Tian-Dong (*Asparagus cochinchinensis*), Bai-He (*Lilium brownii*) and Shan-Zhu-Yu (*Cornus officinalis*) are efficient to inhibit AFB1 induced mutagenicity in Ames test[36]. Cao-Cong-Rong (*Boschniakia rossica*), an herb with traditional efficacy of Yang-tonifying, may inhibit DEN induced hepatocarcinogenesis, increase SOD and GSH-PX (glutathione peroxidase), and down-regulate expression of MDA, GST and mutant p53 and p21 proteins[37,38].

### *Heat-clearing herbs*

Ban-Zi-Lian (*Sculellaria barbata*), an herb with traditional efficacy of clearing heat and detoxifying, has been widely used as an anti-cancer herb. Ban-Zi-Lian has been reported to be able to inhibit experimental hepatocarcinoma and relieve hepatic injures in DEN treated rats[39]. Tu-Fu-Ling (*Smilax glabra* Roxb.), another commonly used clearing heat and detoxifying herb, inhibits hepatocarcinogenesis induced by AFB1[35]. Penta-acetyl geniposide, a component of Zhi-Zi (*Gardenia jasminoides* Ellis), may protect rats from AFB1 induced hepatocarcinogenesis[40]. Berberine, a component of Huang-Lian (*Coptis chinensis* Franch.) or Huang-Bai (*Phellodendron chinense* Schnied.), inhibits hepatocyte proliferation and inducible nitric oxide synthase expression, decreases cytochrome P450 content, inhibits activities of cytochrome P450 2E1 (CYP2E1) and CYP1A2 in DEN plus phenobarbital treated rats[41]. Yin-Chen-Hao (*Artemisia capillaris* Thunb.), an herb for clearing heat, inducing urination and removing jaundice, has been reported to be effective in inhibiting AFB1 induced mutagenicity in Ames test[42].

### *Stasis-dissolving herbs*

Dissolving stasis is another principle for hepatocarcinoma

prevention. Dan-Shen (*Salvia miltiorrhiza* Bunge) may inhibit AFB1 mediated mutagenicity and hepatocarcinogenesis, decrease AFB1-DNA adducts formation and AFB1-induced oxidative DNA damage, and induce glutathione S-transferase Yc-2 expression[35,36,43]. Jiang-Huang (*Curcuma longa* Linn) has beneficial effects on the early and late stages of liver pathogenesis, prevents and delays liver carcinogenesis, and may be related to decreased expression of hepatitis B virus X protein (HBx) and increased expression of p-p53, p21 and cyclin D1 in livers of HBx transgenic mice[44]. Shan-Qi (*Panax notoginseng*), an herb used for hemostasis and dissolving stasis, has been showed to be effective to inhibit DEN induced hepatocarcinogenesis and angiogenesis accompanied by down-regulation of angiopoietin-2, tunica internal endothelial cell kinase 2, hypoxia inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF)[45].

### Other herbs

Wu-Wei-Zi (*Schisandra chinensis*), commonly used for inducing astringency, could inhibit AFB1 induced mutagenicity in Ames test and hepatocarcinogenesis induced by AFB1[35,36]. Gomisin A, an ingredient of Wu-Wei-Zi, is effective to inhibit 3'-methyl-4-dimethylaminoazobenzene induced hepatocarcinogenesis by enhancing the excretion of the carcinogen from the liver and by reversing the normal cytokinesis[46]. Other herbs, such as Bai-Ji (*Bletilla striata*), which may inhibit FAB1 induced hepatocarcinogenesis, Shan-Zha (*Crataegus pinnatifida*), Yi-Yi-Ren (*Coix lacryma-jobi* L.), Xing-Ren (*Prunus armeniaca*) and Wu-Mei (*Prunus mume*), which may inhibit AFB1 induced mutagenicity in Ames test[35,36].

### Clinical prevention against hepatocarcinogenesis

Current treatments for hepatocarcinoma are less than satisfactory. It is important to prevent hepatocarcinogenesis in high risk populations. Jian-Pi-Huo-Xue Formula based herbal treatment has been demonstrated to be effective to inhibit hepatocarcinogenesis in patients with hepatitis, hepatic cirrhosis and low level of AFP[47]. Dan-Shen (*Salvia miltiorrhiza* Bunge) is potent to protect male individuals from hepatocarcinogenesis in a high incidence area of hepatocarcinoma[48]. In Japan, Sho-saiko-to (TJ-9) (Xiao-chai-hu-tang) has been demonstrated to be potent to prevent heapto-carcinogenesis in patients with cirrhosis, particularly in patients without HBs antigen[49].

Taken together, contemporary TCM physicians have adopted classic TCM theory and principles for hepatocarcinoma prevention study, developed A-L tonic capsule, Fu-zheng-hua-yu capsule, Ganfujian, Gan-Zheng oral solution, Herbal compound 861 and other effective herbal formulas/drugs, and confirmed that Nü-Zhen-Zi, Ban-Zi-Lian, Yin-Chen-Hao, Dan-Shen, Wu-Wei-Zi and other herbs could inhibit hepatocarcinogenesis. It is regrettable that there are no more studies on hepatitis virus induced hepatocarcinogenesis models. Related studies will promote TCM to contribute more to protect high-risk individuals from hepatocarcinogenesis. Herbal formulas for hepatocarcinoma prevention are listed in Table 1.

## TCM SYNDROMES IN HEPATOCARCINOMA

TCM syndrome (Zheng) is pathological status that integrates information from clinical symptoms, etiology, disease location and character, tongue picture and TCM theory, and serves as the basis for herbal medication. The clinical manifestation of liver cancer is complex and different patients or different stage of disease may present different TCM syndromes.

### Syndrome differentiation in hepatocarcinoma

In 294 patients preliminarily diagnosed with primary liver cancer, Zhao *et al*[50] found the early symptoms were more observed in the liver and biliary system and digestive tract system. Except for 23.5% of patients without any TCM syndrome, the frequently observed syndromes include dampness, blood-stasis, Qi-stagnation, Qi-deficiency, blood-deficiency, heat, Yang-asthenia and Yin-asthenia. One hundred and eleven cases presented with single syndrome, 76 cases with two combined syndromes, 31 cases with three combined syndromes, 6 cases with four combined syndromes and 1 case with five combined syndromes[50].

In another study, the common syndromes observed in liver cancer patients include blood stasis, spleen-Qi-deficiency, liver-Qi stagnation, spleen deficiency and dampness blocking, disharmony of liver and stomach, dampness-heat of spleen and stomach, liver-Yin-deficiency and kidney-Yin-deficiency. Liver-Qi stagnation and spleen-Qi-deficiency can be found in patients from stages Ⅰ to Ⅲ. Main syndromes in stage Ⅱ are blood stasis, Qi-stagnation, Qi-deficiency and damp-heat. While in stage Ⅲ, Qi-deficiency, Yin-deficiency, blood stasis, Qi-stagnation and retention of water are the most common syndromes. The average numbers of syndromes in stages Ⅰ, Ⅱ and Ⅲ are 2.03, 3.47 and 4.99, respectively. These observations suggest that syndromes are more complicated along with disease progression, and Qi stagnation, blood stasis, Qi-deficiency and Yin-deficiency are the basic syndromes in liver cancer[51,52].

In Guangzhou area, Wang *et al*[53] have found that TCM syndromes distributed in liver cancer patients include spleen deficiency, Qi stagnation, blood stasis and damp-heat. Two overlapped syndromes were observed in 43.5% of patients and 19.9% for three. The overlapped syndromes in patients with stage Ⅱ disease are spleen deficiency and Qi stagnation, Qi stagnation and blood stasis, spleen deficiency and damp-heat, and liver and spleen deficiency. The overlapped syndrome in patients with stage Ⅲ disease is liver and kidney-Yin-deficiency[53].

Syndromes are related to prognosis and QOL to some extent. Yang *et al*[54] analyzed relation between syndrome and survival time. They found that the median survival

**Table 1 Herbal formulas for hepatocarcinoma prevention**

| Herbal formula | Therapeutic principles | Herbs | Models/patients | Effects | Targets/events | Ref. |
|---|---|---|---|---|---|---|
| Ren-shen-bie-jia-jian | Tonifying Qi and dissolving stasis | Ren-Shen (*Panax ginseng*), Bie-Jia (Carapax trionycis), Tao-Ren (Peach seed) and other herbs | DMN induced hepatic precancerous lesion in rats | Inhibit hepatocarcinogenesis | Down-regulation of TGF-βⅡR | [13] |
| Xiao-chai-hu-tang | Soothing liver-Qi stagnation | Chai-Hu (*Bupleurum chinense*), Huang-Qin (*Scutellaria baicalensis* Georgi), Sheng-Jiang (*Zingiber officinale*), Ban-Xia (*Pinellia ternata*), Da-Zao (*Ziziphus jujuba*), Ren-Shen (*Panax ginseng*), Gan-cao (*Glycyrrhiza uralensis*) | NNM induced hepatocarcinogenesis in rats; patients with cirrhosis | Inhibit hepatocarcinogenesis, prevent hepatocarcinogenesis in patients | Increase helper T lymphocytes | [14,49] |
| Shi-quan-da-bu-tang | Tonifying Qi-Blood | Ren-Shen (*Panax ginseng*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Fu-Ling (Poria cocos), Gan-cao (*Glycyrrhiza uralensis*), Chuan-Xiong (*Ligusticum chuanxiong*), Dang Gui (*Angelica sinensis*), Shu-Di-Huang (*Rehmannia glutinosa*), Bai-Shao (*Paeonia lactiflora*), Huang Qi (*Astragalus membranaceous*), Rou-Gui (*Cinnamomum cassia* Presl) | NNM induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Increase IL-2 receptor-positive lymphocytes | [15] |
| Nourishing Yin Decoction | Tonifying liver Yin | Bei-Sha-Shen (*Glehnia littoralis*), Dang-Gui (*Angelica sinensis*), Bai-Shao (*Paeonia lactiflora*), Gou-Qi (*Lycium barbarum*), *etc.* | AFB1 induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Unknown | [16] |
| Removing Toxic Heat Decoction | Detoxifying and tonifying liver Yin | Ban-Zi-Lian (*Sculellaria barbata*), Ban-Bian-Lian (*Lobelia chinensis* Lour.), Mo-Han-Lian (*Eclipta prostrata*), Gu-Ya (rice sprout), *etc.* | AFB1 induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Unknown | [16] |
| A-L tonic capsule | Tonifying liver-Yin and nourishing Qi | Nü-Zhen-Zi (*Ligustrum lucidum* Ait.) and Huang-Qi (*Astragalus membranaceous*) | DEN induced hepatocarcinogenesis in rats | Inhibit DNA content and improve DNA distribution | Unknown | [17] |
| Bu Shen formula | Tonifying kidney | Ba-Ji-Tian (*Morinda officinalis*), Tu-Si-Zi (*Cuscuta chinensis* Lam), Qing-Pi (*Cuscuta chinensis* Lam) and other herbs | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Unknown | [18] |
| Fu-zheng-hua-yu formula | Strengthening body resistance and dissolving stasis | Dong-Chong-Xia-Cao (*Cordyceps sinensis*), Tao-Ren (Peach seed), Dan-shen (*Salvia miltiorrhiza*) and other herbs | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Inhibit cell cycle S phase | [19] |
| Huqi San | Tonifying kidney, nourishing Qi, and dissolving stasis | Hu-Ji-Sheng (*Viscum coloratum*), Huang-Qi (*Astragalus membranaceus*), Yu-Jin (*Radix Curcumae*), Dan-shen (*Salvia miltiorrhiza*) and other herbs | DEN and 2-acetylaminofluorene induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Down-regulation of c-jun, c-fos and c-myc, up-regulation of G-6-Pase, SDH and ATPase | [20,21] |
| Ganfujian | Strengthening spleen | Shan-Yao (Rhizoma Dioscoreae), Shan-Zha (Fructus Crataegi) and Da-Zao (Fructus Ziziphi Jujubae) | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Down-regulation of CDK4, cyclin D1 and PCNA | [22] |
| SRRS recipe | Strengthening spleen, regulating Qi, removing heat, and softening hard lumps and resolving phlegm | Tai-Zi-Shen (*Pseeudostellaria heterophylla*), Zhu-Zi-Shen (*Panax japonicus*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Fu-Ling (*Poria cocos*), Sheng-Mu-Li (raw oyster shell) other herbs | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Unknown | [23] |
| Jianpi Jiedu Recipe | Strengthening spleen and detoxifying | Huang-Qi (*Astragalus membranaceous*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Zhu-Ling (*Polyporus umbrellatus*), Ba-Yue-Zha (*Akebia trifoliata*), Shi-Jian-Chuan (*Salvia chinensis*), Ye-Pu-Tao-Teng (Wild grape stem), Yi-Yi-Ren (*Coix lacryma-jobi L.*) other herbs | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Down-regulating miR-199a and AKT/PI3K, and up-regulating p70s6k | [24,25] |
| Gan Zheng oral solution | Detoxifying and dissolving stasis | Ban-Zi-Lian (*Sculellaria barbata*), Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd.), San-Leng (*Sparganium stoloniferum*), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*) and other herbs | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Down-regulating ICAM-1 | [26] |
| Zao Lian mixture | Detoxifying, dissolving stasis, strengthening spleen, and soothing liver | Zao-Xiu (*Paris Polyphylla*), Ban-Zi-Lian (*Sculellaria barbata*), Hu-Zhang (*Polygonum cuspidatum*), Jiang-Huang (*Carcuma longa*), Huang-Qi (*Astragalus membranaceous*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Fu-Ling (*Poria cocos*), *etc.* | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Improve liver function | [27] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Sb/Bs Remedy | Clearing heat and discharging fire, eliminating dampness and detoxifying, and soothing liver | Huang-Qin (*Scutellaria baicalensis* Georgi) and Chai-Hu (*Bupleurum scorzonerifolfium* Willd) | N-nitrosobis(2-oxopropyl)amine-induced hepatocellular carcinoma in Syrian hamsters | Inhibit hepatocarcinogenesis | Increase TNF-alpha, TGF-beta1, caspase-3 and apoptosis, decrease 8-OHdG expression | [28] |
| Qing Re Fang | Clearing heat and detoxifying | Ban-Zi-Lian (*Sculellaria barbata*), Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd.) and Pu-Gong-Ying (*Taraxacum mongolicum*) | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Up-regulate GAP, down-regulate Ras and Raf1 | [29-31] |
| Huo Xue Fang | Promoting circulation and removing stasis | Chai-Hu (*Bupleurum chinense*), Dan-Shen (*Salvia miltiorrhiza*), Chi-Shao (*Paeonia lactiflora* Pall. or *P. veitchii* Lynch), Ba-Yue-Zha (Caulis Akebiae), Yin-Chen-Hao (*Artemisia capillaris* Thunb.), Yu-Jin ( Radix Curcumae) | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Up-regulate GAP, down-regulate Ras, Grb-2 and Raf 1 | [29-31] |
| Jian Pi Fang | Strengthening spleen | Huang-Qi (*Astragalus membranaceus*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Bai-Shao (*Paeonia lactiflora*), Yi-Yi-Ren ( Semen Coicis), Shen-Qu (medicated leaven), Ban-Xia (*Pinellia ternata*) and Fu-Ling (*Poria cocos*) | DEN induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Up-regulate GAP, down-regulate Ras and SOS | [29-31] |
| Herbal Compound 861 | Dissolving stasis and tonifying Qi | Dan-Shen (*Salvia miltiorrhiza*), Huang-Qi (*Astragalus membranaceus*), Ji-Xue-Teng (*Spatholobus suberectus*) and other herbs | DEN and 2-acetylaminofluorene induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Unknown | [32] |
| New Anti-tumor formula | Tonifying qi, soothing liver and dissolving stasis | Huang-Qi (*Astragalus membranaceus*), Chai-Hu (*Bupleurum chinense*), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*) and other herbs | AFB1 and HBV induced hepatocarcinogenesis in rats | Inhibit hepatocarcinogenesis | Activate SOD and GST, decrease MDA | [33] |
| Jian-Pi-Huo-Xue Formula | Strengthening spleen and dissolving stasis | Huang Qi (*Astragalus membranaceous*), Chi-Shao (*Paeonia anomala*), Dang-Shen (*Codonopsis pilosula*), Dang-Gui (*Angelica sinensis*), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*) and other herbs | Patients with hepatitis, hepatic cirrhosis and low level of AFP | Prevent hepatocarcinogenesis in patients | Unknown | [47] |

DMN: Dimethylnitrosamine; NNM: N-nitrosomorpholine; AFB1: Aflatoxin B1; DEN: Diethylnitrosamine; HBV: Hepatitis B virus; AFP: Radiofrequency ablation; TGF-βⅡR: Alpha fetoprotein; transforming growth factor-β type Ⅱ receptor; IL-2: Interleukin-2; PCNA: Proliferating cell nuclear antigen; CDK4: Cyclin-dependent kinase 4; PI3K/AKT: Phosphatidylinositol-4,5-bisphosphate 3-kinaseV-akt murine thymoma viral oncogene homolog; ICAM-1: Intercellular adhesion molecule 1; TNF: Tumor necrosis factor; Grb-2: Growth factor receptor-bound protein 2; GAP: GTPase-activating protein; SOS: Son of sevenless; SOD: Superoxide dismutase; GST: Glutathione S-transferase; MDA: Malondialdehyde.

times in patients with liver-stagnation and spleen-deficiency, Qi stagnation and blood stasis, damp-heat, dampness and blood stasis, liver and kidney-Yin-deficiency were 14.77 mo, 6.13 mo, 5.27 mo, 4.78 mo and 0.80 mo, respectively[54]. Wan *et al*[55] reported the QOL in patients with sthenia syndrome, such as Qi stagnation, blood stasis and damp-heat, was relatively better than those with asthenia syndrome including Qi-deficiency, Yin-deficiency and blood deficiency[55].

### *The influence of modern treatment on syndromes in hepatocarcinoma*

In addition to disease, treatments such as surgery, TACE and high intensity focus ultrasound (HIFU) treatment would influence syndrome pattern. Before surgery, a large proportion of patients presented blood stasis (22.1%) and damp-heat (20.9%). After operation, blood stasis syndrome decreased while spleen deficiency syndrome increased. The most common syndromes in postoperative patients are damp-heat (21.8%) and spleen deficiency (21.0%)[56]. In a tongue picture study, Ye *et al*[57] reported that the tongues of blood stasis, dampness and Qi-deficiency pattern were commonly observed during the perioperative period of liver cancer, but the tongues of Yin asthenia generating intrinsic heat were increased within 5 d after surgery[57].

TACE is another principal treatment for liver cancer and has been demonstrated to be able to impair Yin, and generate heat and dampness in liver cancer patients[58]. Before interventional treatment, the commonly observed TCM syndromes are liver stagnation, spleen deficiency, damp-heat, blood stasis and Yin-deficiency[59]. After interventional treatment, damp-heat syndrome was increased, and liver stagnation and spleen deficiency decreased. Simultaneous TCM treatment could restore these syndrome changes[60]. In another report, Qi-deficiency and dampness were worsened, while the liver-Qi stagnation and blood stasis were alleviated after TACE. These changes suggest local disease improvement and general impairment[61].

In a systematic observation, Zhang *et al*[62] reported that the syndromes before TACE treatment were blood stasis (86.8%), excess-heat (68.9%), Qi stagnation (58.5%), Qi-deficiency (58.5%), Yin-deficiency (56.6%), blood deficiency (28.3%), Yang-deficiency (17.0%), and fluid and damp syndrome (14.2%). TACE treatment increased Qi-deficiency and Yang-deficiency syndrome. The syndromes found in post-TACE patients were blood stasis (84.0%), Qi-deficiency (82.1%), excess-heat (80.2%), Qi stagnation (49.1%), Yin-deficiency (46.2%), blood deficiency (39.6%), Yang-deficiency (30.2%), and fluid and damp syndrome (18.9%)[62].

HIFU treatment, an important method for liver cancer therapy, may also affect syndrome distribution. It has been reported that syndromes observed before HIFU treatment are Qi-deficiency, blood deficiency, Yin-deficiency, Qi stagnation, blood stasis and dampness. HIFU treatment could relieve Qi stagnation and blood stasis, and aggravate Qi-deficiency and Yin-deficiency[63].

### *Molecular insights into syndromes in hepatocarcinoma*

Contemporary TCM has studied liver cancer syndrome by methods of biomedical science. By using a metabonomics method, Chen *et al*[64] found that amino acid metabolism, lipid metabolism, glycometabolism and energy metabolism are unbalanced or weak in liver cancer patients with Yang-deficiency. Metabolites, including very-low-density lipoprotein/low-density lipoprotein, isoleucine, lactate, lipids, choline and glucose/sugars, were decreased and may be potential biomarkers for diagnosis of Yang-deficiency syndrome in liver cancer patients[64].

Serum protein is a potential source for syndrome study. By using surface enhanced laser desorption ionization time of flight mass spectrometry, Yang *et al*[65] found serum proteins with mass-to-charge ratios (M/Z) of 6589 and 4182 Da (dalton) were down-regulated in liver stagnation syndrome, that with an M/Z of 5710 Da was down-regulated in damp-heat syndrome, that with an M/Z of 6992 Da was down-regulated in Yin-deficiency syndrome, while those with M/Z of 5816 Da and 4297 Da were up-regulated in spleen deficiency and blood stasis syndrome, respectively[65]. Huang *et al*[66] reported that proteins with M/Z of 8576 Da (cytochrome C6) and 8780 Da (cytochrome c oxidase assembly factor 5) were over-expressed in serum from patients with liver stagnation syndrome[66].

By means of GeneChip, Weng *et al*[67] reported that 615 mRNAs were differentially expressed in peripheral blood mononuclear cells from liver cancer patients with liver-kidney Yin-deficiency syndrome. These genes are related to *GO* (gene ontology) of anti-apoptosis, regulation of cell cycle and transmembrane transport, and 26 kyoto encyclopedia of genes and genomes pathways. Among these genes, SEC62 [SEC62 homolog (S. cerevisiae)], cyclin B1 and baculoviral IAP repeat containing 3 (BIRC3) were significantly down-regulated in patients with syndrome of liver-kidney Yin-deficiency compard with those without[67].

TCM syndrome is closely associated with the stage of disease. Su *et al*[68,69] explored the relationship between syndrome differentiation and metastasis potential of hepatocarcinoma cells. The percentage of metastasis in patients was ranked from high to low as follows: liver and kidney-Yin-deficiency, Qi stagnation and blood stasis, damp-heat, and liver stagnation and spleen deficiency, and was related to up-regulation of β-catenin and down-regulation of E-cadherin and matrix metalloproteinase-2 (MMP2)/tissue inhibitor of metalloproteinase-2[68,69].

In addition to clinical studies, it has been confirmed that mouse models can be used for syndrome exploration[70]. Pan *et al*[71] studied gene expression in the adrenal glands of H22 liver cancer bearing mice. In toxicity accumulation and Qi-deficiency syndrome (early stage), xanthine dehydrogenase and other 18 genes were up-regulated, while eukaryotic translation elongation factor 1 alpha 2 and other 8 genes were down-regulated. In Yang-Qi-deficiency syndrome (middle stage), enolase 3 (beta muscle) and other 11 genes were up-regulated, while solute carrier family 32 (GABA vesicular transporter) and other 10 genes were down-regulated. In Qi-Yin-Yang-deficiency syndrome (advanced stage), albumin and other 29 genes were up-regulated, while ATPase, $Na^+/K^+$ transporting, beta 2 polypeptide and other 5 genes were down-regulated. S100 calcium binding protein A8 (calgranulin A) and other 11 genes were gradually up-regulated from early stage to advanced stage[71].

## CLINICAL STUDY OF TCM FOR HEPATOCARCINOMA TREATMENT

TCM has been widely used for liver cancer treatment in combination with surgery, TACE, radiofrequency ablation (RFA), microwave ablation (MWA), HIFU and target therapy, or as monotherapy for disease control and/or alleviating symptoms. TCM have been demonstrated to be effective to inhibit tumor growth, prolong survival time, ameliorate symptoms, and improve QOL and immune function in hepatocarcinoma patients (Table 2). Related studies will further improve clinical efficacy of TCM and benefit more to hepatocarcinoma patients.

### *TCM in combination with surgery*

Surgery is one of the most important treatments for hepatocarcinoma. TCM has been proved potent to prevent recurrence and metastasis after the surgery. Treatment with Jiedu Granule and Cinobufacini injection may postpone tumor recurrence and metastasis, and prolong survival time in postoperative patients with hepatocarcinoma[72]. Long-term use of Ruanjianhugan tablets could prolong overall survival time in postoperative patients with small hepatocarcinoma[73]. Treatment with Jiedu xiaozheng yin for 7 d before surgery and Fuzheng

**Table 2 Clinical study of traditional Chinese medicine for hepatocarcinoma treatment**

| Treatments | TCM therapeutic principles | Herbs/herbl formula | Disease | No. of patients | Effects | Ref. |
|---|---|---|---|---|---|---|
| Surgery, Jiedu Granule and Cinobufacini Injection | Detoxifying and dissipating mass | Jiedu Granule: Shi-Jian-Chuan (*Salvia chinensis*), Mao-Ren-Shen (*Actinidia valvata*), Yi-Yi-Ren (*Semen Coicis*), Shan-Zha (*Fructus Crataegi*), Shen-Qu (*Massa Medicata Fermentata*), *etc.* | Hepatocellular carcinoma after surgical resection | 120 | Postpone tumor recurrence and metastasis, prolong survival time | [72] |
| Surgery, Ruanjianhugan Tablets | Detoxifying, dispersing stasis, tonifying Qi and Yin | Ku-Shen (*Sophora flavescens* Ait.), Xia-Ku-Cao (*Prunella*), Hu-Zhang (*Polygonum cuspidatum*), Nü-Zhen-Zi (*Ligustrum lucidum* Ait), Huang-Qi (*Astragalus membranaceus*), *etc.* | Small hepatocellular carcinoma after surgical resection | 399 | Prolong overall survival time | [73] |
| Surgery, Jiedu Xiaozheng Yin and Fuzheng Yiliu Recipe | Detoxifying and dissipating mass, tonifying Qi and Yin | Jiedu Xiaozheng Yin: Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd), Shan-Ci-Gu (*Pseudobulbus Cremastrae*), Ku-Shen (*Sophora flavescens* Ait.), *et al*. Fuzheng Yiliu Recipe: Huang-Qi (*Astragalus membranaceus*), Nü-Zhen-Zi (*Ligustrum lucidum* Ait), Shan-Yao (*Dioscorea opposita*), *etc.* | Stage Ⅲ hepatocarcinoma before and after surgical resection | 72 | Prolong survival time and prevent recurrence | [74] |
| JDF Granule and TACE | Detoxifying and dispersing stasis | Mao-Ren-Shen (*Actinidia valvata*), Shi-Jian-Chuan (*Salvia chinensis*), Shan-Ci-Gu (*Pseudobulbus Cremastrae*), Ji-Nei-Jin (*gizzard membrane of Gallus gallus domesticus*) | Unresectable hepatocellular carcinoma | 165 | Prolong survival time | [75] |
| Shentao Ruangan Pill and TACE | Detoxifying, dispersing stasis, tonifying Qi and Yang | Yin-Chen-Hao (*Artemisia capillaris* Thunb.), Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd.), Ban-Zi-Lian (*Scutellaria barbata*), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*), Ren-Shen (*Panax ginseng*), *etc.* | Middle-advanced stage large hepatocarcinoma | 85 | Prolong survival time | [76] |
| Ganai No. I and No. II and TACE | Tonifying Qi and Yin, detoxifying and dissipating mass | Ganai No. Ⅰ: Dang-Shen (*Codonopsis pilosula*), Huang Qi (*Astragalus membranaceous*), Nü-Zhen-Zi (*Ligustrum lucidum* Ait), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*), Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd.), *etc.* Ganai No. Ⅱ: Xue-Jie (*Daemonorops draco* BL.), Ru-Xiang (*Boswellia carterii* Birdw), Mo-Yao (*Commiphpra myrrha* Engl.), Zao-Xiu (*Paris polyphylla* Sm.), Long-Kui (*Solanum nigrum* L.), *etc.* | Middle-advanced stage liver cancer | 60 | Prolong survival time, prevent recurrence and alleviate HACE induce leukocytopenia | [77] |
| Jinlong Capsule and TACE or RFA | Detoxifying and dissipating mass | Tian-Long (Gecko), Jin-Qin-Bai-Hua-She (Multibanded krait), Qi-She (Long-nosed pit viper) | Unresectable hepatocarcinoma | 98, 76 | Enhance short-term effect of TACE and improve quality of life; enhance RFA effects, reduce RFA induced liver injury, improve QOL and immune function | [78,89] |
| Syndrome differentiation based TCM therapy and TACE | Syndrome differentiation based TCM therapy | Xiao-Yao-San/Chai-Hu-Shu-Gan-San, Hua-Yu-Xiao-Liu-Tang, Long-Dan-Xie-Gan-Tang, Yi-Guan-Jian + Liu-Wei-Di-Huang-Wan | Middle-advanced stage hepatocarcinoma | 67 | Enhance short-term efficacy and reduce adverse effect of TACE, improve immune function and QOL | [79] |
| Shelian Capsule and TACE | Detoxifying, dissipating mass, tonifying Qi | Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd.), Ban-Zi-Lian (*Scutellaria barbata*), Hu-Zhang (*Polygonum cuspidatum*), Dan-shen (*Salvia miltiorrhiza* Bge.), Ren-Shen (*Panax ginseng*), *etc.* | Early middle and advanced stage hepatocarcinoma | 120 | Enhance short-term efficacy of TACE, improve immune function and QOL | [80] |
| Matrine Injection and TACE | Clearing heat and detoxifying | Matrine, *etc.* | Hepatocarcinoma after TACE | 122 | Protect liver function | [81] |
| Jia Wei Si Jun Zi Tang and TACE | Tonifying spleen, nourishing Qi, soothing liver-Qi stagnation, and dissolving stasis | Dang-Shen (*Codonopsis pilosula*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Huang Qi (*Astragalus membranaceous*), Fo-Shou (*Citrus medica* L. var. *sarcodactylis*), Dan-shen (*Salvia miltiorrhiza* Bge.), *etc.* | Advance stage of liver cancer | 65 | Protect liver function | [82] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Vascular Embolizing Agent | Vascular embolizing | Yu-Jin (*Curcuma aromatic*), Bai-Ji (*Bletilla striata*) | Stage Ⅱ and Ⅲ hepatocarcinoma (Yu-Jin), stage Ⅰ-Ⅲ hepatocarcinoma (Bai-Ji) | 32 (Yu-Jin), 106 (Bai-Ji), 56 (Bai-Ji) | Vascular embolizing agent | [83-85] |
| Aidi Injection and RFA or MWA | Dissipating mass, tonifying Qi and Yin | Ban-Mao (Mylabris), Huang Qi (*Astragalus membranaceous*), *etc.* | Middle-late stage hepatocarcinoma, stage Ⅱ and Ⅲ hepatocarcinoma | 89, 61 | Relieve the impairment of cool-tip radiofrequency ablation on hepatic function, improve immune function and reduce relapse; elevate MWA efficacy, improve QOL, immune and liver function | [86,92] |
| Xiaoaiping Injection and RFA | Dissolving stasis | Xiaoaiping injection | Middle-late stage hepatocarcinoma, advanced hepatocarcinoma | 31, 68 | Enhance RFA effects and reduce non-bacterial inflammatory response; inhibit tumor growth, improve QOL and immune function, increase progression-free survival | [87,99] |
| Tianzhicao Capsule and RFA | Detoxifying, dissolving stasis, tonifying Qi and Yin | Ban-Zi-Lian (*Sculellaria barbata*), Dan-shen (*Salvia miltiorrhiza* Bge.), Huang Qi (*Astragalus membranaceous*), Gou-Qi (*Lycium barbarum*), *etc.* | Small hepatocarcinoma | 90 | Improve QOL and immune function, prolong survival time | [88] |
| Fuzheng Yiliu Recipe and MWA | | Huang Qi (*Astragalus membranaceous*), Ling-Zhi (*Ganoderma lucidum*), Nü-Zhen-Zi (*Ligustrum lucidum* Ait.), *etc.* | Middle-advanced stage hepatocarcinoma | 60 | Enhance MWA efficacy, improve immune and liver function | [90] |
| Shenqi Mixture and MWA | Tonifying Qi | Ren-Shen (*Panax ginseng*), *etc.* | Stage Ⅱ and Ⅲ hepatocarcinoma | 72 | Enhance MWA efficacy, prolong survival time, improve QOL and immune function and relieve symptoms | [91] |
| Qiankun Capsule and HIFU | Tonifying spleen, detoxifying, and dissipating mass | Tai-Zi-Shen (*Pseeudostellaria heterophylla*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Bai-Shao (*Paeonia lactiflora*), Xia-Ku-Cao (*Prunella*), Ban-Zi-Lian (*Sculellaria barbata*), *etc.* | Stage Ⅱ and Ⅲ hepatocarcinoma | 60 | Enhance therapeutic effects and improve immune function | [93] |
| Fu-Zhen-Yang-Yin Formula and HIFU | Tonifying Qi and Yin, detoxifying, and dissipating mass | Huang Qi (*Astragalus membranaceous*), Tai-Zi-Shen (*Pseeudostellaria heterophylla*), Yin-Chen-Hao (*Artemisia capillaris* Thunb.), Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd.), Hu-Zhang (*Polygonum cuspidatum*), *etc.* | Advanced stage hepatocarcinoma | 60 | Alleviate HIFU induced fever and liver function damage | [94] |
| Syndrome Differentiation Based TCM Therapy and Sorafenib | Syndrome differentiation based TCM therapy | Huang Qi (*Astragalus membranaceous*), Shan-Yao (*Dioscorea opposita*), Long-Kui (*Solanum nigrum* L.), Bai-Shao (*Paeonia lactiflora*), *etc.* | Advanced stage hepatocarcinoma | 18 | Enhance therapeutic efficacy of sorafenib and prolong survival time | [95] |
| Cinobufagin tablet and Sorafenib | Dissipating mass | Cinobufagin, *etc.* | Middle-advanced stage hepatocarcinoma | 59 | Enhance effects of sorafenib, improve QOL and liver function and relieve pain | [96] |
| Oleum fructus bruceas intervention, oral intake of Ganji Decoction and external application of Ailitong | Tonifying spleen, soothing liver-Qi stagnation, detoxifying, dissipating mass | Dang-Shen (*Codonopsis pilosula*), Chai-Hu (*Bupleurum scorzonerifolfium* Willd), Bai-Shao (*Paeonia lactiflora*), Tu-Bie-Chong (*Eupolyphaga gasinensis* Walk.), Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd.), *etc.* | Middle-advanced stage hepatocarcinoma | 97 | Prolong survival time, improve QOL and relieve pain | [97] |

| | | | | | |
|---|---|---|---|---|---|
| Hepatic artery perfusion/embolization with turmeric oil microballoon, cinobufotalin and Aidi injection, and Syndrome Differentiation Based TCM Therapy | Tonifying Qi and Yin, and dissipating mass | Yu-Jin (*Curcuma aromatic*), cinobufotalin, Ban-Mao (Mylabris), Huang Qi (*Astragalus membranaceous*), *etc.* | Stage Ⅰ to Ⅲ hepatocarcinoma | 41 | Inhibit tumor growth with fewer adverse reactions, and ameliorate fatigue and anorexia | [98] |
| Cinobufacini Injection | Dissipating masses | Cinobufagin, *etc.* | Middle-advanced stage hepatocarcinoma | 100 | Inhibiting tumor growth, protecting liver function and prolonging survival time | [100] |
| Norcantharidin and herbal decoction | Tonifying spleen and Qi, and dissolving stasis | Huang Qi (*Astragalus membranaceous*), Yin-Chen-Hao (*Artemisia capillaris* Thunb.), Hu-Zhang (*Polygonum cuspidatum*), Fu-Ling (*Poria cocos*), Tian-Long (Gecko), *etc.* | Elderly patients with late stage hepatocarcinoma | 79 | Inhibit tumor growth, prolong survival time and improve QOL | [101] |
| Jianpi Fuzheng Decoction | Tonifying Qi and Yin | Huang Qi (*Astragalus membranaceous*), Yi-Yi-Ren (*Coix lacryma-jobi* L.), Dang-Shen (*Codonopsis pilosula*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Nü-Zhen-Zi (*Ligustrum lucidum* Ait), *etc.* | Late stage hepatocarcinoma | 60 | Ameliorate TCM syndrome, improve QOL and immune function and prolong survival time | [102] |
| Jianpi Yiliu Decoction | Tonifying spleen and Qi, detoxifying, and dissolving stasis | Huang Qi (*Astragalus membranaceous*), Dang-Shen (*Codonopsis pilosula*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Ban-Zi-Lian (*Sculellaria barbata*), Yu-Jin (*Curcuma aromatic*), *etc.* | Late stage hepatocarcinoma | 30 | Ameliorate symptoms, improve QOL and protect liver function | [102] |
| Jiawei Yiguanjian | Tonifying Yin, clearing heat and draining dampness | Bei-Sha-Shen (*Glehnia littoralis*), Mai-Dong (*Ophiopogon japonicus*), Dang-Gui (*Angelica sinensis*), Gou-Qi (*Lycium barbarum*), Chui-Pen-Cao (*Sedum sarmentosum* Bunge), *etc.* | Late stage hepatocarcinoma | 100 | Prolong survival time, ameliorate TCM syndrome, and improve QOL and immune function | [104] |

TCM: Traditional Chinese Medicine; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; MWA: Microwave ablation; QOL: Quality of life; HIFU: High intensity focus ultrasound; HACE: Hepatic artery chemoembolization.

yiliu recipe after operation for 2 years are effective to prolong survival time and prevent recurrence in patients with stage Ⅲ hepatocarcinoma[74].

### *TCM in combination with TACE*

TACE is the principle treatment for unresectable hepatocarcinoma. TCM has been used to improve efficacy of TACE. JDF granule preparation, a traditional Chinese herbal formula, combined with TACE may prolong survival time in patients with unresectable hepatocellular carcinoma[75]. Shentao Ruangan pill based herbal therapy in combination with TACE could prolong survival time in patients with middle-advanced stage liver cancer[76]. Ganai No. Ⅰ and No. Ⅱ in combination with hepatic artery chemoembolization (HACE) have been reported effectively to prolong survival time, prevent recurrence and alleviate HACE induced leukocytopenia in patients with middle-advanced stage hepatocarcinoma[77].

Jinlong Capsule may enhance short-term effect of TACE and improve QOL in patients with unresectable hepatocarcinoma[78]. Syndrome differentiation based TCM therapy could enhance short-term efficacy and reduce adverse effect of TACE, improve immune function and QOL in liver cancer patients[79]. Shelian Capsule, a Chinese patent drug, was reported to effectively enhance short-term efficacy of TACE, improve immune function and QOL in liver cancer patients[80]. Matrine injection and "jia wei si jun zi tang" have been proved effective to protect liver function in liver cancer patients receiving TACE treatment[81,82].

In addition to oral and intravenous administration, some Chinese herbs, such as *curcuma* aromatic and *Bletilla striata*, have been demonstrated to be able to be used as vascular embolizing agents for TACE in liver cancer patients[83-85].

### *TCM in combination with RFA*

RFA is another widely used treatment for hepatocarcinoma. Aidi injection, an herbal injection, has been reported to effectively relieve hepatic function impairment caused by cool-tip RFA, improve immune function and reduce relapse rate in patients with liver cancer[86]. Xiaoaiping injection, an herbal extract injection, could enhance RFA effects and reduce non-bacterial inflammatory response post RFA[87]. Tianzhicao capsule in combination with RFA may improve QOL and immune function, prolong survival time in patients with hepatocellular carcinoma[88]. Jinlong capsule has been demonstrated to enhance RFA effects, reduce RFA induced liver injury, improve QOL and immune function in liver cancer patients[89].

### *TCM in combination with MWA*

In combination with MWA, Fuzheng Yiliu Recipe could enhance MWA efficacy, improve immune and liver function in liver cancer patients[90]. Shenqi mixture has been showed to be effective to enhance MWA efficacy, prolong survival time, improve QOL and immune function and relieve symptoms in patients with hepatocarcinoma[91]. Aidi injection is able to elevate MWA efficacy and improve QOL, immune and liver function in hepatocarcinoma patients[92].

### *TCM in combination with other treatments*

HIFU has become an important treatment method for hepatocarcinoma. In combination with HIFU, Qiankun capsule can enhance therapeutic effects and improve immune function in patients with hepatocarcinoma[93]. Fu-Zhen-Yang-Yin formula could alleviate HIFU induced fever and liver function damage[94].

Sorafenib is a target agent for liver cancer treatment. Syndrome differentiation based TCM therapy could enhance therapeutic efficacy of sorafenib and prolong survival time in patients with advanced hepatocarcinoma[95]. Cinobufagin tablet has been confirmed to enhance effects of sorafenib, improve QOL and liver function and relieve pain in liver cancer patients[96].

### *TCM as a monotherapy*

In addition to intravenous administration, herbal extract injection also can be used as a perfusion/embolization reagent instead of chemotherapeutic drug. Tian *et al*[97] reported that a Chinese medicine comprehensive therapy, *i.e.,* Oleum fructus bruceas intervention combined with oral intake of Ganji Decoction and external application of Ailitong, is safe and effective to prolong survival time, improve QOL and relieve pain compared with conventional TACE in liver cancer patients[97]. Xu *et al*[98] has demonstrated that hepatic artery perfusion/embolization with turmeric oil microballoon, cinobufotalin and Aidi injection is effective with fewer adverse reactions compared with conventional TACE, and could significantly ameliorate fatigue and anorexia in patients with hepatocarcinoma[98].

Chinese patent herbal drugs have been approved as effective treatments for hepatocarcinoma. Xiaoaiping injection is effective to inhibit tumor growth, improve QOL and immune function, increase progression-free survival compared with best supportive treatment in patients with advanced hepatocarcinoma[99]. Cinobufacini injection has been demonstrated to be effective in inhibiting tumor growth, protecting liver function and prolonging survival time in patients with moderate and advanced hepatocarcinoma[100]. Norcantharidin in combination with herbal decoction treatment can inhibit tumor growth, prolong survival time and improve QOL in elderly patients with late stage liver cancer[101].

Herbal decoction is the principle TCM treatment for liver cancer. Jianpi Fuzheng decoction, an herbal formula for tonifying spleen and strengthening body defense, is effective in ameliorating TCM syndrome, improving QOL and immune function and prolonging survival time in patients with advanced liver cancer[102]. Jianpi Yiliu decoction, a formula for tonifying spleen and inhibiting tumor, has been reported to effectively ameliorate symptoms, improve QOL and protect liver function in patients with late stage hepatocarcinoma[103]. Jiawei Yiguanjian, a modified classical herbal formula, has been shown to be effective to prolong survival time, ameliorate TCM syndrome, and improve QOL and immune function in patients with advanced hepatocellular carcinoma[104].

## EFFECTIVE MECHANISM OF CHINESE HERB AGAINST HEPATOCARCINOMA

The effects of Chinese herbs against hepatocarcinoma have been extensively studied, and it has been demonstrated that Chinese herbs are effective to induce apoptosis, autophagy, anoikis and cell senescence, arrest cell cycle, regulate immune function, inhibit metastasis and angiogenesis, reverse drug resistance and enhance effects of chemotherapy. Anti-cancer effects of herbal formulas against hepatocarcinoma are listed in Table 3.

### *Induction of apoptosis*

Apoptosis is one of the most frequently studied filed for elucidating effective mechanism of herbs against hepatocarcinoma. Ren-Shen (*Panax ginseng*) is a commonly used Qi-tonifying herb. 20(R)-ginsenoside Rg3, a component from *Panax ginseng*, may up-regulate TNF-α and induce apoptosis in induced liver tumors in SD rats[105]. Blood tonifying herb or component, including Di-Huang (*Rehmannia glutinosa*) and N-butylidenephthalide from Dang-Gui (*Angelica sinensis*), is effective to induce apoptosis of hepatocarcinoma cells[106,107]. Gou-Qi (*Lycium barbarum*), a liver-Yin tonifying herb, is effective to inhibit proliferation and stimulate p53-mediated apoptosis in liver cancer cells[106]. Icariin, an ingredient from Yang tonifying herb Yin-Yang-Huo (*Epimedium brevicornum* Maxim.), may generate reactive oxygen species (ROS) and up-regulate JNK (c-Jun N-terminal kinase) phosphorylation and B-cell lymphoma 2/Bcl2-associated

**Table 3 Anti-cancer effects of herbal formulas against hepatocarcinoma**

| Herbal formula | Therapeutic principles | Herbs | Models | Effects | Targets/events | Ref. |
|---|---|---|---|---|---|---|
| Fuzheng Yiliu Granule | Tonifying Qi and blood, and dissolving stasis | Hong-Qi (*Hedysarum polybotrys*), Dang-Gui (*Angelica sinensis*), Mu-Tou-Hui (*Patrinia scabra* Bunge), *etc.* | Mouse H22 hepatocarcinoma bearing mice | Inhibit tumor growth, induce apoptosis | p53, caspase-3, mitochondrial membrane potential | [126,127] |
| Fuganchun 6 | Tonifying Qi and Yin, detoxifying and dissipating mass | Huang-Qi (*Astragalus membranaceus*), Nü-Zhen-Zi (*Ligustrum lucidum* Ait), Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd), Bie-Jia (Carapax trionycis), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*), *etc.* | Mouse H22 hepatocarcinoma cells *in vitro* and *in vivo* | Inhibite tumor growth and cell proliferation, and induce apoptosis | Enhance proliferation activity of lymphocyte, NK cells activities and IL-2 production | [128,178] |
| Bushen Jianpi Decoction | Tonifying spleen and kidney | Dang-Shen (Codonopsis pilosula), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Fu-Ling (Poria cocos), Shu-Di-Huang (*Rehmannia glutinosa*), shan-Zhu-Yu (*Cornus officinalis*), Du-Zhong (*Eucommia ulmoides* Oliver), *etc.* | Mouse H22 hepatocarcinoma bearing mice | Inhibit tumor growth, induce apoptosis | VEGF | [129] |
| Warming Yang and Dispersing Stasis Formula | Warming Yang and dispersing stasis | Fu-Zi (*Aconitum carmichaeli* Debx.), Huang-Qi (*Astragalus membranaceus*), Bai-Zhu (*Atractylodes macrocephala* Koidz.), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*), Bai-Shao (*Paeonia lactiflora*), *etc.* | Human BEL-7402 hepatocarcinoma cells | Induce apoptosis | Unknown | [130] |
| Fufangkushen injection | Clearing heat and removing toxicity | Ku-Shen (*Sophora flavescens* Ait.), *etc.* | Human SMMC-7721 hepatocarcinoma cells | Inhibit proliferation, induce apoptosis | Survivin, Bcl-2, caspase-3 | [131] |
| Songyou Yin | Tonifying Qi and blood, dissolving stasis and dissipating mass | Dan-shen (*Salvia miltiorrhiza* Bge.), Huang-Qi (*Astragalus membranaceus* Bge.), Gou-Qi (*Lycium barbarum* L.), Shan-Zha (*Crataegus pinnatifida* Bge.), and Bie-Jia (*Trionyx sinensis* Wiegmann) | Human MHCC97H hepatocarcinoma bearing nude mice; activated rat hepatic stellate cells and rat McA-RH7777 hepatoma cells | Induce apoptosis, inhibit tumor growth, prolong survival; inhibit invasion and metastasis | MMP-2, IL-6, TGF-β1, VEGF, epithelial-mesenchymal transition | [132,167] |
| Huang-lian-jie-du-tang | Clearing heat and removing toxicity | Huang-Lian (*C. chinensis* Franch), Huang-Qin (*Scutellaria baicalensis* Georgi), Huang-Bai (*P. amurense* Rupr) and Zhi-Zi (*Gardenia jasminoides* Ellis) | Human Hep G2 and PLC/PRF/5 hepatomain cells *in vitro* and *in vivo* | Inhibit cell proliferation, induce cell cycle arrest and apoptosis | Cdc2, Cdc25C, cyclin A, cyclin B1, Cdc2, Cdc25C, Bax, Bak, Bcl-2, Bcl-XL, IkappaBalpha | [141] |
| Bu-Zhong-Yi-Qi-Tang | Invigorating spleen-stomach and replenishing qi | Huang-QI (*Astragalus membranaceus*), Ren-Shen (*Panax ginseng*), Gang-Gui (*Angelicae sinensis*), Sheng-Ma (*Cimicifuga foetida* L.), Gan-Cao (*Glycyrrhiza uralensis*), Chen-Pi (*Citrus poonensis*), Chai-Hu (*Bupleurum chinensis*), Sheng-Jiang (*Zingiber officinale*), Da-zhao (*Ziziphs jujuba*), Bai-Shu (*Atractylodes macrocephala* KOIDZUMI) | Human hepatoma Hep3B, HepG2 and HA22T cells | Inhibit cell proliferation, induce apoptosis and arrest cell cycle at G0/G1 phase | Unknown | [142] |
| Jiedu Xiaozheng Yin | Heat-clearing and detoxification | Bai-Hua-She-She-Cao (*Hedyotis diffusa* Willd), Xia-Ku-Cao (*Prunella*), Shan-Ci-Gu (*Pseudobulbus Cremastrae*), Ku-Shen (*Sophora flavescens*) | Human Hep G2 hepatoma cell *in vitro* and *in vivo* | Inhibits the growth of HepG2 cells, arrest cell cycle at the G0/G1 phase | Cyclin D and cyclin E | [143] |
| Liver Yin Tonifying formula | Tonifying liver-Yin, draining dampness and dissipating stasis | Nü-Zhen-Zi (*Ligustrum lucidum* Ait), Zhi-Bie-Jia (processed Carapax trionycis) and Hu-Zhang (*Polygonum cuspidatum*) | Human hepatocarcinoma Bel-7402 cells | Inhibit proliferation, induce apoptosis and cell senescence | Caspases-8, -9 and -3, p16, p21, pRB | [152] |
| Modified Yi Guan Jian | Tonifying liver and kidney-Yin, draining dampness and dissipating stasis | Bei-Sha-Shen (*Glehnia littoralis*), Mai-Dong (*Ophiopogon japonicus*), Dang-Gui (*Angelica sinensis*), Shu-Di (dried *Rehmannia glutinosa*), Gou-Qi (*Lycium barbarum*), Chuan-Lian-Zi (*Melia toosendan* Sieb fruit), and Hu-Zhang (*Polygonum cuspidatum*) | Human hepatocarcinoma Bel-7402 cells | Inhibit proliferation, induce anoikis | Caspases-3, -8 and -9, p38 MAPK | [156] |
| Blejiajian Pill | Tonifying Qi, detoxifying and dissipating mass | Bie-Jia-Jiao(Carapacis trionycis-shell glue), E-Jiao(Colla Corii Asini), Feng-Fang (beehives), Chai-Hu (*Bupleurum chinense*), Huang-Qin (*Scutellaria baicalensis*), Ban-Xia (*Pinellia ternate*), Dang-Shen(*Codonopsis pilosula*), *etc.* | Human Hep G2 hepatoma cells | Inhibit cell proliferation, adhesion and invasion | Unknown | [168] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Compound Astragalus and Salvia miltiorrhiza extract | Tonifying Qi and dissipating stasis | Astragalosides, astragalus polysaccharide and salvianolic acids | Human Hep G2 hepatoma cells | Inhibit TGF-β1 mediated invasion | TGF-β/Smad signaling | [169] |
| Shehuang Xiaoliu Fang | Tonifying spleen, dissolving stasis and detoxifying | She-Xiang (musk), Niu-Huang (Cow-Bezoar), Dan-shen (*Salvia miltiorrhiza* Bge.), Bai-Zhu (*Atractylodes macrocephala* Koidz.), Ban-Zi-Lian (*Sculellaria barbata*), E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin*), *etc.* | Human SMMC-7721 hepatocarcinoma cells | Inhibit invasive and adhesive | nm23-h1 and ICAM-1 | [170] |
| Xiaochaihu Decoction | Relief liver for smooth Qi | Chai-Hu (*Bupleurum chinense*), Huang-Qin (*Scutellaria baicalensis* Georgi), Sheng-Jiang (*Zingiber officinale*), Ban-Xia (*Pinellia ternata*), Da-Zao (*Ziziphus jujuba*), Ren-Shen (*Panax ginseng*), Gan-cao (*Glycyrrhiza uralensis*) | Mouse H22 hepatocarcinoma bearing mice | Inhibit tumor growth | Increase T lymphocytes proliferation, NK cells activities and IL-2 | [175] |
| Mylabris Mixture | Detoxifying and dissipating mass | Ban-Mao (Mylabris), Chen-Pi (*Citrus reticulata* Blanco), Gu-Ya (Setaria italica), *etc.* | Mouse H22 hepatocarcinoma bearing mice | Inhibit tumor growth | Increase $CD4^+$, $CD8^+$ lymphocytes and NK cells, and IFN-γ and IL-4 production | [176] |
| Fuzheng Yiliu Granule-2 | Tonifying Qi and tonifying Yin | Huang-Qi (*Astragalus membranaceus*), Nü-Zhen-Zi (*Ligustrum lucidum* Ait), Ling-Zhi (*Ganoderma lucidum*), and Shan-Yao (*Dioscorea opposita*) | Mouse H22 hepatocarcinoma cells *in vitro* and *in vivo* | Inhibite tumor growth and cell proliferation, and induce apoptosis | Increase $CD4^+$ lymphocyte, IL-2 and TNF-α, and NK cells | [177] |
| Fu-Zheng-Kang-Ai-Tang | Strengthening body resistance and anti-cancer | Ren-Shen (Panax ginseng), Huang-Qin (*Scutellaria baicalensis* Georgi), Ling-Zhi (*Ganoderma lucidum*), Huang-Qi (*Astragalus membranaceus*), *etc.* | Human Bel-7402 hepatocarcinoma bearing nude mice | Inhibit angiogenesis and tumor growth | VEGF and bFGF | [188] |
| Delisheng | Tonifying Qi and resolving masses | Ren-Shen (Panax ginseng), Huang-Qi (Astragalus membranaceus), Cantharidium, *etc.* | Human Hep G2 hepatoma cells | Inhibit tumor growth | Endostatin | [189] |
| QHF | Detoxifying, dissolving stasis and strengthening body resistance | Cinobufagin, Ginsenoside Rg3, Panax Notoginseng Saponins, lentinan | Mouse H22 hepatocarcinoma bearing mice | Inhibit angiogenesis and tumor growth, enhance anti-cancer effects DDP | VEGF, EGFR and MMP-2 | [190,196] |
| Erbie San | Resolving masses | Bie-Jia (Carapax trionycis), *etc.* | Wistar rats bearing Walker-256 liver cancer | Inhibit tumor growth | VEGF and endostatin | [191] |
| Chaiqiyigan Granula | Tonifying spleen, soothing liver, dissolving stasis and detoxifying | Chai-Hu (*Bupleurum chinense*), Huang-Qi (*Astragalus membranaceus*), *etc.* | Human hepG2/EGFP hepatoma bearing nude mice | Enhance anti-cancer effects of Taxol | Bax, p53 and VEGF | [195] |
| Shengmai Injection | Tonifying Qi and Yin | Ren-Shen (*Panax ginseng*), Mai-Dong (*Ophiopogon japonicus*), *etc.* | Mouse H22 hepatocarcinoma bearing mice | Enhance anti-tumor efficacy and reduce toxicity of 5-Fu | Unknown | [197] |

TGF: Transforming growth factor; IL-2: Interleukin-2; ICAM-1: Intercellular adhesion molecule 1; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor; MMP2: Matrix metalloproteinase-2; bFGF: Basic fibroblast growth factor; NK: Natural killer; IFN-γ: Interferon-γ; MAPK: Mitogen-activated protein kinases; Bcl-2: Bcl2-associated X protein; Cdc25B: Cell division cycle 25B.

X protein (Bax/Bcl-2) to induce intrinsic apoptosis in liver cancer cells[108].

Clearing heat and detoxifying herbs are one of the most commonly medicated herbs for liver cancer treatment. Solamargine purified from Long-Kui (*Solanum incanum*) may up-regulate TNF receptor type Ⅰ and induce apoptosis in Hep3B cells[109]. Components from Mao-Ren-Shen (*Actinidia valvata*) may arrested cell cycle at G0/G1 phase and induce apoptosis in H22 cells[110]. Ban-Zi-Lian (*Scutellariae barbata*) is effective in up-regulating Bax/Bcl-2, arresting cell cycle at G2/M phase and inducing apoptosis in hepatocellular carcinoma cells[111]. Ba-Qia (*Smilax glabra* Roxb.) extract could activate p38, JNK and extracellular signal-regulated kinase (ERK) to induce intrinsic apoptosis in liver cancer cells[112]. Essential oil of Qing-Hao (*Artemisia annul* L.) could induce apoptosis in SMMC-7721 cells[113].

Clearing heat and draining dampness herbs are used for damp-heat in the liver and gallbladder. Chui-Pen-Cao (*Sedum sarmentosum* Bunge) can inhibit HepG2 cell

growth and induce apoptosis accompanied by down-regulation of Bcl-2, VEGF and phosphorylated signal transducers and activators of transcription (p-STAT3)[114]. Resveratrol-4-O-D-(2'-galloyl)-glucopyranoside from Hu-Zhang (*Polygonum cuspidatum*) may activate caspases 3 and 9 to induce apoptosis in hepatocellular carcinoma *via* the JNK and ERK pathway[115]. Yin-Chen-Hao (*Artemisia capillaris* Thunb.) is effective to induce apoptosis and G0/G1 cell cycle arrest in SMMC-7721 cells[116].

Dispersing blood stasis is another important principle for liver cancer therapy. Dan-Shen (*Salvia miltiorrhiza*) was reported to effectively inhibit proliferation and induce apoptosis in HepG2 cells coincided with depletion of intracellular glutathione and reduction of mitochondrial membrane potential[117]. Chi-Shao (*Paeoniae Radix*) could inhibit cell growth and induce p53 independent apoptosis coincided with up-regulation Bcl-2/adenovirus E1B 19 kD-interacting protein 3 and down-regulation of ZK1, RAD23 homologue B and heat shock 60 kDa protein 1[118]. Curcumin is a component of Yu-Jin/Jiang-Huang/E-Zhu (*Curcuma kwangsiensis* or *Curcuma phaeocaulis* or *Curcuma wenyujin* or *Curcuma longa*). Resveratrol is a compound that can be isolated from Hu-Zhang (*Polygonum cuspidatum*) or other plants. We have found that curcumin combined with resveratrol may synergistically inhibit X-linked inhibitor of apoptosis protein (XIAP) and survivin expression, up-regulate ROS production, activate caspases-3, -8 and -9 to induce apoptosis in liver cancer cells[119].

Although the TCM pathological factor of phlegm is not directly related to hepatocarcinogenesis, reducing phlegm and/or resolving masses herbs are frequently medicated as anti-cancer herbs in liver cancer. Ban-Xia (*Pinellia ternate*) can inhibit cell proliferation, up-regulate Bax/Bcl-2 and induce apoptosis in Bel-7402 cells[120]. Tian-Nan-Xing (*Arisaema heterophyllum*) may activate caspase-3 and induce apoptosis in hepatocarcinoma cells[121]. Tubeimoside Ⅰ, an ingredient from Tu-Bei-Mu (*Bolbostemma paniculatum*), could up-regulate Bax/Bcl-2 and induce intrinsic apoptosis in hepatoma cells[122]. Toxic Chinese herb or components such as Quan-Xie (scorpio), Norcantharidin and polypeptides from bee venom are potent to induce apoptosis in liver cancer cells[123-125].

Contemporary TCM physicians have developed some new herbal formulas for liver cancer treatment. Fuzheng yiliu granule, a four herb formula for tonifying Qi and blood, and dissolving stasis, is effective to up-regulate p53 and caspase-3 expression and reduce mitochondrial membrane potential to induce apoptosis in H22 hepatocarcinoma[126,127]. Fuganchun 6, a formula for nourishing Qi and Yin, detoxifying and dissipating mass, could induce apoptosis and arrest cell cycle at G0/G1 phase[128]. Bushen jianpi decoction, a formula for tonifying kidney and spleen, was reported to inhibit VEGF expression and induce apoptosis in H22 hepatocarcinoma[129]. Warming yang and dispersing stasis formula may induce apoptosis in hepatocarcinoma Bel-7402 cells[130]. Fufangkushen injection, a patent herbal drug, may inhibit survivin and Bcl-2 expression, increase caspase-3 expression and induce apoptosis in SMMC-7721 cells[131]. Songyou Yin, another patent herbal drug, could induce apoptosis and down-regulation of MMP2 and VEGF to inhibit tumor growth and prolong survival[132] (Figure 1).

### *Arresting cell cycle*

Sustaining proliferative signaling is a hallmark of cancer[133]. Cancer cells present an un-controlled proliferation cycle. To stop cell cycle is an ideal principle for cancer treatment. Some herbs are demonstrated to be potent to arrest cell cycle. Among tonic herbs, extracts of Jiao-Gu-Lan (*Gynostemma pentaphyllum*) can inhibit proliferation and arrest cell cycle at G0/G1 phase in Hep3B cells[134,135]. Triterpene-enriched extracts from Ling-Zhi (*Ganoderma lucidum*) could suppress protein kinase C, activate JNK and p38 mitogen-activated protein kinases (MAPK) to prolong G2 cell cycle phase and inhibit cell growth in Huh-7 cells[136].

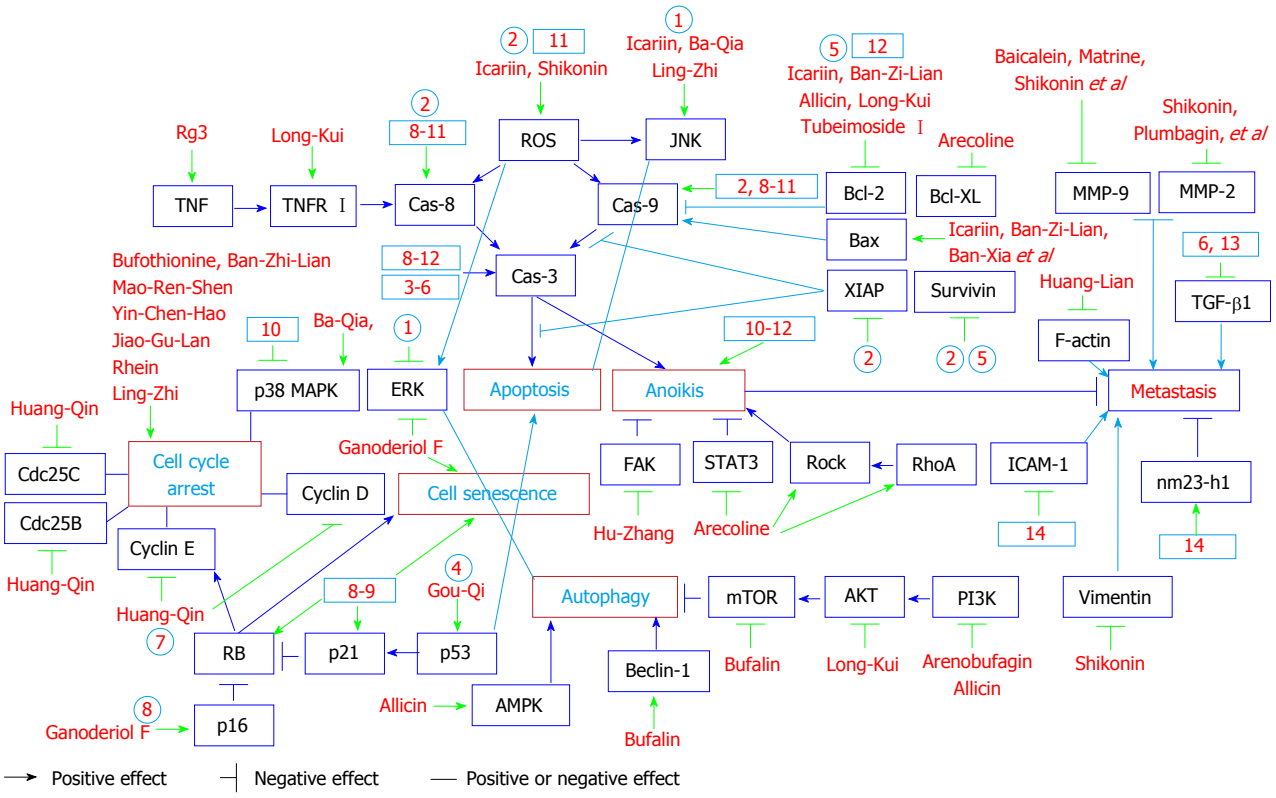
Ban-Zhi-Lian (*Scutellaria barbata*) is able to inhibit cell proliferation, decrease the number of cells in S-phase and increase the number of cells in G0/G1-phase and induce apoptosis in HepG2 cells [137]. Huang-Qin (*Scutellaria baicalensis*) is effective to inhibit cell growth and G2/M phase arrest accompanied by increased Cyclin E and down-regulation of p53, ETS1 (V-ets avian erythroblastosis virus E26 oncogene homolog 1), cell division cycle 25B (Cdc25B), p63, epidermal growth factor receptor (EGFR), ERK1/2, XIAP, HIF-2alpha, and Cdc25C[138]. Bufothionine also can inhibit cell proliferation and arrest cell cycle at G2/M phase in liver cancer cells[139]. Rhein, a component from Da-Huang (*Rheum palmatum* L. or *Rheum tanguticum* Maxim. ex Balf.), is potent to inhibit cell growth, induce apoptosis and arrest cell cycle at S phase[140].

Huang-lian-jie-du-tang, a classic herbal formula for clearing heat and removing toxicity, was reported to effectively inhibit cell proliferation, induce cell cycle arrest and apoptosis in hepatocarcinoma *in vitro* and *in vivo*[141]. Bu-Zhong-Yi-Qi-Tang, a Qi tonifying herbal formula, is confirmed effective to inhibit cell proliferation, induce apoptosis and arrest cell cycle at G0/G1 phase[142]. Jiedu Xiaozheng Yin, a modern herbal formula, could inhibit the growth of HepG2 cells and arrest cell cycle at the G0/G1 phase coincidence with up-regulation of cyclin D and cyclin E[143] (Figure 1).

### *Induction of autophagy*

Autophagy, type Ⅱ programmed cell death, is a process in which organelles and proteins are sequestered and subsequently degraded through fusion with lysosomes, and has been recognized as a target for hepatocarcinoma treatment[144,145]. Long-Kui (*Solanum nigrum* L.), a frequently used anti-cancer herb, may induce apoptosis and down-regulate Bcl-2 and AKT to induce autophagy in hepatocarcinoma cells[146].

Arenobufagin, a natural bufadienolide from toad




1: Resveratrol-4-O-D-(2'-galloyl)-glucopyranoside; 2: Curcumin and resveratrol; 3: Tian-Nan-Xing (*Arisaema heterophyllum*); 4: Fuzheng yiliu granule; 5: Fufangkushen injection; 6: Songyou Yin; 7: Jiedu Xiaozheng Yin; 8: Liver-Yin tonifying formula; 9: Nü-zhen-zi (*Ligustrum lucidum* Ait. Fruit); 10: Modified Yi Guan Jian; 11: Hu-Zhang (*Polygonum cuspidatum*); 12: Arecoline; 13: Compound Astragalus and Salvia miltiorrhiza extract; 14: Shehuang Xiaoliu Fang.

**Figure 1 Therapeutic targets of Chinese herb(s) in hepatocarcinoma cells.** TNF: Tumor necrosis factor; ROS: Reactive oxygen species; JNK: C-Jun N-terminal kinase; Bax/Bcl-2: B-cell lymphoma 2/Bcl2-associated X protein; XIAP: X-linked inhibitor of apoptosis protein; MMP-2: Matrix metalloproteinase-2; TGF-β: Transforming growth factor-β; ICAM-1: Intercellular adhesion molecule 1; mTOR: Mechanistic target of rapamycin; AMPK: AMP-activated protein kinase; Cdc25B: Cell division cycle 25B; TNFR Ⅰ: Tumor necrosis factor receptor type Ⅰ; ERK: Extracellular signal-regulated kinase; FAK: Focal adhesion kinase; PI3K/Akt: Phosphatidylinositol-4,5-bisphosphate 3-kinaseV-akt murine thymoma viral oncogene homolog; STAT3: Signal transducers and activators of transcription 3.

venom, is potent to induce apoptosis and autophagy by down-regulation of PI3K/AKT/mechanistic target of rapamycin (mTOR) pathway in HepG2/ADM hepatoma cells, and thus inhibit xenograft tumor growth[147]. Bufalin, a component from toad skin, has been demonstrated to induce AMP-activated protein kinase (AMPK) dependent autophagy accompanied by enhanced Beclin-1 expression and LC3-Ⅰ to LC3-Ⅱ conversion, and decreased p62 expression and mTOR signaling in HepG2 cells[148].

Allicin, a major phytochemical of crushed garlic, is effective to induce autophagy in HepG2 cells by decreasing the level of cytoplasmic p53, the PI3K/mTOR signaling pathway, and Bcl-2 and up-regulating the expression of AMPK/tuberous sclerosis protein 2 and Beclin-1[149]. Shikonin, a naphthoquinone from Zi-Cao (*Lithospermum erythrorhizon*), could induce autophagy and reactive oxygen species generation which further activates ERK[150] (Figure 1).

### *Induction of cell senescence*

Cell senescence is a state of stable, irreversible cell cycle arrest provoked by a variety of stimuli. Pro-senescence has been suggested for hepatocellular carcinoma treatment[151]. We have established a liver-Yin tonifying formula (LYTF) for liver-Yin-deficiency in patients with hepatocarcinoma. LYTF could activate caspases-8, -9 and -3 to induce apoptosis, and up-regulate p16 and p21 and down-regulate RB phosphorylation to induce cell senescence in Bel-7402 cells[152]. Ganoderiol F, a tetracyclic triterpene from *Ganoderma* amboinense, may activate ERK and up-regulate p16 to induce cell senescence in hepatoma HepG2 cells[153]. Nü-zhen-zi (*Ligustrum lucidum* Ait. Fruit), an herb for tonifying liver-Yin, is potent to up-regulate p21, activate caspases-8, -9 and -3 to induce apoptosis and down-regulate RB phosphorylation to induce cell senescence in hepatocarcinoma cells[154] (Figure 1).

### *Induction of anoikis*

Anoikis, an apoptotic process occurring when cells detach from the extracellular matrix, is associated with metastasis of hepatocarcinoma[155,156]. We have found modified Yi Guan Jian, an herbal formula for hepatocarcinoma, may activate caspase-3, -8 and -9, inhibit the expression and phosphorylation of p38 MAPK, and induce anoikis in human hepatocarcinoma Bel-7402 cells[156]. Hu-Zhang (*Polygonum cuspidatum*), an herb for draining dampness and dissipating stasis, could activate caspase-3 and -9 and induce anoikis in

human hepatocarcinoma Bel-7402 cells accompanied by ROS generation and focal adhesion kinase down-regulation[157]. Arecoline, an alkaloid from Bing-Lang (*Areca catechu* L.), may induce anoikis in HA22T/VGH cells involving inhibition of STAT3 and increased RhoA/Rock (Ras homolog family member A/Rho-associated, coiled-coil containing protein kinase) activation[158] (Figure 1).

### *Inhibition of metastasis*

In addition to induction of anoikis, Chinese herbs also could inhibit metastasis potential in hepatocarcinoma cells, such as adhesion, migration, invasion and metastasis. Among heat-clearing herbs, *Coptidis* Rhizoma (Huang-Lian) may reduce F-actin polymerization and damage to cytoskeleton network to inhibit hepatocarcinoma cell migration[159]. Baicalein, a compound from *Scutellaria baicalensis* Georgi (Huang-Qin), could inhibit migration and invasion in human hepatocellular carcinoma SMMC-7721 cells accompanied by down-regulation of ezrin, VEGF, and MMP-9[160]. Matrine, a component of *Sophora flavescens* Ait. (Ku-Shen), was reported to be effective to inhibit MMP-9 and nuclear factor κB (NF-κB) to inhibit invasion of liver cancer cells[161]. Shikonin, an ingredient of *Lithospermum erythrorhizon* (Zi-Cao), is potent to inhibit the migratory ability of hepatocarcinoma cells through downregulation of vimentin, MMP-2 and MMP-9[162].

Another frequently studied herbal type is dissolving stasis herbs. Gekko sulfated polysaccharide-protein complex has been demonstrated to be effective in inhibiting hepatocellular carcinoma cell migration through calcium-mediated regulation of the actin cytoskeleton reorganization[163]. Ardipusilloside, a compound from *Ardisia japonica* (Thunb) Blume (Zi-Jin-Niu), has the potential to inhibit liver cancer survival, invasion and metastasis by down-regulation of MMP-9 and MMP-2 and activating Ras-related C3 botulinum toxin substrate 1 (Rac 1) to enhance E-cadherin activity[164]. Plumbagin, a constituent of Plumbago zeylanica L. (Bai-Hua-Dan), could suppress the proliferation and invasiveness in SK-hep-1 cells by up-regulation of p21 and down-regulation of MMP-2 and MMP-9[165]. Tanshinone Ⅱ-A, a component from Dan-Shen (*Salvia miltiorrhiza*), could effectively inhibit invasion and metastasis of hepatocaxrcinoma cells partly by inhibiting MMP-2 and MMP-9 activities and blocking NF-κB activation[166].

Some herbal formulas have been confirmed to be effective against hepatocarcinoma metastasis. In addition to inhibiting liver cancer growth, Songyou Yin also could down-regulate activated hepatic stellate cells secreted IL-6, TGF-β1 and VEGF to reduce MMP-2 expression and reverse epithelial-mesenchymal transition, and thus inhibit invasion and metastasis in hepatocarcinoma[167]. Biejiajian pill, a classical herbal formula, could effectively inhibit HepG2 cell proliferation, adhesion and invasion[168]. Compound *Astragalus* and *Salvia miltiorrhiza* extract, an herbal component formula made up of astragalosides, astragalus polysaccharide and salvianolic acids, may inhibit TGF-β1 mediated invasion in HepG2 hepatoma cells through modulating TGF-beta/Smad signaling[169]. Shehuang Xiaoliu Fang, an herbal formula for detoxifying and dissipating masses, is potent to inhibit invasive and adhesive ability of SMMC-7721 cells accompanied by increased nm23-h1 expression and reduced ICAM-1 expression[170] (Figure 1).

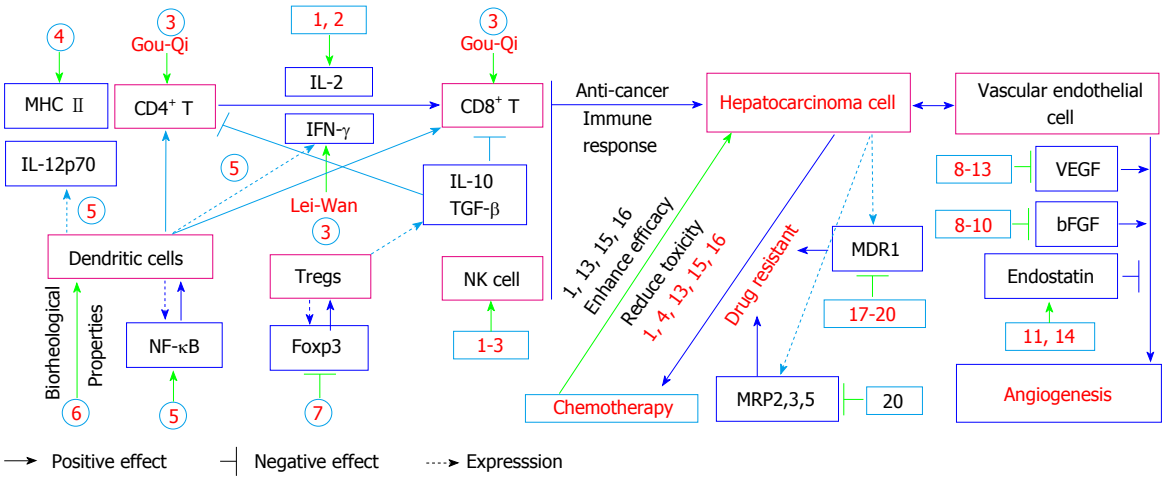
### *Regulation of immune function*

$CD4^+$ and $CD8^+$ T lymphocytes are the major cell populations for cellular immunity against cancer. *Lycium barbarum* (Gou-Qi) polysaccharides could increase $CD4^+$ and $CD8^+$ T cells in tumor-infiltrating lymphocytes in H22 hepatoma[171]. Dong-Chong-Xia-Cao (*Cordyceps sinensis*), a commonly used herb for tonifying kidney, may increase the expression of major histocompatibility complex class Ⅱ antigens on human hepatoma HA22T/VGH cells[172]. Ban-Zi-Lian (*Scutellaria barbata*), an effective anti-cancer herb for hepatocarcinoma, could increase the thymus and spleen index, lymphocytes proliferation activities, natural killer (NK) cell activities and IL-2 production in splenocytes[173]. The proteins extracted from mycelia of Lei-Wan (*Omphalia lapidesces*) are effective in increasing spleen mass and interferon-γ (IFN-γ) production in H22 hepatocarcinoma bearing mice[174].

Xiaochaihu decoction, a classical herbal formula for liver disease, is effective in inhibiting tumor growth and increasing T lymphocyte proliferation, NK cells activities and IL-2 level in H22 bearing mice[175]. Mylabris Mixture, a modern anti-hepatoma herbal formula, may increase $CD4^+$, $CD8^+$ lymphocytes and NK cells, and IFN-γ and IL-4 production in H22 cancer-bearing mice[176]. Fuzheng Yiliu Granule, an effective pro-apoptosis contemporary herbal formula, may increase $CD4^+$ lymphocytes and related cytokines IL-2 and TNF-α, and NK cells in H22 tumor-bearing mice[177]. Fuganchun 6, a modern herbal formula, can inhibit tumor growth and enhance proliferation activity of lymphocytes, NK cell activities and IL-2 production in H22 hepatoma bearing mice[178].

Dendritic cells (DCs) are important antigen present cells for anti-cancer immunity. *Lycium barbarum* (Gou-Qi) polysaccharides could promote DCs to stimulate allogeneic lymphocyte proliferation, produce IL-12p70 and IFN-γ and may relate to NF-κB expression[179]. Chen *et al*[180] found that human hepatocellular carcinoma SMMC-7721 cells may impair the biorheological properties of DCs, such as cell deformability, migration, and electrophoresis mobility, and changed organizations of cytoskeletal proteins. Gekko (Tian-Long) sulfated polysaccharide-protein complex could partially restore the defective biorheological characteristics of DCs mediated by SMMC-7721 cells[180].

$CD4^+$ $CD25^+$ regulatory T cells (Tregs) are originated from $CD4^+$ Th0 cells upon the stimulation of TGF-β and Foxp3 expression. Tregs may produce IL-10 and function as a negative immune regulator. *Radix Glycyrrhizae* (Gan-Cao) polysaccharides were demonstrated to down-regulate Tregs, related cytokines IL-10 and TGF-β,




1: Ban-Zi-Lian (*Sculellaria barbata*); 2: Xiaochaihu decoction; 3: Mylabris Mixture; 4: Dong-Chong-Xia-Cao (*Cordyceps sinensis*); 5: Gou-Qi (*Lycium barbarum*) polysaccharide; 6: Gekko (Tian-Long) sulfated polysaccharide-protein complex; 7: *Radix Glycyrrhizae* (Gan-Cao) polysaccharide; 8: Tian-Long (Gekko Chinenis); 9: Gekko-sulfated glycopeptide; 10: Fu-Zheng-Kang-Ai-Tang; 11: Erbie San; 12: Huaier; 13: QHF; 14: Delisheng; 15: Chaiqiyigan granula; 16: Shengmai Injection; 17: *Astragalus* polysaccharides; 18: Astragaloside Ⅱ; 19: Green tea catechins; 20: Tetramethylpyrazine.

**Figure 2 Therapeutic targets of Chinese herb(s) in regulating immune function, inhibiting angiogenesis and combinational treatment with chemotherapy in hepatocarcinoma.** TGF-β: Transforming growth factor-β; IFN-γ: Interferon γ; IL-6: Interleukin 6; VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor; MRP2: Multi-drug resistance protein 2; NF-κB: Nuclear factor-κB; NK: Natural killer.

and Foxp3 expression, and increased IL-2 and IL-12p70 levels in serum in H22 hepatocarcinoma bearing mice[181] (Figure 2).

### *Inhibition of angiogenesis*

Angiogenesis, the process of new blood vessel generation from existing vessels, plays a crucial role in tumor growth and metastasis, and has been suggested as a potential target for hepatocarcinoma prevention and treatment[182,183].

Tian-Long (Gekko Chinenis) has been showed to be effective to inhibit tumor growth, induce apoptosis, and inhibit angiogenesis accompanied by down-regulation of VEGF and basic fibroblast growth factor (bFGF) in H22 hepatocarcinoma[184]. Gekko-sulfated glycopeptide may inhibit angiogenesis by decreasing bFGF secretion, and binding to heparin/heparan sulfate in liver cancer[185]. Bai-Ji (*Bletilla* colloid) could inhibit endothelial cell growth and angiogenesis after TACE[186]. Huaier may inhibit VEGF expression and angiogenesis, induce apoptosis and inhibit tumor growth in hepatocarcinoma[187].

Fu-Zheng-Kang-Ai-Tang, an herbal formula for strengthening body defense and anti-cancer, could inhibit VEGF and bFGF expression, angiogenesis and tumor growth in hepatocarcinoma[188]. Delisheng, an herbal formula for tonifying Qi and resolving masses, may inhibit cell growth and increase endostatin expression in hepatocellular carcinoma HepG2 cells[189]. Qingrejiedu, huoxuehuayu and fuzhengguben (QHF) formula, an herbal component formula, is potent to inhibit VEGF, EGFR and MMP-2, angiogenesis and tumor growth[190]. Erbie San, a patent herbal drug for resolving masses, was demonstrated to be effective to down-regulate VEGF/endostatin and inhibit tumor growth in Walker-256 liver cancer[191] (Figure 2).

### *Combination with chemotherapy*

Chemotherapy is not conventionally used in hepatocarcinoma, but chemotherapeutic drugs based TACE is a major treatment for liver cancer. Ban-Zi-Lian (*Sculellaria barbata*) can significantly enhance 5-fluorouracil (5-Fu) to inhibit tumor growth and prolong survival time, improve immune function, and reduce the toxic effects of 5-Fu in the H22 tumor-bearing mice[192]. Ling-Zhi (*Ganoderma lucidum*) is able to inhibit hepatocarcinoma cell proliferation and protect hepatocytes from chemotherapy induced damage[193]. Dong-Chong-Xia-Cao (*Cordyceps sinensis*) may protect H22 hepatocarcinoma bearing mice from chemotherapy induced immunosuppression[194].

Chaiqiyigan granula is potent to enhance inhibitory effects of taxol on hepatocarcinoma growth accompanied by up-regulation of Bax and down-regulation of p53 and VEGF[195]. QHF, an herbal component formula, could reduce cisplatin (DDP)-induced leucopenia, spleen and thymus atrophy, enhance tumor growth inhibition and prolong survival time in H22 bearing mice[196]. Shengmai Injection, a patent herbal drug, is effective to enhance anti-tumor efficacy and reduce toxicity of 5-Fu in H22 hepatocarcinoma[197] (Figure 2).

### *Reversal of drug resistance*

Drug resistance contributes to chemotherapy refractoriness in hepatocarcinoma[198]. To seek effective herbs or herbal components to reverse drug resistance has become one of research focuses in liver cancer study. *Astragalus* polysaccharides have been reported to be potent to enhance anti-tumor effects of adriamycin in H22 hepatocarcinoma by up-regulating IL-1α, IL-2, IL-6, and TNF-α, down-regulating IL-10 and multidrug resistance protein 1 (MDR1)[199]. Astragaloside Ⅱ, another component from Huang-Qi (*Astragalus membranaceus*),

is effective to increase 5-Fu cytotoxicity toward 5-Fu-resistant Bel-7402/Fu cells accompanied by down-regulation of P-gp (P-glycoprotein), phosphorylation of ERK1/2, p38 and JNK[200].

Green tea catechins have been reported to effectively inhibit MDR1 expression, increase intracellular doxorubicin (DOX) accumulation and enhance DOX-induced cell killing activities against BEL-7404/DOX cells[201]. Tetramethylpyrazine, a bioactive constituent isolated from the root of *Ligusticum chuanxiong* Hort, could down-regulate MDR1, multidrug resistance protein 2 (MRP2), MRP3 and MRP5 in adriamycin resistant HepG2 cells[202]. Pseudolaric acid B, polyphyllin D and *Eclipta alba* (Mo-Han-Lian) have been demonstrated to increase drug sensitivity in drug resistant HepG2 hepatoma cells[203-205] (Figure 2).

## CONCLUSION

Hepatocarcinoma patients may present Qi-stagnation, damp-heat, blood stasis, Qi-deficiency, Yin-deficiency and other TCM syndromes. Modern treatments such as surgery, TACE and HIFU and other treatments would influence syndrome manifestation in liver cancer patients. Modern technologies have been exploited to elucidate the relation between syndromes and biomedical sciences. Further studies are needed to use those information for herb medication and formulation to improve clinical efficacy of TCM treatment for hepatocarcinoma.

Since TCM pathological factors and syndromes in hepatocarcinoma patients, such as Qi-stagnation, damp-heat, blood stasis, Qi and Yin-deficiency, are also seen in other disease, hepatocarcinoma needs to be recognized and treated as a malignant disease. In addition to syndrome differentiation based treatment, anti-cancer therapy or anti-cancer herb application should be reinforced in the treatment of hepatocarcinoma. Moreover, since most of liver cancer patients receive comprehensive therapy, TCM treatment in those patients should consider the received therapy and accordingly adjust herb medication.

Classic TCM theory and principles have been adopted for hepatocarcinoma prevention study. Chinese herbs with traditional efficacy of tonifying Qi, blood and Yin, soothing liver-Qi stagnation, clearing heat and detoxifying and dissolving stasis, have been demonstrated to be potent to prevent hepatocarcinogenesis. Some classical and modern herbal formulas have been demonstrated to have preventive effects against hepatocarcinogenesis. Further clinical studies are needed to use these achievements to protect high-risk individuals from hepatocarcinogenesis.

Clinical practices have confirmed that TCM is effective to alleviate clinical symptoms, improve QOL and immune function, prevent recurrence and metastasis, delay tumor progression, and prolong survival time in hepatocarcinoma patients. Experimental studies have demonstrated that Chinese herbs and/or herbal formula are potent to induce apoptosis, autophagy, anoikis and cell senescence, arrest cell cycle, regulate immune function, inhibit metastasis and angiogenesis, reverse drug resistance and enhance effects of chemotherapy in hepatocarcinoma.

How to use experimental results to improve clinical efficacy has become an important research subject. For commonly used traditional herbs with anti-cancer effects, the property of Chinese herbs should be firstly considered and medicated by the guidance of TCM principles. For traditionally uncommonly used anti-cancer herbs, such as Mu-Tou-Hui (*Patrinia scabra* Bunge) and She-Mei [*Duchesnea indica* (Andr.) Focke], the property of Chinese herbs and anti-cancer characters of those herbs need to be further studied. In addition, there is a great need to explore the compatibility or combinational application rule of anti-cancer herbs to further improve clinical efficacy of TCM treatment for hepatocarcinoma.

## 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 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

3 **Chen JG**, Chen WQ, Zhang SW, Zheng RS, Zhu J, Zhang YH. [Incidence and mortality of liver cancer in China: an analysis on data from the National Registration System between 2003 and 2007]. *Zhonghua Liuxingbingxue ZaZhi* 2012; **33**: 547-553 [PMID: 22883257]

4 **Fan JH**, Wang JB, Jiang Y, Xiang W, Liang H, Wei WQ, Qiao YL, Boffetta P. Attributable causes of liver cancer mortality and incidence in china. *Asian Pac J Cancer Prev* 2013; **14**: 7251-7256 [PMID: 24460283 DOI: 10.7314/APJCP.2013.14.12.7251]

5 **de Kretser TA**, Lee GT, Thorne HJ, Jose DG. Monoclonal antibody CI-panHu defines a pan-human cell-surface antigen unique to higher primates. *Immunology* 1986; **57**: 579-585 [PMID: 2420708 DOI: 10.1016/j.jnci.2013.08.002]

6 **Tang ZY**. Combination of traditional Chinese medicine and western medicine in the treatment of liver cancer. *Linchuang Gandanbing Zazhi* 2011; **27**: 449-450

7 **Wu MC**. [Traditional Chinese medicine in prevention and treatment of liver cancer: function, status and existed problems]. *Zhongxiyi Jiehe Xuebao* 2003; **1**: 163-164 [PMID: 15339547 DOI: 10.3736/jcim20030302]

8 **Li M**, Qiao C, Qin L, Zhang J, Ling C. Application of traditional Chinese medicine injection in treatment of primary liver cancer: a review. *J Tradit Chin Med* 2012; **32**: 299-307 [PMID: 23297547 DOI: 10.1016/S0254-6272(13)60029-1]

9 **Wu P**, Dugoua JJ, Eyawo O, Mills EJ. Traditional Chinese Medicines in the treatment of hepatocellular cancers: a systematic review and meta-analysis. *J Exp Clin Cancer Res* 2009; **28**: 112 [PMID: 19674474 DOI: 10.1186/1756-9966-28-112]

10 **Hu B**, Du Q, Shen KP, Xu L. Principles and scientific basis of Traditional Chinese Medicine in cancer treatment. *J Bioanal Biomed* 2012; **S6**: 005 [DOI: 10.4172/1948-593X.S6-005]

11 **Du Q**, Hu B, Shen KP, An HM. Progress in TCM pathogenesis and treatment of liver cancer. *Shijie Zhongxiyi Jiehe Zazhi* 2010; **5**: 814-817

12 **Hu B**, An HM, Shen KP. Syndrome differential anti-cancer and scientific anti-cancer. *Zhongguo Minzu Minjian Yiyao* 2009; **18**: 33-34

13 **Wang Y**, Ji G, Wang YQ, Zhang W, Xing LJ, Dong GH, Wang YN, Lin SH. Effects of traditional Chinese medicine on TGF-βIIR expression in DMN induced hepatic precancerous lesion in rats. *Zhejiang Zhongxiyi Jiehe Zazhi* 2001; **11**: 23-24

14 **Tatsuta M**, Iishi H, Baba M, Nakaizumi A, Uehara H. Inhibition by xiao-chai-hu-tang (TJ-9) of development of hepatic foci induced by N-nitrosomorpholine in Sprague-Dawley rats. *Jpn J Cancer Res* 1991; **82**: 987-992 [PMID: 1682293 DOI: 10.1111/j.1349-7006.1991.tb01932.x]

15 **Tatsuta M**, Iishi H, Baba M, Nakaizumi A, Uehara H. Inhibition by shi-quan-da-bu-tang (TJ-48) of experimental hepatocarcinogenesis induced by N-nitrosomorpholine in Sprague-Dawley rats. *Eur J Cancer* 1994; **30A**: 74-78 [PMID: 8142169 DOI: 10.1016/S0959-8049(05)80022-X]

16 **Zou KC**, Song WM, Xu FX, Lin SM, Guo GY, Wang HW, Liu L, Zhou JP, Zhang HF, Sun YQ. Effect of Nourishing-Yin Decoction and Removing-Toxic-Heat Decoction on histological observation in rats Liver during canceration. *Zhongguo Zhongxiyi Jiehe Zazhi* 1995; **15**: 238-240, 396

17 **Guan Y**, Zhou Z, Zhang C, Ruan Y, Wu Z. Effect of A-L tonic capsule on DNA content in rat experimental hepatocarcinogenesis. *J Huazhong Univ Sci Technolog Med Sci* 2002; **22**: 12-13, 16 [PMID: 12658771 DOI: 10.1007/BF02904776]

18 **Zou J**, Chen JJ. Preventive effect of prescription of Nourishing Shen on rat hepatocarcinoma induced by Diethylnitrosamine. *Zhongxiyi Jiehe Ganbing Zazhi* 2003; **13**: 278-280

19 **Liu C**, Liu P, Wang XS, Xu LM, Hu YY. Experimental study of Fu-zheng-hua-yu formula for liver cancer prevention. *Zhongguo Zhongxiyi Jiehe Piwei Zazhi* 1995; **33**: 163-166

20 **Li X**, Shi ZM, Feng P, Wen ZY, Wang XJ. Effect of Qi-protecting powder (Huqi San) on expression of c-jun, c-fos and c-myc in diethylnitrosamine-mediated hepatocarcinogenesis. *World J Gastroenterol* 2007; **13**: 4192-4198 [PMID: 17696247]

21 **Wen Z**, Shi Z, Feng P, Xue X, Dong K, Wang X. Modulation of energy metabolic enzyme expression in N-nitrosodiethylamine-mediated hepatocarcinogenesis by Chinese herbs, Huqi San. *Biofactors* 2008; **34**: 303-312 [PMID: 19850985 DOI: 10.3233/BIO-2009-1084]

22 **Qian Y**, Ling CQ. Preventive effect of Ganfujian granule on experimental hepatocarcinoma in rats. *World J Gastroenterol* 2004; **10**: 755-757 [PMID: 14991955]

23 **Qiu JX**. [The effect of "SRRS" recipe on the formation of liver cancer in the rat]. *Zhongxiyi Jiehe Zazhi* 1988; **8**: 734-735, 710 [PMID: 3248335]

24 **Zhang B**, Li Q, Yin PH, Zhao CG, Li XQ, Gao H, Sun J, Fan ZZ. Involvement of miR-199a downregulation in the preventive effects of Jianpi Jiedu Recipe against experimental hepatocarcinoma in rats. *Shijie Huaren Xiaohua Zazhi* 2010; **18**: 125-131

25 **Zhang B**, Li Q, Yin PH, Zhao CG, Gao H, Sun J, Fan ZZ. Study on AKT/ PI3K signaling pathway and prevenion effect of Jianpi Jiedu Recipe in rats with experimental hepatocarcinogenesis. *Zhongguo Zhongxiyi Jiehe Xiaohua Zazhi* 2010; **18**: 4-9

26 **Gao HS**, Yao SK, Yin F. The effect of Ganzheng solution on the ICAM-1 expression in liver tissues of rat in the diethylnitrosamine induced hepatocarcinogenesis. *Hebei Zhongyi* 2007; **29**: 848-850

27 **Nie G**, Long AH, Fu QL, Li HM, Ming AP, Xiao L, Yan XS, Wang BX. Zao-Lian mixture prevents Diethylnitrosamine induced hepatocirrhosis and hepatocaecinogenesis in rats. *Zhongxiyi Jiehe Ganbing Zazhi* 1998; **8**: 107-109

28 **Lee CY**, Hsu YC, Wang JY, Chen CC, Chiu JH. Chemopreventive effect of selenium and Chinese medicinal herbs on N-nitrosobis(2-oxopropyl)amine-induced hepatocellular carcinoma in Syrian hamsters. *Liver Int* 2008; **28**: 841-855 [PMID: 18346132 DOI: 10.1111/j.1478-3231.2008.01698.x]

29 **Fang ZQ**, Guan DY, Liang SH, Qin HY, Li HY. Comparative study on functions of different therapies on liver cancer in mice. *Shanghai Zhongyiyao Daxue Xuebao* 2002; **16**: 42-46

30 **Guan DY**, Lu HX, Fang ZQ. Study on the regulation of different therapies on some signal moleculars transcriptions in the pathway of Ras/MAPK. *Zhongguo Zhongyi Jichu Yixue Zazhi* 2002; **8**: 36-38

31 **Guan D**, Fang Z, Lu H, Li H. Preliminary investigation on regulating effects of different TCM treatments on transcription of the correlated genes of liver cancer in rats. *J Tradit Chin Med* 2003; **23**: 62-66 [PMID: 12747207]

32 **Cui R**, Wang B, Zhang F, Chen G, Yin S. Suppressive effect of herbal compound 861 on chemical hepatocarcinogenesis in rats. *Chin Med J* (Engl) 2003; **116**: 378-382 [PMID: 12781041]

33 **Zhu MJ**, Zhang H, Gao SS, Zhang XS. Effects of new anti-tumor formula on protecting against synergistic hepatocarcinogenesis of hepatitis B virus expression and aflatoxin B1 and free radicals metabolism in HBV large envelope transgenic mice. *Liaoning Zhongyi Xueyuan Xuebao* 2003; **5**: 263-264

34 **Li X**, Wu XG. [Effects of ginseng on hepatocellular carcinoma in rats induced by diethylnitrosamine--a further study]. *J Tongji Med Univ* 1991; **11**: 73-80 [PMID: 1816418]

35 **Yan RQ**, Chen ZY, Qin GZ, Qin LL. Effects of twelve herbs on Aflatoxin B1 induced hepatocarcinogensis in rats. *Guangxi Yixueyuan Xuebao* 1986; **3**: 21-23

36 **Ruan CC**, Liang Y, Liu ZH. Inhibition of 12 Chinese Traditional Medicinal herbs on mutagenic effects induced by Aflatoxin B1. *Ai Zheng* 1987; **8**: 29-31

37 **Shen M**, Yin Z. [Effect of Boschniakia rossica extract on antioxidative activities in rat hepatic preneoplasia induced by diethylnitrosamine]. *Zhongguo Zhongyao Zazhi* 1999; **24**: 746-78, 765 [PMID: 12205985]

38 **Yin ZZ**, Jin HL, Li TZ, Lee JJ, Kim YH, Lee CH, Lee KH, Hyun BH. [Inhibitory effect of methanol extract of Boschniakia rossica Fedtsch. et Flerov on rat hepatic preneoplastic lesions induced by diethylnitrosamine]. *Zhongguo Zhongyao Zazhi* 1998; **23**: 424-426, 448- inside back cover [PMID: 11601354]

39 **Dai ZJ**, Wang XJ, Ji ZZ, Liu XX, Kang HF, Diao Y, Ma XB, Liu L, Ren HT. [Inhibitory effects of Scutellaria barbate extracts on diethylnitrosamine-induced hepatocarcinoma in rats]. *Zhong Yao Cai* 2009; **32**: 568-571 [PMID: 19645244]

40 **Lin YL**, Hsu JD, Chou FP, Lee MJ, Shiow SJ, Wang CJ. Suppressive effect of penta-acetyl geniposide on the development of gamma-glutamyl transpeptidase foci-induced by aflatoxin B(1) in rats. *Chem Biol Interact* 2000; **128**: 115-126 [PMID: 11024451 DOI: 10.1016/S0009-2797(00)00193-9]

41 **Zhao X**, Zhang JJ, Wang X, Bu XY, Lou YQ, Zhang GL. Effect of berberine on hepatocyte proliferation, inducible nitric oxide synthase expression, cytochrome P450 2E1 and 1A2 activities in diethylnitrosamine- and phenobarbital-treated rats. *Biomed Pharmacother* 2008; **62**: 567-572 [PMID: 17418998 DOI: 10.1016/j.biopha.2007.02.009]

42 **Luo RX**, Lin RT, Lu YB, Cai L, Yi YN. Inhibition of Artemisa capillaris Thunb on mutagenic effects induced by Aflatoxin B1. *Fujian Yixueyuan Xuebao* 1990; **24**: 352-354

43 **Liu J**, Yang CF, Wasser S, Shen HM, Tan CE, Ong CN. Protection of salvia miltiorrhiza against aflatoxin-B1-induced hepatocarcinogenesis in Fischer 344 rats dual mechanisms involved. *Life Sci* 2001; **69**: 309-326 [PMID: 11441922 DOI: 10.1016/S0024-3205(01)01116-X]

44 **Kim J**, Ha HL, Moon HB, Lee YW, Cho CK, Yoo HS, Yu DY. Chemopreventive effect of Curcuma longa Linn on liver pathology in HBx transgenic mice. *Integr Cancer Ther* 2011; **10**: 168-177 [PMID: 21190953 DOI: 10.1177/1534735410380613]

45 **Deng W**, Xiang Q, Li B. Effects of prescription of Sanqi on rats with hepatocarcinoma induced by diethylnireosamine. *Guangxi Yixue* 2013; **35**: 1442-1446

46 **Ohtaki Y**, Nomura M, Hida T, Miyamoto K, Kanitani M, Aizawa T, Aburada M. Inhibition by gomisin A, a lignan compound, of hepatocarcinogenesis by 3'-methyl-4-dimethylaminoazobenzene in rats. *Biol Pharm Bull* 1994; **17**: 808-814 [PMID: 7951143 DOI: 10.1248/bpb.17.808]

47 **Zhang YH**, WuJP. Effects of Jian-Pi-Huo-Xue Formula on in patients with hepatitis, hepatic cirrhosis and low level of AFP. *Zhongxiyi Jiehe Ganbing Zazhi* 2000; **10**: 13-14

48 **Zhang ZQ**, Liu QF, Zhong SC, Huang TR, Wu YD, Wang TC, Huang NF, Liang RX. Epidemiological studies on Salvia miltiorrhiza for liver cancer prevention. *Guangxi Yixue* 1995; **17**: 179-181

49 **Oka H**, Yamamoto S, Kuroki T, Harihara S, Marumo T, Kim

SR, Monna T, Kobayashi K, Tango T. Prospective study of chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). *Cancer* 1995; **76**: 743-749 [PMID: 8625175]
50 **Zhao G**, Lang QB, Ling CQ. Characteristics of 294 cases of primary liver cancer. *Shijie Zhongxiyi Jiehe Zazhi* 2008; **3**: 275-277
51 **Li YJ**, Fang ZQ, Tang CL, Ma J, Guan DY, Chen DS. Clinical epidemiological investigation and research of syndrome distribution law in Chinese medicine of 2060 cases of primary liver carcinoma. *Zhongguo Yiyao Xuebao* 2003; **18**: 144-146
52 **Fang ZQ**, Li YJ, Tang CL, Ma J, Guan DY, Chen DS. Analysis on characteristics of syndromes in 2060 cases of primary hepatic cancer. *Zhongyi Zazhi* 2004; **45**: 53-54
53 **Wang CJ**, Hu Y, Fu TT, Huang XH, Dai XB, Shi L, Li YC, Xioang J. Syndrome distribution of primary liver cancer in Guangzhou area. *Zhonghua Zhongyiyao Xuekan* 2010; **28**: 2314-2318
54 **Yang XB**, Long SQ, Wu WY, Deng H, Pan ZQ, He WF, Zhou YS, Liao GY, Ouyang YS, Li QP, Huang L, Hu XJ, Xiao SJ, Cai JZ. [The distribution of Chinese medicine syndrome types in primary liver cancer and their differences of the survival time: a clinical study]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2013; **33**: 911-914 [PMID: 24063211]
55 **Wan X**, Zhang YH. Relation betwwen syndrome and quality of life in patients with liver cancer. *Guiyang Zhongyi Xueyuan Xuebao* 2008; **30**: 27-28
56 **Zhang YH**, Qin X, Xu J, Sun Z, Lang QB, Yue XQ, Zhai XF. A preliminary study on TCM compound syndrome characteristics of 58 patients with liver cancer before and after operation. *Zhongxiyi Jiehe Ganbing Zazhi* 2012; **22**: 75-80
57 **Ye Y**, Qin LP, Yue XQ, Zhai XF, Xu J, Xiao KY, Huang F, Li B. Tongue changes in 103 perioperative patients with primary liver cancer. *Zhongyi Zazhi* 2014; **55**: 48-52
58 **Li YJ**, Fang ZQ, Tang ZL, Ma J, Guan DY. Comparative study of TCM syndromes in liver cancer patients after TACE and surgery. *Zhongyiyao Xuekan* 2003; **21**: 504-504
59 **Yang SF**, Lin YL. TCM Syndrome of Primary Liver Carcinoma before Interventional Treatment by Cluster Analysis. *Zhongyiyao Xinxi Zazhi* 2012; **19**: 11-12
60 **Zeng PH**, Liu WS, Pan MQ, Xu K, Wu WY, Chang G, Jiang YL, Cai M. Study on development of syndrome in Chinese medicine during transcatheter arterial intervention in primary hepatic carcinoma. *Hunan Zhongyiyao Daxue Xuebao* 2008; **28**: 54-56
61 **Meng J**, Qin LP, Liu L, Lang QB, Li B. Impact of TACE on Syndromes in PHC. *Zhejiang Zhongyiyao Daxue Xuebao* 2012; **36**: 1194-1198
62 **Zhang YH**, Qin X, Xu J. [Analysis of Chinese medical syndrome features of patients with primary liver cancer before and after transcatheter arterial chemoembolization]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2012; **32**: 1171-1174 [PMID: 23185752]
63 **He D**. Study on change of TCM syndrome in patients with primary liver cancer before and after high intensity focus ultrasound treatment. *Jianyan Yixue Yu Linchuang* 2012; **9**: 2676-2677
64 **Chen QW**, Huang XQ, Yang GJ, Dai WX, Lang QB, Yan SK, Zhou J, Zhang WD, Ling CQ. Preliminary study of serum metabonomics on Yang-deficiency Syndrome of patients with primary liver cancer. *Zhonghua Zhongyiyao Xuekan* 2012; **30**: 526-529
65 **Yang SF**, Qiu SP, Liu QH. [Observation of serum protein fingerprinting in primary liver cancer patients of different Chinese medical syndromes before and after interventional treatment]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2013; **33**: 1352-1355 [PMID: 24432678]
66 **Huang ZG**, Zhang XQ, Ye YB, Chen HQ, Chen Q, Yang AL. Initial study on application of protein fingerprinting in Zheng differentiation-classification of primary liver cancer. *Xiandai Zhongxiyi Jiehe Zazhi* 2012; **21**: 3660-3664
67 **Weng L**, Du J, He WT, Ling CQ. [Characteristic genomics of peripheral blood mononuclear cells of hepatocellular carcinoma patients with liver-kidney yin deficiency syndrome]. *Zhongxiyi Jiehe Xuebao* 2012; **10**: 406-415 [PMID: 22500714 DOI: 10.3736/jcim20120409]
68 **Su XK**, Wu YS, Li YH, Yang XW, Lu YY, Chi JA. Relationship between TCM Syndrome Differentiation of liver cancer and adhesion degree of cancer Cell. *Zhongguo Zhongxiyi Jiehe Waike Zazhi* 2009; **15**: 10-12
69 **Su XK**, Cheng YX, Lin SH. Correlation of Syndrome Differentiation of liver cancer with matrix degradation. *Xin Zhongyi* 2008; **40**: 37-39
70 **Pan ZQ**, Fang ZQ, Lu WL. Comparative research on syndrome manifestation features in mice with H22 liver cancer and Lewis lung carcinoma. *Beijing Zhongyiyao Daxue Xuebao* 2008; **31**: 184-188
71 **Pan ZQ**, Fang ZQ, Lu WL, Liang C, Wu ZH, Liu XM, Hou L, Zhang H, Zhuo SY, Liao MJ, Gao BF. [Differentially expressed genes in adrenal gland of H22 liver cancer mice with different syndromes and in different stages]. *Zhongxiyi Jiehe Xuebao* 2008; **6**: 843-851 [PMID: 18664355 DOI: 10.3736/jcim20080814]
72 **Chen Z**, Chen HY, Lang QB, Li B, Zhai XF, Guo YY, Yue XQ, Ling CQ. Preventive effects of jiedu granules combined with cinobufacini injection versus transcatheter arterial chemoembolization in post-surgical patients with hepatocellular carcinoma: a case-control trial. *Chin J Integr Med* 2012; **18**: 339-344 [PMID: 22549390]
73 **Sun Z**, Liang ST, Zhai XF, Lang QB, Zhou QH, Yue XQ, He J, Xu J, Zhu Y, Ling CQ. A traditional Chinese herbal medicine compound preparation versus interventional therapy after resection of small hepatocellular carcinoma: 22-year follow-up. *J Tradit Chin Med* 2012; **32**: 156-163 [PMID: 22876437 DOI: 10.1016/S0254-6272(13)60005-9]
74 **Chen LW**, Lin J, Chen W, Zhang W. [Effect of Chinese herbal medicine on patients with primary hepatic carcinoma in III stage during perioperational period: a report of 42 cases]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2005; **25**: 832-834 [PMID: 16250104]
75 **Yu Y**, Lang Q, Chen Z, Li B, Yu C, Zhu D, Zhai X, Ling C. The efficacy for unresectable hepatocellular carcinoma may be improved by transcatheter arterial chemoembolization in combination with a traditional Chinese herbal medicine formula: a retrospective study. *Cancer* 2009; **115**: 5132-5138 [PMID: 19672999 DOI: 10.1002/cncr.24567]
76 **Lin LZ**, Zhou DH, Liu K, Wang FJ, Lan SQ, Ye XW. [Analysis on the prognostic factors in patients with large hepatocarcinoma treated by shentao ruangan pill and hydroxycamptothecine]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2005; **25**: 8-11 [PMID: 15719740]
77 **Shao ZX**, Cheng ZG, Yin X. [Clinical study on treatment of middle-advanced stage liver cancer by combined treatment of hepatic artery chemoembolization with gan'ai no. I and no. II]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2001; **21**: 168-170 [PMID: 12577328]
78 **Wu GL**, Zhang L, Li TY, Chen J, Yu GY, Li JP. Short-term effect of combined therapy with Jinlong Capsule and transcatheter arterial chemoembolization on patients with primary hepatic carcinoma and its influence on serum osteopontin expression. *Chin J Integr Med* 2010; **16**: 109-113 [PMID: 20473734 DOI: 10.1007/s11655-010-0109-9]
79 **Hou EC**, Lu YX. [Primary hepatocarcinoma treated by traditional Chinese medicine combined with transcatheter arterial chemoembolization]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2009; **29**: 225-227 [PMID: 19548439]
80 **Gao WB**, Han JD, Du M. [Observation on efficiency of shelian capsule as adjuvant of embolismic chemotherapy on primary hepatic carcinoma]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2005; **25**: 980-982 [PMID: 16355611]
81 **Lao Y**. [Clinical study on effect of matrine injection to protect the liver function for patients with primary hepatic carcinoma after trans-artery chemo-embolization (TAE)]. *Zhong Yao Cai* 2005; **28**: 637-638 [PMID: 16252735]
82 **Zhang B**, Huang G, Zhang Y, Chen X, Hu P, Xu B. [Clinical observation on prevention of "jia wei si jun zi tang" from damage of hepatic reserving function after intervention of liver cancer]. *Zhong Yao Cai* 2004; **27**: 387-389 [PMID: 15376398]
83 **Cheng JH**, Chang G, Wu WY. [A controlled clinical study between hepatic arterial infusion with embolized curcuma aromatic

oil and chemical drugs in treating primary liver cancer]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2001; **21**: 165-167 [PMID: 12577327]
84 **Zheng C**, Feng G, Liang H. Bletilla striata as a vascular embolizing agent in interventional treatment of primary hepatic carcinoma. *Chin Med J* (Engl) 1998; **111**: 1060-1063 [PMID: 11263363]
85 **Zheng C**, Feng G, Zhou R. [New use of Bletilla striata as embolizing agent in the intervention treatment of hepatic carcinoma]. *Zhonghua Zhongliu Zazhi* 1996; **18**: 305-307 [PMID: 9387329]
86 **Lou HZ**, Pan HM, Jin W. [Clinical study on treatment of primary liver cancer by Aidi injection combined with cool-tip radiofrequency ablation]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2007; **27**: 393-395 [PMID: 17650788]
87 **Wang WR**, Cui SD, Zeng LM. Clinical observation on treatment of mid-advanced patients with hepatocarcinoma by Xiao'aiping combined with ultrasound guided percutaneous radio-frequency. *Zhongguo Zhongxiyi Jiehe Zazhi* 2003; **2**: 19-21
88 **Zhang YW**. Combinational treatment with ultrasound-guided percutaneous radiofrequency and Tianzhicao capsule in 90 cases patients with hepatocarcinoma. *Dangdai Yixue* 2010; **16**: 6-8
89 **Liu XT**, Liu JB, Zhang ZQ, Liu SY. Jinlong capsule clinical observation of radiofrequency ablation technique for the treatment of primary liver cancer. *Shoudu Yiyao* 2013; (**10**): 47-48
90 **Zhao HJ**, Du J, Chen X. [Clinical study of Fuzheng Yiliu Recipe combined with microwave ablation on hepatocellular carcinoma]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2012; **32**: 32-34 [PMID: 22500387]
91 **Lin JJ**, Jin CN, Zheng ML, Ouyang XN, Zeng JX, Dai XH. Clinical study on treatment of primary hepatocellular carcinoma by Shenqi mixture combined with microwave coagulation. *Chin J Integr Med* 2005; **11**: 104-110 [PMID: 16150196 DOI: 10.1007/BF02836465]
92 **Guo ZJ**, Lin JJ, Jin CY, Zhong YS. Clinical observation of combinational treatment with Aidi injection and microwave ablation in hepatocarcinoma patients. *Zhongyiyao Tongbao* 2006; **5**: 51-54
93 **Zhang SC**, Li J, Yang LJ, Fan LH, Wang CX, Wang RC, He YS. Effect of Qiankun capsule combined with high-intensity focused ultrasound on the immunological function of patients with primary liver cancer. *Zhongguo Zhongyi Jichu Yixue Zazhi* 2005; **11**: 367-380
94 **Pan JY**, Ma J, Jia L, Zhou YJ, Li YY. Effects of Fu-Zhen-Yang-Yin formula combination with high-intensity focused ultrasound in advanced liver cancer patients. *Shijie Huaren Xiaohua Zazhi* 2004; **12**: 1750-1751
95 **Wang XW**, Chan RH. Effects of Syndrome differentiation based herbal therapy combination with sorafenib in patients with advanced hepatocarcinoma. *Zhongguo Yiyao Daobao* 2008; **5**: 48-49
96 **Feng LH**, Chen YD, Zheng ZG, Gao YQ. Clinical observation of patients with primary liver cancer treated by sorafenib combined cinobufagin tablets. *Zhongguo Aizheng Zazhi* 2012; **22**: 856-859
97 **Tian HQ**, Li HL, Wang B, Liang GW, Huang XQ, Huang ZQ, Lang JM, Zhang YP, Chen XZ, Chen YS. Treatment of middle/late stage primary hepatic carcinoma by Chinese medicine comprehensive therapy: A prospective randomized controlled study. *Chin J Integr Med* 2010; **16**: 102-108 [PMID: 20473733 DOI: 10.1007/s11655-010-0102-3]
98 **Xu K**, Luo HY, Li LN. [Clinical study on comprehensive treatment of primary liver cancer mainly with chinese medicinal perfusion/embolization]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2005; **25**: 299-302 [PMID: 15892270]
99 **Huang Z**, Wang Y, Chen J, Wang R, Chen Q. Effect of Xiaoaiping injection on advanced hepatocellular carcinoma in patients. *J Tradit Chin Med* 2013; **33**: 34-38 [PMID: 23596809 DOI: 10.1016/S0254-6272(13)60097-7]
100 **Chen Z**, Zhai XF, Su YH, Wan XY, Li J, Xie JM, Gao B. [Clinical observation of cinobufacini injection used to treat moderate and advanced primary liver cancer]. *Zhongxiyi Jiehe Xuebao* 2003; **1**: 184-186 [PMID: 15339556 DOI: 10.3736/jcim20030311]
101 **Chu YP**, Shen L, Bai Y. Efficacy observation of liver cancer at the later stage for the elderly treated with integration of Norcantharidin and Chinese medicine. *Shijie Zhongxiyi Jiehe Xuebao* 2012; **7**: 765-767
102 **Xu RQ**, Huang ZF, Li HZ, Wei JS, Zhang ZJ, Xiang LY, Gui HT, Tan QQ. Effects of Jianpi Fuzheng decoction on symptom and immune function in patients with advanced liver cancer. *Zhongxiyi Jiehe Ganbing Zazhi* 2013; **23**: 73-75
103 **Chen QS**, ChenY, Pei RQ, Huang WZ, Li CY, Zhou B, Chen YY. Clinical observation of 30 cases of late-stage primary hepatic cancer treated with Jianpi Yiliu Decoction. *Shijie Zhongxiyi Jiehe Zazhi* 2013; **8**: 368-370
104 **Yang YK**, Qiu DZ, Wang GH, Gu XX, Fan Y, Tang CL. Clinical study on Jiawei Yiguanjian for improvement of the survival rate in advanced hepatocellular carcinoma patients. *Zhongguo Linchuang Yixue* 2011; **18**: 818-820
105 **Li X**, Guan YS, Zhou XP, Sun L, Liu Y, He Q, Fu L, Mao YQ. [Anticarcinogenic effect of 20(R)-ginsenoside Rg3 on induced hepatocellular carcinoma in rats]. *Sichuan Daxue Xuebao Yixueban* 2005; **36**: 217-220 [PMID: 15807271]
106 **Chao JC**, Chiang SW, Wang CC, Tsai YH, Wu MS. Hot water-extracted Lycium barbarum and Rehmannia glutinosa inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells. *World J Gastroenterol* 2006; **12**: 4478-4484 [PMID: 16874858 DOI: 10.3748/wjg.v12.i28.4478]
107 **Chen YL**, Jian MH, Lin CC, Kang JC, Chen SP, Lin PC, Hung PJ, Chen JR, Chang WL, Lin SZ, Harn HJ. The induction of orphan nuclear receptor Nur77 expression by n-butylenephthalide as pharmaceuticals on hepatocellular carcinoma cell therapy. *Mol Pharmacol* 2008; **74**: 1046-1058 [PMID: 18577687 DOI: 10.1124/mol.107.044800]
108 **Li S**, Dong P, Wang J, Zhang J, Gu J, Wu X, Wu W, Fei X, Zhang Z, Wang Y, Quan Z, Liu Y. Icariin, a natural flavonol glycoside, induces apoptosis in human hepatoma SMMC-7721 cells via a ROS/JNK-dependent mitochondrial pathway. *Cancer Lett* 2010; **298**: 222-230 [PMID: 20674153 DOI: 10.1016/j.canlet.2010.07.009]
109 **Hsu SH**, Tsai TR, Lin CN, Yen MH, Kuo KW. Solamargine purified from Solanum incanum Chinese herb triggers gene expression of human TNFR I which may lead to cell apoptosis. *Biochem Biophys Res Commun* 1996; **229**: 1-5 [PMID: 8954074 DOI: 10.1006/bbrc.1996.1748]
110 **Zhang YN**, Liu L, Ling CQ. [Inhibition effect of active fraction from Actinidia valvata on growth of transplanated mouse tumor cells and preliminary study of its mechanism]. *Zhongguo Zhongyao Zazhi* 2006; **31**: 918-920 [PMID: 17048634]
111 **Lin JM**, Liu Y, Luo RC. [Inhibition activity of Scutellariae barbata extracts against human hepatocellular carcinoma cells]. *Nanfang Yike Daxue Xuebao* 2006; **26**: 591-593 [PMID: 16762857]
112 **Sa F**, Gao JL, Fung KP, Zheng Y, Lee SM, Wang YT. Anti-proliferative and pro-apoptotic effect of Smilax glabra Roxb. extract on hepatoma cell lines. *Chem Biol Interact* 2008; **171**: 1-14 [PMID: 17996228 DOI: 10.1016/j.cbi.2007.08.012]
113 **Li Y**, Li MY, Wang L, Jiang ZH, Li WY, Li H. [Induction of apoptosis of cultured hepatocarcinoma cell by essential oil of Artemisia Annul L]. *Sichuan Daxue Xuebao Yixueban* 2004; **35**: 337-339 [PMID: 15181829]
114 **Huang D**, Zhang W, Huang D, Wu J. Antitumor activity of the aqueous extract from Sedum sarmentosum Bunge in vitro. *Cancer Biother Radiopharm* 2010; **25**: 81-88 [PMID: 20187800 DOI: 10.1089/cbr.2009.0632]
115 **Xie Q**, Yang Y, Wang Z, Chen F, Zhang A, Liu C. Resveratrol-4-O-D-(2'-galloyl)-glucopyranoside isolated from Polygonum cuspidatum exhibits anti-hepatocellular carcinoma viability by inducing apoptosis via the JNK and ERK pathway. *Molecules* 2014; **19**: 1592-1602 [PMID: 24473215 DOI: 10.3390/molecules19021592]
116 **Hu YQ**, Tan RX, Chu MY, Zhou J. Apoptosis in human hepatoma cell line SMMC-7721 induced by water-soluble macromolecular components of Artemisia capillaris Thunberg. *Jpn J Cancer Res* 2000; **91**: 113-117 [PMID: 10744052 DOI: 10.1111/j.1349-7006.2000.tb00867.x]

117 **Liu J**, Shen HM, Ong CN. Salvia miltiorrhiza inhibits cell growth and induces apoptosis in human hepatoma HepG(2) cells. *Cancer Lett* 2000; **153**: 85-93 [PMID: 10779635]

118 **Lee SM**, Li ML, Tse YC, Leung SC, Lee MM, Tsui SK, Fung KP, Lee CY, Waye MM. Paeoniae Radix, a Chinese herbal extract, inhibit hepatoma cells growth by inducing apoptosis in a p53 independent pathway. *Life Sci* 2002; **71**: 2267-2277 [PMID: 12215374 DOI: 10.1016/S0024-3205(02)01962-8]

119 **Du Q**, Hu B, An HM, Shen KP, Xu L, Deng S, Wei MM. Synergistic anticancer effects of curcumin and resveratrol in Hepa1-6 hepatocellular carcinoma cells. *Oncol Rep* 2013; **29**: 1851-1858 [PMID: 23446753 DOI: 10.3892/or.2013.2310]

120 **Chen F**, Yang L, Wang XK, Zhang Y. Effects of the Alkaloids of Pinellia ternata on Proliferation of Human Hepatocarcinoma Cell Strain Bel-7402. *Zhongguo Yaofang* 2011; **43**: 4048-4050

121 **Yang ZH**, Yin JY, Wei ZR, Yan WQ. Extract of Tian-Nan-Xing (Arisaema heterophyllum) induces apoptosis in human hepatocarcinoma SMMC-7721 cells and its mechanism. *Zhongguo Laonianxue Zazhi* 2007; **27**: 142-144

122 **Wang Y**, Deng L, Zhong H, Wang Y, Jiang X, Chen J. Natural plant extract tubeimoside I promotes apoptosis-mediated cell death in cultured human hepatoma (HepG2) cells. *Biol Pharm Bull* 2011; **34**: 831-838 [PMID: 21628880 DOI: 10.1248/bpb.34.831]

123 **Kwon KB**, Kim EK, Lim JG, Jeong ES, Shin BC, Jeon YS, Kim KS, Seo EA, Ryu DG. Molecular mechanisms of apoptosis induced by Scorpio water extract in human hepatoma HepG2 cells. *World J Gastroenterol* 2005; **11**: 943-947 [PMID: 15742393 DOI: 10.3748/wjg.v11.i7.943]

124 **Chang C**, Zhu YQ, Mei JJ, Liu SQ, Luo J. Involvement of mitochondrial pathway in NCTD-induced cytotoxicity in human hepG2 cells. *J Exp Clin Cancer Res* 2010; **29**: 145 [PMID: 21059274 DOI: 10.1186/1756-9966-29-145]

125 **Hu H**, Chen D, Li Y, Zhang X. Effect of polypeptides in bee venom on growth inhibition and apoptosis induction of the human hepatoma cell line SMMC-7721 in-vitro and Balb/c nude mice in-vivo. *J Pharm Pharmacol* 2006; **58**: 83-89 [PMID: 16393467 DOI: 10.1211/jpp.58.1.0010]

126 **Song Q**, Zhao JX, Zhu YZ. [Effect of fuzheng yiliu granule on H22 tumor cell apoptosis, p53 and caspase-3 gene expression in mice]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2005; **25**: 338-341 [PMID: 15892280]

127 **Zhao JX**, Guo FL, Bai DC, Wang XX. [Effects of fuzheng yiliu granules on apoptotic rate and mitochondrial membrane potential of hepatocellular carcinoma cell line H22 from mice]. *Zhongxiyi Jiehe Xuebao* 2006; **4**: 271-274 [PMID: 16696914 DOI: 10.3736/jcim20060310]

128 **Zhang YY**, Liu FY, Zhang HL. Study on Apoptosis of hepatica carcinoma cell induced by Fuganchun 6 in Vitro. *Hebei Beifang Xueyuan Xuebao Yixueban* 2009; **26**: 6-10

129 **Zhong Y**, Luo CL, An-Jun Z. [Effect of bushen jianpi decoction and its disassemble recipes on tumor growth in mice with transplanted primary hepatic carcinoma]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2011; **31**: 213-217 [PMID: 21425577]

130 **Zhang X**, Liu DQ, Liu YZ, Wang P, Chen XY. [Preliminary study on apoptosis of BEL-7402 cells induced by Chinese herbs for warming yang and dispersing stasis]. *Zhongguo Zhongyao Zazhi* 2000; **25**: 428-430 [PMID: 12515227]

131 **Jiang ZY**, Hua HQ, Yang AZ, Qin SK. Effects of Fufangkushen Injection in combination with oxaliplatin and recombinant human endostatin injection on apoptosis in human hepatoma cell line SMMC-7721. *Shijie Huaren Xiaohua Zazhi* 2010; **18**: 3396-3401

132 **Huang XY**, Wang L, Huang ZL, Zheng Q, Li QS, Tang ZY. Herbal extract "Songyou Yin" inhibits tumor growth and prolongs survival in nude mice bearing human hepatocellular carcinoma xenograft with high metastatic potential. *J Cancer Res Clin Oncol* 2009; **135**: 1245-1255 [PMID: 19277711 DOI: 10.1007/s00432-009-0566-8]

133 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

134 **Tsai YC**, Wu WB, Chen BH. Preparation of carotenoids and chlorophylls from Gynostemma pentaphyllum (Thunb.) Makino and their antiproliferation effect on hepatoma cell. *J Med Food* 2010; **13**: 1431-1442 [PMID: 20946022 DOI: 10.1089/jmf.2010.1165]

135 **Tsai YC**, Lin CL, Chen BH. Preparative chromatography of flavonoids and saponins in Gynostemma pentaphyllum and their antiproliferation effect on hepatoma cell. *Phytomedicine* 2010; **18**: 2-10 [PMID: 21036575 DOI: 10.1016/j.phymed.2010.09.004]

136 **Lin SB**, Li CH, Lee SS, Kan LS. Triterpene-enriched extracts from Ganoderma lucidum inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. *Life Sci* 2003; **72**: 2381-2390 [PMID: 12639703 DOI: 10.1016/S0024-3205(03)00124-3]

137 **Lin JM**, Liu Y, Luo RC. [Effect of Scutellaria barbata extract against human hepatocellular Hep-G2 cell proliferation and its mechanism]. *Nanfang Yike Daxue Xuebao* 2006; **26**: 975-977 [PMID: 16864090]

138 **Ye F**, Che Y, McMillen E, Gorski J, Brodman D, Saw D, Jiang B, Zhang DY. The effect of Scutellaria baicalensis on the signaling network in hepatocellular carcinoma cells. *Nutr Cancer* 2009; **61**: 530-537 [PMID: 19838925 DOI: 10.1080/01635580902803719]

139 **Xie RF**, Li ZC, Gao B, Shi ZN, Zhou X. Bufothionine, a possible effective component in cinobufocini injection for hepatocellular carcinoma. *J Ethnopharmacol* 2012; **141**: 692-700 [PMID: 22210051 DOI: 10.1016/j.jep.2011.12.018]

140 **Shi P**, Huang Z, Chen G. Rhein induces apoptosis and cell cycle arrest in human hepatocellular carcinoma BEL-7402 cells. *Am J Chin Med* 2008; **36**: 805-813 [PMID: 18711776 DOI: 10.1142/S0192415X08006259]

141 **Hsu YL**, Kuo PL, Tzeng TF, Sung SC, Yen MH, Lin LT, Lin CC. Huang-lian-jie-du-tang, a traditional Chinese medicine prescription, induces cell-cycle arrest and apoptosis in human liver cancer cells in vitro and in vivo. *J Gastroenterol Hepatol* 2008; **23**: e290-e299 [PMID: 18522681 DOI: 10.1111/j.1440-1746.2008.05390.x]

142 **Kao ST**, Yeh CC, Hsieh CC, Yang MD, Lee MR, Liu HS, Lin JG. The Chinese medicine Bu-Zhong-Yi-Qi-Tang inhibited proliferation of hepatoma cell lines by inducing apoptosis via G0/G1 arrest. *Life Sci* 2001; **69**: 1485-1496 [PMID: 11554610 DOI: 10.1016/S0024-3205(01)01226-7]

143 **Cao Z**, Lin W, Huang Z, Chen X, Zhao J, Zheng L, Ye H, Liu Z, Liao L, Du J. Ethyl acetate extraction from a Chinese herbal formula, Jiedu Xiaozheng Yin, inhibits the proliferation of hepatocellular carcinoma cells via induction of G0/G1 phase arrest in vivo and in vitro. *Int J Oncol* 2013; **42**: 202-210 [PMID: 23165653 DOI: 10.3892/ijo.2012.1703]

144 **Deng S**, Hu B, An HM. Traditional Chinese Medicinal Syndromes and treatment in colorectal cancer. *J Cancer Ther* 2012; **3**: 888-897 [DOI: 10.4236/jct.2012.326114]

145 **Wang Z**, Han W, Sui X, Fang Y, Pan H. Autophagy: A novel therapeutic target for hepatocarcinoma (Review). *Oncol Lett* 2014; **7**: 1345-1351 [PMID: 24765136 DOI: 10.3892/ol.2014.1916]

146 **Lin HM**, Tseng HC, Wang CJ, Chyau CC, Liao KK, Peng PL, Chou FP. Induction of autophagy and apoptosis by the extract of Solanum nigrum Linn in HepG2 cells. *J Agric Food Chem* 2007; **55**: 3620-3628 [PMID: 17419635 DOI: 10.1021/jf062406m]

147 **Zhang DM**, Liu JS, Deng LJ, Chen MF, Yiu A, Cao HH, Tian HY, Fung KP, Kurihara H, Pan JX, Ye WC. Arenobufagin, a natural bufadienolide from toad venom, induces apoptosis and autophagy in human hepatocellular carcinoma cells through inhibition of PI3K/Akt/mTOR pathway. *Carcinogenesis* 2013; **34**: 1331-1342 [PMID: 23393227 DOI: 10.1093/carcin/bgt060]

148 **Miao Q**, Bi LL, Li X, Miao S, Zhang J, Zhang S, Yang Q, Xie YH, Zhang J, Wang SW. Anticancer Effects of Bufalin on Human Hepatocellular Carcinoma HepG2 Cells: Roles of Apoptosis and Autophagy. *Int J Mol Sci* 2013; **14**: 1370-1382 [PMID: 23344047]

149 **Chu YL**, Ho CT, Chung JG, Rajasekaran R, Sheen LY. Allicin induces p53-mediated autophagy in Hep G2 human liver cancer cells. *J Agric Food Chem* 2012; **60**: 8363-8371 [PMID: 22860996 DOI: 10.1021/jf301298y]

150 **Gong K**, Zhang Z, Chen Y, Shu HB, Li W. Extracellular signal-

regulated kinase, receptor interacting protein, and reactive oxygen species regulate shikonin-induced autophagy in human hepatocellular carcinoma. *Eur J Pharmacol* 2014; **738**: 142-152 [PMID: 24886888 DOI: 10.1016/j.ejphar.2014.05.034]

151 **Muehlich S**, Gudermann T. Pro-senescence therapy for hepatocellular carcinoma. *Aging* (Albany NY) 2013; **5**: 639-640 [PMID: 24057649]

152 **Hu B**, An HM, Shen KP, Xu L, DU Q, Deng S, Wu Y. Liver Yin deficiency tonifying herbal extract induces apoptosis and cell senescence in Bel-7402 human hepatocarcinoma cells. *Exp Ther Med* 2012; **3**: 80-86 [PMID: 22969849 DOI: 10.3892/etm.2011.364]

153 **Chang UM**, Li CH, Lin LI, Huang CP, Kan LS, Lin SB. Ganoderiol F, a ganoderma triterpene, induces senescence in hepatoma HepG2 cells. *Life Sci* 2006; **79**: 1129-1139 [PMID: 16635496 DOI: 10.1016/j.lfs.2006.03.027]

154 **Hu B**, Du Q, Deng S, An HM, Pan CF, Shen KP, Xu L, Wei MM, Wang SS. Ligustrum lucidum Ait. fruit extract induces apoptosis and cell senescence in human hepatocellular carcinoma cells through upregulation of p21. *Oncol Rep* 2014; **32**: 1037-1042 [PMID: 25017491 DOI: 10.3892/or.2014.3312]

155 **Cao L**, Han L, Zhang Z, Li J, Qu Z, Du J, Liang X, Liu Y, Liu H, Shi Y, Liu S, Gao L, Sun W. Involvement of anoikis-resistance in the metastasis of hepatoma cells. *Exp Cell Res* 2009; **315**: 1148-1156 [PMID: 19073174 DOI: 10.1016/j.yexcr.2008.11.010]

156 **Hu B**, An HM, Shen KP, Xu L, Du Q, Deng S, Wu Y. Modified Yi Guan Jian, a Chinese herbal formula, induces anoikis in Bel-7402 human hepatocarcinoma cells in vitro. *Oncol Rep* 2011; **26**: 1465-1470 [PMID: 21822542 DOI: 10.3892/or.2011.1414]

157 **Hu B**, An HM, Shen KP, Song HY, Deng S. Polygonum cuspidatum Extract Induces Anoikis in Hepatocarcinoma Cells Associated with Generation of Reactive Oxygen Species and Downregulation of Focal Adhesion Kinase. *Evid Based Complement Alternat Med* 2012; **2012**: 607675 [PMID: 23008742 DOI: 10.1155/2012/607675]

158 **Cheng HL**, Su SJ, Huang LW, Hsieh BS, Hu YC, Hung TC, Chang KL. Arecoline induces HA22T/VGH hepatoma cells to undergo anoikis - involvement of STAT3 and RhoA activation. *Mol Cancer* 2010; **9**: 126 [PMID: 20507639 DOI: 10.1186/1476-4598-9-126]

159 **Wang N**, Feng Y, Lau EP, Tsang C, Ching Y, Man K, Tong Y, Nagamatsu T, Su W, Tsao S. F-actin reorganization and inactivation of rho signaling pathway involved in the inhibitory effect of Coptidis Rhizoma on hepatoma cell migration. *Integr Cancer Ther* 2010; **9**: 354-364 [PMID: 21106616 DOI: 10.1177/1534735410379121]

160 **Xiao XL**, Wang XY, Pu X, Guo QX, Long HA. Effect of Baicalein on the in Vitro migration and invasion of human hepatocellular carcinoma cell line SMMC-7721. *Zhongguo Zhongliu Linchuang* 2012; **39**: 305-309

161 **Yu HB**, Zhang HF, Li DY, Zhang X, Xue HZ, Zhao SH. Matrine inhibits matrix metalloproteinase-9 expression and invasion of human hepatocellular carcinoma cells. *J Asian Nat Prod Res* 2011; **13**: 242-250 [PMID: 21409686 DOI: 10.1080/10286020.2010.551641]

162 **Wei PL**, Tu CC, Chen CH, Ho YS, Wu CT, Su HY, Chen WY, Liu JJ, Chang YJ. Shikonin suppresses the migratory ability of hepatocellular carcinoma cells. *J Agric Food Chem* 2013; **61**: 8191-8197 [PMID: 23899086 DOI: 10.1021/jf4009586]

163 **Chen D**, Yao WJ, Zhang XL, Han XQ, Qu XY, Ka WB, Sun DG, Wu XZ, Wen ZY. Effects of Gekko sulfated polysaccharide-protein complex on human hepatoma SMMC-7721 cells: inhibition of proliferation and migration. *J Ethnopharmacol* 2010; **127**: 702-708 [PMID: 19969061 DOI: 10.1016/j.jep.2009.12.003]

164 **Lou L**, Ye W, Chen Y, Wu S, Jin L, He J, Tao X, Zhu J, Chen X, Deng A, Wang J. Ardipusilloside inhibits survival, invasion and metastasis of human hepatocellular carcinoma cells. *Phytomedicine* 2012; **19**: 603-608 [PMID: 22349030 DOI: 10.1016/j.phymed.2012.01.003]

165 **Cao XC**, Wang H, Zhang HM, Liu YW, Lu ZX. The effects of plumbagin on proliferation and metastasis in human liver cancer SK-hep-1 cells. *Zhongguo Aizheng Zazhi* 2013; **23**: 721-727

166 **Yuxian X**, Feng T, Ren L, Zhengcai L. Tanshinone II-A inhibits invasion and metastasis of human hepatocellular carcinoma cells in vitro and in vivo. *Tumori* 2009; **95**: 789-795 [PMID: 20210245 DOI: 10.1700/467.5521]

167 **Jia QA**, Wang ZM, Ren ZG, Bu Y, Xie XY, Wang YH, Zhang L, Zhang QB, Xue TC, Deng LF, Tang ZY. Herbal compound "Songyou Yin" attenuates hepatoma cell invasiveness and metastasis through downregulation of cytokines secreted by activated hepatic stellate cells. *BMC Complement Altern Med* 2013; **13**: 89 [PMID: 23622143 DOI: 10.1186/1472-6882-13-89]

168 **Cheng Y**, He SQ, Zhu Y, Fan Q, Yang S, Chen R. [Blejiajian pill inhibited the proliferation, adhesion, and invasion of hepatoma carcinoma cells: an experimental research]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2013; **33**: 664-667 [PMID: 23905388]

169 **Liu X**, Yang Y, Zhang X, Xu S, He S, Huang W, Roberts MS. Compound Astragalus and Salvia miltiorrhiza extract inhibits cell invasion by modulating transforming growth factor-beta/Smad in HepG2 cell. *J Gastroenterol Hepatol* 2010; **25**: 420-426 [PMID: 19793165 DOI: 10.1111/j.1440-1746.2009.05981.x]

170 **Ding XM**, Cheng ZR. Study on inhibitory effect of Shehuang Xiaoliu Fang on invasive and adhesive ability of liver cancer cells. *Zhongxiyi Jiehe Ganbing Zazhi* 2006; **16**: 96-98

171 **He YL**, Ying Y, Xu YL, Su JF, Luo H, Wang HF. [Effects of Lycium barbarum polysaccharide on tumor microenvironment T-lymphocyte subsets and dendritic cells in H22-bearing mice]. *Zhongxiyi Jiehe Xuebao* 2005; **3**: 374-377 [PMID: 16159572 DOI: 10.3736/jcim20050511]

172 **Chiu JH**, Ju CH, Wu LH, Lui WY, Wu CW, Shiao MS, Hong CY. Cordyceps sinensis increases the expression of major histocompatibility complex class II antigens on human hepatoma cell line HA22T/VGH cells. *Am J Chin Med* 1998; **26**: 159-170 [PMID: 9799968 DOI: 10.1142/S0192415X9800021X]

173 **Dai ZJ**, Liu XX, Tang W, Xue Q, Wang XJ, Ji ZZ, Kang HF, Diao Y. [Antitumor and immune-modulating effects of Scutellaria barbata extract in mice bearing hepatocarcinoma H22 cells-derived tumor]. *Nanfang Yike Daxue Xuebao* 2008; **28**: 1835-1837 [PMID: 18971184]

174 **Chen YT**, Lin MA, Cheng DQ, Shi ZJ, Zhu JL, Wu J. [Effect of proteins extracted from mycelia of Omphalia lapidescens on inhibiting H, liver cancer in mice and regulating immune function]. *Zhongyaocai* 2009; **32**: 1870-1874 [PMID: 20432906]

175 **Li J**, Xie M, Gan Y. [Effect of Xiaochaihu decoction and different herbal formulation of component on inhibiting H22 liver cancer in mice and enhancing immune function]. *Zhongguo Zhongyao Zazhi* 2008; **33**: 1039-1044 [PMID: 18652353]

176 **Zhou AG**, Zhang Y, Kong DY, Wang Y, Zhang HZ, Wang SJ, Kui G, Hong S, Ge HL, Ren QH, Mao XM, Dong JR. [Tumor inhibiting and immunoloregulation effects of Mylabris Mixture on H22 cancer-bearing mice]. *Zhongxiyi Jiehe Xuebao* 2006; **4**: 504-508 [PMID: 16965746 DOI: 10.3736/jcim20060513]

177 **Cao ZY**, Chen XZ, Liao LM, Peng J, Hu HX, Liu ZZ, Du J. Fuzheng Yiliu Granule inhibits the growth of hepatocellular cancer by regulating immune function and inducing apoptosis in vivo and in vitro. *Chin J Integr Med* 2011; **17**: 691-697 [PMID: 21910071 DOI: 10.1007/s11655-011-0847-3]

178 **Li XJ**, Mao YX, Zhang HL, Wang JX, Liu FY. [Study on effects of anticancer and immunoregulation of Fuganchun 6 on hepatoma of mouse]. *Zhongguo Zhongyao Zazhi* 2006; **31**: 1622-1625 [PMID: 17165590]

179 **Chen JR**, Li EQ, Dai CQ, Yu B, Wu XL, Huang CR, Chen XY. The inducible effect of LBP on maturation of dendritic cells and the related immune signaling pathways in hepatocellular carcinoma (HCC). *Curr Drug Deliv* 2012; **9**: 414-420 [PMID: 22640039 DOI: 10.2174/156720112801323107]

180 **Chen D**, Zhang X, Du Y, Jia B, Ka W, Sun D, Yao W, Wen Z. Effects of Gekko sulfated polysaccharide-protein complex on the defective biorheological characters of dendritic cells under tumor microenvironment. *Cell Biochem Biophys* 2012; **62**: 193-201 [PMID: 21913004 DOI: 10.1007/s12013-011-9282-5]

181 **He X**, Li X, Liu B, Xu L, Zhao H, Lu A. Down-regulation of Treg cells and up-regulation of TH1/TH2 cytokine ratio were induced by polysaccharide from Radix Glycyrrhizae in H22 hepatocarcinoma

bearing mice. *Molecules* 2011; **16**: 8343-8352 [PMID: 21963624 DOI: 10.3390/molecules16108343]
182 **Deng S**, Hu B, An HM, Du Q, Xu L, Shen KP, Shi XF, Wei MM, Wu Y. Teng-Long-Bu-Zhong-Tang, a Chinese herbal formula, enhances anticancer effects of 5--Fluorouracil in CT26 colon carcinoma. *BMC Complement Altern Med* 2013; **13**: 128 [PMID: 23758730 DOI: 10.1186/1472-6882-13-128]
183 **Bishayee A**, Darvesh AS. Angiogenesis in hepatocellular carcinoma: a potential target for chemoprevention and therapy. *Curr Cancer Drug Targets* 2012; **12**: 1095-1118 [PMID: 22873221 DOI: 10.2174/15680096112091095]
184 **Song P**, Wang XM, Xie S. [Experimental study on mechanisms of lyophilized powder of fresh gekko Chinenis in inhibiting H22 hepatocarcinoma angiogenesis]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2006; **26**: 58-62 [PMID: 16466175]
185 **Zhang SX**, Zhu C, Ba Y, Chen D, Zhou XL, Cao R, Wang LP, Ren Y, Wu XZ. Gekko-sulfated glycopeptide inhibits tumor angiogenesis by targeting basic fibroblast growth factor. *J Biol Chem* 2012; **287**: 13206-13215 [PMID: 22371501 DOI: 10.1074/jbc.M111.321521]
186 **Feng GS**, Li X, Zheng CS, Zhou CK, Liu X, Wu HP. [Mechanism of inhibition of tumor angiogenesis by Bletilla colloid: an experimental study]. *Zhonghua Yixue Zazhi* 2003; **83**: 412-416 [PMID: 12820920]
187 **Ren J**, Zheng C, Feng G, Liang H, Xia X, Fang J, Duan X, Zhao H. Inhibitory effect of extract of fungi of Huaier on hepatocellular carcinoma cells. *J Huazhong Univ Sci Technolog Med Sci* 2009; **29**: 198-201 [PMID: 19399404 DOI: 10.1007/s11596-009-0212-3]
188 **Liu SJ**, Shen WL, Liu XL, Wen J, Cui YZ. Anti-angiogenesis effects of Fu-Zheng-Kang-Ai-Tang in the treatment of hepatocarcinoma. *Shijie Huaren Xiaohua Zazhi* 2005; **13**: 908-910
189 **Cui J**, Nan KJ, Tian T, Guo YH, Zhao N, Wang L. Chinese medicinal compound delisheng has satisfactory anti-tumor activity, and is associated with up-regulation of endostatin in human hepatocellular carcinoma cell line HepG2 in three-dimensional culture. *World J Gastroenterol* 2007; **13**: 5432-5439 [PMID: 17907285 DOI: 10.3748/wjg.v13.i41.5432]
190 **Chen T**, Fu YL, Gong ZP, Deng LR, Hu YQ. Studies on the anti-angiogenic mechanism of the formula of Chinese medicine active ingredients combined with small dose cisplatin in mice of hepatocellular carcinoma. *Zhongguo Shiyan Fangji Xue Zazhi* 2010; **16**: 157-160
191 **Wang S**, Xu X, Zhang W, Li A, Dong W. [Effects of Erbie San on Walker-256 liver cancer and adjustment to unbalance of VEGF/endostatin in rats]. *Zhongguo Zhongyao Zazhi* 2009; **34**: 89-91 [PMID: 19382459]
192 **Dai Z**, Liu X, Ji Z, Liu L, Kang H, Wang X, Diao Y. The effect-enhancing and toxicity-reducing action of the extract of herba Scutellariae barbatae for chemotherapy in hepatoma H22 tumor-bearing mice. *J Tradit Chin Med* 2008; **28**: 205-210 [PMID: 19004205 DOI: 10.1016/S0254-6272(08)60048-5]
193 **Wang DH**, Weng XC. [Antitumor activity of extracts of Ganoderma lucidum and their protective effects on damaged HL-7702 cells induced by radiotherapy and chemotherapy]. *Zhongguo Zhongyao Zazhi* 2006; **31**: 1618-1622 [PMID: 17165589]
194 **Sun Y**, Guan J, Wang Q. The influence of Cordyceps sinensis on the immunologic function of the rats with liver cancer (H22) after chemotherapy. *Zhongguo Jiceng Yiyao* 2002; **9**: 127-128
195 **You M**, Luo M, Liao W, Hu S, Xu W, Jing L. [Chaiqiyigan granula enhances Taxol-induced growth inhibition of hepatocellular carcinoma xenografts in nude mice: an in vivo fluorescence imaging study]. *Nanfang Yike Daxue Xuebao* 2012; **32**: 1042-1045 [PMID: 22820595]
196 **Chen T**, Li D, Fu YL, Hu W. Screening of QHF formula for effective ingredients from Chinese herbs and its anti-hepatic cell cancer effect in combination with chemotherapy. *Chin Med J (Engl)* 2008; **121**: 363-368 [PMID: 18304471]
197 **Chen Z**, Wang P, Huang WX, Liu LM. [Experimental study on effects of shengmai injection: enhancing 5-FU anti-tumor efficacy and reducing its toxicity]. *Zhongxiyi Jiehe Xuebao* 2005; **3**: 476-479 [PMID: 16282061 DOI: 10.3736/jcim20050615]
198 **Yau T**, Chan P, Epstein R, Poon RT. Evolution of systemic therapy of advanced hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 6437-6441 [PMID: 19030192 DOI: 10.3748/wjg.14.6437]
199 **Tian QE**, Li HD, Yan M, Cai HL, Tan QY, Zhang WY. Astragalus polysaccharides can regulate cytokine and P-glycoprotein expression in H22 tumor-bearing mice. *World J Gastroenterol* 2012; **18**: 7079-7086 [PMID: 23323011 DOI: 10.3748/wjg.v18.i47.7079]
200 **Huang C**, Xu D, Xia Q, Wang P, Rong C, Su Y. Reversal of P-glycoprotein-mediated multidrug resistance of human hepatic cancer cells by Astragaloside II. *J Pharm Pharmacol* 2012; **64**: 1741-1750 [PMID: 23146037 DOI: 10.1111/j.2042-7158.2012.01549.x]
201 **Liang G**, Tang A, Lin X, Li L, Zhang S, Huang Z, Tang H, Li QQ. Green tea catechins augment the antitumor activity of doxorubicin in an in vivo mouse model for chemoresistant liver cancer. *Int J Oncol* 2010; **37**: 111-123 [PMID: 20514403 DOI: 10.3892/ijo_00000659]
202 **Wang XB**, Wang SS, Zhang QF, Liu M, Li HL, Liu Y, Wang JN, Zheng F, Guo LY, Xiang JZ. Inhibition of tetramethylpyrazine on P-gp, MRP2, MRP3 and MRP5 in multidrug resistant human hepatocellular carcinoma cells. *Oncol Rep* 2010; **23**: 211-215 [PMID: 19956884 DOI: 10.3892/or_00000625]
203 **Wong VK**, Chiu P, Chung SS, Chow LM, Zhao YZ, Yang BB, Ko BC. Pseudolaric acid B, a novel microtubule-destabilizing agent that circumvents multidrug resistance phenotype and exhibits antitumor activity in vivo. *Clin Cancer Res* 2005; **11**: 6002-6011 [PMID: 16115945 DOI: 10.1158/1078-0432.CCR-05-0209]
204 **Ong RC**, Lei J, Lee RK, Cheung JY, Fung KP, Lin C, Ho HP, Yu B, Li M, Kong SK. Polyphyllin D induces mitochondrial fragmentation and acts directly on the mitochondria to induce apoptosis in drug-resistant HepG2 cells. *Cancer Lett* 2008; **261**: 158-164 [PMID: 18082940 DOI: 10.1016/j.canlet.2007.11.005]
205 **Chaudhary H**, Jena PK, Seshadri S. Evaluation of hydro-alcoholic extract of Eclipta alba for its multidrug resistance reversal potential: an in vitro study. *Nutr Cancer* 2013; **65**: 775-780 [PMID: 23859045 DOI: 10.1080/01635581.2013.789116]

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# Chronic hepatitis B infection in pregnancy

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## Abstract

There are no standard guidelines to follow when a patient with chronic hepatitis B infection becomes pregnant or desires pregnancy. Topics to consider include which patients to treat, when to start treatment, what treatment to use and when to stop treatment. Without any prophylaxis or antiviral therapy, a hepatitis B surface antigen and E antigen positive mother has up to a 90% likelihood of vertical transmission of hepatitis B virus (HBV) to child. Standard of care in the United States to prevent perinatal transmission consists of administration of hepatitis B immune globulin and HBV vaccination to the infant. The two strongest risk factors of mother to child transmission (MTCT) of HBV infection despite immunoprophylaxis are high maternal HBV viral load and high activity of viral replication. The goal is to prevent transmission of HBV at birth by decreasing viral load and/or decreasing activity of the virus. Although it is still somewhat controversial, most evidence shows that starting antivirals in the third trimester is effective in decreasing MTCT without affecting fetal development. There is a growing body of literature supporting the safety and efficacy of antiviral therapies to reduce MTCT of hepatitis B. There are no formal recommendations regarding which agent to choose. Tenofovir, lamivudine and telbivudine have all been proven efficacious in decreasing viral load at birth without known birth defects, but final decision of which antiviral medication to use will have to be determined by physician and patient. The antivirals may be discontinued immediately if patient is breastfeeding, or within first four weeks if infant is being formula fed.

**Key words:** Chronic hepatitis B infection; Pregnancy; Hepatitis B immune globulin; Hepatitis B virus vaccine; Antivirals



**Core tip:** In pregnant patients chronically infected with hepatitis B, determining which patients require treatment is not well understood. In this concise review, we discuss four important questions to consider when faced with this patient population: who to treat, when to treat, what medication in which to treat and when to stop treatment.

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## INTRODUCTION

Chronic infection with hepatitis B virus (HBV) is a relatively well-understood and manageable disease process. With current available medications, suppression of the virus can be achieved in most patients. Practice guidelines are available for beginning medical therapy in chronic HBV infection. However, in certain specific circumstances, treatment of HBV infection becomes less clear. One of those circumstances is chronic HBV infection in pregnancy. In fact, of the 50 million people newly infected with hepatitis B every year worldwide, the majority of this transmission occurs from mother to child transmission (MTCT)[1]. In this concise review, we discuss four important questions to consider when faced with this patient population: who to treat, when to treat, what medication in which to treat and when to stop treatment.

## WHO TO TREAT?

It is first important to note the difference between acute and chronic HBV infection in pregnancy. Generally, patients acutely infected with HBV during pregnancy should be monitored closely and managed conservatively. Unless the pregnant mother develops evidence of acute liver failure, antivirals are not generally indicated[2].

Although MTCT is not the most common transmission route of HBV infection in the United States, it remains extremely high risk if the mother and child are not managed properly. Without any prophylaxis or antiviral therapy, a hepatitis B surface antigen (HBsAg) and E antigen (HBeAg) positive mother has up to a 90% likelihood of vertical transmission of HBV to child[3]. Standard of care in the United States to prevent perinatal transmission consists of administration of hepatitis B immune globulin (HBIG) and HBV vaccination to the infant[4,5]. Despite receiving immunoprophylaxis, up to 10%-15% of infants develop chronic HBV infection through MTCT.

There are two indications to treat chronic hepatitis B in a pregnant mother; chronic liver disease in mother and prevention of MTCT. We will not discuss chronic liver disease in the mother in detail in this review.

Risk factors that increase the risk of perinatal transmission of HBV to the infant have been determined. The two strongest risk factors are high maternal HBV viral load and high activity of viral replication[6,7]. Other risk factors including amniocentesis, preterm premature rupture of membranes and breast feeding carry a much lower risk for MTCT of HBV[8].

Not every pregnant woman with chronic HBV infection needs antiviral therapy due to concern of drug related adverse events[9]. The decision to start antivirals must be discussed in detail between patient and physician. However, it should be strongly considered in HBsAg positive pregnant patients with high viral load and/or active viral disease.

High viral load is defined as > $10^6$ or > $10^8$ copies per milliliter (20 million IU/mL) in previous studies[10,11]. High activity of viral replication can be defined in several different manners and has been proposed with positive HBeAg (prior to seroconversion) or elevated alanine aminotransferase level and viral load of > 20000 IU/mL[9].

## WHEN TO TREAT?

Again, immunoprophylaxis is given to all infants born to HBsAg positive mothers. The passive immunization, the HBIG, is given to the infant within 12 h of birth. The active immunization, which is the first dose of HBV vaccine, is also given in these first few hours of life. The remainder of the dosing for HBV vaccine follows standard protocol; the second dose is given at 1 mo and the last dose is given at 6 mo of age and no later than 9 mo of age[5].

Maternal HBsAg is checked during early pregnancy and again in the third trimester of pregnancy. If the HBsAg status of mother is unknown at the time of birth, the infant should receive first dose of HBV vaccine. If confirmatory testing is positive for HBsAg in the mother, then HBIG should be administered to the infant as well, and the HBV vaccine should be completed[12].

If the decision is made to treat with antivirals, due to either maternal high viral load and/or evidence of active disease, the timing of administration of these medications is noteworthy. The goal is to prevent transmission of HBV at birth by decreasing viral load and/or decreasing activity of the virus. Although it is still somewhat controversial, most evidence shows that starting antivirals in the third trimester is effective in decreasing MTCT without affecting fetal development. Starting medication earlier in the pregnancy is not necessary to decrease MTCT and puts the fetus at higher risk due to the longer exposure to a non-approved medication. In fact, many studies have shown the efficacy of starting antivirals well into the third trimester, such as 29 wk, 32 wk or even 34 wk[13-15].

## WHAT MEDICATION IN WHICH TO TREAT?

As previously mentioned, MTCT accounts for more than one-third of all HBV transmission worldwide. With the use of HBV vaccination and HBIG, the rate of MTCT may be reduced from 90% to 10%[16]. The greatest risk exists in children born to mothers with high viral loads. In this particular population, there are several studies examining the use of antiviral agents to further reduce the risk of MTCT. The following paragraphs will review the data surrounding the use antiviral agents available for the treatment of chronic hepatitis B during pregnancy.

Currently, there are six therapies approved for the treatment of chronic HBV infection. None is approved for use during pregnancy (Table 1). Current registry data does not suggest any increased risk of major

**Table 1 Food and Drug Administration pregnancy categories for nucleos(t)ide analogues for hepatitis B**

| Drug | Pregnancy category |
|---|---|
| LdT | B |
| TDF | B |
| LAM | C |
| Entecavir | C |
| Adefovir | C |
| (Pegylated) interferon | C |

LdT: Telbivudine; TDF: Tenofovir; LAM: Lamivudine.

birth defects in women exposed to tenofovir (TDF) or lamivudine (LAM) during pregnancy compared with large population controls[17].

In early studies of LAM in pregnancy, 8 women with HBV DNA levels > 1.2 x $10^9$ copies/mL were treated with 150 mg daily beginning at 34 wk of gestation. Viral serologies of their offspring were measured at 0, 3, 6, and 12 mo. Twenty-five children born to untreated women served as controls. All children received active and passive immunization at birth. At 12 mo of age, only 1 of the 8 children (12.5%) in the treatment group remained HBsAg positive with measurable HBV DNA levels. In the control group, MTCT occurred in 7 of 25 children (28%)[15]. No adverse events were noted. A double blind, randomized control trial examined the use of LAM 100 mg daily and active-passive immunization *vs* placebo and active-passive immunization in Chinese women from a gestational age of 32 wk to 12 wk after delivery. All women had an HBV DNA level > $10^9$ copies/mL. At week 52, infants in the LAM group had a significant decrease in HBsAg positivity (18% *vs* 39%) and HBV DNA compared with placebo. With sensitivity analyses to account for dropouts in the placebo group, these differences were not statistically significant. One congenital anomaly was noted in the LAM group[18].

Telbivudine (LdT) has also been studied for the prevention of MTCT of HBV. In an open label study, 135 women with HBV DNA levels > $10^7$ copies/mL received LdT 600 mg daily from week 20 to week 32 of gestation. Ninety-four women served as controls. All infants received standard active-passive immunization. Seven months after delivery, MTCT was significantly lower in the infants born to the LdT treated mothers than to controls (0% *vs* 8%). No congenital abnormalities were identified[14]. Similar efficacy and safety data has been provided by other studies, wherein the rate of MTCT was 8.6% in the placebo group *vs* 0% in the treated group[19]. In a larger Chinese study, 648 women with high viral load were randomized to receive LAM, LdT, or placebo from 28 wk gestation until 4 wk postpartum. On treatment analysis indicated 0% of HBsAg positive infants in the treated group *vs* 2.84% in the placebo group. There were no safety concerns identified in this large study[20].

The utility of TDF for the prevention of MTCT was first demonstrated in a small case series, in which women with greater than $10^7$ copies/mL of HBV DNA were given TDF 300 mg daily in the third trimester. The median duration of TDF use was 10 wk. All infants received active-passive immunization; all infants were HBsAg negative 28-36 wk after birth[13]. A larger multicenter prospective study demonstrated that MTCT was reduced to 2% in a group of high viral load women treated with TDF 300 mg daily for a mean of 58 d before delivery. Within the same study population, the rate of MTCT was 20% in the controls and 0% in the LAM cohort[21]. Although there have been limited studies regarding TDF and hepatitis B in pregnancy, there is significant safety data within human immunodeficiency virus/HBV coinfection cohorts supporting safe use of TDF in pregnancy[22].

In summary, there is a growing body of literature supporting the safety and efficacy of antiviral therapies to reduce MTCT of hepatitis B. This is especially efficacious in women with known risk factors for MTCT as discussed elsewhere in this paper. There are no formal recommendations regarding which agent to choose. As listed in Table 1, LdT and TDF are the United States Food and Drug Administration (FDA) pregnancy category B medications (animal studies without demonstrable risk to the fetus), while LAM is a category C medication (adverse risk to fetus in animal studies). Table 2 further summarizes the articles that report on medication options discussed above. Based on available evidence, the previously mentioned drugs have minimal reported side effects to mothers or newborns, but have not yet been approved for standard use in pregnancy. Interferon is contraindicated during pregnancy and the safety of entecavir (a FDA category C medication) is unknown. If indicated, antiviral therapy should be initiated in the early third trimester as previously discussed.

## WHEN TO STOP TREATMENT?

The dosing and administration of immunoprophylaxis has been previously discussed, the third and last dose of HBV vaccine should be completed by 9 mo of age[5].

Concerning mothers that began antiviral medication due to above listed indications during the third trimester, discontinuation of the antivirals is not necessarily intuitive. If the initial indication for antivirals was due to high viral load or active disease, then the medication can be continued, following the current guidelines for treatment of chronic HBV infection[23,24]. If the indication for therapy was to simply reduce MTCT, the antivirals may be discontinued immediately if patient is breastfeeding, or within first four weeks if infant is being formula fed[9].

At this time, HBsAg positivity is not a contraindication to breastfeeding as evidence has shown that the risk of transmission is low[25]. Although the active metabolite of most antivirals is not expressed in breast milk, breastfeeding on antiviral medication is not recommended[12].

Pregnancy that occurs in women on long term

**Table 2 Landmark study results of nucleos(t)ide analogues in hepatitis B virus infection and pregnancy**

| Ref. | Medication | Treatment timing | Major adverse events | % of transmission | |
|---|---|---|---|---|---|
| | | | | Treatment group | Control group |
| Han *et al*[14] | LdT 600 mg daily | 20 to 32 wk | 0% | 0% | 8% |
| van Zonneveld *et al*[15] | LAM 150 mg daily | 34 wk | 0% | 12.5% | 28% |
| Pan *et al*[19] | LdT 300 mg daily | 23 wk | 0% | 0% | 8.6% |
| Zhang *et al*[20] | LdT 600 mg daily or LAM 100 mg daily | 28 wk | 0% | 0% | 2.8% |
| Greenup *et al*[21] | TDF 300 mg daily | 32 wk | 0% | 1.1% | 20% |

LAM: Lamivudine; LdT: Telbivudine; TDF: Tenofovir.

treatment for chronic HBV infection is a topic not yet discussed in this review. Due to concern for adverse events from antiviral therapy early in pregnancy, consideration to stop the medication during the first two trimesters must be made. However, if the patient that has become pregnant has significant chronic liver disease due to HBV infection, risk of discontinuation of antiviral is of larger concern and thus antiviral is typically continued throughout pregnancy[25].

There is a theoretical risk of a postpartum HBV flare with the withdrawal of antiviral therapy. This flare is typically defined as a significant rise in transaminases above upper limit of normal. Nguyen *et al*[26] studied the effects of continuing antivirals two weeks after delivery *vs* twelve weeks after delivery, compared to patients that opted out of antiviral therapy altogether. In this study, there was no significant difference in occurrence of postpartum flares among the three groups and spontaneous resolution of the flare occurred equally among the groups as well. Another retrospective cohort study revealed similar findings[27]. It can therefore safely be concluded that the decision to start or stop antiviral therapy should not be made based on concern for possible postpartum HBV flare. Nonetheless, it is important to monitor postpartum mothers closely for at least 6 mo, especially those who are HBeAg-positive or have stopped antiviral therapy[28].

## CONCLUSION

In conclusion, it is important to remember that patients chronically infected with HBV that become pregnant will need additional care and consideration. All infants in the United States should receive active and passive immunization at birth, as recommended by the Centers for Disease Control and Prevention[5]. Pregnant women with chronic HBV infection that have high viral loads or elevated transaminases suggesting active viral replication are at risk of MTCT despite immunoprophylaxis. This patient population should consider taking antiviral medication in the third trimester of pregnancy to decrease risk of MTCT. TDF, LAM and LdT have all been proven efficacious in decreasing viral load at birth without known birth defects, but final decision of which antiviral medication to use will have to be determined by physician and patient.

Despite its prevalence and the availability of safe treatment, there are no current consensus guidelines regarding prevention of MTCT. Large randomized studies should be conducted to further characterize the most effective method to minimize the transmission of HBV to future generations. In the interim, although it is not a frequent scenario for most gastroenterologists to encounter, with these brief recommendations; management of chronic HBV in pregnancy should be less daunting.

## REFERENCES


1 **Patton H**, Tran TT. Management of hepatitis B during pregnancy. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 402-409 [PMID: 24686270 DOI: 10.1038/nrgastro.2014.30]

2 **Degertekin B**, Lok AS. Indications for therapy in hepatitis B. *Hepatology* 2009; **49**: S129-S137 [PMID: 19399799 DOI: 10.1002/hep.22931]

3 **Dyson JK**, Waller J, Turley A, Michael E, Moses S, Valappil M, Hudson M, Bassendine M, McPherson S. Hepatitis B in pregnancy. *Frontline Gastroenterol* 2014; **5**: 111-117 [PMID: 24683447 DOI: 10.1136/flgastro-2013-100361]

4 **Sarkar M**, Terrault NA. Ending vertical transmission of hepatitis B: the third trimester intervention. *Hepatology* 2014; **60**: 448-451 [PMID: 24668789 DOI: 10.1002/hep.27145]

5 **Mast EE**, Margolis HS, Fiore AE, Brink EW, Goldstein ST, Wang SA, Moyer LA, Bell BP, Alter MJ. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. *MMWR Recomm Rep* 2005; **54**: 1-31 [PMID: 16371945]

6 **Chen HL**, Lin LH, Hu FC, Lee JT, Lin WT, Yang YJ, Huang FC, Wu SF, Chen SC, Wen WH, Chu CH, Ni YH, Hsu HY, Tsai PL, Chiang CL, Shyu MK, Lee PI, Chang FY, Chang MH. Effects of maternal screening and universal immunization to prevent mother-to-infant transmission of HBV. *Gastroenterology* 2012; **142**: 773-781.e2 [PMID: 22198276 DOI: 10.1053/j.gastro.2011.12.035]

7 **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]

8 **Shi Z**, Yang Y, Wang H, Ma L, Schreiber A, Li X, Sun W, Zhao X, Yang X, Zhang L, Lu W, Teng J, An Y. Breastfeeding of newborns by mothers carrying hepatitis B virus: a meta-analysis and systematic review. *Arch Pediatr Adolesc Med* 2011; **165**: 837-846 [PMID: 21536948 DOI: 10.1001/archpediatrics.2011.72]

9 **Whittaker G**, Herrera JL. Hepatitis B in pregnancy. *South Med J* 2014; **107**: 195-200 [PMID: 24937341 DOI: 10.1097/SMJ.0000000000000077]

10 **Xu DZ**, Yan YP, Choi BC, Xu JQ, Men K, Zhang JX, Liu ZH, Wang FS. Risk factors and mechanism of transplacental transmission of hepatitis B virus: a case-control study. *J Med Virol* 2002; **67**: 20-26 [PMID: 11920813 DOI: 10.1002/jmv.2187]

11 **Wiseman E**, Fraser MA, Holden S, Glass A, Kidson BL, Heron LG, Maley MW, Ayres A, Locarnini SA, Levy MT. Perinatal transmission of hepatitis B virus: an Australian experience. *Med J*

*Aust* 2009; **190**: 489-492 [PMID: 19413519]
12 **Buchanan C**, Tran TT. Management of chronic hepatitis B in pregnancy. *Clin Liver Dis* 2010; **14**: 495-504 [PMID: 20638027 DOI: 10.1016/j.cld.2010.05.008]
13 **Pan CQ**, Mi LJ, Bunchorntavakul C, Karsdon J, Huang WM, Singhvi G, Ghany MG, Reddy KR. Tenofovir disoproxil fumarate for prevention of vertical transmission of hepatitis B virus infection by highly viremic pregnant women: a case series. *Dig Dis Sci* 2012; **57**: 2423-2429 [PMID: 22543886 DOI: 10.1007/s10620-012-2187-3]
14 **Han GR**, Cao MK, Zhao W, Jiang HX, Wang CM, Bai SF, Yue X, Wang GJ, Tang X, Fang ZX. A prospective and open-label study for the efficacy and safety of telbivudine in pregnancy for the prevention of perinatal transmission of hepatitis B virus infection. *J Hepatol* 2011; **55**: 1215-1221 [PMID: 21703206 DOI: 10.1016/j.jhep.2011.02.032]
15 **van Zonneveld M**, van Nunen AB, Niesters HG, de Man RA, Schalm SW, Janssen HL. Lamivudine treatment during pregnancy to prevent perinatal transmission of hepatitis B virus infection. *J Viral Hepat* 2003; **10**: 294-297 [PMID: 12823596 DOI: 10.1046/j.1365-2893.2003.00440.x]
16 **del Canho R**, Grosheide PM, Mazel JA, Heijtink RA, Hop WC, Gerards LJ, de Gast GC, Fetter WP, Zwijneberg J, Schalm SW. Ten-year neonatal hepatitis B vaccination program, The Netherlands, 1982-1992: protective efficacy and long-term immunogenicity. *Vaccine* 1997; **15**: 1624-1630 [PMID: 9364693 DOI: 10.1016/S0264-410X(97)00080-7]
17 **Brown RS**, Verna EC, Pereira MR, Tilson HH, Aguilar C, Leu CS, Buti M, Fagan EA. Hepatitis B virus and human immunodeficiency virus drugs in pregnancy: findings from the Antiretroviral Pregnancy Registry. *J Hepatol* 2012; **57**: 953-959 [PMID: 22766470 DOI: 10.1016/j.jhep.2012.06.031]
18 **Xu WM**, Cui YT, Wang L, Yang H, Liang ZQ, Li XM, Zhang SL, Qiao FY, Campbell F, Chang CN, Gardner S, Atkins M. Lamivudine in late pregnancy to prevent perinatal transmission of hepatitis B virus infection: a multicentre, randomized, double-blind, placebo-controlled study. *J Viral Hepat* 2009; **16**: 94-103 [PMID: 19175878 DOI: 10.1111/j.1365-2893.2008.01056.x]
19 **Pan CQ**, Han GR, Jiang HX, Zhao W, Cao MK, Wang CM, Yue X, Wang GJ. Telbivudine prevents vertical transmission from HBeAg-positive women with chronic hepatitis B. *Clin Gastroenterol Hepatol* 2012; **10**: 520-526 [PMID: 22343511 DOI: 10.1016/j.cgh.2012.01.019]
20 **Zhang H**, Pan CQ, Pang Q, Tian R, Yan M, Liu X. Telbivudine or lamivudine use in late pregnancy safely reduces perinatal transmission of hepatitis B virus in real-life practice. *Hepatology* 2014; Epub ahead of print [PMID: 25227594 DOI: 10.1002/hep.27034]
21 **Greenup AJ**, Tan PK, Nguyen V, Glass A, Davison S, Chatterjee U, Holdaway S, Samarasinghe D, Jackson K, Locarnini SA, Levy MT. Efficacy and safety of tenofovir disoproxil fumarate in pregnancy to prevent perinatal transmission of hepatitis B virus. *J Hepatol* 2014; **61**: 502-507 [PMID: 24801414 DOI: 10.1016/j.jhep.2014.04.038]
22 **Wang L**, Kourtis AP, Ellington S, Legardy-Williams J, Bulterys M. Safety of tenofovir during pregnancy for the mother and fetus: a systematic review. *Clin Infect Dis* 2013; **57**: 1773-1781 [PMID: 24046310 DOI: 10.1093/cid/cit601]
23 **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]
24 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]
25 **Bzowej NH**. Optimal Management of the Hepatitis B Patient Who Desires Pregnancy or Is Pregnant. *Curr Hepat Rep* 2012; **11**: 82-89 [PMID: 22707918 DOI: 10.1007/s11901-012-0130-x]
26 **Nguyen V**, Tan PK, Greenup AJ, Glass A, Davison S, Samarasinghe D, Holdaway S, Strasser SI, Chatterjee U, Jackson K, Locarnini SA, Levy MT. Anti-viral therapy for prevention of perinatal HBV transmission: extending therapy beyond birth does not protect against post-partum flare. *Aliment Pharmacol Ther* 2014; **39**: 1225-1234 [PMID: 24666381 DOI: 10.1111/apt.12726]
27 **ter Borg MJ**, Leemans WF, de Man RA, Janssen HL. Exacerbation of chronic hepatitis B infection after delivery. *J Viral Hepat* 2008; **15**: 37-41 [PMID: 18088243]
28 **Chang ML**, Liaw YF. Hepatitis B flares in chronic hepatitis B: pathogenesis, natural course, and management. *J Hepatol* 2014; **61**: 1407-1417 [PMID: 25178562 DOI: 10.1016/j.jhep.2014.08.033]

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MINIREVIEWS

# Anticoagulation and antiplatelets as prophylaxis for hepatic artery thrombosis after liver transplantation

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## Abstract

Hepatic artery thrombosis (HAT) is the most serious vascular complication after liver transplantation. Multiple risk factors have been identified to impact its development. Changes in haemostasis associated with end stage liver disease and the disturbance of the coagulation and anticoagulation cascades play an important role in development of this lethal complication. Early recognition and therapeutic intervention is mandatory to avoid its consequences. Pharmacological prophylaxis, by the use of antiplatelet or anticoagulant agents, is an important tool to reduce its incidence and prevent graft loss. Only a few studies have shown a clear benefit of antiplatelet agents in reducing HAT occurrence, however, these studies are limited by being retrospective and by inhomogeneous populations. The use of anticoagulants such as heparin is associated with an improvement in the outcomes mainly when used for a high-risk patients like living related liver recipients. The major concern when using these agents is the tendency to increase bleeding complications in a setting of already unstable haemostasis. Hence, monitoring of their administration and careful selection of patients to be treated are of great importance. Well-designed clinical studies are still needed to further explore their effects and to formulate proper protocols that can be implemented safely.

**Key words:** Hepatic artery thrombosis; Haemostasis; Anticoagulation; Liver transplantation; Antiplatelets; Heparin



**Core tip:** Hepatic artery thrombosis (HAT) is the most serious vascular complication after liver transplantation. Changes in haemostasis associated with liver disease play a role in its development. Pharmacological prophylaxis may reduce its incidence and prevent graft loss. Few studies have shown a clear benefit of antiplatelets in reducing HAT occurrence, however, these studies have several limitations. The use of anticoagulants showed an improvement in the outcomes when used for high-risk patients. Their major concern is the tendency to increase bleeding complication. Hence, monitoring of their administration and careful selection of patients to be treated are of great importance.

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## INTRODUCTION

Vascular complications after liver transplantation (LT) are common despite the progressive improvement and innovations in anastomotic vascular techniques. They frequently result in hepatic failure and graft loss and thus the need for re-transplantation. Hepatic artery thrombosis (HAT) after LT remains the most serious vascular complication with an overall incidence varies from 2% to 9%[1]. It is usually associated with around 50% rate of mortality or re-transplantation[2].

The mechanism of HAT development is not fully understood. It is believed to be multifactorial including both operative and non-operative factors (Table 1)[3,4]. All these factors are involved in causing recipient hypercoagulability and to disturb the unstable rebalanced haemostasis that usually occurs in liver disease patients leading to a rise in the incidence of HAT[5,6].

An early diagnosis with immediate treatment is the key to proper management of HAT. Therapeutic options for managing HAT include either revascularization of the transplanted graft or re transplantation[7]. Revascularization can be achieved through arterial reconstruction, surgical thrombectomy, or radiologically guided thrombolysis. Shortage of organs and poor patients conditions may lead physicians to consider revascularization, which can achieve good success rates[8]. Re-transplantation is still the gold standard treatment for HAT but is confounded by both graft availability and the patient's general condition.

Prophylaxis against HAT in the early postoperative period by usage of antiplatelet or anticoagulant agents is thought to be hazardous by many surgeons because of the risk of postoperative bleeding. However, post-operative pharmacological prophylaxis can reduce the risk of arterial anastomosis thrombosis when used in vascular surgical procedures[9]. The adoption of this in LT could help in reducing the incidence of HAT.

## HAEMOSTASIS IN END STAGE LIVER DISEASE

In liver transplant recipient with cirrhosis, considerable changes in the haemostatic system are often found[10]. These changes include thrombocytopenia, platelet functional defects, decreased circulating levels of coagulative factors and decreased fibrinolytic proteins. Although routine diagnostic tests of haemostasis are commonly abnormal, interpretation of these tests is more difficult in patients with a complex haemostatic disorder as in cirrhotic patients. It is now well established that patients with cirrhosis and abnormal routine coagulation tests might not have an increased bleeding tendency and that thrombotic complications may still occur[11]. This happens through a state of a rebalanced haemostasis where the changes in the pro-haemostatic pathways are associated with changes in the anti-haemostatic pathways (Figure 1)[12]. The changes that promote bleeding include thrombocytopenia, platelet function defects, enhanced production of nitric oxide and prostacyclin, low levels of factors Ⅱ, Ⅴ, Ⅶ, Ⅸ, Ⅹ, and Ⅺ, vitamin K deficiency, dysfibrinogenemia, low levels of a2-antiplasmin, factor Ⅻ and thrombin-activatable fibrinolysis inhibitor and elevated tissue plasminogen activator level. Those alterations are encountered by others that counteract bleeding such as: elevated levels of von Willebrand factor, decreased levels of ADAMTS-13 (von Willebrand factor cleaving protease), elevated levels of factor Ⅷ, decreased levels of protein C, protein S, antithrombin, a2-macroglobulin and heparin cofactor Ⅱ and low levels of plasminogen[5,12].

The resultant effect of all these changes is a rebalanced haemostasis. Although it is a functional system, it is unstable compared to the haemostatic balance in healthy individuals, explaining the potential occurrence of both bleeding and thrombotic complications in patients with cirrhosis[12].

## CHANGES IN THE HAEMOSTATIC BALANCE AFTER LT

In the first days after LT, multiple transplantation-related triggers initiate pro-coagulation, anticoagulation and pro-fibrinolytic cascades. Theses mechanisms might not be balanced properly leading to thrombosis in the area of anastomosis (Figure 1). The triggers include substantial surgical damage, stasis as a result of clamping of major vessels, release of activators from the donor liver and systemic inflammatory responses. Other factors are shown in Table 1[6].

Intraoperatively, the graft endothelium gets activated due to cold ischemia and reperfusion effects. As soon as the reperfusion happens, platelets adhere to the sinusoidal endothelium, which contributes to endothelial cell apoptosis causing more ischemia/reperfusion damage. This process will probably expose additional pro-coagulant triggers causing further platelet activation and initiation of coagulation[13]. As long as these pro-coagulant triggers go on, thrombosis can easily occur. However, the activated fibrinolytic system removes the clots immediately as they are formed. When this balance is disturbed, a state of hypercoagulability arises and a clinically evident thrombus might form in the anastomotic areas[14].

In addition, an acquired hypercoagulability may increase the risk for immediate graft thrombosis. It can be due to transplanting a liver from a donor with factor Ⅴ Leiden mutation or infections like cytomegalovirus

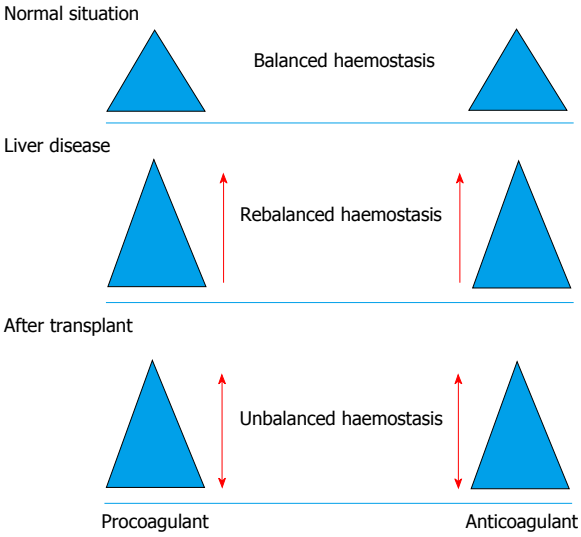


**Figure 1 Changes in haemostasis in normal situation, liver disease and after liver transplantation.**

(CMV). Perioperative haemostatic agent such as fresh frozen plasma, platelets, recombinant factor Ⅶa, and anti-fibrinolytics such as aprotinin and aminocaproic acid plays role as well (Table 2)[12].

Mechanisms of late thrombosis of the graft vascular anastomosis or even systemic thrombosis are less obvious. It could be because of pro-thrombotic side effect of immunosuppressant or as consequences of medical comorbidities like hypertension, hypercholesterolemia and diabetes mellitus. It could happen due to viral infection as well especially CMV[15].

Liver diseases are classically portrayed as having a substantial bleeding tendency. For this reasons, the postoperative use of anticoagulants or antiplatelets has been restricted, in order to limit bleeding complications. However, recent data showed that the bleeding diathesis of patients with liver disease is not only a result of poor haemostasis[16]. Other factors may play a significant role and augment bleeding tendency. Portal hypertension is an example of that. The minimal blood loss and the decreasing requirements of coagulation products in nowadays transplant support this concept. Moreover, improvements in surgical techniques and a restrictive fluid and transfusion protocols has led to a reduction in perioperative transfusion requirements[17]. Considering this fact, the limited use of anticoagulants or antiplatelets in the postoperative period is reconsidered.

## USE OF ANTIPLATELETS IN LT

In the immediate periods after LT, the constant platelet activation and aggregation result in development of thromboxane leading to fibrinogen activation, which contribute to arterial thrombosis and graft failure[18]. Because of this pivotal role of platelets, antiplatelets therapy by inhibiting this platelet-activation may lead to reduction in the incidence of such complication. Aspirin, which is the most frequent used antiplatelet

**Table 1 Risk factors of hepatic artery thrombosis**

| Operative factors | Non operative factors |
|---|---|
| Surgical technique | Donor age of more than 60 yr |
| Number of anastomosis | Long cold ischemia time |
| Use of conduits | Preservation damage |
| Vessels kinking | Lack of blood group compatibility |
| Small vessels size | Cytomegalovirus-positive donor status |
| | Hypercoagulable recipient status |
| | Recipient cigarette smoking |
| | Rejection |
| | Primary sclerosing cholangitis |

**Table 2 Factors involved in development of hypercoagulability after liver transplantation**

| Endogenous | Acquired |
|---|---|
| Substantial surgical damage | Donor with factor V Leiden mutation |
| Stasis as a result of clamping of major vessels | Infections |
| Release of activators from the donor liver | Viral infection like cytomegalovirus |
| Systemic inflammatory responses | Perioperative haemostatic agent |
| The quality of the graft | Fresh frozen plasma |
| The length of surgery | Platelets |
| The technique of graft preservation | Recombinant factor Ⅶa |
| Cold ischemia/reperfusion effect | Anti-fibrinolytics |
| | Aprotinin Aminocaproic acid |

agents, interferes with platelet aggregation, which in turn leads to an endothelial cell-mediated inhibition of the coagulation cascade. This happens through irreversible inhibition of cyclooxygenase 1 and hence, inhibition of thromboxane generation[19].

Very few studies focused on the efficacy and safety of antiplatelet therapy in patients after LT. In 1997, in a retrospective study, Wolf *et al*[20] found no significant difference in the incidence of early HAT (3.7%) *vs* (4.0%) between patients who had a prophylaxis aspirin (354 of 529 patients) and those who did not. Bleeding events occurred in 1.1% of patients treated with aspirin compared to 0.6% of the control group. The effect of aspirin prophylaxis on late HAT was not investigated[20].

Vivarelli *et al*[21] reported a single centre retrospective study where they looked to the effect of long-term aspirin administration (100 mg) on the incidence of late HAT in a large number of patients. In this study, they categorize the patients into high and low risk groups based on their finding on a previous study. One of two independent risk factors was used to categorize the patients into either group: grafts retrieved from donors who died from a cerebrovascular accident or the use of an arterial iliac conduit. Late HAT incidence was 3.6% (12 of 338) of the high-risk patients who were not given aspirin, whilst it was seen in only 0.6% (1 of 160) of the high-risk patients who received aspirin prophylaxis. The relative risk reduction was 82%. In the low-risk group, 1 of 330 patients developed late HAT and he was in the non-prophylaxis arm (254 patients). Out of 236 patients

who received aspirin, there was no recorded episode of any bleeding complications throughout the follow up period (median of 1704 d). They could not assess the effect of antiplatelets on early HAT due to their inability to start patients immediately on aspirin with a known impaired coagulative function and a high risk of bleeding. This obvious benefit of aspirin on late HAT occurrence should be considered specifically in patients with an absence of bleeding complications.

Shay *et al*[22] showed that aspirin prophylaxis is safe and effective in decreasing early HAT in adult recipients. The incidence of overall HAT was found to be significantly higher at 4.9% in the control group *vs* 3.0% in the treated group. Early HAT incidence dropped from 3.9% in the control group to 1.8% with aspirin prophylaxis. Also the incidence of early HAT causing graft loss decreased significantly from 3.6% to 0% with the use of early aspirin prophylaxis. There was no difference in bleeding complications between the two groups. The main difference between these studies is the dosage and the time of initiation of therapy. Shay *et al*[22] used a higher dosage at 325 mg/d, initiated immediately after surgery with no evidence of significant bleeding.

There are several limitations to these studies that should be considered. First, not all of them include paediatric patients, where the incidence of HAT is higher and survival is better. Secondly, they were all retrospective studies with a higher risk of bias. Also the compared groups were mostly from different time periods where surgical techniques, donor selection, and postoperative management are different. However, these studies showed evidence of benefit from the use of antiplatelet agents as a prophylaxis for early and late HAT without increasing the risk of bleeding or other surgical complications. The current usage of antiplatelet agents in treatment of cardiovascular and peripheral vascular disease might support this conclusion. A further well-designed randomized study to explore this field would be appropriate.

## USE OF ANTICOAGULANTS IN LT

The plasma concentration of coagulant and anti-coagulant proteins is disturbed after LT. This imbalance leads to hypercoagulability condition that contributes to vascular thrombosis and possible loss of the graft[14]. The reducing level of antithrombin Ⅲ and protein C are believed to be responsible for the development of this hypercoagulability state[23]. For this reason, supplementation of protein C and augmenting the effect of antithrombin Ⅲ could help to overcome this haemostatic disturbance.

Fresh frozen plasma (FFP) is usually the source of protein C along with other clotting factors. Hashikura *et al*[24] reported that infusing FFP was helpful in reducing incidence of HAT and maintaining coagulation haemostasis. In contrast, Mazzaferro *et al*[25] found a higher incidence of HAT in paediatric liver recipient who received more FFP compared to those who did not receive FFP intraoperatively. Hatano *et al*[26] found a similar result where the occurrence of HAT was associated with a higher intraoperative infusion of FFP. Although the use of FFP might be associated with an increased risk of HAT, its usage is still warranted to maintain haemostasis and to reduce bleeding complication. Excessive use of FFP should be avoided as it might induce a state of hypercoagulability and could result in graft thrombosis.

Heparin appears to be the method of choice to anticoagulate liver transplant recipients. Most liver transplant centres have developed their own protocols for heparin infusions and the monitoring of its activity. It is important to note that the rationales of these protocols remain unclear, as they are designed based on empirical rules. The Shinshu group demonstrated that intensive anticoagulation should be established in living related liver transplantation (LDLT) in a paediatric Population[24]. Sugawara *et al*[27] showed a similar result in the setting of living donor LT. The reports on usage of heparin in cadaveric liver transplant are very limited to date. The anti-coagulatory effect of the unfractionated heparin (UFH) is known to be through its antithrombin Ⅲ activity. The low molecular weight heparin (LMWH) selectively inhibit clotting factor Ⅹ a and to a lesser extent augment antithrombin Ⅲ activity[28].

Bleeding complication can occur with the unmonitored use of heparin. Kaneko *et al*[28] reported that 9% of their living related liver recipients who used UFH developed haemorrhagic complications that required surgical treatment. In contrast to UFH, using LMWH is believed to be useful to reduce haemorrhagic complications due to its selective inhibition of coagulation factor Xa and because of its reduced ability to bind to endogenous plasma proteins such as platelet factor 4 and von Willebrand factor[29]. In addition, LMWH lessen liver damage in ischemia-reperfusion injury and hyperperfusion[30]. For these reasons, LMWH is more advantageous than UFH for intraoperative and postoperative anticoagulant therapy in LT. The major problem for the prophylactic use of LMWH is determining the optimal dose and monitoring serum factor Ⅹa activity. The route of administration of LMWH depends on the indication. In patients with a high bleeding tendency, such as liver transplant recipients, an adjustable continuous infusion may be recommended to avoid increased plasma levels and to cope with continuous pathophysiological changes in the coagulation cascade[31].

Antithrombin is plasma glycoprotein synthesized in the liver and plays a major role in the coagulation after LT. The level of antithrombin Ⅲ drops after transplant and remains low for up to two weeks. Replacing this drop might help to gain an adequate anticoagulation effect[32]. Kaneko *et al*[32] in his pilot study found that the combined use of AT and UFH might reduce fibrin

degradation product D-dimer levels and prevent a postoperative drop in the platelet count. Taniai *et al*[33] concluded in his study that adding antithrombin to heparin could be the best approach. Further research should be done to prove its benefit and safety in LT[33].

Monitoring of anticoagulation after LT assures an adequate level without an increase in the bleeding complications. Activated clotting time (ACT) is the most commonly used monitoring method. Linkins *et al*[34] showed that UFH is more sensitive than LMWH in regards to ACT level. This indicates that the ACT cannot monitor the activity of factor Xa but is still a valuable tool in monitoring the anticoagulant effects of LMWH. The lower level of antithrombin activity in the LMWH compared to the anti-Xa activity could explain this lower sensitivity[29]. It has been shown that ACT can monitor the anticoagulatory effect of LMWH in coronary intervention procedures[35]. Uchikawa *et al*[31] showed that ACT measurement is a simple, reliable method for bedside monitoring of LMWH anticoagulant effects for LDLT. It is assumed that ACT level should be kept within the normal range in order to prevent haemorrhagic complication. Thus, the anticipated value of ACT is between 140 and 150 s, which is the upper limit of the normal range. However, Kaneko *et al*[32] tested the ACT level by measuring plasmin-alpha2 plasmin inhibitor complex, thrombin-antithrombin Ⅲ complex and fibrin degradation product pre and postoperatively with measurement of complete blood count, ACT, activated partial thromboplastin time and prothrombin time international normalized ratio for two weeks after surgery. They conclude that frequent monitoring of ACT is necessary to keep the ACT level in the target range in the first postoperative week. Because of hyperfibrinolytic condition and the high rate of haemorrhagic complications after the first week, the dose of heparin should be adjusted to maintain lower ACT levels during this period.

In contrast to UFH, LMWH are cleared by renal route. Hence in patients with impaired renal functions, as commonly seen following LT, monitoring and adjustment of the dose according to the degree of renal injury are required. Observational studies have shown more bleeding complications in renal impaired patients[36]. Prophylactic LMWH doesn't appear to increase the bleeding tendency and therefore might not need monitoring or adjustment[37]. Therapeutic LMWH bio accumulates and cause more bleeding if left unadjusted[38]. This bioaccumulation is more evident when creatinine clearance is less than 30 mL/min[38]. No clear guidelines are available for dose adjustment and further clinical and pharmacological studies are required for dosage guidance[39].

It is clear that a further study looking to the appropriate protocols of anticoagulation and the proper monitoring tools is needed. The rarity of studies and the empirical assumption of tailoring the protocols make drawing a conclusion difficult.

## CONCLUSION

Pharmacological prophylaxis is probably beneficial in reducing the incidence of HAT. It is relatively safe if used carefully with a continuous monitoring and adjustment. Proper protocols need to be developed based on proper well-designed clinical studies.

## REFERENCES


1 **Mourad MM**, Liossis C, Gunson BK, Mergental H, Isaac J, Muiesan P, Mirza DF, Perera MT, Bramhall SR. Etiology and management of hepatic artery thrombosis after adult liver transplantation. *Liver Transpl* 2014; **20**: 713-723 [PMID: 24652787 DOI: 10.1002/lt.23874]

2 **Stange BJ**, Glanemann M, Nuessler NC, Settmacher U, Steinmüller T, Neuhaus P. Hepatic artery thrombosis after adult liver transplantation. *Liver Transpl* 2003; **9**: 612-620 [PMID: 12783404 DOI: 10.1053/jlts.2003.50098]

3 **Bekker J**, Ploem S, de Jong KP. Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. *Am J Transplant* 2009; **9**: 746-757 [PMID: 19298450 DOI: 10.1111/j.1600-6143.2008.02541.x]

4 **Grodzicki M**, Anysz-Grodzicka A, Remiszewski P, Cieślak B, Kotulski M, Kalinowski P, Paluszkiewicz R, Rowiński O, Krawczyk M. Treatment of early hepatic artery thrombosis after liver transplantation. *Transplant Proc* 2011; **43**: 3039-3042 [PMID: 21996219 DOI: 10.1016/j.transproceed.2011.08.028]

5 **Tripodi A**, Primignani M, Chantarangkul V, Dell'Era A, Clerici M, de Franchis R, Colombo M, Mannucci PM. An imbalance of pro- vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology* 2009; **137**: 2105-2111 [PMID: 19706293 DOI: 10.1053/j.gastro.2009.08.045]

6 **Pastacaldi S**, Teixeira R, Montalto P, Rolles K, Burroughs AK. Hepatic artery thrombosis after orthotopic liver transplantation: a review of nonsurgical causes. *Liver Transpl* 2001; **7**: 75-81 [PMID: 11172388 DOI: 10.1053/jlts.2001.22040]

7 **Tian MG**, Tso WK, Lo CM, Liu CL, Fan ST. Treatment of hepatic artery thrombosis after orthotopic liver transplantation. *Asian J Surg* 2004; **27**: 213-217; discussion 218 [PMID: 15564163 DOI: 10.1016/S1015-9584(09)60035-X]

8 **Boyvat F**, Aytekin C, Harman A, Sevmiş S, Karakayali H, Haberal M. Endovascular stent placement in patients with hepatic artery stenoses or thromboses after liver transplant. *Transplant Proc* 2008; **40**: 22-26 [PMID: 18261538 DOI: 10.1016/j.transproceed.2007.12.027]

9 **Cadroy Y**, Bossavy JP, Thalamas C, Sagnard L, Sakariassen K, Boneu B. Early potent antithrombotic effect with combined aspirin and a loading dose of clopidogrel on experimental arterial thrombogenesis in humans. *Circulation* 2000; **101**: 2823-2828 [PMID: 10859288]

10 **Lisman T**, Leebeek FW, de Groot PG. Haemostatic abnormalities in patients with liver disease. *J Hepatol* 2002; **37**: 280-287 [PMID: 12127437]

11 **Lisman T**, Leebeek FW. Hemostatic alterations in liver disease: a review on pathophysiology, clinical consequences, and treatment. *Dig Surg* 2007; **24**: 250-258 [PMID: 17657149 DOI: 10.1159/000103655]

12 **Lisman T**, Porte RJ. Antiplatelet medication after liver transplantation: does it affect outcome? *Liver Transpl* 2007; **13**: 644-646 [PMID: 17457859 DOI: 10.1002/lt.21063]

13 **Sindram D**, Porte RJ, Hoffman MR, Bentley RC, Clavien PA. Platelets induce sinusoidal endothelial cell apoptosis upon reperfusion of the cold ischemic rat liver. *Gastroenterology* 2000; **118**: 183-191 [PMID: 10611167]

14 **Gologorsky E**, De Wolf AM, Scott V, Aggarwal S, Dishart M, Kang Y. Intracardiac thrombus formation and pulmonary thromboembolism immediately after graft reperfusion in 7 patients undergoing liver transplantation. *Liver Transpl* 2001; **7**: 783-789 [PMID: 11552212 DOI: 10.1053/jlts.2001.26928]

15 **Johnston SD**, Morris JK, Cramb R, Gunson BK, Neuberger J. Cardiovascular morbidity and mortality after orthotopic liver transplantation. *Transplantation* 2002; **73**: 901-906 [PMID: 11923689]

16 **Lisman T**, Caldwell SH, Leebeek FW, Porte RJ. Is chronic liver disease associated with a bleeding diathesis? *J Thromb Haemost* 2006; **4**: 2059-2060 [PMID: 16961613 DOI: 10.1111/j.1538-7836.2006.02075.x]

17 **de Boer MT**, Molenaar IQ, Hendriks HG, Slooff MJ, Porte RJ. Minimizing blood loss in liver transplantation: progress through research and evolution of techniques. *Dig Surg* 2005; **22**: 265-275 [PMID: 16174983 DOI: 10.1159/000088056]

18 **Patrono C**, Rocca B. Drug insight: aspirin resistance--fact or fashion? *Nat Clin Pract Cardiovasc Med* 2007; **4**: 42-50 [PMID: 17180149 DOI: 10.1038/ncpcardio0728]

19 **Fritsma GA**, Ens GE, Alvord MA, Carroll AA, Jensen R. Monitoring the antiplatelet action of aspirin. *JAAPA* 2001; **14**: 57-58, 61-62 [PMID: 11523339]

20 **Wolf DC**, Freni MA, Boccagni P, Mor E, Chodoff L, Birnbaum A, Miller CM, Schwartz ME, Bodenheimer HC. Low-dose aspirin therapy is associated with few side effects but does not prevent hepatic artery thrombosis in liver transplant recipients. *Liver Transpl Surg* 1997; **3**: 598-603 [PMID: 9404960]

21 **Vivarelli M**, La Barba G, Cucchetti A, Lauro A, Del Gaudio M, Ravaioli M, Grazi GL, Pinna AD. Can antiplatelet prophylaxis reduce the incidence of hepatic artery thrombosis after liver transplantation? *Liver Transpl* 2007; **13**: 651-654 [PMID: 17457885 DOI: 10.1002/lt.21028]

22 **Shay R**, Taber D, Pilch N, Meadows H, Tischer S, McGillicuddy J, Bratton C, Baliga P, Chavin K. Early aspirin therapy may reduce hepatic artery thrombosis in liver transplantation. *Transplant Proc* 2013; **45**: 330-334 [PMID: 23267805 DOI: 10.1016/j.transproceed.2012.05.075]

23 **Stahl RL**, Duncan A, Hooks MA, Henderson JM, Millikan WJ, Warren WD. A hypercoagulable state follows orthotopic liver transplantation. *Hepatology* 1990; **12**: 553-558 [PMID: 2401460]

24 **Hashikura Y**, Kawasaki S, Okumura N, Ishikawa S, Matsunami H, Ikegami T, Nakazawa Y, Makuuchi M. Prevention of hepatic artery thrombosis in pediatric liver transplantation. *Transplantation* 1995; **60**: 1109-1112 [PMID: 7482717]

25 **Mazzaferro V**, Esquivel CO, Makowka L, Belle S, Kahn D, Koneru B, Scantlebury VP, Stieber AC, Todo S, Tzakis AG. Hepatic artery thrombosis after pediatric liver transplantation--a medical or surgical event? *Transplantation* 1989; **47**: 971-977 [PMID: 2472028]

26 **Hatano E**, Terajima H, Yabe S, Asonuma K, Egawa H, Kiuchi T, Uemoto S, Inomata Y, Tanaka K, Yamaoka Y. Hepatic artery thrombosis in living related liver transplantation. *Transplantation* 1997; **64**: 1443-1446 [PMID: 9392309]

27 **Sugawara Y**, Kaneko J, Akamatsu N, Imamura H, Kokudo N, Makuuchi M. Anticoagulant therapy against hepatic artery thrombosis in living donor liver transplantation. *Transplant Proc* 2002; **34**: 3325-3326 [PMID: 12493462]

28 **Kaneko J**, Sugawara Y, Tamura S, Togashi J, Matsui Y, Akamatsu N, Kishi Y, Makuuchi M. Coagulation and fibrinolytic profiles and appropriate use of heparin after living-donor liver transplantation. *Clin Transplant* 2005; **19**: 804-809 [PMID: 16313329 DOI: 10.1111/j.1399-0012.2005.00425.x]

29 **Weitz JI**. Low-molecular-weight heparins. *N Engl J Med* 1997; **337**: 688-698 [PMID: 9278467 DOI: 10.1056/NEJM199709043371007]

30 **Harada N**, Okajima K, Uchiba M. Dalteparin, a low molecular weight heparin, attenuates inflammatory responses and reduces ischemia-reperfusion-induced liver injury in rats. *Crit Care Med* 2006; **34**: 1883-1891 [PMID: 16641616 DOI: 10.1097/01.CCM.0000220764.10155.03]

31 **Uchikawa Y**, Ikegami T, Masuda Y, Ohno Y, Mita A, Urata K, Nakazawa Y, Terada M, Miyagawa S. Administration of dalteparin based on the activated clotting time for prophylaxis of hepatic vessel thrombosis in living donor liver transplantation. *Transplant Proc* 2009; **41**: 3784-3790 [PMID: 19917388 DOI: 10.1016/j.transproceed.2009.04.011]

32 **Kaneko J**, Sugawara Y, Tamura S, Togashi J, Matsui Y, Makuuchi M. Antithrombin effect on coagulation and fibrinolytic profiles after living donor liver transplantation: a pilot study. *Int J Lab Hematol* 2009; **31**: 81-86 [PMID: 18727651 DOI: 10.1111/j.1751-553X.2007.01008.x]

33 **Taniai N**, Onda M, Tajiri T, Akimaru K, Yoshida H, Mamada Y, Yokomuro S, Mineta S, Yoshioka M, Hirakata A, Kawano Y, Mizuguchi Y. Anticoagulant therapy in living-related liver transplantation. *Transplant Proc* 2002; **34**: 2788-2790 [PMID: 12431612]

34 **Linkins LA**, Julian JA, Rischke J, Hirsh J, Weitz JI. In vitro comparison of the effect of heparin, enoxaparin and fondaparinux on tests of coagulation. *Thromb Res* 2002; **107**: 241-244 [PMID: 12479885]

35 **Marmur JD**, Anand SX, Bagga RS, Fareed J, Pan CM, Sharma SK, Richard MF. The activated clotting time can be used to monitor the low molecular weight heparin dalteparin after intravenous administration. *J Am Coll Cardiol* 2003; **41**: 394-402 [PMID: 12575965]

36 **Gerlach AT**, Pickworth KK, Seth SK, Tanna SB, Barnes JF. Enoxaparin and bleeding complications: a review in patients with and without renal insufficiency. *Pharmacotherapy* 2000; **20**: 771-775 [PMID: 10907967]

37 **Chan KE**, Thadhani RI, Maddux FW. No difference in bleeding risk between subcutaneous enoxaparin and heparin for thromboprophylaxis in end-stage renal disease. *Kidney Int* 2013; **84**: 555-561 [PMID: 23677243 DOI: 10.1038/ki.2013.152]

38 **Lim W**, Dentali F, Eikelboom JW, Crowther MA. Meta-analysis: low-molecular-weight heparin and bleeding in patients with severe renal insufficiency. *Ann Intern Med* 2006; **144**: 673-684 [PMID: 16670137]

39 **Lai S**, Coppola B. Use of enoxaparin in end-stage renal disease. *Kidney Int* 2013; **84**: 433-436 [PMID: 23989356 DOI: 10.1038/ki.2013.163]


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MINIREVIEWS

# New prognostic markers in liver cirrhosis

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## Abstract

Determining the prognosis of cirrhotic patients is not an easy task. Prognostic scores, like Child-Pugh and Model of End-stage Liver Disease scores, are commonly used by hepatologists, but do not always reflect superimposed events that may strongly influence the prognosis. Among them, bacterial intestinal translocation is a key phenomenon for the development of cirrhosis-related complications. Several biological variables (C-reactive protein, serum free cortisol, copeptin, von Willebrand factor antigen) are surrogates of "inflammatory stress" and have recently been identified as potential prognostic markers in cirrhotic patients. Most of these above mentioned markers were investigated in pilot studies with sometimes a modest sample size but allow us to catch a glimpse of the pathophysiological mechanisms leading to the worsening of cirrhosis. These new data should generate further well-designed studies to better assess the benefit for liver function of preventing intestinal bacterial translocation and microvascular thrombosis. The control of infection is vital and among all actors of immunity, vitamin D also appears to act as an anti-infective agent and therefore has probably a prognostic value.

**Key words:** Cirrhosis; C-reactive protein; Copeptin; Vitamin D; Serum free cortisol; Von Willebrand factor antigen



**Core tip:** This review provides new insights on the prognosis of cirrhotic patients. Several biological markers account for events that strongly impact on prognosis but are not taken into account by common prognosis scores such as Child-Pugh or Model of End-stage Liver Disease. The rationale for the use of these markers is discussed on the basis of the most recent available data.

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## INTRODUCTION

The prognosis of patients with cirrhosis depends on several factors such as the etiology and severity of liver disease, the presence of associated complications and comorbidities. Several prognostic scores have been developed to estimate the survival of patients in a simple and reliable manner, thus allowing to better adapting their management. Child-Pugh and Model

of End-stage Liver Disease (MELD) scores, commonly used in clinical practice, mainly reflect the degree of liver failure but do not account for events superimposed to liver failure that are strongly involved in the onset of extrahepatic organ failures. Therefore, beyond a certain severity of liver failure, MELD and Child-Pugh scores appear limited for accurately predict short-term death. In critically ill cirrhotic patients admitted to intensive care unit (ICU), for instance, general ICU scores such as Acute Physiology and Chronic Health Evaluation (APACHE) Ⅱ, Sequential Organ Failure Assessment (SOFA), its derivative from the Chronic Liver Failure Consortium of the European Association for the Study of the Liver and simplified acute physiology score (SAPS) Ⅱ scores provide better prediction of short-term death than liver specific scores[1]. The purpose of this review is to introduce readers to some new recently published biomarkers that may provide additional prognostic information to that given by the usual prognostic scores (Child-Pugh and MELD) and their derivatives (MELD-Na, iMELD, MESO index) even out of the context of multiorgan failure. Such variables, combined with the MELD score should be useful to better sort patients awaiting a liver transplant. The list of these variables of interest discussed in this article is probably not exhaustive.

## C-REACTIVE PROTEIN

C-reactive protein (CRP) is a protein of the acute phase of inflammation. Its hepatic synthesis is primarily stimulated by interleukin-6 (IL-6), a proinflammatory cytokine, and maintained even in the context of advanced liver failure[2,3]. The value of CRP reflects the degree of systemic inflammation, regardless of its cause. The physiological role of CRP is to bind to apoptotic cells and microorganisms through the recognition of different molecular patterns. This binding results in activating the complement system and stimulating phagocytosis. Recent studies also suggest a role of CRP in the induction of endothelial dysfunction[4] (Figure 1). Serum CRP increases in the event of systemic inflammatory response syndrome (SIRS) (even in the absence of overt bacterial infection). It was able to predict the risk of death in different populations of non-cirrhotic patients. In cirrhotic patients, SIRS is associated with the occurrence of complications such as hepatic encephalopathy, kidney failure and death[5]. However, the diagnostic criteria of SIRS may be modified in the context of cirrhosis, making the interpretation difficult. Hypersplenism can hide leukocytosis or increase leukopenia; subclinical hepatic encephalopathy can increase the respiratory rate and favor hypocapnia; hyperkinetic syndrome can increase heart rate in cirrhotic patients whereas beta-blockers may mask tachycardia. This is why the measurement of CRP could be both easier and more reliable than the SIRS criteria to identify a systemic inflammation that may adverse the prognosis of cirrhotic patients. We investigated the prognostic value of CRP in a prospective series of 148 cirrhotic patients hospitalized for clinical decompensation (Child Pugh $\geqslant$ 8), without hepatocellular carcinoma. The CRP levels were not correlated to the MELD score and had a strong prognostic significance[6]. High CRP levels were indeed associated with an independent risk of mortality at 6 mo, especially when considered the subgroup of 32 patients in whom the value of CRP remained above 29 mg/L during the first 15 d of hospitalization despite the resolution of any overt bacterial infection initially documented. The prognostic value of CRP remained significant when the analysis was restricted to patients without bacterial infection or alcoholic hepatitis at baseline. Taking into account the MELD, the existence of comorbidities, and the variation of CRP levels during the first 15 d, it was possible to build a prognostic model that was able to predict the mortality at 6 mo with a performance of 0.80 (AUROC) *vs* 0.67 for the MELD alone. Recently, the CANONIC study including 1343 cirrhotic patients from 29 European centers showed that the risk of organ failure and death was significantly associated with the value of CRP, even when the analyzes were restricted to uninfected patients[7]. When we analyzed the subgroup of 583 patients from the CANONIC study in which serial measures of CRP were available, our prognostic model was still relevant[8].

## SERUM FREE CORTISOL

The existence of a cortisol deficiency in cirrhosis remains uncertain and the reported prevalence of adrenal insufficiency (AI) (approximately one third of patients "hemodynamically stable" and up to 77% of septic patients) was overestimated by the measure of total serum cortisol concentrations that closely depends on its two main carrier proteins (CBG and albumin) synthesized by the liver and generally lowered in cirrhotic patient. Hence, total serum cortisol is low in case of decreased serum concentrations of CBG and albumin, whereas the free fraction of total cortisol that corresponds to the biologically active hormone is increased[9]. In addition, the lack of specificity of symptoms (fatigue, malaise, reduced muscle strength) renders the clinical diagnosis of AI difficult, especially in the setting of cirrhosis, in which malnutrition is common. Conventionally, the diagnosis of AI is made when the plasma cortisol measured at 8:00 AM is < 83 nmol/L and/or when adrenal stimulation exhibits poor adrenal reactivity (cortisol < 500 nmol/L 30 or 60 min after the intravenous injection of 250 mcg adrenocorticotropic hormone (Synacthen). In patients under stress (especially with septic shock), adrenal dysfunction is defined by a delta cortisol < 250 nmol/L or a random total cortisol < 276 nmol/L[10]. In cirrhotic patients, the mechanisms involved in the onset of AI are not well understood but may involve an exhaustion of the adrenal gland by lack of substrate (high density lipoprotein-cholesterol) for the synthesis of cortisol, or a corticoresistance induced by pro-inflammatory cytokines.

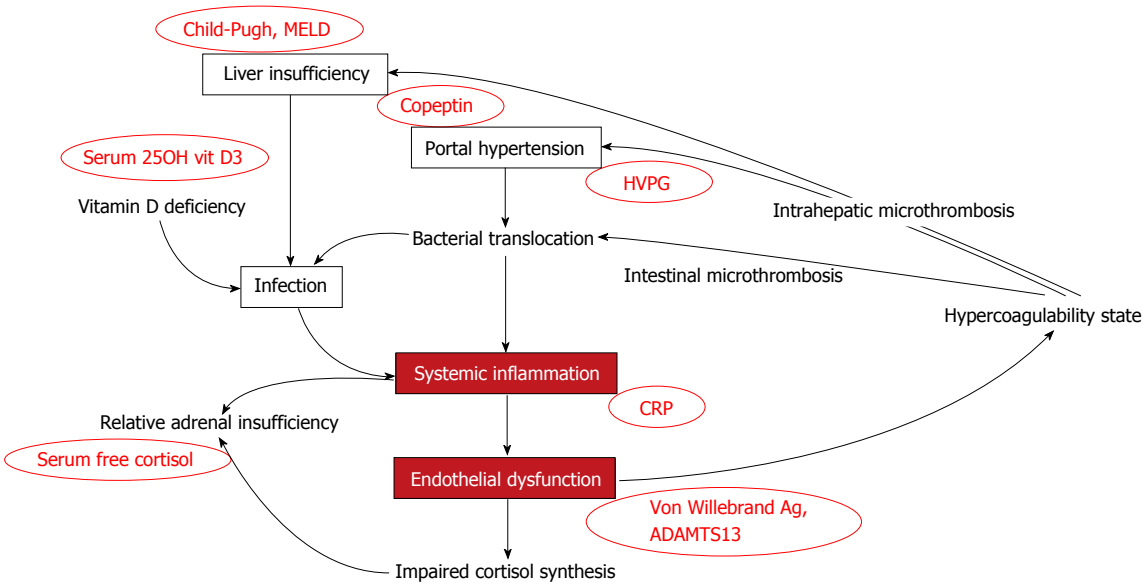


**Figure 1 Mechanisms involved in the poor prognosis of cirrhosis (favoring the occurrence of multiorgan failures) and their related markers.** Systemic inflammation and endothelial dysfunction appear as high relevant factors not reflected by Child-Pugh, MELD score, or measure of the HVPG. They worsen liver function, increase portal hypertension, and self-sustain the processes. HVPG: Hepatic venous pressure gradient; CRP: C-reactive protein; ADAMTS13: A metalloprotease with decreased activity.

In cirrhotic patients, the study of adrenal function remains difficult because the definition of AI is lacking. Due to changes in cortisol binding proteins concentrations related to liver impairment, the "normal" levels of total serum cortisol are not determined, and measuring serum free cortisol (SFC) is challenging and not routinely feasible. In a recent study we conducted, however, the determination of SFC provided unexpected results regarding its prognosis implications[11]. When the 1 mcg synacthen test was performed in 95 hemodynamically stable cirrhotic patients, a poor prognosis was associated with high levels of SFC (deaths occurred in 26.2% of patients with SFC $\geqslant$ 79 nmol/L *vs* 3.4% of patients with SFC < 79 nmol/L, $P = 0.027$ by log-rank test). By adjusting on the severity of cirrhosis (MELD) and on serum albumin levels, the risk of death of patients with SFC $\geqslant$ 79 nmol/L was increased by 5 times but the relationship was no longer significant probably due to the small sample size of this pilot study. Another interesting finding of this study was that concentrations of SFC were positively correlated with CRP levels, suggesting that SFC increases in the context of systemic inflammation. Further studies with sufficient numbers and serial determinations of cortisol are warranted to clarify the prognostic role of SFC by distinguishing different categories of cirrhotic patients (compensated, decompensated without sepsis, and septic). From our results, the concept of "hepatoadrenal syndrome"[12] mimicking the hepatorenal syndrome (*i.e.*, AI occurring in end-stage liver disease and impacting on mortality) is probably wrong or at least largely overestimated. Recent experimental data using deuterated cortisol-tracer technique show that the cortisol synthesis is increased by 83% in critically ill patients and that the conversion of cortisol to cortisone is reduced in order to maintain high levels of serum cortisol[13]. The levels of pro-inflammatory cytokines (IL-6 and tumor necrosis factor alpha) were positively correlated with cortisol production, and patients with a SIRS had 90% higher cortisol production than those who did not have SIRS. These new data allow us to better understand the discrepancies between the two landmark studies which evaluated the impact of hydrocortisone administration on survival in patients with septic shock[14,15]. Conversely to these findings, a rationale for a true "hepatoadrenal syndrome" in end-staged cirrhotic patients still persists. It has been shown that inflammation may cause endothelial dysfunction, leading to impaired cortisol synthesis[16] (Figure 1). Thus, in patients with compensated cirrhosis, although AI may be a latent condition, the proinflammatory cytokines appropriately stimulate the hypothalamic-pituitary adrenal axis leading to an increase of cortisol. When the stress becomes more intense (and associated with an excessive production of pro-inflammatory cytokines), a steroid-resistance may occur leading to an insufficient cortisol production regarding the clinical situation. This concept of relative AI is probably of high relevance in cirrhotic patients, particularly in the event of septic shock. It was a rationale to conduct a randomized controlled trial to assess the effect of hydrocortisone on survival in 75 cirrhotic patients with septic shock. Despite initial hemodynamic improvement, an interim analysis found no difference in 28-d mortality between the two groups, and the trial was thus interrupted[17].

## VITAMIN D

Vitamin D (calciferol) is essential to the maintenance of phosphate homeostasis of the body. It comes from

food and skin. Under the action of sunlight (UV-B), skin 7-dehydrocholesterol is converted to previtamin D3 then vitamin D3 and is subsequently transported to the liver where hydroxylation to 25-OH vitamin D3 occurs. Then, 25-OH D3 undergoes a second hydroxylation in the kidney thereby resulting in 1,25-(OH)2-vitamin D3 (calcitriol), which is the biologically active hormonal form of vitamin D3 (rarely measured in clinical practice). Serum 25-OH vitamin D3 (the storage form of the vitamin) concentration is nearly 1000 times higher than that of serum 1,25-(OH)2 vitamin D3. The measure of serum 25-OH vitamin D3 is thus appropriate for the diagnosis of vitamin D deficiency[18]. In cirrhotic patients, however, because of low albumin and vitamin D binding protein levels, recent reports highlighted the overestimation of vitamin D deficiency and suggested that the measure of free 25-OH D3 would be more appropriate[19].

The role of vitamin D is not confined to its skeletal actions. Vitamin D also regulates immune defenses and is able to modulate the differentiation and proliferation of certain cell types. Its deficiency has been associated with an increased risk of cancer, cardiovascular disease, autoimmune diseases and infectious diseases[18]. The impact of vitamin D on immune system may be relevant for the prognosis of cirrhotic patients. Indeed, vitamin D it is commonly deficient in this population and it's easy to hypothesize that it favors bacterial infections, which increase by four-fold the risk of death in cirrhotic patients[20] (Figure 1). Because of the antiproliferation, pro-differentiation, pro-apoptosis, anti-inflammation, and immune regulation properties of vitamin D, Vitamin D deficiency may also contribute to the development of hepatocellular carcinoma. Although the epidemiologic evidence regarding the association of vitamin D and hepatocellular carcinoma is still inconclusive, biochemical evidence clearly indicates that hepatocellular carcinoma cells are responsive to the inhibitory effect of vitamin D and its analogs[21] and genetic determinants have been identified for the role of vitamin D to modulate the development of hepatitis C associated hepatocellular carcinoma (HCC)[22]. Unfortunately, the therapeutic use of vitamin D analogs for HCC provided disappointing results to date.

The reasons why vitamin D is deficient in patients with cirrhosis are multiple and include solar underexposure of these patients, malnutrition, malabsorption of vitamin D by a lack of bile acids and/or impairment of the hepatic hydroxylation[23]. In a recent study, low vitamin D levels (< 6 ng/mL) were associated with increased mortality (OR = 6.3, $P$ = 0.024) in cirrhotic patients regardless of their MELD score, and sepsis were the cause of death in the majority of these patients[24]. Similar findings were observed in 75 cirrhotic patients from Austria, in which negative correlations were observed between 25-OH vitamin D concentrations and MELD scores ($r$ = -0.34, $P$ = 0.003)[25]. By distributing vitamin D levels into tertiles, the authors observed an increased risk (OR = 6.37, $P$ = 0.005) of hepatic decompensation (ascites, encephalopathy, gastrointestinal bleeding, hepatorenal syndrome) in patients with the lowest values of vitamin D (1st tertile) compared to those with the highest values (3rd tertile). Patients of the 1st tertile had also a higher risk of death compared with patients of the 3rd tertile in multivariate analysis adjusted for age and sex (HR = 4.31, $P$ = 0.012). However, the adjustment on the Child-Pugh score and/or MELD score no longer allowed retaining vitamin D as a significant prognostic marker *per se*. A more recent study conducted in 324 patients with alcoholic liver disease showed that low vitamin D levels (< 10 ng/mL) were associated with: (1) cirrhosis; (2) high hepatic venous pressure gradients; (3) high Child-Pugh and MELD scores; (4) occurrence of portal hypertension-related complications; and (5) one-year mortality[26]. Vitamin D deficiency may contribute to deterioration of liver functions by increasing liver inflammation and fibrosis. Indeed, experimental models have shown a reduction in inflammatory and profibrotic activity of hepatic stellate cells after vitamin D[27]. An unresolved issue is whether vitamin D supplementation could improve liver function and survival of cirrhotic patients. Anyway, screening and treatment of vitamin D deficiency in patients with cirrhosis is already justified given the high prevalence (12%-86%) of osteoporosis and fracture risk (5%-20%) in these patients. Further studies should investigate the *free* faction of vitamin D and may determine more in depth the true spectrum of vitamin D deficiency in cirrhosis and its clinical implications.

## COPEPTIN

In cirrhotic patients, intestinal bacterial translocation is responsible for overproduction of nitric oxyde (NO) *via* activation of monocytes and lymphocytes and increase in circulating levels of proinflammatory cytokines. NO increases splanchnic vasodilation that stimulates compensatory systems to restore adequate blood volume: sympathetic nervous system, renin-angiotensin-aldosterone system and arginine vasopressin (AVP, also called antidiuretic hormone). It has been shown that AVP concentrations increase with deterioration of liver function and this biological marker may thus have a prognostic value. However, its measurement is difficult and not routinely available. Copeptin, the pre-pro-AVP C terminal fragment, is released into the serum in equimolar quantities than AVP. Hence, copeptin concentrations closely reflect the production of AVP, either in healthy subjects or in stressful situations such as sepsis[28]. The main interest of copeptin is its serum stability, conversely to AVP. Copeptin is thus easy to measure. Moreover, its concentration increases much more than cortisol in the event of stress. The prognostic value of copeptin was recently mentioned in several diseases: high concentrations of copeptin were associated with unfavorable outcomes in patients with chronic heart failure, pulmonary infections, and in patients with transient ischemic stroke[28]. We studied this marker in a cohort of 125 cirrhotic patients including

34 Child-Pugh A, 29 Child-Pugh B, 32 Child-Pugh C and 30 infected patients with Child-Pugh score > B8[29]. Copeptin concentrations were higher in infected patients (18.81 pmol/L *vs* 6.64 pmol/L in patients without infection, $P = 0.0007$), patients with ascites (13.27 pmol/L *vs* 6.06 pmol/L in others, $P < 0.0001$) and patients with renal impairment (44.67 pmol/L *vs* 8.40 pmol/L in patients with normal renal function, $P = 0.0018$). Copeptin concentrations were positively correlated with Child-Pugh, MELD scores ($r = 0.43$, $P < 0.0001$) and CRP levels ($r = 0.49$, $P < 0.0001$). After a median follow-up of 12 mo, 8 patients were transplanted and 28 (24%) patients had died. In univariate analysis, patients who died or were transplanted had higher baseline copeptin concentrations compared with others (15.02 pmol/L *vs* 6.68 pmol/L; $P = 0.0006$), and nearly three quarters of patients who had died belonged to the two highest quintiles regarding copeptin concentrations. Survival analysis showed excess mortality in patients with copeptin values > 13 pmol/L. In multivariate analysis, high value (> 13 pmol/L) of copeptin kept its detrimental impact on prognosis after adjustment on CRP and MELD score. This study suggests that copeptin could be a good marker of stress during cirrhosis. Its impact on survival warrants confirmation by larger studies.

## THE VON WILLEBRAND FACTOR ANTIGEN

The vascular endothelium plays a critical role in the regulation of vascular tone through its ability to release vasoactive substances, including NO and prostacyclin (vasodilators) and endothelin and thromboxane A2 (vasoconstrictors). In cirrhotic patients, an endothelial dysfunction is responsible for abnormal vascular reactivity which is involved in portal hypertension both by increasing intrahepatic vascular resistance (due to intrahepatic vasoconstriction) and portal flow (by increase in cardiac output to compensate for systemic arteriolar vasodilatation). The measurement of hepatic venous pressure gradient (HVPG) identifies cirrhotic patients at high risk of complications (mainly esophageal varices) and death when HVPG exceeds 10 mmHg but this measure is not feasible in routine. The Von Willebrand antigen (VWF Ag) is a glycoprotein synthesized by activated endothelial cells and can be used as a marker of endothelial dysfunction. Endotoxemia activates endothelial cells and is well correlated with the levels of serum VWF Ag[30]. In 42 cirrhotic patients with severe portal hypertension (HVPG $\geqslant$ 12 mmHg), the team from Barcelona has shown a positive correlation between serum VWF Ag and MELD score ($r = 0.34$, $P = 0.032$), and between serum VWF Ag and HVPG ($r = 0.47$, $P < 0.001$)[31]. However, given the small sample size and the inclusion of patients with HVPG $\geqslant$ 12 mmHg, the prognostic value of VWF Ag could not be determined convincingly. A more recent study[32] has come to fill this gap by including 286 patients with cirrhosis at different stages of severity (Child-Pugh 148 A, 104 B and 34 C). These authors confirmed the previous results and showed a good correlation ($r = 0.68$, $P < 0.001$) between VWF Ag and HVPG[32]. A nice consequence for clinical practice was that levels of VWF Ag > 241% accurately predicted the risk of variceal bleeding, as well as HVPG $\geqslant$ 12 mmHg did. In addition, VWF Ag > 315 % was able to predict death (HR = 2.92, $P < 0.001$) regardless of the HVPG, the Child-Pugh, the MELD, and the presence of hepatocellular carcinoma. This spectacular result suggests that marked endothelial dysfunction, as assessed by high levels of VWF Ag, has a strong detrimental prognostic influence *per se*. During cirrhosis, VWF Ag may remain high due to the presence of circulating endotoxins but also due to a decreased activity of a metalloprotease (ADAMTS13), which cleaves the multimers of Willebrand factor. A recent study suggests that a low enzymatic activity of ADAMTS13 predicts the risk of death at 1 year and 2 years, as well as Child-Pugh and MELD scores do[33]. The mechanisms involved in the pejorative influence of high levels of VWF Ag are unknown. VWF Ag plays a key role in both primary hemostasis (mediator of platelet adhesion to sub-endothelium) and coagulation (plasma factor Ⅷ carrier). It is likely that elevated serum VWF Ag levels participate to a hypercoagulable state that is often underestimated in severe cirrhotic patients. This hypercoagulable state may contribute to deterioration of liver functions by inducing thrombosis in the hepatic microcirculation, as well as it may increase portal hypertension as hypothesized by Wanless twenty years ago[34] (Figure 1). It is also possible that these thrombotic events, when located in the intestinal microcirculation, favor enterocyte ischemia and intestinal bacterial translocation subsequently. This self-perpetuates the phenomenon and causes systemic inflammation.

## CONCLUSION

Risk assessments of cirrhotic patients is sometimes tricky but remains crucial for optimal identifications of liver transplant candidates. Even commonly used for a long time in clinical practice, Child-Pugh and MELD scores lack finesse, whereas general scores (such as SAPS Ⅱ, APACHE or SOFA), are inappropriate outside of the context of intensive care. New biological variables (CRP, serum free cortisol, copeptin, vitamin D, or Willebrand antigen) account for well identified event which impact on prognosis of cirrhotic patients and deserve further research to build a new prognostic score, easy to use but more powerful than Child-Pugh, MELD score, or its derivatives created to date.

## REFERENCES

1 **Levesque E**, Hoti E, Azoulay D, Ichaï P, Habouchi H, Castaing D, Samuel D, Saliba F. Prospective evaluation of the prognostic scores for cirrhotic patients admitted to an intensive care unit. *J Hepatol* 2012; **56**: 95-102 [PMID: 21835136 DOI: 10.1016/j.jhep.2011.06.024]

2 **Bota DP**, Van Nuffelen M, Zakariah AN, Vincent JL. Serum levels of C-reactive protein and procalcitonin in critically ill patients with cirrhosis of the liver. *J Lab Clin Med* 2005; **146**: 347-351 [PMID: 16310518]

3 **Park WB**, Lee KD, Lee CS, Jang HC, Kim HB, Lee HS, Oh MD, Choe KW. Production of C-reactive protein in Escherichia coli-infected patients with liver dysfunction due to liver cirrhosis. *Diagn Microbiol Infect Dis* 2005; **51**: 227-230 [PMID: 15808312]

4 **Devaraj S**, Kumaresan PR, Jialal I. C-reactive protein induces release of both endothelial microparticles and circulating endothelial cells in vitro and in vivo: further evidence of endothelial dysfunction. *Clin Chem* 2011; **57**: 1757-1761 [PMID: 21980169 DOI: 10.1373/clinchem.2011.169839]

5 **Thabut D**, Massard J, Gangloff A, Carbonell N, Francoz C, Nguyen-Khac E, Duhamel C, Lebrec D, Poynard T, Moreau R. Model for end-stage liver disease score and systemic inflammatory response are major prognostic factors in patients with cirrhosis and acute functional renal failure. *Hepatology* 2007; **46**: 1872-1882 [PMID: 17972337]

6 **Cervoni JP**, Thévenot T, Weil D, Muel E, Barbot O, Sheppard F, Monnet E, Di Martino V. C-reactive protein predicts short-term mortality in patients with cirrhosis. *J Hepatol* 2012; **56**: 1299-1304 [PMID: 22314431 DOI: 10.1016/j.jhep.2011.12.030]

7 **Moreau R**, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437, 1437.e1-9 [PMID: 23474284 DOI: 10.1053/j.gastro.2013.02.042]

8 **Cervoni JP**, Amoros A, Moreau R, Arroyo V, Jalan R, Saliba F. Prognostic value of C-reactive protein in patients with cirrhosis: external validation from the CANONIC cohort. *Hepatology* 2014; **60** (Suppl): 495A

9 **Thevenot T**, Borot S, Remy-Martin A, Sapin R, Cervoni JP, Richou C, Vanlemmens C, Cleau D, Muel E, Minello A, Tirziu S, Penfornis A, Di Martino V, Monnet E. Assessment of adrenal function in cirrhotic patients using concentration of serum-free and salivary cortisol. *Liver Int* 2011; **31**: 425-433 [PMID: 21281437 DOI: 10.1111/j.1478-3231.2010.02431.x]

10 **Marik PE**, Pastores SM, Annane D, Meduri GU, Sprung CL, Arlt W, Keh D, Briegel J, Beishuizen A, Dimopoulou I, Tsagarakis S, Singer M, Chrousos GP, Zaloga G, Bokhari F, Vogeser M. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. *Crit Care Med* 2008; **36**: 1937-1949 [PMID: 18496365 DOI: 10.1097/CCM.0b013e31817603ba]

11 **Thevenot T**, Dorin R, Monnet E, Qualls CR, Sapin R, Grandclement E, Borot S, Sheppard F, Weil D, Degand T, Di Martino V, Kazlauskaite R. High serum levels of free cortisol indicate severity of cirrhosis in hemodynamically stable patients. *J Gastroenterol Hepatol* 2012; **27**: 1596-1601 [PMID: 22647073 DOI: 10.1111/j.1440-1746.2012.07188.x]

12 **Fede G**, Spadaro L, Tomaselli T, Privitera G, Germani G, Tsochatzis E, Thomas M, Bouloux PM, Burroughs AK, Purrello F. Adrenocortical dysfunction in liver disease: a systematic review. *Hepatology* 2012; **55**: 1282-1291 [PMID: 22234976 DOI: 10.1002/hep.25573]

13 **Boonen E**, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, Vanwijngaerden YM, Spriet I, Wouters PJ, Vander Perre S, Langouche L, Vanhorebeek I, Walker BR, Van den Berghe G. Reduced cortisol metabolism during critical illness. *N Engl J Med* 2013; **368**: 1477-1488 [PMID: 23506003 DOI: 10.1056/NEJMoa1214969]

14 **Annane D**, Sébille V, Charpentier C, Bollaert PE, François B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troché G, Chaumet-Riffaud P, Bellissant E. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002; **288**: 862-871 [PMID: 12186604]

15 **Sprung CL**, Annane D, Keh D, Moreno R, Singer M, Freivogel K, Weiss YG, Benbenishty J, Kalenka A, Forst H, Laterre PF, Reinhart K, Cuthbertson BH, Payen D, Briegel J. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; **358**: 111-124 [PMID: 18184957 DOI: 10.1097/CCM.0B013E3181659610]

16 **Kanczkowski W**, Chatzigeorgiou A, Grossklaus S, Sprott D, Bornstein SR, Chavakis T. Role of the endothelial-derived endogenous anti-inflammatory factor Del-1 in inflammation-mediated adrenal gland dysfunction. *Endocrinology* 2013; **154**: 1181-1189 [PMID: 23364949 DOI: 10.1210/en.2012-1617]

17 **Arabi YM**, Aljumah A, Dabbagh O, Tamim HM, Rishu AH, Al-Abdulkareem A, Knawy BA, Hajeer AH, Tamimi W, Cherfan A. Low-dose hydrocortisone in patients with cirrhosis and septic shock: a randomized controlled trial. *CMAJ* 2010; **182**: 1971-1977 [PMID: 21059778 DOI: 10.1503/cmaj.090707]

18 **Holick MF**. Vitamin D deficiency. *N Engl J Med* 2007; **357**: 266-281 [PMID: 17634462]

19 **Lai JC**, Terrault N, Bikle D, Lizaola BL, Schwartz JB. Total 25(OH) Vitamin D is Not an Accurate Marker of Vitamin D Status in Patients with Cirrhosis and Synthetic Dysfunction. *Hepatology* 2014; **60** (suppl): 304A

20 **Arvaniti V**, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010; **139**: 1246-1256, 1256.e1-5 [PMID: 20558165 DOI: 10.1053/j.gastro.2010.06.019]

21 **Chiang KC**, Yeh CN, Chen MF, Chen TC. Hepatocellular carcinoma and vitamin D: a review. *J Gastroenterol Hepatol* 2011; **26**: 1597-1603 [PMID: 21880026 DOI: 10.1111/j.1440-1746.2011.06892.x]

22 **Lange CM**, Miki D, Ochi H, Nischalke HD, Bojunga J, Bibert S, Morikawa K, Gouttenoire J, Cerny A, Dufour JF, Gorgievski-Hrisoho M, Heim MH, Malinverni R, Müllhaupt B, Negro F, Semela D, Kutalik Z, Müller T, Spengler U, Berg T, Chayama K, Moradpour D, Bochud PY. Genetic analyses reveal a role for vitamin D insufficiency in HCV-associated hepatocellular carcinoma development. *PLoS One* 2013; **8**: e64053 [PMID: 23734184 DOI: 10.1371/journal.pone.0064053]

23 **Zúñiga S**, Firrincieli D, Housset C, Chignard N. Vitamin D and the vitamin D receptor in liver pathophysiology. *Clin Res Hepatol Gastroenterol* 2011; **35**: 295-302 [PMID: 21440524 DOI: 10.1016/j.clinre.2011.02.003]

24 **Stokes CS**, Krawczyk M, Reichel C, Lammert F, Grünhage F. Vitamin D deficiency is associated with mortality in patients with advanced liver cirrhosis. *Eur J Clin Invest* 2014; **44**: 176-183 [PMID: 24236541 DOI: 10.1111/eci.12205]

25 **Putz-Bankuti C**, Pilz S, Stojakovic T, Scharnagl H, Pieber TR, Trauner M, Obermayer-Pietsch B, Stauber RE. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. *Liver Int* 2012; **32**: 845-851 [PMID: 22222013 DOI: 10.1111/j.1478-3231.2011.02735.x]

26 **Trépo E**, Ouziel R, Pradat P, Momozawa Y, Quertinmont E, Gervy C, Gustot T, Degré D, Vercruysse V, Deltenre P, Verset L, Gulbis B, Franchimont D, Devière J, Lemmers A, Moreno C. Marked 25-hydroxyvitamin D deficiency is associated with poor prognosis in patients with alcoholic liver disease. *J Hepatol* 2013; **59**: 344-350 [PMID: 23557869 DOI: 10.1016/j.jhep.2013.03.024]

27 **Potter JJ**, Liu X, Koteish A, Mezey E. 1,25-dihydroxyvitamin D3 and its nuclear receptor repress human α1 (I) collagen expression and type I collagen formation. *Liver Int* 2013; **33**: 677-686 [PMID: 23413886 DOI: 10.1111/liv.12122]

28 **Morgenthaler NG**, Struck J, Jochberger S, Dünser MW. Copeptin: clinical use of a new biomarker. *Trends Endocrinol Metab* 2008; **19**: 43-49 [PMID: 18291667 DOI: 10.1016/j.tem.2007.11.001]

29 **Moreno JP**, Grandclement E, Monnet E, Clerc B, Agin A, Cervoni JP, Richou C, Vanlemmens C, Dritsas S, Dumoulin G, Di Martino V, Thevenot T. Plasma copeptin, a possible prognostic marker in cirrhosis. *Liver Int* 2013; **33**: 843-851 [PMID: 23560938 DOI: 10.1111/liv.12175]

30 **Ferro D**, Quintarelli C, Lattuada A, Leo R, Alessandroni M,

Mannucci PM, Violi F. High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia. *Hepatology* 1996; **23**: 1377-1383 [PMID: 8675154]

31 **La Mura V**, Reverter JC, Flores-Arroyo A, Raffa S, Reverter E, Seijo S, Abraldes JG, Bosch J, García-Pagán JC. Von Willebrand factor levels predict clinical outcome in patients with cirrhosis and portal hypertension. *Gut* 2011; **60**: 1133-1138 [PMID: 21427197 DOI: 10.1136/gut.2010.235689]

32 **Ferlitsch M**, Reiberger T, Hoke M, Salzl P, Schwengerer B, Ulbrich G, Payer BA, Trauner M, Peck-Radosavljevic M, Ferlitsch A. von Willebrand factor as new noninvasive predictor of portal hypertension, decompensation and mortality in patients with liver cirrhosis. *Hepatology* 2012; **56**: 1439-1447 [PMID: 22532296 DOI: 10.1002/hep.25806]

33 **Takaya H**, Uemura M, Fujimura Y, Matsumoto M, Matsuyama T, Kato S, Morioka C, Ishizashi H, Hori Y, Fujimoto M, Tsujimoto T, Kawaratani H, Toyohara M, Kurumatani N, Fukui H. ADAMTS13 activity may predict the cumulative survival of patients with liver cirrhosis in comparison with the Child-Turcotte-Pugh score and the Model for End-Stage Liver Disease score. *Hepatol Res* 2012; **42**: 459-472 [PMID: 22292786 DOI: 10.1111/j.1872-034X.2011.00950.x]

34 **Wanless IR**, Wong F, Blendis LM, Greig P, Heathcote EJ, Levy G. Hepatic and portal vein thrombosis in cirrhosis: possible role in development of parenchymal extinction and portal hypertension. *Hepatology* 1995; **21**: 1238-1247 [PMID: 7737629]

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MINIREVIEWS

# Valproic acid and nonalcoholic fatty liver disease: A possible association?

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## Abstract

Valproic acid (VPA) is one of the most prescribed drugs in children with newly diagnosed epilepsy. Weight gain and obesity have been observed as side effects of VPA. These are often linked with other metabolic disturbances such as development of insulin resistance, dyslipidemia, metabolic syndrome (MetS) and non-alcoholic fatty liver disease or nonalcoholic fatty liver disease (NAFLD). NAFLD refers to a group of liver disorders with marked hepatic steatosis. It is associated with an increased incidence of cardiovascular diseases and overall reduced life expectancy. NAFLD occurs in 20%-25% of the general population and it is known to be the most common cause of chronic liver disease. NAFLD therefore represents a major public health issue worldwide. This study reviews and summarizes relevant literature that supports the existence of an association between VPA therapy and the development of NAFLD in children. Long-term VPA-therapy appears to be associated with an increased risk of developing NAFLD. Further studies are needed to clarify the pathogenic mechanisms that lie behind this association and to standardize the options for the use of this drug in overweight patients and in those with risks for developing MetS and NAFLD.

**Key words:** Nonalcoholic fatty liver disease; Valproic acid; Obesity; Insulin resistance



**Core tip:** Nonalcoholic fatty liver disease (NAFLD) is a major medical issue worldwide. It affects 20%-25% of the general population including children. The term NAFLD covers a wide spectrum of hepatic diseases. These diseases include simple hepatic steatosis, inflammation, cirrhosis and the development of hepatocellular carcinoma. Valproic acid (VPA), one of the most used anti-epileptic drugs, has been investigated as a contributing factor for the development of NAFLD. This association seems stronger with long term VPA therapy. Further studies are required to determine the mechanism of this association.

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## NONALCOHOLIC FATTY LIVER DISEASE: DEFINITION

Nonalcoholic fatty liver disease (NAFLD) is a group of diseases characterized by steatosis and the absence of a secondary cause for hepatic fat accumulation. Steatosis must be documented histologically or with sonography[1]. Secondary causes of hepatic fat accumulation such as hereditary syndromes, infective diseases, significant alcohol use or steatogenic drugs should be excluded. NAFLD has two subsets. These are nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFL is a benign condition characterized by the presence of intrahepatic fat accumulation (micro or macro-vesicular steatosis) without evidence of hepatic injury. NASH has worse lesions like hepatic steatosis with associated inflammation and hepatic injury with or without fibrosis. NASH also poses a risk for the development of cirrhosis and hepatic malignancy. NAFLD can be suspected in findings of slightly elevated liver enzymes. Such blood exam results correlate well with NAFLD in obese patients. Ultrasound and other non-invasive imaging techniques can diagnose the presence of NAFLD[1,2]. However, a histologic evaluation is necessary to accurately assess the degree of steatosis and confirm the distinct inflammatory lesions that characterize NASH. Biopsy of the liver is therefore needed to distinguish simple steatosis from other conditions like steatosis with inflammation, fibrosis, cirrhosis and cancer. The spectrum of lesions associated with NAFLD is presented in Table 1 which shows a validated semi-quantitative scoring system to assess the severity of inflammation (grading), fibrosis (staging) and steatosis in NAFLD. This system is simple and useful in managing both adults and children with any degree of NAFLD. Biopsy samples with scores $\geqslant$ 5 are diagnosed as having NASH while scores less than 3 are not considered as NASH[3,4]. The estimated prevalence of NAFLD in the general population is about 20%-25%[1,2]. In children, it has been estimated to be 9%-10%. Several studies have demonstrated that NAFLD is more common in adolescents than in children with a male predominance ratio of 2:1[5,6]. Factors that may explain the higher rate of NAFLD in adolescents include increased circulating hormones, puberty related insulin resistance (IR), diet preferences and having a sedentary lifestyle[6]. Patients with NAFLD are usually asymptomatic[2]. Patients with NASH have a general sensation of being unwell and have an upper right abdominal discomfort[7]. Physical examination may document the presence of hepatomegaly and, rarely, splenomegaly. NAFLD is associated with obesity, dyslipidaemia, type 2 diabetes, IR, metabolic syndrome (MetS). There are also emerging associations between NAFLD and polycystic ovary syndrome, hypothyroidism, hypopituitarism, hypogonadism, obstructive sleep apnea and pancreatico-duodenal resection. Adults with a diagnosis of NAFLD in childhood have a higher risk to develop cardiovascular diseases, MetS, cirrhosis and hepatocellular carcinoma[6]. There are no current specific therapeutic indications for NAFLD. Since most NAFLD patients are overweight or obese, experts recommend lifestyle modification which include proper dieting and exercise programs[8]. It is a primary intervention with the intention of maintaining ideal body weight in this set of patients. For patients with poor compliance or unresponsive to this conservative approach, a pharmacological approach can be considered. Dietary supplementation with docosahexaenoic acid has been shown to improve liver steatosis and insulin sensitivity in children with NAFLD[9,10]. Other therapeutic approaches for NAFLD may include administration of antioxidants such as alpha-tocopherol (vitamin E), insulin sensitizers like metformin, cytoprotective agents like ursodeoxycholic acid, probiotic therapy and bariatric surgery[8,11,12].

## VALPROIC ACID: MAIN SIDE EFFECTS

Valproic acid (VPA) is a widely used antiepileptic drug (AED). It is effective against many types of seizure disorders either alone or as a component of a multidrug regimen. It was commercialized in 1969 in France and 1978 in United States. VPA has a broad spectrum of activity against generalized and partial epilepsy in both adults and children. VPA is generally regarded as a first-choice agent for newly diagnosed epilepsy[13]. Recently, it has been used for other medical conditions such as neuropathic pain, in prophylaxis for migraine headaches and as a mood stabilizer for specific psychiatric disorders. VPA is sometimes used for controlling behavioral disturbances in dementia patients and in treating spinal muscular atrophy[14]. It has been estimated that more than one million people in the world are taking VPA every day. It may as well be the most widely prescribed antiepileptic drug worldwide[15]. VPA is available in oral (immediate release, enteric-coated and delayed-release) and parenteral preparations. The side effects of this drug are listed in Table 2[16,17].

In 1978, several clinical studies revealed the existence of VPA-related biochemical abnormalities in the liver. Hepatotoxicity associated with VPA may manifest as one of the following conditions: (1) Hyperammonaemia: It can be observed as an isolated biochemical finding discovered on a routine blood examination. It can be a symptomatic condition with progressive impairment of consciousness and ataxia that is usually preceded by gastrointestinal symptoms like nausea, vomiting, anorexia and diarrhea. Generally, hyperammonaemia and its related symptoms resolve after one to three days of drug discontinuation[18]; (2) Hepatitis-like syndrome: It is characterized by a dose-dependent elevation of

**Table 1 Nonalcoholic steato-hepatitis Clinical Research Network Scoring System (adapted from ref. [3])**

| Item | | Definition | Score/code |
|---|---|---|---|
| Steatosis | | | |
| | Grade | Low-to medium-power evaluation of parenchymal involvement by steatosis | |
| | | < 5% | 0 |
| | | 5%-33% | 1 |
| | | > 33%-66% | 2 |
| | | > 66% | 3 |
| | Location | Predominant distribution pattern | |
| | | Zone 3 | 0 |
| | | Zone 1 | 1 |
| | | Azonal | 2 |
| | | Panacinar | 3 |
| | Microvesicular steatosis | Contiguous patches | |
| | | Not present | 0 |
| | | Present | 1 |
| Fibrosis | | | |
| | Stage | None | 0 |
| | | Perisinusoidal or periportal | 1 |
| | | Mild, zone 3, perisinusoidal | 1A |
| | | Moderate, zone 3, perisinusoidal | 1B |
| | | Portal/periportal | 1C |
| | | Perisinusoidal and portal/periportal | 2 |
| | | Bridging fibrosis | 3 |
| | | Cirrhosis | 4 |
| Inflammation | | | |
| | Lobular inflammation | Overall assessment of all inflammatory foci | |
| | | No foci | 0 |
| | | < 2 foci per 200 × field | 1 |
| | | 2-4 foci per 200 × field | 2 |
| | | > 4 foci per 200 × field | 3 |
| | Microgranulomas | Small aggregates of macrophages | |
| | | Absent | 0 |
| | | Present | 1 |
| | Large lipogranulomas | Usually in portal areas or adjacent to central veins | |
| | | Absent | 0 |
| | | Present | 1 |
| | Portal inflammation | Assessed from low magnification | |
| | | None to minimal | 0 |
| | | Greater than minimal | 1 |
| Liver cell injury | | | |
| | Ballooning | None | 0 |
| | | Few balloon cells | 1 |
| | | Many cells/prominent ballooning | 2 |
| | Acidophil bodies | None to rare | 0 |
| | | Many | 1 |
| | Pigmented macrophages | None to rare | 0 |
| | | Many | 1 |
| | Megamitochondria | None to rare | 0 |
| | | Many | 1 |
| Other findings | | | |
| | Mallory's hyaline | Visible on routine stains | |
| | | None to rare | 0 |
| | | Many | 1 |
| | Glycogenated nuclei | Contiguous patches | |
| | | None to rare | 0 |
| | | Many | 1 |

serum aminotransferases. Majority of the patients are asymptomatic but a few can manifest symptoms of malaise, anorexia and lethargy. Biochemical abnormalities and clinical aspects usually normalize after discontinuing the drug[16,18]; (3) Reye's-like syndrome: It is a dangerous, rare and idiosyncratic condition. Patients usually manifest with acute onset of high fever, vomiting, anorexia, lethargy, loss of consciousness and cerebral edema[16,18]; and (4) NAFLD: The steatogenic effect of VPA has been demonstrated since the beginning of the 1980s. This was documented by histology of liver tissue taken from patients considered to have died from VPA hepatotoxicity[19] and by experimental studies on animals[20,21].

The pathology behind the development of hepatotoxicity, hyperammonaemia and NAFLD in relation to

**Table 2 Main side effects of valproic acid**

| |
|---|
| GI disorders: an increase of liver enzymes is common, particularly in early treatment, and it may be transient. Nausea and diarrhea occur frequently at the beginning of treatment, but disappear after a few days without discontinuing treatment. Rare cases of pancreatitis have been reported |
| Nervous system disorders: transient side effects such as dizziness, headache, tremor, diplopia and sedation have been evaluated and they can lead to the reduction or the discontinuation of the drug |
| Weight gain: being overweight at the beginning of treatment may be a significant predictor of further weight gain with VPA |
| Blood dyscrasias: different studies demonstrated the association of VPA with pro and anticoagulatory effects and they are dose-dependent |
| Endocrinological disorders: there is a correlation between hypothyroidism and treatment with VPA in monotherapy; moreover, VPA increases the synthesis of Testosterone and decreases its conversion to Estradiol, leading to the PCOS with amenorrhea and irregular periods |
| Hair loss: it is usually transient and sometimes dose-related. Regrowth normally begins within 6 months after the end of the therapy |
| Hypersensitivity: this effect is rare and dose, time, frequency-independent |
| Teratogenicity: despite VPA can induce teratogenic effects during pregnancy, United States Food and Drugs Administration considers acceptable the risk/benefit ratio. The most common teratogenic effect is the delay/impaired development |

VPA: Valproic acid; PCOS: Polycystic ovary syndrome.

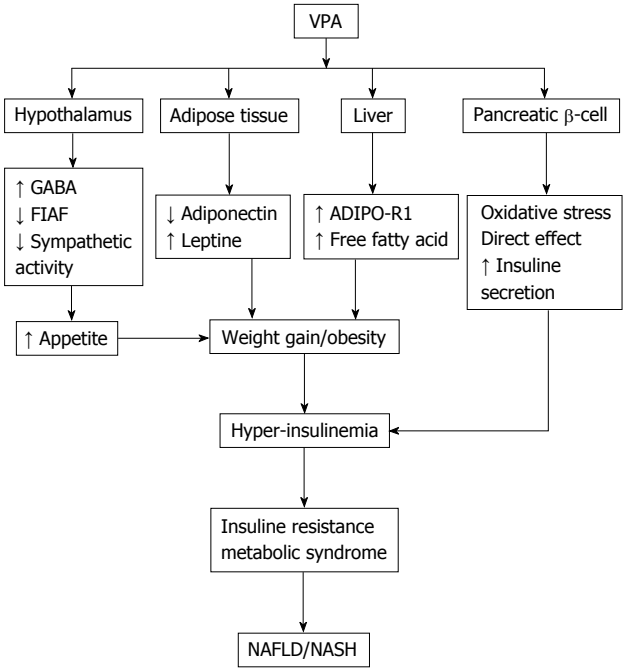


**Figure 1 Pathogenic mechanisms of valproic acid-induced nonalcoholic fatty liver disease/nonalcoholic steato-hepatitis.** VPA: Valproic acid; GABA: Gamma-aminobutyric acid; FIAF: Fasting-induced adipose factor; ADIPO-R1: Adiponectin receptor expression; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steato-hepatitis.

VPA therapy is still poorly understood.

## VPA, WEIGHT GAIN AND INSULIN RESISTANCE

The association between VPA-therapy and weight gain was first proved in a study performed in 1981[22]. The frequency of developing obesity in children treated with VPA ranged from 10% to 70%[23]. Most affected patients were peri-pubertal girls who underwent a prolonged therapy and were already overweight from the beginning of treatment[23]. The pathogenic mechanisms of VPA-induced weight gain remains unclear and is most likely multifactorial. It is regulated centrally and peripherally by various neuropeptides and cytokines (Figure 1). These regulatory substances include resistin, fasting-induced adipose factor (FIAF), adiponectin, leptin, ghrelin and visfatin[23]. VPA may increase appetite and craving by enhancing gamma-aminobutyric acid (GABA) transmission in the hypothalamic pathways. VPA may also modify gene expression of adipokines such as resistin and FIAF. This could lead to leptin and insulin resistance and eventually, obesity. These mechanisms have been suggested in patients treated with VPA[24-26]. Adiponectin is a protein that belongs to the adipokine family. It plays an important role in the modulation of lipid metabolism and insulin sensitivity and therefore in body-weight regulation[27]. VPA has been shown to down-regulate adiponectin gene expression in adipocytes[28] and increase the gene expression of its receptor (adipoR1) in liver cells. This contributes to the dysregulation of fatty acid oxidation[29]. Leptin, another protein of the adipokine group, suppresses hunger and increases fatty acid metabolism within the adipocytes[30]. Its serum concentration and mRNA expression in adipose tissue is directly related to fasting insulin serum concentrations and to the obesity severity[31]. Increased serum leptin and leptin resistance was frequently reported in obese patients undergoing long term VPA therapy. Normal leptin levels were observed in non-obese patients[31,32]. The leptin levels and resistance may not be directly affected by VPA but could be a consequence of the abundance in adipose tissue[31,32]. Ghrelin is an orexigenic hormone which acts in increasing appetite. Plasma ghrelin levels increase before meals and decrease post-prandial[33]. Moreover, ghrelin regulates the secretion of leptin and insulin and preferentially consumes carbohydrates over lipids[33]. There is an increase in ghrelin levels in the early period of VPA-treatment[34]. Hyperinsulinaemia has been extensively reported during VPA-therapy[26,35]. It is known that this alteration is often associated with obesity, dyslipidaemia and insulin resistance. Some authors do not consider insulin resistance as an effect of weight gain. Rather, they consider it as a trigger for the development of weight gain itself[35,36]. This is supported by the observation that VPA-induced obesity is related to an increase in insulin levels and a decrease in glucose levels[23]. This could stimulate appetite and therefore

lead to obesity. A study on the prevalence of MetS among a group of obese Chinese patients treated with VPA for epilepsy revealed that the homeostasis model assessment index was higher in the VPA-obese patients than in the non-VPA treated obese control group[36]. This supports the hypothesis that VPA may be the trigger for IR. These findings have also been confirmed by a study which showed that both obese and lean patients taking VPA had higher serum insulin levels compared to their respective control groups with similar body mass index (BMI) values[35]. Different hypotheses regarding the role of VPA in determining hyperinsulinaemia and IR have been formulated: (1) VPA is a GABA agonist which may increase insulin levels directly stimulating GABA receptors of pancreatic beta-cells[37,38]; (2) VPA and its metabolites may increase oxidative stress and consequently cause pancreatic beta-cells dysfunction[39,40]; (3) VPA can affect the sympathetic response to glucose load[41]; (4) VPA metabolism can compete with fatty acid mitochondrial oxidation thus increasing free fatty acid plasma levels and therefore increase insulin release from pancreatic beta-cells[42,43]; and (5) VPA can impair insulin signal transduction pathway by inhibiting GLUT-1 mRNA expression[44]. GLUT-1 is a cell membrane carrier involved in the insulin transduction signal pathway.

On the other side of the spectrum, some others authors believe that IR and MetS are the consequences and not the trigger for increased body fat. Supporting this theory, a recent 2010 study reported that about 40% of patients who became obese during treatment with VPA developed MetS and IR, while patients who did not gain weight did not manifest such metabolic changes[45].

In summary, irrespective of the mechanism involved, VPA significantly increases body-weight and therefore the likelihood of obesity, IR and MetS. All these conditions are important risk factors for the development of cardiovascular complications, diabetes, dyslipidemia and intrahepatic fat accumulation (NAFLD). The choice of initiating VPA-therapy is a decision that should thus be made after evaluating the individual risks and benefits posed by the treatment especially in children[23].

## VPA AND NAFLD

Despite the growing body of literature regarding NAFLD, the role of VPA-therapy in the development of this liver disease is poorly understood. Luef *et al*[46] in 2004 published a pilot study. In a group of forty-five non-diabetic, non-obese, epileptic patients treated with VPA or carbamazepine (CBZ) monotherapy for at least two years, ultrasound features of fatty liver disease were found in 61% of the VPA-treated patients compared to 23% of CBZ-treated patients[46]. In 2009, the same authors led a cross-sectional controlled study where sixty-eight non-diabetic and non-obese epileptic patients received either VPA, CBZ or lamotrigine (LTG) as monotherapy for at least two years. They were compared with sixteen healthy controls. All the patients were evaluated by abdominal ultrasound and by measurement of serum fasting insulin and glucose, serum lipids, liver function parameters and anthropometric data. They demonstrated ultrasound characteristics of NAFLD in 60.9% of patients treated with VPA, in 22.7% with CBZ, in 8.7% with LTG and in 12.5% of healthy controls. The mean BMI was significantly higher in the VPA-treated group compared to LTG group and controls ($P$ = 0.015 and $P$ = 0.049 respectively). The authors emphasized the importance of regular ultrasound monitoring as well as measuring serum lipids and BMI during treatment with VPA and other AEDs[47]. In 2005, a Spanish group reported three cases of adult NAFLD in patients receiving long term VPA-therapy for epilepsy. NAFLD was diagnosed by ultrasound evaluation and by histological findings in one case. At the time of diagnosis the patients were asymptomatic but obese, dyslipidemic and with slightly elevated serum aminotransferases. Treatment with VPA was interrupted and interestingly, some months later, aminotransferase levels normalized and the ultrasound evidence of NAFLD disappeared[48]. The first pediatric case of an eleven year old pre-pubertal girl who developed NAFLD after one year of treatment with VPA was reported in 2004. At the beginning of the treatment, the girl had a normal body mass index (between 50$^{th}$-75$^{th}$ percentiles) and normal routine biochemical serum laboratory exams. Her serum exams included albumin, total proteins, alanine-aminotransferase, aspartate-aminotransferase, gamma-glutamyltransferase, ammonia and lipid profile. No major changes were noted during the first six months of therapy with VPA. After twelve months the patient showed a clear increase in BMI (95$^{th}$ percentile) and a threefold increase in serum aminotransferases values. At this time an ultrasound scan of the abdomen revealed NAFLD. The VPA treatment was replaced with LEV. Three months later, serum liver enzymes returned to normal values and NAFLD disappeared[49]. In 2011, the same authors published a cross sectional double-controlled study to highlight the prevalence of NAFLD in adolescents receiving VPA. Eighty-six adolescents with epilepsy who had received VPA monotherapy for at least twelve months and had a normal weight (BMI < 85$^{th}$ percentile) before starting VPA were enrolled. During the same period, two groups of subjects for comparison were taken. Sixty seven age and sex-matched adolescents with normal weight (normal weight controls) and forty three age, sex and BMI-matched adolescents (weight-matched controls) were included. The occurrence of NAFLD was significantly higher in the VPA-treated patients than in the normal weight control group (36% *vs* 7.5% respectively, $P$ < 0.001) but was surprisingly similar between the VPA-treated and weight-matched control (36% *vs* 34.9% respectively, $P$ > 0.05). Considering these data, the authors hypothesized that even if there is a clear association between NAFLD and VPA-treatment, the development of NAFLD may not be the consequence of the action of the VPA-metabolites *per se*[50]. It may be the consequence of the weight

gain, MetS and IR induced by VPA. Finally, a recent 2012 cross-sectional controlled study performed by an Egyptian group evaluated the presence of NAFLD in thirty-eight children and adolescents treated with VPA, CBZ or in combination for at least eight months. Interestingly, they found characteristics of NAFLD in 42.8% of VPA, in 21.4% of CBZ, in 60% of combination therapy and none in the healthy control group[51]. In this study, the diagnosis of hepatic steatosis was made by an abdominal computer tomography scan. This diagnostic modality is not normally recommended due to the unjustified radiation exposure especially for the pediatric population[12].

## CONCLUSION

Long term VPA-therapy is associated with an increased development of NAFLD. Evidences from the current literature indicate that the association is particularly strong in patients who are initially overweight with presenting features of MetS. The pathogenic mechanisms through which VPA leads to obesity, MetS and increased risk of NAFLD are still an issue for debate. Further studies are needed to clarify the chronology and sequencing of events between NAFLD, weight gain, MetS and VPA therapy and to determine if any VPA metabolite is involved. Patients in VPA-treatment who gain weight or develop features of MetS should be closely monitored to prevent or detect the early development of NAFLD. Monitoring with abdominal ultrasound, liver function tests, lipid profile and fasting blood glucose determination is strongly recommended in patients taking VPA alone or as part of a multi-AED treatment. Finally, the option to replace or retain VPA-therapy in obese patients, in patients who quickly gain weight during the treatment and in patients with risks of developing MetS and NAFLD requires standardization.

## 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 Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

2 **Loria P**, Adinolfi LE, Bellentani S, Bugianesi E, Grieco A, Fargion S, Gasbarrini A, Loguercio C, Lonardo A, Marchesini G, Marra F, Persico M, Prati D, Baroni GS. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. *Dig Liver Dis* 2010; **42**: 272-282 [PMID: 20171943 DOI: 10.1016/j.dld.2010.01.021]

3 **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]

4 **Schwimmer JB**, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641-649 [PMID: 16116629 DOI: 10.1002/hep.20842]

5 **Barshop NJ**, Francis CS, Schwimmer JB, Lavine JE. Nonalcoholic fatty liver disease as a comorbidity of childhood obesity. *Ped Health* 2009; **3**: 271-281 [PMID: 20556232 DOI: 10.2217/phe.09.21]

6 **Giorgio V**, Prono F, Graziano F, Nobili V. Pediatric non alcoholic fatty liver disease: old and new concepts on development, progression, metabolic insight and potential treatment targets. *BMC Pediatr* 2013; **13**: 40 [PMID: 23530957 DOI: 10.1186/1471-2431-13-40]

7 **Chopra S**. Patient information. Nonalcoholic steatohepatitis (NASH) (Beyond the basics). [Up date 2013]. Available from: URL: http://www.uptodate.com/contents/nonalcoholic-fatty-liver-disease-nafld-including-nonalcoholic-steatohepatitis-nash-beyond-the-basics

8 **Nobili V**, Alisi A, Raponi M. Pediatric non-alcoholic fatty liver disease: preventive and therapeutic value of lifestyle intervention. *World J Gastroenterol* 2009; **15**: 6017-6022 [PMID: 20027672 DOI: 10.3748/wjg.15.6017]

9 **Nobili V**, Bedogni G, Alisi A, Pietrobattista A, Risé P, Galli C, Agostoni C. Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomised controlled clinical trial. *Arch Dis Child* 2011; **96**: 350-353 [PMID: 21233083 DOI: 10.1136/adc.2010.192401]

10 **Nobili V**, Carpino G, Alisi A, De Vito R, Franchitto A, Alpini G, Onori P, Gaudio E. Role of docosahexaenoic acid treatment in improving liver histology in pediatric nonalcoholic fatty liver disease. *PLoS One* 2014; **9**: e88005 [PMID: 24505350 DOI: 10.1371/journal.pone.0088005]

11 **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]

12 **Nobili V**, Svegliati-Baroni G, Alisi A, Miele L, Valenti L, Vajro P. A 360-degree overview of paediatric NAFLD: recent insights. *J Hepatol* 2013; **58**: 1218-1229 [PMID: 23238106 DOI: 10.1016/j.jhep.2012.12.003]

13 **Guerrini R**. Valproate as a mainstay of therapy for pediatric epilepsy. *Paediatr Drugs* 2006; **8**: 113-129 [PMID: 16608372 DOI: 10.2165/00148581-200608020-00004]

14 **Tyrer P**, Bateman AW. Drug treatment for personality disorders. *Advances in Psychiatric Treatment* 2004; **10**: 389-398 [DOI: 10.1192/apt.10.5.389]

15 **Perucca E**. Pharmacological and therapeutic properties of valproate: a summary after 35 years of clinical experience. *CNS Drugs* 2002; **16**: 695-714 [PMID: 12269862 DOI: 10.2165/00023210-200216100-00004]

16 **Gerstner T**, Bell N, König S. Oral valproic acid for epilepsy--long-term experience in therapy and side effects. *Expert Opin Pharmacother* 2008; **9**: 285-292 [PMID: 18201150 DOI: 10.1517/14656566.9.2.285]

17 **Nanau RM**, Neuman MG. Adverse drug reactions induced by valproic acid. *Clin Biochem* 2013; **46**: 1323-1338 [PMID: 23792104 DOI: 10.1016/j.clinbiochem.2013.06.012]

18 **Powell-Jackson PR**, Tredger JM, Williams R. Hepatotoxicity to sodium valproate: a review. *Gut* 1984; **25**: 673-681 [PMID: 6428980 DOI: 10.1136/gut.25.6.673]

19 **Zimmerman HJ**, Ishak KG. Valproate-induced hepatic injury: analyses of 23 fatal cases. *Hepatology* 1982; **2**: 591-597 [PMID: 6811394 DOI: 10.1002/hep.1840020513]

20 **Lewis JH**, Zimmerman HJ, Garrett CT, Rosenberg E. Valproate-induced hepatic steatogenesis in rats. *Hepatology* 1982; **2**: 870-873 [PMID: 6815046 DOI: 10.1002/hep.1840020622]

21 **Olson MJ**, Handler JA, Thurman RG. Mechanism of zone-specific hepatic steatosis caused by valproate: inhibition of ketogenesis in periportal regions of the liver lobule. *Mol Pharmacol* 1986; **30**: 520-525 [PMID: 3097499]

22 **Egger J**, Brett EM. Effects of sodium valproate in 100 children with special reference to weight. *Br Med J (Clin Res Ed)* 1981; **283**: 577-581 [PMID: 6790086 DOI: 10.1136/bmj.283.6291.577]

23 **Verrotti A**, D'Egidio C, Mohn A, Coppola G, Chiarelli F. Weight gain following treatment with valproic acid: pathogenetic mechanisms and clinical implications. *Obes Rev* 2011; **12**: e32-e43

[PMID: 20880119 DOI: 10.1111/j.1467-789X.2010.00800.x]
24 **Brown R**, Imran SA, Ur E, Wilkinson M. Valproic acid and CEBPalpha-mediated regulation of adipokine gene expression in hypothalamic neurons and 3T3-L1 adipocytes. *Neuroendocrinology* 2008; **88**: 25-34 [PMID: 18212493 DOI: 10.1159/000113927]
25 **Howard JK**, Flier JS. Attenuation of leptin and insulin signaling by SOCS proteins. *Trends Endocrinol Metab* 2006; **17**: 365-371 [PMID: 17010638 DOI: 10.1016/j.tem.2006.09.007]
26 **Verrotti A**, Basciani F, De Simone M, Trotta D, Morgese G, Chiarelli F. Insulin resistance in epileptic girls who gain weight after therapy with valproic acid. *J Child Neurol* 2002; **17**: 265-268 [PMID: 12088081]
27 **Kawano J**, Arora R. The role of adiponectin in obesity, diabetes, and cardiovascular disease. *J Cardiometab Syndr* 2009; **4**: 44-49 [PMID: 19245516 DOI: 10.1111/j.1559-4572.2008.00030.x]
28 **Qiao L**, Schaack J, Shao J. Suppression of adiponectin gene expression by histone deacetylase inhibitor valproic acid. *Endocrinology* 2006; **147**: 865-874 [PMID: 16282359 DOI: 10.1210/en.2005-1030]
29 **Greco R**, Latini G, Chiarelli F, Iannetti P, Verrotti A. Leptin, ghrelin, and adiponectin in epileptic patients treated with valproic acid. *Neurology* 2005; **65**: 1808-1809 [PMID: 16344528 DOI: 10.1212/01.wnl.0000187074.27586.d1]
30 **Aydin K**, Serdaroglu A, Okuyaz C, Bideci A, Gucuyener K. Serum insulin, leptin, and neuropeptide y levels in epileptic children treated with valproate. *J Child Neurol* 2005; **20**: 848-851 [PMID: 16417885 DOI: 10.1177/08830738050200101501]
31 **Verrotti A**, Basciani F, Morresi S, de Martino M, Morgese G, Chiarelli F. Serum leptin changes in epileptic patients who gain weight after therapy with valproic acid. *Neurology* 1999; **53**: 230-232 [PMID: 10408570 DOI: 10.1212/WNL.53.1.230]
32 **Hamed SA**. Leptin and insulin homeostasis in epilepsy: relation to weight adverse conditions. *Epilepsy Res* 2007; **75**: 1-9 [PMID: 17499974 DOI: 10.1016/j.eplepsyres.2007.04.001]
33 **Asakawa A**, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, Kasuga M. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 2003; **52**: 947-952 [PMID: 12801949 DOI: 10.1136/gut.52.7.947]
34 **Gungor S**, Yücel G, Akinci A, Tabel Y, Ozerol IH, Yologlu S. The role of ghrelin in weight gain and growth in epileptic children using valproate. *J Child Neurol* 2007; **22**: 1384-1388 [PMID: 18174556 DOI: 10.1177/0883073807307096]
35 **Pylvänen V**, Knip M, Pakarinen A, Kotila M, Turkka J, Isojärvi JI. Serum insulin and leptin levels in valproate-associated obesity. *Epilepsia* 2002; **43**: 514-517 [PMID: 12027912 DOI: 10.1046/j.1528-1157.2002.31501.x]
36 **Fang J**, Chen S, Tong N, Chen L, An D, Mu J, Zhou D. Metabolic syndrome among Chinese obese patients with epilepsy on sodium valproate. *Seizure* 2012; **21**: 578-582 [PMID: 22743100 DOI: 10.1016/j.seizure.2012.06.001]
37 **Luef GJ**, Lechleitner M, Bauer G, Trinka E, Hengster P. Valproic acid modulates islet cell insulin secretion: a possible mechanism of weight gain in epilepsy patients. *Epilepsy Res* 2003; **55**: 53-58 [PMID: 12948616 DOI: 10.1016/S0920-1211(03)00091-3]
38 **Shi Y**, Kanaani J, Menard-Rose V, Ma YH, Chang PY, Hanahan D, Tobin A, Grodsky G, Baekkeskov S. Increased expression of GAD65 and GABA in pancreatic beta-cells impairs first-phase insulin secretion. *Am J Physiol Endocrinol Metab* 2000; **279**: E684-E694 [PMID: 10950838]
39 **Aycicek A**, Iscan A. The effects of carbamazepine, valproic acid and phenobarbital on the oxidative and antioxidative balance in epileptic children. *Eur Neurol* 2007; **57**: 65-69 [PMID: 17179706 DOI: 10.1159/000098053]
40 **Evans JL**, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 2003; **52**: 1-8 [PMID: 12502486]
41 **Meeker RB**, Myers RD. GABA and glutamate: possible metabolic intermediaries involved in the hypothalamic regulation of food intake. *Brain Res* 1980; **5**: 253-259 [DOI: 10.1016/0361-9230(80)90042-8]
42 **McGarry JD**. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 2002; **51**: 7-18 [PMID: 11756317 DOI: 10.2337/diabetes.51.1.7]
43 **Yoshikawa H**, Tajiri Y, Sako Y, Hashimoto T, Umeda F, Nawata H. Effects of free fatty acids on beta-cell functions: a possible involvement of peroxisome proliferator-activated receptors alpha or pancreatic/duodenal homeobox. *Metabolism* 2001; **50**: 613-618 [PMID: 11319727 DOI: 10.1053/meta.2001.22565]
44 **Wong HY**, Chu TS, Lai JC, Fung KP, Fok TF, Fujii T, Ho YY. Sodium valproate inhibits glucose transport and exacerbates Glut1-deficiency in vitro. *J Cell Biochem* 2005; **96**: 775-785 [PMID: 16149077 DOI: 10.1002/jcb.20555]
45 **Verrotti A**, Manco R, Agostinelli S, Coppola G, Chiarelli F. The metabolic syndrome in overweight epileptic patients treated with valproic acid. *Epilepsia* 2010; **51**: 268-273 [PMID: 19682024 DOI: 10.1111/j.1528-1167.2009.02206.x]
46 **Luef GJ**, Waldmann M, Sturm W, Naser A, Trinka E, Unterberger I, Bauer G, Lechleitner M. Valproate therapy and nonalcoholic fatty liver disease. *Ann Neurol* 2004; **55**: 729-732 [PMID: 15122714 DOI: 10.1002/ana.20074]
47 **Luef G**, Rauchenzauner M, Waldmann M, Sturm W, Sandhofer A, Seppi K, Trinka E, Unterberger I, Ebenbichler CF, Joannidis M, Walser G, Bauer G, Hoppichler F, Lechleitner M. Non-alcoholic fatty liver disease (NAFLD), insulin resistance and lipid profile in antiepileptic drug treatment. *Epilepsy Res* 2009; **86**: 42-47 [PMID: 19464851 DOI: 10.1016/j.eplepsyres.2009.04.004]
48 **Ortiz-Sáenz de Santa María R**, Santiago-Fernández C, Cano-Del Pozo M, Pérez-Gil O, Soto-Alvarez MI, Barriuso-Pérez E, Moche-Loeri JA. [Treatment with valproate and hepatic steatosis]. *Rev Neurol* 2005; **41**: 766-767 [PMID: 16355364]
49 **Verrotti A**, Di Marco G, la Torre R, Pelliccia P, Chiarelli F. Nonalcoholic fatty liver disease during valproate therapy. *Eur J Pediatr* 2009; **168**: 1391-1394 [PMID: 19184102 DOI: 10.1007/s00431-009-0927-y]
50 **Verrotti A**, Agostinelli S, Parisi P, Chiarelli F, Coppola G. Nonalcoholic fatty liver disease in adolescents receiving valproic acid. *Epilepsy Behav* 2011; **20**: 382-385 [PMID: 21256090 DOI: 10.1016/j.yebeh.2010.12.012]
51 **Saleh DA**, Ismail MA, Ibrahim AM. Non alcoholic fatty liver disease, insulin resistance, dyslipidemia and atherogenic ratios in epileptic children and adolescents on long term antiepileptic drug therapy. *Pak J Biol Sci* 2012; **15**: 68-77 [PMID: 22545359]

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MINIREVIEWS

# Bone changes in alcoholic liver disease

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## Abstract

Alcoholism has been associated with growth impairment, osteomalacia, delayed fracture healing, and aseptic necrosis (primarily necrosis of the femoral head), but the main alterations observed in the bones of alcoholic patients are osteoporosis and an increased risk of fractures. Decreased bone mass is a hallmark of osteoporosis, and it may be due either to decreased bone synthesis and/or to increased bone breakdown. Ethanol may affect both mechanisms. It is generally accepted that ethanol decreases bone synthesis, and most authors have reported decreased osteocalcin levels (a "marker" of bone synthesis), but some controversy exists regarding the effect of alcohol on bone breakdown, and, indeed, disparate results have been reported for telopeptide and other biochemical markers of bone resorption. In addition to the direct effect of ethanol, systemic alterations such as malnutrition, malabsorption, liver disease, increased levels of proinflammatory cytokines, alcoholic myopathy and neuropathy, low testosterone levels, and an increased risk of trauma, play contributory roles. The treatment of alcoholic bone disease should be aimed towards increasing bone formation and decreasing bone degradation. In this sense, vitamin D and calcium supplementation, together with biphosphonates are essential, but alcohol abstinence and nutritional improvement are equally important. In this review we study the pathogenesis of bone changes in alcoholic liver disease and discuss potential therapies.

**Key words:** Alcoholism; Liver disease; Osteoporosis; Vitamin D; Bone fractures



**Core tip:** Alcoholism is associated with an increased risk of fractures and a higher prevalence of bone disease, particularly osteoporosis. While ethanol has a direct toxic effect on bone, other factors such as malnutrition, increased levels of proinflammatory cytokines, alcoholic myopathy and neuropathy, and an increased risk of trauma also contribute to bone disease. It is noteworthy that alterations in bone metabolism have been described as reversible so that the mainstay of treatment should be alcohol abstinence. Treatment with vitamin D, calcium, and biphosphonates have been studied in the general population but further trials are needed in alcoholic patients.

González-Reimers E, Quintero-Platt G, Rodríguez-Rodríguez E, Martínez-Riera A, Alvisa-Negrín J, Santolaria-Fernández F. Bone changes in alcoholic liver disease. *World J Hepatol* 2015; 7(9): 1258-1264 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i9/1258.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i9.1258

## INTRODUCTION

Heavy alcohol consumption has classically been associated with an increased risk of bone fractures. In 1977, Oppenheim[1] described the "battered alcoholic syndrome" in patients with three or more fractures in different stages of healing. In that study, Oppenheim suggests that alcohol itself has direct negative effects on bone metabolism. In fact, other authors have found a similarity between alcoholic patients and the postmenopausal and geriatric population due to an increased incidence of fractures and a lower bone density. In 1965, Saville[2] described that the bone density measured in the left iliac crest of alcoholics below 45 years of age was similar to that of non-alcoholic men and women older than 70 years. Snell[3] described in 1971 that the pattern of fractures seen in alcoholics is similar to that seen in patients 20 years older. And in a classic study published in 1980, Israel *et al*[4] found an increased prevalence of rib and vertebral fractures in alcoholic patients and therefore suggest using routine chest X rays to identify problem drinking and to screen the general population for alcoholism.

Although alcoholics may have an increased risk of trauma and subsequent fractures, alcohol itself has a negative impact on bone metabolism. Alcoholism has been associated with growth impairment, osteoporosis, osteomalacia, fractures, delayed fracture healing, and aseptic necrosis (primarily necrosis of the femoral head)[5,6]. The mechanisms that explain the loss of structural integrity of bone are partially known. These include a direct toxic effect of ethanol on bone synthesis and systemic alterations such as malnutrition and malabsorption, liver disease, altered hormonal and cytokine profiles, alcoholic myopathy, and neuropathy (Figure 1)[6]. In this review we will outline each of these mechanisms.

## ALTERED BONE SYNTHESIS. DIRECT EFFECTS OF ETHANOL

As mentioned above, in 1965 Saville[2] had already described decreased bone density among alcoholic patients. In 1989 Diamond *et al*[7] found that drinkers had significantly less osteoblastic activity than tee-totallers and suggested that reduced bone formation and mineralization is due to osteoblastic dysfunction. This has been reproduced in a study by Gonzalez-Calvín *et al*[8] where they found that chronic ethanol consumption reduces bone mineral density regardless of liver cirrhosis. In recent decades osteocalcin, a polypeptide bone matrix protein also known as bone gamma-carboxyglutamic acid-containing protein or BGLAP, has been described as a specific marker of osteoblast function and bone synthesis[9]. In the same study by Diamond *et al*[7], they described decreased osteocalcin levels in alcoholics. The same finding was also described in a more recent study by Santori *et al*[10] in 2008.

Giuliani *et al*[11] found that in murine bone marrow cultures, ethanol and acetaldehyde (the main metabolite of ethanol) significantly reduced osteoblast proliferation and decreased the number of colony forming units for fibroblasts. This may explain the decreased bone mineral density (BMD) in alcoholics. In fact, Torricelli *et al*[12] found in an *in vitro* model that osteoblast proliferation is affected more significantly by exposure to alcohol than by estrogen deficiency. Therefore, chronic alcoholism may have even more deleterious effects on bone metabolism than menopause.

These findings contrast with those described by Lau *et al*[13] in which they found that ethanol had a dose-dependent effect on bone formation. These authors found that low levels of alcohol increased bone proliferation but that alcohol might interfere with the normal bone remodeling process[13]. Similar results were reported by Marrone *et al*[14] where postmenopausal moderate alcohol consumers were found to have increased BMD and reduced bone turnover markers. However, in several studies the amount of alcohol consumed and the presence of osteopenia adopts a J-shaped curve where heavy drinking clearly has detrimental effects on BMD and the risk of fractures[15,16].

Other studies have also suggested that the inhibition of bone formation in chronic alcoholism may be mediated by the accumulation of reactive oxygen species (ROS). Chen *et al*[17] found that in a rat model ethanol up-regulated NADPH oxidase expression in osteoblasts. Administration of antioxidants such as N-acetyl cysteine prevented bone loss in rats exposed to ethanol[17]. This was also shown in a study by Lee *et al*[18] and Choi *et al*[19] where an NADPH oxidase inhibitor called apocynin protected osteoblasts from oxidative damage and increased cell survival, calcium deposition and osteoprotegerin release. Consistent with these findings, Chen *et al*[20] showed that ethanol-induced TNF-α secretion also promotes differentiation of mesenchymal cells towards adipose tissue. All of these effects are in line with the observation performed by Maurel *et al*[21] regarding osteocyte apoptosis suffered by osteocytes exposed to ethanol.

However, the changes in bone metabolism are not permanent and bone disorders in alcoholic patients have been considered by some authors as reversible[22,23]. In fact, in the study mentioned above by Gonzalez-Calvin they found that after just 7 d of abstinence, osteocalcin levels increased significantly[8]. We found similar results among 60 patients who were followed-up after admission and it was found that those who had stopped

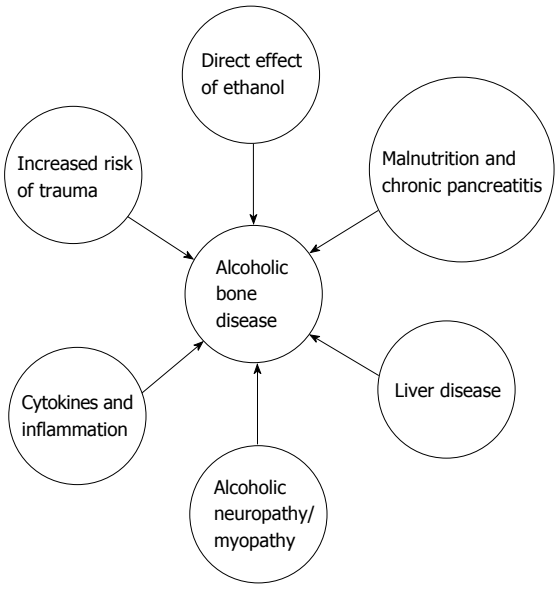


**Figure 1 Factors that contribute to bone disease in alcoholic patients.**

drinking showed an increase in total BMD as measured by bone densitometry[24].

While there are several studies that describe the effect of alcohol on bone metabolism in adults, adolescence is an important period in determining bone mass. In a study performed in rats by Turner *et al*[25] in 1988, it was found that tibial length was lower in animals exposed to ethanol and that there was an increase in resorption and a loss of trabecular bone. While bone disorders associated with alcoholism have been reported to be reversible in adults, osteopenia was shown to not be completely reversible in growing Sprague-Dawley rats exposed to ethanol[26]. We found among two sets of patients that those who began to drink before 18 years of age were shorter than controls. We also found that these patients had Harris lines in their right tibiae, a condition associated with episodes of stunted growth and subsequent recovery[27].

## ALTERED HORMONE LEVELS MUSCLE ATROPHY

The effects of alcohol administration have been described in rats where it resulted in the stimulation of adrenal steroid production that did not occur when rats were hypophysectomised[28]. Smith[28] found that stimulation of the adrenal cortex led to increased excretion of calcium in urine which may contribute to bone demineralization in alcoholics. In a study by Badrick *et al*[29] a 3% increase in cortisol levels per unit of alcohol consumed was found. They also showed a reduced control of the hypothalamic-pituitary-adrenal (HPA) axis. However, what is usually described among alcoholics is a Pseudo-Cushing's syndrome in which it has been suggested that there is increased secretion of corticotropin releasing hormone with a normal HPA axis[30-33]. While these patients usually have physical signs of hypercortisolism, they can be differentiated because Cushing's syndrome is associated with a higher cortisol midnight:morning ratio, higher midnight serum cortisol levels, and higher levels of corticotropin-releasing hormone/stimulated cortisol after dexamethasone suppression[34]. While cortisol may theoretically reduce BMD, we have failed to find a relationship between cortisol and BMD in several studies[27].

Muscle mass and strength are determinants of bone mass and muscle atrophy and bone loss have already been described as interdependent. In a study by Lloyd it has been found that in mice subjected to mechanical unloading, muscle atrophy precedes bone loss and contributes to trabecular bone loss[35]. In this sense, muscle atrophy associated with alcoholism is yet another factor that contributes to decreased BMD in alcoholics. Muscle atrophy in alcoholics is, in turn, associated with vitamin D deficiency and low testosterone levels.

Decreased vitamin D levels have been described in patients with chronic liver disease[36]. In a study by Fisher *et al*[37] it was found that cirrhotic patients had a higher prevalence of vitamin D deficiency (defined as levels below 50 nmol/L) and that these levels were significantly lower in Child-Pugh class C patients. A correlation between serum vitamin D levels and INR and albumin was also found. In addition, vitamin D has an important effect on muscle and a specific vitamin D receptor has already been described in muscle fibers[38-40].

On the other hand, hypogonadism has been classically described in men with chronic alcoholism and this has been attributed to a defect in testicular androgen production or to defects in luteinizing hormone secretion[41]. Testosterone increases muscle mass so that testosterone deficiency in alcoholics contributes to the muscle atrophy that is seen in these patients.

Muscle function affects bone through the activation of the Wnt/β-catenin pathway mediated by the load exerted by muscle contraction. The Wnt signaling cascade was described in the 1980s and its role in bone formation has been extensively studied in recent years[42]. This cascade regulates osteogenesis through the repression of mesenchymal differentiation towards adipocytes and chondrocytes, favoring osteoblast differentiation while also blocking osteoblast apoptosis[42]. In a study by Kramer *et al*[43] it was shown that β-catenin deficient mice had a low bone mass associated with increased osteoclast activity. Independently on the effects of muscle mass, Chen *et al*[44] described that ethanol inhibits the Wnt1/β-catenin signaling pathway, leading to a shift of differentiation of bone marrow cells towards adipogenesis instead towards osteoblasts. Li *et al*[45] have shown that sclerostin could antagonize Wnt signaling by binding to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6). In this sense, loss of sclerostin should be associated with an increased bone mass but low sclerostin levels have been described in osteoporotic women[46]. In a preliminary study on alcoholic patients we have found increased levels of sclerostin which could

explain decreased BMD[47].

## INCREASED BREAKDOWN THE ROLE OF CYTOKINES AND THE OPG/RANK-L SYSTEM

Bone resorption depends on osteoclasts and osteoclast differentiation is in turn mediated through the receptor activator of nuclear factor-kappaB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) signaling pathway which was described in the late 1990s[48]. This pathway regulates the formation and activation of osteoclasts. On the other hand, OPG binds to RANKL and prevents it from binding to RANK. Osteoprotegerin was first described by Simonet *et al*[49] in 1997 as a novel member of the TNF receptor superfamily that is able to block osteoclast formation. Therefore, the OPG/RANKL ratio is an important marker of bone formation/degradation and is also regulated by the Wnt/bcatenin canonical pathway. This pathway up-regulates OPG expression and down-regulates RANKL expression, thus suppressing osteoclastogenesis[50].

Hofbauer *et al*[51] report that proinflammatory cytokines such as interleukin (IL)-1beta and tumor necrosis factor (TNF)-α but not IL-6 increase osteoprotegerin ligand levels (whose effects are prevented by its receptor, osteoprotegerin) which may stimulate the formation of osteoclasts. This may explain reduced BMD among alcoholics in which there are increased levels of TNF and IL-6 due to the effect of activated Kuppfer cells mediated by gut-derived endotoxin[52,53]. Proinflammatory cytokine levels are also increased in alcoholics due to associated infections that are common among these patients. Therefore, the effects of increased TNF-α levels involve both decreased osteoblast differentiation and increased bone resorption. In accordance with the resorptive effect of TNF-α (a molecule which induces increased ROS production), NADPH oxidase derived ROS are also involved in increased osteoclastogenesis[54].

However, despite these experimental evidence, in alcoholic patients the effects of ethanol on osteoclast activity is less clear[55] and while some authors report increased bone turnover in alcoholic cirrhosis, others find no differences with controls. In one study we found increased levels of OPG in alcoholic patients which may be interpreted as a compensatory mechanism in the context of osteopenia[56].

## MALNUTRITION AND VITAMIN D DEFICIENCY IN ALCOHOLICS

Malnutrition is one of the main factors that contributes to decreased bone mass among alcoholic patients and is associated with decreased caloric intake, impaired absorption, and decreased hepatic synthesis of proteins. Alcoholism-associated malnutrition is multifactorial and is related to irregular feeding habits, cirrhosis with ascites, high ethanol intake and social factors[57]. Interestingly, we have shown that low BMD is not related to the intensity of alcoholism or calciotropic hormone levels but to malnutrition[27].

Specific nutritional deficiencies such as lower zinc levels have also been described among chronic alcoholics[58]. Zinc deficiency in these patients may be due to impaired absorption, increased urinary excretion or decreased affinity of albumin for zinc[59]. As described by Yamaguchi[60], zinc stimulates osteoblastic bone formation and mineralization while its deficiency is associated with decreased bone mass. This suggests a possible role of zinc supplementation in the prevention of osteoporosis in alcoholic patients.

Bone changes in alcoholic patients are also related to deranged vitamin D metabolism. As reviewed previously by Pitts *et al*[61], low vitamin D levels in alcoholics are due to reduced hepatic 25-hydroxylase activity, malabsorption, irregular feeding habits and lack of sun exposure. Low vitamin D levels also have an effect on calcium and phosphate homeostasis due to malabsorption of calcium and resultant hypocalcemia. Laitinen *et al*[62] described in 1991 that moderate drinking elevates parathyroid hormone (PTH) levels while acute ingestion of alcohol leads to a transient decrease in PTH. This was reproduced in an animal model studied by Chen *et al*[63] where it was found that long term treatment of hamsters with ethanol lead to stimulation of the secretory activity of the parathyroid gland. Another effect of ethanol on calcium homeostasis was described by Kalbfleisch *et al*[64] in 1965 when it was described that the administration of ethanol increased urinary excretion of calcium and magnesium.

## POTENTIAL THERAPIES FOR ALCOHOL-INDUCED BONE DISEASE

As illustrated throughout this review, alcohol has a toxic effect on bone mediated by decreased bone formation and increased bone degradation that are, in turn, influenced by nutritional, hormonal, and proinflammatory factors. Therefore, the treatment of osteoporosis in alcoholic patients should be aimed towards increasing osteoblastic and decreasing osteoclastic activity (Figure 2). As mentioned above, the supplementation of alcoholic patients with micronutrients such as zinc may also have an important role in the prevention of bone disease. In this sense, we have shown that in Sprague-Dawley rats zinc supplementation increases osteoid area but randomized trials in patients are still needed[58].

Some studies have shown that vitamin D supplementation in mice exposed to ethanol may have beneficial effects on bone metabolism[65] and this has also been shown in animal models of acute alcohol consumption (equivalent to binge drinking)[66]. Once again, further studies in humans are needed to determine the impact of vitamin D supplementation and treatment with

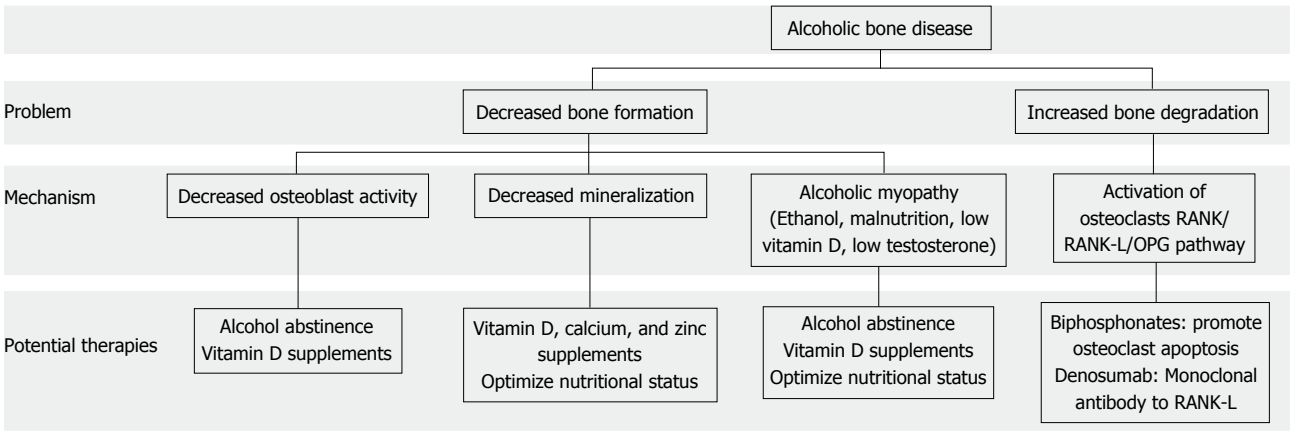


**Figure 2 Pathogenesis of alcoholic bone disease and potential therapies.** Bone disease in alcoholic patients is due basically to decreased bone formation (decreased osteoblast activity and poor mineralization) and increased bone degradation (increased osteoclast activity). Potential therapies should counteract these effects but the mainstay of treatment should be alcohol abstinence. RANK: Receptor activator of nuclear factor kappa-B; RANK-L: RANK ligand; OPG: Osteoprotegerin.

biphosphonates among alcoholic patients.

Biphosphonates inhibit bone resorption and have been widely used to treat osteoporosis in postmenopausal women. They promote the apoptosis of osteoclasts through the inhibition of farnesyl pyrophosphate synthase, an enzyme that is involved in the production of isoprenoid lipids. This impairs the isoprenylation of proteins that are involved in the survival of osteoclasts[67]. In a meta-analysis that studied 11 trials performed on postmenopausal women receiving alendronate, it was found that it reduced the risk of both vertebral and non vertebral fractures. It also increased bone density and this was dose and time dependent[68].

Biphosphonates have also been studied in patients with chronic liver disease. In a recent Cochrane meta-analysis it was found that treatment of patients with primary biliary cirrhosis with biphosphonates (etidronate or alendronate) did not decrease the risk of fractures compared with those who received placebo. They also had no significant effect on bone mineral density compared with placebo. In the trials analyzed they found that biphosphonates reduced the concentration of urinary amino telopeptides of collagen and serum osteocalcin[69]. Despite these findings, biphosphonates are well tolerated and are considered the mainstay of treatment in bone disease associated with both cholestatic and parenchymal liver disease.

Other classes of drugs that have been shown to be effective in postmenopausal osteoporosis and could theoretically benefit alcohol-induced osteoporosis are selective estrogen receptor modulators such as raloxifene and molecules that inhibit the activation of the RANK/RANKL pathway such as Denosumab. Raloxifene was studied in postmenopausal women in the MORE trial and it was found that continued therapy reduced the incidence of vertebral fractures, preserved bone mineral density and lowered markers of bone turnover[70]. On the other hand, Denosumab is a human monoclonal antibody to the RANKL, thus inhibiting its binding to RANK and decreasing osteoclast activity and subsequent bone resorption. Denosumab reduced the risk of vertebral, non vertebral, and hip fractures in postmenopausal women compared to placebo[71]. Once again, these results could be extrapolated to alcoholic patients.

## REFERENCES


1 **Oppenheim WL**. The "battered alcoholic syndrome". *J Trauma* 1977; **17**: 850-856 [PMID: 915961 DOI: 10.1097/00005373-197711000-00006]

2 **Saville PD**. Changes in bone mass with age and alcoholism. *J Bone Joint Surg Am* 1965; **47**: 492-499 [PMID: 14275168]

3 **Snell W**. The chronic alcoholic and his fractures. *J Bone Joint Surg* (Am) 1971; **53**-A: 1655

4 **Israel Y**, Orrego H, Holt S, Macdonald DW, Meema HE. Identification of alcohol abuse: thoracic fractures on routine chest X-rays as indicators of alcoholism. *Alcohol Clin Exp Res* 1980; **4**: 420-422 [PMID: 7004243 DOI: 10.1111/j.1530-0277.1980.tb04842.x]

5 **Arlot ME**, Bonjean M, Chavassieux PM, Meunier PJ. Bone histology in adults with aseptic necrosis. Histomorphometric evaluation of iliac biopsies in seventy-seven patients. *J Bone Joint Surg Am* 1983; **65**: 1319-1327 [PMID: 6361038]

6 **Chakkalakal DA**. Alcohol-induced bone loss and deficient bone repair. *Alcohol Clin Exp Res* 2005; **29**: 2077-2090 [PMID: 16385177 DOI: 10.1097/01.alc.0000192039.21305.55]

7 **Diamond T**, Stiel D, Lunzer M, Wilkinson M, Posen S. Ethanol reduces bone formation and may cause osteoporosis. *Am J Med* 1989; **86**: 282-288 [PMID: 2784031 DOI: 10.1016/0002-9343(89)90297-0]

8 **Gonzalez-Calvín JL**, Garcia-Sanchez A, Bellot V, Muñoz-Torres M, Raya-Alvarez E, Salvatierra-Rios D. Mineral metabolism, osteoblastic function and bone mass in chronic alcoholism. *Alcohol Alcohol* 1993; **28**: 571-579 [PMID: 8274181]

9 **Neve A**, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. *J Cell Physiol* 2013; **228**: 1149-1153 [PMID: 23139068 DOI: 10.1002/jcp.24278]

10 **Santori C**, Ceccanti M, Diacinti D, Attilia ML, Toppo L, D' Erasmo E, Romagnoli E, Mascia ML, Cipriani C, Prastaro A, Carnevale V, Minisola S. Skeletal turnover, bone mineral density, and fractures in male chronic abusers of alcohol. *J Endocrinol Invest* 2008; **31**: 321-326 [PMID: 18475050]

11 **Giuliani N**, Girasole G, Vescovi PP, Passeri G, Pedrazzoni M. Ethanol and acetaldehyde inhibit the formation of early osteoblast progenitors in murine and human bone marrow cultures. *Alcohol Clin Exp Res* 1999; **23**: 381-385 [PMID: 10069572 DOI: 10.1111/

j.1530-0277.1999.tb04126.x]
12 **Torricelli P**, Fini M, Giavaresi G, Borsari V, Rimondini L, Rimondini R, Carrassi A, Giardino R. Intermittent exposure to ethanol vapor affects osteoblast behaviour more severely than estrogen deficiency does in vitro study on rat osteoblasts. *Toxicology* 2007; **237**: 168-176 [PMID: 17590496 DOI: 10.1016/j.tox.2007.05.010]
13 **Lau JR**, Farley R, Fitzsimmons AK. An in vitro investigation of effects of ethanol on bone formation and bone resorption. *Orthopaedic Research Society* 1985; **9**: 278-279
14 **Marrone JA**, Maddalozzo GF, Branscum AJ, Hardin K, Cialdella-Kam L, Philbrick KA, Breggia AC, Rosen CJ, Turner RT, Iwaniec UT. Moderate alcohol intake lowers biochemical markers of bone turnover in postmenopausal women. *Menopause* 2012; **19**: 974-979 [PMID: 22922514 DOI: 10.1097/gme.0b013e31824ac071]
15 **Sommer I**, Erkkilä AT, Järvinen R, Mursu J, Sirola J, Jurvelin JS, Kröger H, Tuppurainen M. Alcohol consumption and bone mineral density in elderly women. *Public Health Nutr* 2013; **16**: 704-712 [PMID: 22800300 DOI: 10.1017/S136898001200331X]
16 **Berg KM**, Kunins HV, Jackson JL, Nahvi S, Chaudhry A, Harris KA, Malik R, Arnsten JH. Association between alcohol consumption and both osteoporotic fracture and bone density. *Am J Med* 2008; **121**: 406-418 [PMID: 18456037 DOI: 10.1016/j.amjmed.2007.12.012]
17 **Chen JR**, Lazarenko OP, Shankar K, Blackburn ML, Lumpkin CK, Badger TM, Ronis MJ. Inhibition of NADPH oxidases prevents chronic ethanol-induced bone loss in female rats. *J Pharmacol Exp Ther* 2011; **336**: 734-742 [PMID: 21098090 DOI: 10.1124/jpet.110.175091]
18 **Lee YS**, Choi EM. Apocynin stimulates osteoblast differentiation and inhibits bone-resorbing mediators in MC3T3-E1 cells. *Cell Immunol* 2011; **270**: 224-229 [PMID: 21683946 DOI: 10.1016/j.cellimm.2011.05.011]
19 **Choi EM**, Lee YS. Protective effect of apocynin on antimycin A-induced cell damage in osteoblastic MC3T3-E1 cells. *J Appl Toxicol* 2012; **32**: 714-721 [PMID: 21538410 DOI: 10.1002/jat.1689]
20 **Chen Y**, Gao H, Yin Q, Chen L, Dong P, Zhang X, Kang J. ER stress activating ATF4/CHOP-TNF-α signaling pathway contributes to alcohol-induced disruption of osteogenic lineage of multipotential mesenchymal stem cell. *Cell Physiol Biochem* 2013; **32**: 743-754 [PMID: 24080827 DOI: 10.1159/000354476]
21 **Maurel DB**, Pallu S, Jaffré C, Fazzalari NL, Boisseau N, Uzbekov R, Benhamou CL, Rochefort GY. Osteocyte apoptosis and lipid infiltration as mechanisms of alcohol-induced bone loss. *Alcohol Alcohol* 2012; **47**: 413-422 [PMID: 22596044 DOI: 10.1093/alcalc/ags057]
22 **Lindholm J**, Steiniche T, Rasmussen E, Thamsborg G, Nielsen IO, Brockstedt-Rasmussen H, Storm T, Hyldstrup L, Schou C. Bone disorder in men with chronic alcoholism: a reversible disease? *J Clin Endocrinol Metab* 1991; **73**: 118-124 [PMID: 2045462 DOI: 10.1210/jcem-73-1-118]
23 **Peris P**, Parés A, Guañabens N, Del Río L, Pons F, Martínez de Osaba MJ, Monegal A, Caballería J, Rodés J, Muñoz-Gómez J. Bone mass improves in alcoholics after 2 years of abstinence. *J Bone Miner Res* 1994; **9**: 1607-1612 [PMID: 7817807]
24 **Alvisa-Negrín J**, González-Reimers E, Santolaria-Fernández F, García-Valdecasas-Campelo E, Valls MR, Pelazas-González R, Durán-Castellón MC, de Los Angeles Gómez-Rodríguez M. Osteopenia in alcoholics: effect of alcohol abstinence. *Alcohol Alcohol* 2009; **44**: 468-475 [PMID: 19535494 DOI: 10.1093/alcalc/agp038]
25 **Turner RT**, Aloia RC, Segel LD, Hannon KS, Bell NH. Chronic alcohol treatment results in disturbed vitamin D metabolism and skeletal abnormalities in rats. *Alcohol Clin Exp Res* 1988; **12**: 159-162 [PMID: 3279849 DOI: 10.1111/j.1530-0277.1988.tb00152.x]
26 **Sampson HW**, Spears H. Osteopenia due to chronic alcohol consumption by young actively growing rats is not completely reversible. *Alcohol Clin Exp Res* 1999; **23**: 324-327 [PMID: 10069563 DOI: 10.1111/j.1530-0277.1999.tb04117.x]
27 **Santolaria F**, González-Reimers E, Pérez-Manzano JL, Milena A, Gómez-Rodríguez MA, González-Díaz A, de la Vega MJ, Martínez-Riera A. Osteopenia assessed by body composition analysis is related to malnutrition in alcoholic patients. *Alcohol* 2000; **22**: 147-157 [PMID: 11163122 DOI: 10.1016/S0741-8329(00)00115-4]
28 **Smith J**. The effect of alcohol on the ascorbic acid and cholesterol of the rat. *J Clin Endoc* 1951; **11**: 792-797
29 **Badrick E**, Bobak M, Britton A, Kirschbaum C, Marmot M, Kumari M. The relationship between alcohol consumption and cortisol secretion in an aging cohort. *J Clin Endocrinol Metab* 2008; **93**: 750-757 [PMID: 18073316 DOI: 10.1210/jc.2007-0737]
30 **Ceroni L**, Cota D, Pasquali R. [Pseudo-Cushing syndrome. Physiopathologic aspects and differential diagnosis]. *Minerva Endocrinol* 2000; **25**: 47-54 [PMID: 11189791]
31 **Kapcala LP**. Alcohol-induced pseudo-Cushing's syndrome mimicking Cushing's disease in a patient with an adrenal mass. *Am J Med* 1987; **82**: 849-856 [PMID: 3565439 DOI: 10.1016/0002-9343(87)90028-3]
32 **Lamberts SW**, Klijn JG, de Jong FH, Birkenhäger JC. Hormone secretion in alcohol-induced pseudo-Cushing's syndrome. Differential diagnosis with Cushing disease. *JAMA* 1979; **242**: 1640-1643 [PMID: 39183 DOI: 10.1001/jama.242.15.1640]
33 **Kirkman S**, Nelson DH. Alcohol-induced pseudo-Cushing's disease: a study of prevalence with review of the literature. *Metabolism* 1988; **37**: 390-394 [PMID: 2833680 DOI: 10.1016/0026-0495(88)90141-2]
34 **Alwani RA**, Schmit Jongbloed LW, de Jong FH, van der Lely AJ, de Herder WW, Feelders RA. Differentiating between Cushing's disease and pseudo-Cushing's syndrome: comparison of four tests. *Eur J Endocrinol* 2014; **170**: 477-486 [PMID: 24394725 DOI: 10.1530/EJE-13-0702]
35 **Lloyd SA**, Lang CH, Zhang Y, Paul EM, Laufenberg LJ, Lewis GS, Donahue HJ. Interdependence of muscle atrophy and bone loss induced by mechanical unloading. *J Bone Miner Res* 2014; **29**: 1118-1130 [PMID: 24127218 DOI: 10.1002/jbmr.2113]
36 **Miroliaee A**, Nasiri-Toosi M, Khalilzadeh O, Esteghamati A, Abdollahi A, Mazloumi M. Disturbances of parathyroid hormone-vitamin D axis in non-cholestatic chronic liver disease: a cross-sectional study. *Hepatol Int* 2010; **4**: 634-640 [PMID: 21063488 DOI: 10.1007/s12072-010-9194-2]
37 **Fisher L**, Fisher A. Vitamin D and parathyroid hormone in outpatients with noncholestatic chronic liver disease. *Clin Gastroenterol Hepatol* 2007; **5**: 513-520 [PMID: 17222588 DOI: 10.1016/j.cgh.2006.10.015]
38 **Bischoff HA**, Borchers M, Gudat F, Duermueller U, Theiler R, Stähelin HB, Dick W. In situ detection of 1,25-dihydroxyvitamin D3 receptor in human skeletal muscle tissue. *Histochem J* 2001; **33**: 19-24 [PMID: 11352397]
39 **Girgis CM**, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin D in skeletal muscle: form, function, and metabolism. *Endocr Rev* 2013; **34**: 33-83 [PMID: 23169676 DOI: 10.1210/er.2012-1012]
40 **Ceglia L**. Vitamin D and skeletal muscle tissue and function. *Mol Aspects Med* 2008; **29**: 407-414 [PMID: 18727936 DOI: 10.1016/j.mam.2008.07.002]
41 **Bannister P**, Losowsky MS. Ethanol and hypogonadism. *Alcohol Alcohol* 1987; **22**: 213-217 [PMID: 3304308]
42 **Krishnan V**, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest* 2006; **116**: 1202-1209 [PMID: 16670761 DOI: 10.1172/JCI28551]
43 **Kramer I**, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, Feng JQ, Bonewald LF, Kneissel M. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. *Mol Cell Biol* 2010; **30**: 3071-3085 [PMID: 20404086 DOI: 10.1128/MCB.01428-09]
44 **Chen Y**, Chen L, Yin Q, Gao H, Dong P, Zhang X, Kang J. Reciprocal interferences of TNF-α and Wnt1/β-catenin signaling axes shift bone marrow-derived stem cells towards osteoblast lineage after ethanol exposure. *Cell Physiol Biochem* 2013; **32**: 755-765 [PMID: 24080828 DOI: 10.1159/000354477]
45 **Li X**, Zhang Y, Kang H, Liu W, Liu P, Zhang J, Harris SE, Wu D. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J Biol Chem* 2005; **280**: 19883-19887 [PMID: 15778503 DOI: 10.1074/jbc.M413274200]

46 **Sheng Z**, Tong D, Ou Y, Zhang H, Zhang Z, Li S, Zhou J, Zhang J, Liao E. Serum sclerostin levels were positively correlated with fat mass and bone mineral density in central south Chinese postmenopausal women. *Clin Endocrinol* (Oxf) 2012; **76**: 797-801 [PMID: 22151063 DOI: 10.1111/j.1365-2265.2011.04315.x]

47 **González-Reimers E**, Martín-González C, de la Vega-Prieto MJ, Pelazas-González R, Fernández-Rodríguez C, López-Prieto J, Alvisa-Negrín J, Santolaria-Fernández F. Serum sclerostin in alcoholics: a pilot study. *Alcohol Alcohol* 2013; **48**: 278-282 [PMID: 23296214 DOI: 10.1093/alcalc/ags136]

48 **Boyce BF**, Xing L. Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Res Ther* 2007; **9** Suppl 1: S1 [PMID: 17634140 DOI: 10.1186/ar2165]

49 **Simonet WS**, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309-319 [PMID: 9108485 DOI: 10.1016/S0092-8674(00)80209-3]

50 **Takahashi N**, Maeda K, Ishihara A, Uehara S, Kobayashi Y. Regulatory mechanism of osteoclastogenesis by RANKL and Wnt signals. *Front Biosci* (Landmark Ed) 2011; **16**: 21-30 [PMID: 21196156 DOI: 10.2741/3673]

51 **Hofbauer LC**, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Interleukin-1beta and tumor necrosis factor-alpha, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. *Bone* 1999; **25**: 255-259 [PMID: 10495128]

52 **Fukui H**. Relation of endotoxin, endotoxin binding proteins and macrophages to severe alcoholic liver injury and multiple organ failure. *Alcohol Clin Exp Res* 2005; **29**: 172S-179S [PMID: 16344605 DOI: 10.1097/01.alc.0000189278.30237.e9]

53 **Nakatani Y**, Fukui H, Kitazawa T, Fujimoto M, Yamao J, Uemura M. Effect of alcohol on the secretion of tumor necrosis factor-alpha by macrophages in the presence of rat serum. *Alcohol Clin Exp Res* 2002; **26**: 81S-85S [PMID: 12198381 DOI: 10.1097/01.ALC.0000026982.94557.1A]

54 **Mercer KE**, Sims CR, Yang CS, Wynne RA, Moutos C, Hogue WR, Lumpkin CK, Suva LJ, Chen JR, Badger TM, Ronis MJ. Loss of functional NADPH oxidase 2 protects against alcohol-induced bone resorption in female p47phox-/- mice. *Alcohol Clin Exp Res* 2014; **38**: 672-682 [PMID: 24256560 DOI: 10.1111/acer.12305]

55 **Turner RT**. Skeletal response to alcohol. *Alcohol Clin Exp Res* 2000; **24**: 1693-1701 [PMID: 11104117 DOI: 10.1111/j.1530-0277.2000.tb01971.x]

56 **García-Valdecasas-Campelo E**, González-Reimers E, Santolaria-Fernández F, De la Vega-Prieto MJ, Milena-Abril A, Sánchez-Pérez MJ, Martínez-Riera A, Gómez-Rodríguez Mde L. Serum osteoprotegerin and RANKL levels in chronic alcoholic liver disease. *Alcohol Alcohol* 2006; **41**: 261-266 [PMID: 16476762 DOI: 10.1093/alcalc/agl004]

57 **Bunout D**. Nutritional and metabolic effects of alcoholism: their relationship with alcoholic liver disease. *Nutrition* 1999; **15**: 583-589 [PMID: 10422091 DOI: 10.1016/S0899-9007(99)00090-8]

58 **González-Reimers E**, Durán-Castellón MC, Martín-Olivera R, López-Lirola A, Santolaria-Fernández F, De la Vega-Prieto MJ, Pérez-Ramírez A, García-Valdecasas Campelo E. Effect of zinc supplementation on ethanol-mediated bone alterations. *Food Chem Toxicol* 2005; **43**: 1497-1505 [PMID: 15964119 DOI: 10.1016/j.fct.2005.04.003]

59 **McClain CJ**, Su LC. Zinc deficiency in the alcoholic: a review. *Alcohol Clin Exp Res* 1983; **7**: 5-10 [PMID: 6342450 DOI: 10.1111/j.1530-0277.1983.tb05402.x]

60 **Yamaguchi M**. Role of nutritional zinc in the prevention of osteoporosis. *Mol Cell Biochem* 2010; **338**: 241-254 [PMID: 20035439 DOI: 10.1007/s11010-009-0358-0]

61 **Pitts TO**, Van Thiel DH. Disorders of divalent ions and vitamin D metabolism in chronic alcoholism. *Recent Dev Alcohol* 1986; **4**: 357-377 [PMID: 3754648]

62 **Laitinen K**, Välimäki M. Alcohol and bone. *Calcif Tissue Int* 1991; **49** Suppl: S70-S73 [PMID: 1933604 DOI: 10.1007/BF02555094]

63 **Chen H**, Hayakawa D, Emura S, Tamada A, Jamali M, Ozawa Y, Taguchi H, Yano R, Ohtake N, Shoumura S. Effects of long-term treatment with ethanol on the ultrastructure of the golden hamster parathyroid gland. *Histol Histopathol* 1999; **14**: 343-349 [PMID: 10212795]

64 **Kalbfleisch JM**, Lindeman RD, Ginn HE, Smith WO. Effects of ethanol administration on urinary excretion of magnesium and other electrolytes in alcoholic and normal subjects. *J Clin Invest* 1963; **42**: 1471-1475 [PMID: 14060991 DOI: 10.1172/JCI104831]

65 **Mercer KE**, Wynne RA, Lazarenko OP, Lumpkin CK, Hogue WR, Suva LJ, Chen JR, Mason AZ, Badger TM, Ronis MJ. Vitamin D supplementation protects against bone loss associated with chronic alcohol administration in female mice. *J Pharmacol Exp Ther* 2012; **343**: 401-412 [PMID: 22892342 DOI: 10.1124/jpet.112.197038]

66 **Wezeman FH**, Juknelis D, Himes R, Callaci JJ. Vitamin D and ibandronate prevent cancellous bone loss associated with binge alcohol treatment in male rats. *Bone* 2007; **41**: 639-645 [PMID: 17643361 DOI: 10.1016/j.bone.2007.06.002]

67 **Drake MT**, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. *Mayo Clin Proc* 2008; **83**: 1032-1045 [PMID: 18775204 DOI: 10.4065/83.9.1032]

68 **Wells GA**, Cranney A, Peterson J, Boucher M, Shea B, Robinson V, Coyle D, Tugwell P. Alendronate for the primary and secondary prevention of osteoporotic fractures in postmenopausal women. *Cochrane Database Syst Rev* 2008; (**1**): CD001155 [PMID: 18253985 DOI: 10.1002/14651858.CD001155.pub2]

69 **Rudic JS**, Giljaca V, Krstic MN, Bjelakovic G, Gluud C. Bisphosphonates for osteoporosis in primary biliary cirrhosis. *Cochrane Database Syst Rev* 2011; (**12**): CD009144 [PMID: 22161446 DOI: 10.1002/14651858.CD009144.pub2]

70 **Ettinger B**, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Glüer CC, Krueger K, Cohen FJ, Eckert S, Ensrud KE, Avioli LV, Lips P, Cummings SR. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. *JAMA* 1999; **282**: 637-645 [PMID: 10517716 DOI: 10.1001/jama.282.7.637]

71 **Cummings SR**, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009; **361**: 756-765 [PMID: 19671655 DOI: 10.1056/NEJMoa0809493]


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# Hepatitis B among Inuit: A review with focus on Greenland Inuit

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## Abstract

Hepatitis B virus (HBV) infection is a disease with a highly variable course. Chronic HBV infection may cause end-stage liver disease including cirrhosis and hepatocellular carcinoma, which is the 3rd most common cause of cancer related death due to the poor prognosis. The prevalence of HBV infection is low in many countries. Still, it remains important due to the potential consequences of the disease. HBV is endemic in the Arctic with serologic markers of chronic HBV infection in up to 29% of the population in some areas in Greenland. Interestingly, Inuit populations rarely show signs of liver disease despite the fact that around half of all Inuit has been exposed to HBV and around 8% of Inuit are chronically infected with HBV. These findings have been consistent in surveys conducted for more than four decades among Arctic Inuit. We thus review HBV infection in the Arctic with focus on Greenland Inuit and compared with Inuit in Canada, Alaska and Siberia. The aspects described include epidemiology and monitoring of the disease, as well as treatment and the risk of liver cancer.

**Key words:** Hepatitis B; Hepatitis D; Monitoring disease; Prevention; Liver cancer; Inuit; Greenland; Arctic



**Core tip:** Hepatitis B virus (HBV) is endemic in the Arctic with serologic markers of chronic HBV infection in up to 29% of the population in some areas in Greenland. Interestingly, Inuit populations in Greenland and Canada rarely show signs of liver disease as opposed to Alaskan Inuit where hepatocellular carcinoma was common before introduction of vaccination. Wheather this is related to a special favorable genotype or other host or environmental factors remains to be clarified. This paper is a review of present status.

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## INTRODUCTION

Chronic hepatitis B virus (HBV) infection is recognized as a disease with several stages ranging from immune tolerant and an inactive carrier state to cirrhosis with end-stage liver disease and liver cancer. The course of the disease is influenced by a number of factors such as hepatitis B e-antigen (HBeAg) seroconversion, HBV-DNA level, HBV genotype, pre-core (pre-C) and core promoter mutations, reactivation with flares, co-infections, *etc*. Also, age at infection, disease duration, environmental and genetic factors, ethnicity and gender influences the disease[1]. Still, HBV causes a potentially lethal infection and a number of aspects of the disease remain unknown with lessons to be learned from populations with endemic disease occurrence.

More than 350 million people are chronically infected with HBV globally and HBV will continue to pose a health problem for decades[2,3]. In general, the risk of chronic disease is 90% in children vertically infected, while the risk is approximately 30% in horizontally infected children under the age of five and less than 10% in adults[3,4]. Chronic HBV infection relates to end-stage liver disease, including cirrhosis and hepatocellular carcinoma (HCC), and it causes 1 million deaths per year[2]. Hence, HCC is now the 6$^{th}$ most common cause of cancer in the world, and the third most common cause of cancer death due to the poor prognosis[5].

Earlier studies in the circumpolar region have shown HBV infection to be endemic in the various indigenous populations with the highest prevalence among Arctic Inuit[6-9].

This paper will address HBV epidemiology, monitoring, treatment and consequences of chronic HBV infection among Inuit. The focus is on Greenland Inuit, which is a major Inuit population, and the perspective is based on comparisons with Inuit in Canada, Alaska and Russia.

## EPIDEMIOLOGY

### *Greenland Inuit*

The population in Greenland is around 57000 people and most are of Inuit descent. Around 7000 are born outside Greenland, most in Denmark, and around 18000 persons in Denmark are of Greenlandic descent[10,11].

Greenland has for many years been recognized as a high-risk area for hepatitis B[12]. Markers of present or previous HBV infection have been found to vary from 42% to 88% in studies of different age groups and from different areas in Greenland[8,9,13-15].

Studies have shown a prevalence of hepatitis B surface antigen (HBsAg) ranging from 12% to 29% among Inuit in East Greenland, 6.2% to 17% in Northwest Greenland and between 1.2% and 7.3% among Inuit in Southwest Greenland (Table 1)[9,12,13]. A comprehensive study among a population aged 12 years and above in Greenland's second largest town showed that 75% of population had signs of present or previous HBV infection at the age of 25 and a rise in HBV markers of 40% was seen between the ages of 15 and 25 years[8]. The highest incidence of acute HBV infection was observed in that same age interval. This suggested that sexual transmission is an important mode of transmission[8] in addition to other modes of horizontal spread. Also, extensive horizontal transmission between the ages of 5 and 20 years rather than from mother to child has been reported among Inuit in Greenland[13,15-17].

In a later study among individuals aged 7 through 79 years Langer found 7% ($n$ = 35) HBsAg positive (Table 1). HBeAg was positive in 6% of these and 94% were anti-HBe positive. HBV-DNA could be measured in 49% and genotype could be determined in 11 persons of which 9 had genotype D and 2 had genotype A[15].

In a more recent study among individuals aged 50 through 69 years, HBsAg was positive in 20% ($n$ = 86). This study found a difference with geography as HBsAg was positive in 28.9% in East Greenland and 2.7% in West Greenland[9]. HBeAg was positive in 1%, five percent were both HBeAg and anti-HBe positive, and 94% were anti-HBe positive only. HBV-DNA could be measured in 71% of HBsAg positive individuals. The dominating genotype B was found in 47 individuals, 4 had genotype D and 1 had both B and D. Genotype B was subtyped to be genotype B6[9].

In a comprehensive study covering all age groups in most of West Greenland, Børresen *et al*[18] describes in her thesis 650 (7.3%) HBsAg positive subjects out of a total of 8879 participants.

Based on this study and other population based studies it can be estimated that there are between 3000 to 4000 carriers of HBsAg in Greenland, and that only 17% of the Inuit population in Greenland has never been exposed to HBV[9].

### *Greenland Inuit in Denmark*

As described above Denmark hosts a greater part of Inuit migrants from Greenland and their descendants. This provides an opportunity to gain insight into some aspects of the influence of migration on the prevalence and consequences of hosting an HBV infection. Rex *et al*[19] showed that the occurrence of markers of HBV infection was similar among Inuit in Denmark and in Greenland. Accordingly, 54.5% of this group aged 40 through 69 years had serological signs of HBV exposure. Present HBV infection was found in 4% of individuals (Table 1) and all were anti-HBe positive[20]. Thus, there was no difference between Inuit in Denmark and in Greenland even though a major group of those migrated had lived in Denmark for decades. These data suggest that migration does not influence the prevalence of HBV infection.

**Table 1 Studies of hepatitis B virus markers among Inuit populations**

| Country | Ref. | HBsAg % | Anti-HBs % | Anti-HBc % | HBeAg % | Anti-HBe % | Participants Inuit/*n* | Year |
|---|---|---|---|---|---|---|---|---|
| Greenland | | | | | | | | |
| | Skinhoj *et al*[12] | 7.1 | | | | | 2904/2904 | 1974 |
| | Skinhoj *et al*[13] | 16.6[1] | 45.8[1] | 41.6[1] | 6[2] | 30[3] | 1450/1450 | 1977 |
| | Skinhøj *et al*[16] | 16.7 | 23.6 | | 0.0 | 2.6 | 191/254 | 1979 |
| | Melby *et al*[17] | 0-20[4] | 0-35[4] | | | | 193/371 | 1984 |
| | Olsen *et al*[8] | 11.5[5] | | | | | NA/1893 | 1989 |
| | Langer *et al*[15] | 7.0 | | 42.0 | 0.4 | | 503/503 | 1997 |
| | Krarup *et al*[9] | 20 | | | | | 434/434 | 2008 |
| | Børresen *et al*[14] | 18.9 | | 62.6 | 4.3 | | 185/185 | 2010 |
| | Børresen *et al*[18] | 7.8 | | 37.4 | | | NA/8879 | 2011 |
| | Rex *et al*[20] | 4.4 | | | 0.0 | | 136/136 | 2012 |
| Canada | | | | | | | | |
| | Minuk *et al*[21] | 4.0[5] | | | 0.0 | 3.8[3] | 671/720 | 1982 |
| | Larke *et al*[7] | 3.9 | 24.5 | | 8.8[2] | | 8282/14198 | 1987 |
| | Baikie *et al*[22] | 6.9 | | 26.4 | 0.0[2] | | 766/2156 | 1989 |
| Alaska | | | | | | | | |
| | Schreeder *et al*[23] | 6.4 | 17.8 | | | | 3053/3053 | 1983 |
| Russia | | | | | | | | |
| | Ohba *et al*[24] | 11.8[5] | 35.9[5] | | | | NA/348 | 1999 |

[1]Calculated from randomly selected samples; [2]Among all HBsAg positive tested for HBeAg; [3]Among all HBsAg positive tested for anti-HBe; [4]Age specific prevalences; [5]Among all studied. HBeAg: Hepatitis B e-antigen; HBsAg: Hepatitis B surface antigen; Anti-HBc: Hepatitis B core antibody; Anti-HBs: Hepatitis B surface antibody; Anti-HBe: Hepatitis B e antibody; NA: Not analyzed.

### *Inuit in Canada, Alaska and Russia*

A survey in 1980 that included 720 people (93% Canadian Inuit) found serologic markers of HBV infection in 27%, an increase with age, and an overall prevalence rate of HBsAg of 4% in Nunavut[21]. The 29 HBsAg carriers were adults who were anti-HBe positive[21]. A comprehensive study in the Northwest Territories in Canada in the early 1980-ties included 51% (*n* = 8282) of the Inuit population[7]. Among Inuit, they found a prevalence of 3.9% (*n* = 309) for HBsAg and 24.5% (*n* = 2031) for anti-HBs. Among the 14198 participants 428 were HBsAg positive and 37 HBeAg positive[7]. Baikie *et al*[22] reported HBsAg carrier rate among Inuit in seven northern Labrador communities to be 6.9% (Table 1) and none were HBeAg positive. Furthermore, they found that 85% had a marker of HBV exposure. These reports suggest a similar pattern of HBV exposure and infection among Canadian Inuit compared to Greenland Inuit.

Studies in Alaska have found HBsAg in 6.4% of 3053 residents of 12 Inuit villages (Table 1)[23]. Evidence of HBV exposure as defined by either positive HBsAg or anti-HBs was suggested in 5% to 70% of those surveyed. The occurrence differed between villages and age groups with a rise with age[23].

Data on hepatitis from the Russian Arctic are sparse. However, Ohba *et al*[24] reported the prevalence of HBV infection in 348 Siberian natives who lived in the Kamchatka Peninsula of Russia. HBsAg was found in 41 of 348 (12%) and anti-HBs was found in 125 of 348 (36%)[24]. Though the data are limited the occurrence may be comparable to that seen among the Inuit in Alaska, Canada and Greenland.

## CO-INFECTION WITH HBV AND HDV, HCV OR HUMAN IMMUNODEFICIENCY VIRUS

A limited number of studies have focused on co-infection of HBV with HDV, HCV or human immunodeficiency virus (HIV) among Inuit. Nevertheless, co-infection with HDV could become a problem in Greenland as superinfection in chronic carriers of HBV has caused severe liver disease even in children. An outbreak of hepatitis D infection was reported in one settlement in West Greenland[14] but also sporadic cases have been identified from the remaining parts of the west coast (Krarup, personal communication). Still, HDV was rare in a systematic testing of adults aged 50 through 70 years in East or West Greenland[20]. However, there is limited data available for Greenland Inuit.

Olsen *et al*[8] found no HIV positive in their study among Greenlanders.

Spradling *et al*[25] found that co-infection with HIV or HCV was uncommon and HIV was found in only 3 of 300 HBV infected Alaska natives with defined risk factors for HIV[25]. Also HDV co-infection prevalence was stated to be low[25].

There are very limited data published on HBV co-infections among Inuit in Canada and the Russian Arctic. In Canada co-infection with HDV was rare in a study of several ethnic groups[26] but the number of Inuit was not specified.

The high number of Inuit with HBV infection in Greenland and the other Arctic countries poses a risk to the Inuit societies. Should HDV spread in the population as a super infection it is a threat as it has been related

to more severe accelerated fulminant acute hepatitis, chronic active hepatitis, cirrhosis and HCC in other populations[27,28] as well as in a single outbreak in Greenland[14].

## VACCINATION AND MONITORING OF HBV

### *Greenland*

All health care services are publicly funded in Greenland. This includes free vaccination, treatment and all visits to health care providers. Such free services support high coverage of health care and reliability of programs to monitor populations.

Screening for hepatitis B has been carried out among pregnant women in Greenland since 1992[18]. Universal hepatitis B vaccination of newborns was introduced in 2010 as part of the childhood immunization program[18]. Also, the Greenlandic Hepatitis B database (HB database) has operated since 1992. It records the results of HBV testing in Greenland, performed at Queen Ingrid's Hospital, Nuuk[18]. Thus, recording of HBV among Greenland Inuit is ongoing.

Legislation enforces reporting of acute and chronic hepatitis, and HBV infections are notifiable to the Chief Medical Officer in Greenland. Laboratory findings of persons tested positive for hepatitis are reported to the Chief Medical Officer. Cases of viral hepatitis are also identified through the mandatory screening of blood donors, pregnant women and through the diagnostic workup of patients undergoing routine clinical investigations. Besides, screening for HBV infection is recommended in Greenland in all patients referred for chemotherapy and immunosuppressive treatment, including tumor necrosis factor-inhibitors and patient on corticosteroid for more than one month, in patients with HIV and in those who have elevated alanine aminotransferases. Despite these case finding measures, hepatitis B infection is still likely to be underdiagnosed and underreported in Greenland.

The National Board of Health in Greenland collects surveillance data using the unique 10-digit civil registration number assigned to all individuals in Greenland. Data on several infectious diseases, including HBV, and data on childhood vaccination can be retrieved through the national electronic patient record systems.

A new childhood vaccination program against hepatitis B was introduced in Greenland 2010. This includes four vaccinations in all, at birth and at 1, 2 and 12 mo. Also, children born before the initiation of the program are offered three vaccinations after the age of twelve. Recording of vaccinations changed from being based on pen and paper to electronic reporting. This was believed to improve the quality of the data reported. However, the records were incomplete in 2010 and 2011, and it has not been possible to estimate vaccination coverage for these years. Thus, results are available for 2012 only[11].

Based on the electronic registration it seems that fewer children were vaccinated than previously. However, data may be lacking.

Data from 2012 showed that the universal hepatitis B vaccination at birth covers 55%, and that 38.5% receives the fourth hepatitis vaccination. The vaccination at the age of 12 mo is only given to children who have had the first three HBV vaccinations.

Unlike Alaska, Greenland has no systematic follow-up on patients with chronic HBV infection despite knowledge on HBV status. There are intentions to initiate such a program but as yet this has not been established. Furthermore, there are no national guidelines for diagnostic work-up and follow-up of HBV infection in Greenland. Treatment is in accordance with international guidelines. Both recording of vaccination and follow-up on chronic carriers may be optimised as a new nation-wide electronic patient record system is scheduled for implementation.

HDV infection is not a notifiable disease in Greenland as is the case in most of the countries in the Arctic. Hence, limited information on hepatitis D infection is available.

### *Canada, Alaska and Russia*

Canada has a universal vaccination program against hepatitis B. However, the vaccination schedules are reported to differ between provinces and the children and adolescents may not be vaccinated if they move between provinces at certain ages[29,30]. As a result a nation-wide HBV vaccination programme for newborns and children does not provide full coverage[31,32] and it may be calculated that among adult Canadians approximately 39.7% are immunized[33].

A surveillance system of hepatitis B has been operating in Canada since 1998, including The Northwest Territories from 2009. This surveillance system provides clinical and laboratory data of new cases of acute and chronic hepatitis B[34]. Also, follow-up of newborns occurs in all regions of the North[30].

Alaska has been the lead in childhood vaccination against hepatitis B. Alaska natives received universal HBV vaccination since 1984, and since 1994 The State of Alaska has had a policy of universal hepatitis B vaccination of all newborns[34]. This included all children up to age of 18 years from 1997 and was free of charge. The universal hepatitis B vaccination program coverage in Alaska is at the moment approximately 59.4% for newborns and 92.1% for 24 mo old children[35]. Surveillance of HBV is a State responsibility in Alaska, and laboratories and health care providers are required by law to report cases of acute hepatitis B[4]. In 2003 there were 1300 Alaska Natives followed for chronic hepatitis B[4].

In Russia HBV vaccination of newborns and 13-year-old children was introduced in 1998[30]. Universal infant immunization was added from 2001, which included school-based catch up programs[4]. The childhood vaccination programs in Russia have an average coverage

rate of over 95%[36,37].

A surveillance program for infection with HBV operating in Russia includes acute and chronic HBV infection as well as the carrier state[30,34].

## TREATMENT

Vaccination and immunoglobulin treatment of newborns has been shown to have a huge impact on the incidence of chronic hepatitis among the circumpolar populations as documented also in other areas[38]. This supports the benefit of vaccination. However, a unique characteristic among Greenland Inuit has been the lack of apparent disease among those infected with HBV. Hence, there has been little attention to the need for treatment of HBV infection. Still, a few patients have been identified with signs of moderate fibrosis on liver biopsy (Krarup, personal communication). These patients were initially treated with lamivudine and since 2009 with tenofovir. Furthermore, patients co-infected with hepatitis D have been treated with interferon-alpha, and in recent years this has been as pegylated interferon. Still, systematic follow-up on these patients has not been undertaken yet.

The Greenlandic guidelines on treatment of chronic hepatitis are similar to the Danish guidelines[39]. All hepatitis B infected Greenlanders in need of diagnostic work-up or treatment are consulted by one central unit in Greenland. The consultants at the Department of Internal Medicine at Queen Ingrid's Hospital in Nuuk are further in close consultancy with hepatitis experts in Denmark. This structure supports an updated treatment in Greenland.

Even though subpopulations in Canada host HBV infections with endemic prevalence there are no data regarding the effects of anti-viral therapy in Canadian circumpolar populations infected with viral hepatitis[40]. Treatment of HBV infection in Alaska is in accordance with the AASLD guidelines[41].

## LIVER CANCER

The long term clinical outcome of hepatitis B infection is variable. HCC has in general demonstrated a low occurrence among Inuit in Greenland compared to what would be expected based on the prevalence of HBsAg. Similar findings are available for other populations in the Arctic region, specifically in Canada. In 1978 Skinhøj *et al*[42] reported an occurrence of HCC in Greenland Inuit similar to that seen in Northern European populations. This report was based on official mortality statistics and biopsies and necropsies, and it covered the time period from 1951 through 1975. They recognised that death statistics were not comprehensive. In addition, the greater part of this time span was prior to westernisation and the referral to hospital and travel from the small settlements scattered along the vast coastline of the world's largest island was likely limited. This probably hampered the diagnosis of HCC.

Similar limitations account for the study by Melbye *et al*[17] using the same methods. They identified 12 cases in the same area between 1960 and 1981 with a 15 year overlap between the two studies. Thus, the diagnosis of HCC was probably not comprehensive in this study either.

Nielsen and Storm reported a total of 3255 incident cancers diagnosed from 1969 to 1988 in 85000-110000 individuals among circumpolar Inuit in Greenland, Canada, Alaska and Russia. Among these inhabitants of the circumpolar region primary liver cancer was reported in 53 men with 37 from Alaska, 15 from Greenland, and 1 from Canada[43]. There were 16 cases among women with 6 from Alaska, 7 from Greenland, and 3 from Canada. They also reported an increasing incidence in Greenland Inuit and a decreasing incidence among Alaska Inuit. This coincided with the introduction of the vaccination program in Alaska. Indirect standardization showed excess risk of cancer of the liver and stomach compared to populations in the lower states in United States, in Canada and in Denmark[43]. The study by Nielsen was more comprehensive than previous studies in Greenland, and a gradual improvement of the validity of the registries may be anticipated.

Friborg *et al*[44] retrieved information of liver cancer cases among a total of 72331 individuals born in Greenland and alive from 1973 to 1997. These individuals were tracked in the Danish Cancer Registry by the use of their Civil Registration Number. Cancers among Inuit living in Denmark at the time of diagnosis were included. Standardized incidence ratio for liver cancers in Greenlandic men was 1.9 (95%CI: 1.0-3.5) compared with Denmark from 1988 to 1997[44]. This conforms to the notion that liver cancer was not a prominent disease in Greenland at least up until 15 years ago.

A more recent study on the occurrence of liver related disease among Greenland Inuit was based on more valid hospital statistics. The study was conducted as a follow-up in 2007 on studies performed in 1987 and 1998[18]. A main finding was a lower frequency of hospitalization due to liver disease than what would be expected even when compared to areas with a low HBV prevalence[18]. Also, the authors identified 15 cases of HCC. This may still not cover all cases of HCC in Greenland due to logistics, patient choice and the aggressive nature of HCC. However, the number is low, and the data suggest that HBV infection in Greenland may be associated with a lower risk of liver cancer and liver disease than in other parts of the world[18]. Further investigations based on this finding are of interest as new aspects of the infection may present themselves and contribute to an understanding of what causes a benign or non-benign outcome of the disease.

The overall low incidence HCC in chronically HBV-infected Inuit has suggested a more benign course in Greenland than in other parts of the world[9,18,43]. This finding is not restricted to Greenland Inuit. HCC is also uncommon in Canadian Inuit[45]. The incidence is only

marginally higher in Inuit compared to Danes who have a low occurrence of HBV. Among Alaskan Native people infected with HBV, genotypes F and C associate with a higher incidence of HCC. This outcome may be associated with the genomic variability of the predominant HBV genotype in each country[46].

Hepatitis B vaccination programs and especially universal infant vaccinations have demonstrated success in reducing both incidence of disease and chronic carriers but indeed also in reducing HCC occurrence in children[38,47,48]. Even though the incidence is low in Inuit the vaccination programmes in the Arctic should prevent the few cases that may be anticipated.

## FUTURE ASSESSMENT

Health care workers, health care providers and the population of Greenland and other Arctic countries must be advised on the burden of hepatitis. A nation-wide approach that acts according to international guidelines on the monitoring of hepatitis should be implemented. For economical, educational, feasibility and technical reasons monitoring should be performed through readily available IT-programs to reduce technical problems among health-care staff in a geographical area with huge infrastructural challenges due to sparse populations in vast areas. Also, patients should be given the opportunity to have blood samples drawn whenever they come to a town. Patients with clinical and/or biochemical abnormalities should go through the usual clinical work-up through contact to physicians at the medical department at the local hospital.

## CONCLUSION

Infection with HBV is endemic in Greenland Inuit as in other Inuit populations across the Arctic. Still, studies seem to confirm a benign course of the disease among Inuit both in Greenland and Canada, but not in Alaska. This has contributed to a late initiation of a universal HBV vaccination program in Greenland in contrast to Alaskan, Canadian and Russian Arctic. Thus, a monitoring program for viral hepatitis in Greenland is relevant and the inclusion in an electronic patient file system covering all of Greenland is warranted and scheduled.

## REFERENCES


1 **McMahon BJ**. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. *Hepatol Int* 2009; **3**: 334-342 [PMID: 19669359 DOI: 10.1007/s12072-008-9112-z]

2 **Lee WM**. Hepatitis B virus infection. *N Engl J Med* 1997; **337**: 1733-1745 [PMID: 9392700 DOI: 10.1056/NEJM199712113372406]

3 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2001; **34**: 1225-1241 [PMID: 11732013 DOI: 10.1053/jhep.2001.29401]

4 **McMahon BJ**. Viral hepatitis in the Arctic. *Int J Circumpolar Health* 2004; **63** Suppl 2: 41-48 [PMID: 15736621 DOI: 10.3402/ijch.v63i0.17784]

5 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

6 **McMahon BJ**, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, Maynard JE. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985; **151**: 599-603 [PMID: 3973412 DOI: 10.1093/infdis/151.4.599]

7 **Larke RP**, Froese GJ, Devine RD, Petruk MW. Extension of the epidemiology of hepatitis B in circumpolar regions through a comprehensive serologic study in the Northwest Territories of Canada. *J Med Virol* 1987; **22**: 269-276 [PMID: 3625175 DOI: 10.1002/jmv.1890220311]

8 **Olsen OR**, Skinhøj P, Krogsgaard K, Baek L. [Hepatitis B--an endemic sexually transmitted infection in a local community in Greenland]. *Ugeskr Laeger* 1989; **151**: 1668-1670 [PMID: 2781630]

9 **Krarup HB**, Andersen S, Madsen PH, Okkels H, Hvingel BH, Laurberg P. Benign course of long-standing hepatitis B virus infection among Greenland Inuit? *Scand J Gastroenterol* 2008; **43**: 334-343 [PMID: 18266176 DOI: 10.1080/00365520701712198]

10 **Greenlanders living in Denmark**. Report by The North Atlantic Group in the Danish Parlament; 2007. [Cited 2014-11-07]. Available from: URL: http://www.udsattegroenlaendere.dk/wp-content/uploads/dnag-groenlaendere-20i-20danmark-201-1.pdf

11 Statistics Greenland. [Cited 2014-11-07]. Available from: URL: http://www.stat.gl/dialog/topmain.asp?lang=en&subject=Population&sc=BE

12 **Skinhoj P**, McNair A, Andersen ST. Hepatitis and hepatitis B-antigen in Greenland. *Am J Epidemiol* 1974; **99**: 50-57 [PMID: 4204315]

13 **Skinhoj P**. Hepatitis and hepatitis B-antigen in Greenland. II: Occurrence and interrelation of hepatitis B associated surface, core, and "e" antigen-antibody systems in a highly endemic area. *Am J Epidemiol* 1977; **105**: 99-106 [PMID: 835567]

14 **Børresen ML**, Olsen OR, Ladefoged K, McMahon BJ, Hjuler T, Panum I, Simonetti J, Jones C, Krarup H, Koch A. Hepatitis D outbreak among children in a hepatitis B hyper-endemic settlement in Greenland. *J Viral Hepat* 2010; **17**: 162-170 [PMID: 19780937 DOI: 10.1111/j.1365-2893.2009.01159.x]

15 **Langer BC**, Frösner GG, von Brunn A. Epidemiological study of viral hepatitis types A, B, C, D and E among Inuits in West Greenland. *J Viral Hepat* 1997; **4**: 339-349 [PMID: 9310933 DOI: 10.1046/j.1365-2893.1997.00063.x]

16 **Skinhøj P**. Hepatitis B virus infection in children. A sero-epidemiological study in three endemic areas. *Trans R Soc Trop Med Hyg* 1979; **73**: 549-552 [PMID: 531908 DOI: 10.1016/0035-9203(79)90049-X]

17 **Melbye M**, Skinhøj P, Nielsen NH, Vestergaard BF, Ebbesen P, Hansen JP, Biggar RJ. Virus-associated cancers in Greenland: frequent hepatitis B virus infection but low primary hepatocellular carcinoma incidence. *J Natl Cancer Inst* 1984; **73**: 1267-1272 [PMID: 6096620]

18 **Børresen ML**, Koch A, Biggar RJ, Andersson M, Wohlfahrt J, Ladefoged K, Melbye M. Hepatocellular carcinoma and other liver disease among Greenlanders chronically infected with hepatitis B virus: a population-based study. *J Natl Cancer Inst* 2011; **103**: 1676-1685 [PMID: 22021665 DOI: 10.1093/jnci/djr405]

19 **Rex KF**, Krarup HB, Laurberg P, Andersen S. Inuit Living in Greenland and Greenland Inuit migrated to Denmark have similar high prevalence of markers of viral hepatitis. *Int J Circumpolar Health* 2013; **72**: 22447

20 **Rex KF**, Krarup HB, Laurberg P, Andersen S. Population-based comparative epidemiological survey of hepatitis B, D, and C among Inuit migrated to Denmark and in high endemic Greenland. *Scand J Gastroenterol* 2012; **47**: 692-701 [PMID: 22229496 DOI: 10.3109/00365521.2011.634026]

21 **Minuk GY**, Nicolle LE, Postl B, Waggoner JG, Hoofnagle JH. Hepatitis virus infection in an isolated Canadian Inuit (Eskimo) population. *J Med Virol* 1982; **10**: 255-264 [PMID: 7161596 DOI: 10.1002/jmv.1890100405]

22 **Baikie M**, Ratnam S, Bryant DG, Jong M, Bokhout M. Epidemiologic features of hepatitis B virus infection in northern Labrador. *CMAJ* 1989; **141**: 791-795 [PMID: 2790622]

23 **Schreeder MT**, Bender TR, McMahon BJ, Moser MR, Murphy BL, Sheller MJ, Heyward WL, Hall DB, Maynard JE. Prevalence of hepatitis B in selected Alaskan Eskimo villages. *Am J Epidemiol* 1983; **118**: 543-549 [PMID: 6637981]

24 **Ohba K**, Mizokami M, Kato T, Ueda R, Gurtsenvitch V, Senyuta N, Syrtsev A, Zoya K, Yamashita M, Hayami M. Seroprevalence of hepatitis B virus, hepatitis C virus and GB virus-C infections in Siberia. *Epidemiol Infect* 1999; **122**: 139-143 [PMID: 10098797 DOI: 10.1017/S0950268898001940]

25 **Spradling PR**, Bulkow L, Teshale EH,Negus S, Homan C, Simons B, McMahon BJ. McMahon. Prevalence and Causes of Elevated Serum Aminotransferase Levels in a Population-based Cohort of Persons with Chronic HBV Infection. *J Hepatol* 2014; **6**: 785-791 [DOI: 10.1016/j.jhep.2014.05.045]

26 **Cheng HH**, Wang D, Minuk GY, Anands CM, Stowe TC, Buchan KA. The Prevalence of Antibody to Delta Virus in Western Canada. *Clin Invest Med* 1986; **9**: 156-159

27 **World Health Organization**. Global Alert and Response. Hepatitis D. [Cited 2014-11-07]. Available from: URL: http://www.who.int/csr/disease/hepatitis/whocdscsrncs20011/en/index3.html

28 **Ji J**, Sundquist K, Sundquist J. A population-based study of hepatitis D virus as potential risk factor for hepatocellular carcinoma. *J Natl Cancer Inst* 2012; **104**: 790-792 [PMID: 22423008 DOI: 10.1093/jnci/djs168]

29 **Canadian Liver Foundation**. Position Statement. Hepatitis B Vaccination. 2010. Available from: URL: http://www.liver.ca/files/PDF/Position_Statements/CLF_Position_Statements_-_Hepatitis_B_vaccination_FINAL.pdf

30 **Tulisov A**, McMahon BJ, Koch A, Minuk G, Chulanov V, Bruce MG, Uhanova J, Børresen M, Williams J, Osiowy C, Gelvan A, Alexeeva M, Larke B, Watt K. Viral hepatitis in the Arctic. A review from a Circumpolar Workshop on Viral hepatitis, ICCH13. *Alaska Med* 2007; **49**: 193-203 [PMID: 17929632]

31 **Public Health Agency of Canada**. Publicly funded Immunization Programs in Canada - Routine Schedule for Infants and Children including special programs and catch-up programs (as of September, 2014). [Cited 2014-11-07]. Available from: URL: http://www.phac-aspc.gc.ca/im/ptimprog-progimpt/table-1-eng.php

32 Immunize Canada. [Cited 2014-11-07]. Available from: URL: http://www.immunize.ca/en/publications-resources/links.aspx

33 **Public Health Agency of Canda**. Vaccine coverage amongst adult Canadians: Results from the 2012 adult National Immunization Coverage (aNIC) survey. [Cited 2014-11-07]. Available from: URL: http://www.phac-aspc.gc.ca/im/nics-enva/vcac-cvac-eng.php

34 **FitzSimons D**, McMahon B, Hendrickx G, Vorsters A, Van Damme P. Burden and prevention of viral hepatitis in the Arctic region, Copenhagen, Denmark, 22-23 March 2012. *Int J Circumpolar Health* 2013; **72**: eCollection 2013 [PMID: 23971016]

35 **Centers for Disease Control and Prevention**. Vaccination Coverage Rates and Data. [Cited 2014-11-07]. Available from: URL: http://www.cdc.gov/vaccines/imz-managers/coverage/index.html

36 GIDEON Guides to Infectious Diseases and Microbiology. Available from: URL: http://www.gideononline.com/ebooks/

37 **Klushkina VV**, Kozhanova TV, Bazhazhina PG, Popova OE, Il'chenko LIu, Kiuregian KK, Mikhaĭlov MI. [Evaluation of the effect of mass vaccination against hepatitis B in the Russian Federation]. *Zh Mikrobiol Epidemiol Immunobiol* 2012; (**5**): 42-48 [PMID: 23163035]

38 **Harpaz R**, McMahon BJ, Margolis HS, Shapiro CN, Havron D, Carpenter G, Bulkow LR, Wainwright RB. Elimination of new chronic hepatitis B virus infections: results of the Alaska immunization program. *J Infect Dis* 2000; **181**: 413-418 [PMID: 10669320 DOI: 10.1086/315259]

39 **Christensen PB**, Clausen MR, Krarup H, Laursen AL, Schlichting P, Weis N. Treatment for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection - Danish national guidelines 2011. *Dan Med J* 2012; **59**: C4465 [PMID: 22677253]

40 **Minuk GY**, Uhanova J. Chronic hepatitis B infection in Canada. *Can J Infect Dis* 2001; **12**: 351-356 [PMID: 18159362]

41 **Lok ASF**, McMahon BJ. Chronic hepatitis B: Update 2009. *Hepatology* 2009; **50**: 661-662 [DOI: 10.1002/hep.23190]

42 **Skinhøj P**, Hart Hansen JP, Nielsen NH, Mikkelsen F. Occurrence of cirrhosis and primary liver cancer in an Eskimo population hyperendemically infected with hepatitis B virus. *Am J Epidemiol* 1978; **108**: 121-125 [PMID: 707473]

43 **Nielsen NH**, Storm HH, Gaudette LA, Lanier AP. Cancer in Circumpolar Inuit 1969-1988. A summary. *Acta Oncol* 1996; **35**: 621-628 [PMID: 8813071 DOI: 10.3109/02841869609096996]

44 **Friborg J**, Koch A, Wohlfarht J, Storm HH, Melbye M. Cancer in Greenlandic Inuit 1973-1997. *Int J Circumpolar Health* 2004; **63** Suppl 2: 195-198 [PMID: 15736651]

45 **Minuk GY**, Macrury S, Uhanova J, Caouette S, Coleman N, Cummings K, Larke B, Vardy L, Triet Huyn C, Osiowy C. A paucity of liver disease in Canadian Inuit with chronic hepatitis B virus, subgenotype B6 infection. *J Viral Hepat* 2013; **20**: 890-896 [PMID: 24304459 DOI: 10.1111/jvh.12121]

46 **Kowalec K**, Minuk GY, Børresen ML, Koch A, McMahon BJ, Simons B, Osiowy C. Genetic diversity of hepatitis B virus genotypes B6, D and F among circumpolar indigenous individuals. *J Viral Hepat* 2013; **20**: 122-130 [PMID: 23301547 DOI: 10.1111/j.1365-2893.2012.01632.x]

47 **Mahoney FJ**, Woodruff BA, Erben JJ, Coleman PJ, Reid EC, Schatz GC, Kane MA. Effect of a hepatitis B vaccination program on the prevalence of hepatitis B virus infection. *J Infect Dis* 1993; **167**: 203-207 [PMID: 8418167]

48 **Chang MH**, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, Liang DC, Shau WY, Chen DS. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997; **336**: 1855-1859 [PMID: 9197213]


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# Search for a cure for chronic hepatitis B infection: How close are we?

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## Abstract

Chronic hepatitis B (CHB) remains a significant unmet medical need, with 240 million chronically infected persons worldwide. It can be controlled effectively with either nucleoside/nucleotide-based or interferon-based therapies. However, most patients receiving these therapies will relapse after treatment withdrawal. During recent years, the advances in molecular biology and immunology have enabled a better understanding of the viral-host interaction and inspired new treatment approaches to achieve either elimination of the virus from the liver or durable immune control of the infection. This review aims to provide a brief overview on the potential new therapies that may overcome the challenge of persistent CHB infection in the near future.

**Key words:** Hepatitis B; Antiviral therapy; Covalently closed circular DNA; Drug target; Immunomodulator



**Core tip:** Current hepatitis B treatments can only control the disease, but they rarely lead to substantial rates of hepatitis B surface antigen loss and seroconversion. Several new therapeutic approaches are being developed so as to attain the elusive goal of successful functional cure of chronic hepatitis B infection.

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## INTRODUCTION

Chronic hepatitis B (CHB) remains a global health challenge. More than 240 million persons have been infected with CHB virus. CHB has become one of the most common causes of liver cirrhosis and hepatocellular carcinoma, and has led to more than 780000 deaths per year[1,2]. At this point, there is no cure for CHB, even though potent drugs are available to control the virus and prevent complications. In the last few years, several new drug classes targeting the various stages of hepatitis B replication cycle are under investigation. Whether and which of these agents could become clinically useful therapies are still unclear[3]. The review

will discuss these new investigational approaches for the treatment of hepatitis B virus (HBV) infection, and their respective stages of development in clinical trials.

## WHY DO WE NEED NEW DRUGS FOR CHB?

The Food and Drug Administration-approved chronic hepatitis B treatment of choice is either an immunomodulators [standard and pegylated interferon (Peg-IFN)] or nucleoside/nucleotide analogues (lamivudine, adefovir, telbivudine, entecavir and tenofovir). The latter class is less expensive and orally available. These drugs have minimal side-effects comparing to interferon and can be used for decompensated cirrhosis and after liver transplantation[4]. However, they have to be taken on a long-term basis in general, and result in hepatitis B e antigen (HBeAg) seroconversion rate of 20%-25% and HBV antigen (HBsAg) loss of 1% or less[5]. This is because they are designed to be reversible transcriptase inhibitors, a crucial step in viral replication, but not to eliminate the HBV minichromosomes [HBV covalently closed circular DNA (cccDNA), Figure 1] persisting in the nucleus of the hepatocytes[6]. There is a significant risk of HBV reactivation and sometimes HBV flare after withdrawal of the antiviral agents. Drug resistance may evolve after long-term therapy[3]. In contrast, the interferon-alfa therapy is of finite duration and results in 30%-40% HBeAg seroconversion, 5%-10% HBsAg seroclearance, with no risk of drug resistance. However, its systemic adverse effects limit its usefulness, especially in patients with decompensated liver cirrhosis[4].

Modifications to existing therapies are being investigated, such as combining nucleoside/nucleotide analogues with interferon, switching treatments, and extending Peg-IFN. Twenty-five HBeAg negative patients were given an extended (96-wk) course of Peg-IFN plus adefovir in a study by Cao *et al*[7]. All patients achieved HBV DNA of less than 500 copies/mL, and HBsAg seroconversion rates were 12%, 28%, and 32% at weeks 48, 96 and 120 respectively. HBeAg positive patients who had been on entecavir for 9 to 36 mo, with HBV DNA of < 1000 copies/mL and HBeAg < 100 PEIU/mL, randomized to switch to Peg-IFN or to continue entecavir for 48 wk in OSST trial. The trial showed that switching to Peg-IFN led to a higher HBeAg seroconversion at week 48 (14.9% *vs* 6.1%, $P = 0.0467$). Among Peg-IFN-treated patients with HBeAg loss and HBsAg < 1500 IU/mL at randomization, as high as 22.2% achieved HBsAg loss[8]. Larger studies for the optimal combination regimen are ongoing, but HBsAg loss much higher than 30% is unlikely using current modalities available.

## WHAT ARE THE POTENTIAL TARGETS IN THE VIRAL LIFE CYCLE?

### *Viral life cycle*

Understanding the HBV life cycle is essential before attempting to discuss the mechanisms of the new targets. HBV, belonging to *hepadnaviridae* family, is 42 nm in diameter comprising of approximately 3.2 kb double-stranded relaxed coiled DNA (rcDNA) formed by the reverse transcription of pregenomic RNA (pgRNA)[9]. HBV is hepatotrophic and hepatocytes are the only cells that support HBV replication in the human body[10]. Availability of the cell lines susceptible to HBV infection has led to the better understanding of the early stages of its life cycle, starting from viral attachment, entry and translocation of rcDNA into nucleus to form cccDNA, as well as the later stages viral replication, including transcription of viral RNA, reverse transcription to form daughter DNA, assembly of viral particles and secretion out of the cells[11] (Figure 1).

HBV virion enters the hepatocyte *via* endocytosis by its N-terminal region of large (L) envelope (preS1) binding to the sodium taurocholate cotransporting polypeptides (NTCP) receptor on the plasma membrane of hepatocyte[12]. A peptide derived from this preS1 region is a possible therapeutic target to inhibit viral entry by binding to its receptors[13]. After uncoating and releasing into the cytoplasm, nucleocapsid containing rcDNA is transported to the nucleus to form cccDNA[3]. This formation is mediated by the viral polymerase that completes the incomplete plus-strand of viral rcDNA after which the polymerase is removed by cellular enzymes, leading to the formation of cccDNA by covalent ligation of both DNA strand[14]. cccDNA is also known as episomal minichromosome and is crucial for the persistence of the virus in the host hepatocytes and cause chronic infection[15]. It acts as the sole DNA template for the formation of 4 groups of viral RNA, namely precore mRNA (pre-C); pgRNA; mRNA coding for surface (S), middle (M) and large (L) envelope proteins; and mRNA coding for X protein[11]. Pre-C mRNA is processed into HBeAg that can be detected in the circulation with commercial assays, which reflects infectivity of the HBV infection. The pgRNA serves as a template for viral DNA by reverse transcription, DNA polymerase and viral capsid protein. pgRNA, together with core protein and DNA polymerase, are self-assembled and encapsidated[3]. Inside the nucleocapsid, pgRNA is reverse transcribed into rcDNA, enveloped and is either secreted out of the hepatocyte[16] or shunted back into the nucleus to replenish the HBV cccDNA pool[17].

### *HBV entry inhibitor*

PreS1 region of viral L protein is required to bind to cell surface receptor for viral entry. The functional receptor is the heparan sulphate proteoglycans, specifically NTCP on the surface of hepatocytes[13,19]. With this knowledge, the scientists discovered that a synthetic myristoylated lipopeptide derived from HBV envelop protein[13], myrcludex-B, reversibly inhibits HBV entry into the naïve hepatocyte[19]. Six weeks of subcutaneous treatment of Myrcludex B to humanized mice infected with HBV reduced amplification of existing intrahepatic cccDNA as well as the spread of infection[20,21], without interfering with viral replication[19]. The drug has also been tested in

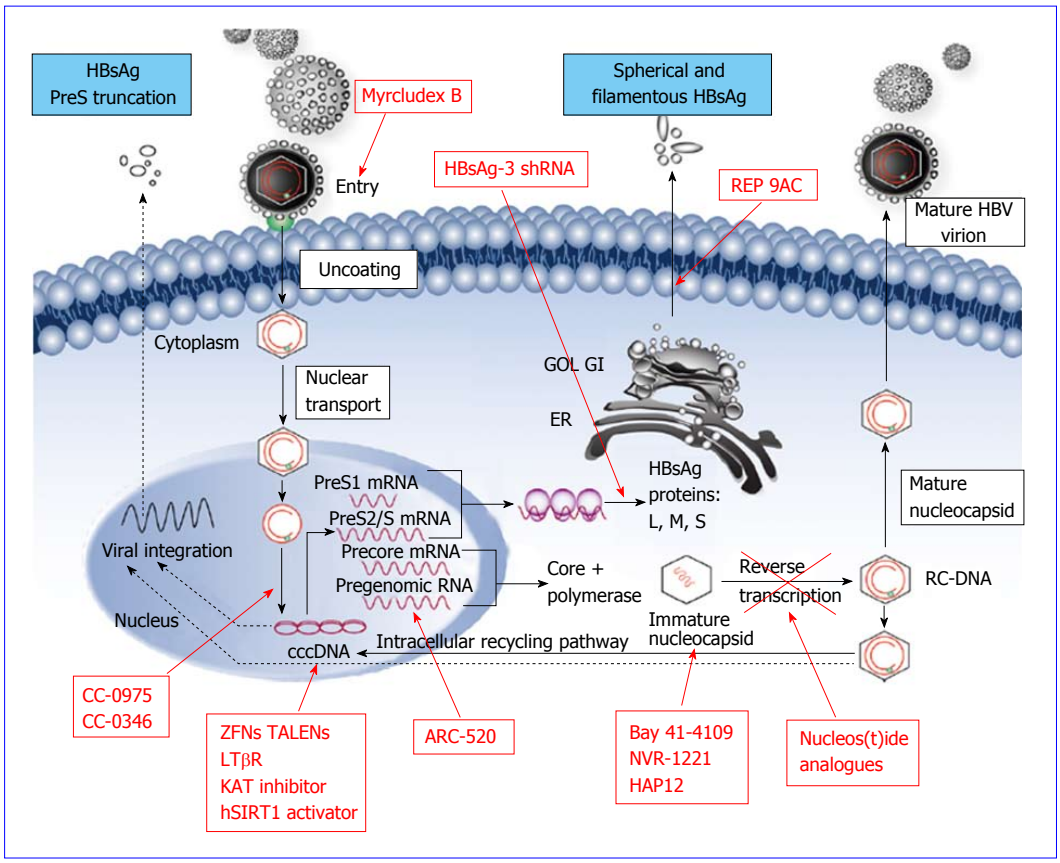


**Figure 1 Replicative cycle of hepatitis B virus and the respective targets of the current and experimental therapeutic agents.** Modified from Chan *et al*[18], with permission granted by Elsevier (Copyright owner). ER: Endoplasmic reticulum; HAP12: Heteroaryldihydropyrimidines 12; HBs protein L, M, S: HBs protein Large, Medium, Small; HBV: Hepatitis B virus; HBsAg: Hepatitis B virus antigen; RC-DNA: Relaxed circular DNA; RNAi: RNA interference; shRNA: Short/small hairpin RNA; cccDNA: Covalently closed circular DNA; KAT: Kynurenine aminotransferase; LTβR: Lymphotoxin beta receptor; TALEN: Transcription activator-like effector nuclease; ZFN: Zinc finger nuclease.

chronic HBV infected subjects showing good tolerability and lack of serious side-effects with doses up to 5 mg intravenous and 0.8 mg subcutaneous[22]. Moreover, this entry inhibitor could be used as a treatment option for infected patient and high risk neonates. The application could potentially be extended clinically to post-liver transplantation and post-immunosuppression therapy to prevent HBV reactivation or flare[19]. In order to achieve the optimal outcome, the entry inhibitor was suggested to be used together with existing antivirals[20].

### *Targeting the HBV cccDNA*

Persistence of HBV cccDNA in patients after successful long-term viral suppression by antiviral agents suggests that the key to HBV eradication in established CHB infection lies in the elimination of the reservoir of HBV minichromosomes from the hepatocyte[23]. Efforts in this area are still in early pre-clinical phases. This can be achieved by inhibiting cccDNA synthesis and maintenance, which include inhibition of its establishment, silencing its activity by transcription inhibitors, direct deactivation of cccDNA using engineered nucleases and activation of host innate immune response.

**Blocking of HBV cccDNA formation:** Recently, two novel compounds were reported that block conversion of relaxed circular HBV DNA into cccDNA at micromolar concentrations. Broadly known as distributed sulphonamide (DSS) compounds, they have phosphodiesterase or protease inhibitor activity, and can inhibit the conversion to cccDNA from rcDNA in human and duck hepatocytes through direct inhibition of deproteinization of rcDNA. The compounds were identified through a cell-based high throughput screen and neither the mechanism nor the target for these compounds is currently known. Among DSS, CCC-0975 and CCC-0346 were found to be the most potent in duck hepatocytes. Further studies are needed to improve their potency in order to obtain optimal benefits[24].

**Promoting HBV cccDNA loss:** One of the most exciting finding in 2014 in the field of HBV was the work by Lucifora *et al*[25], which described how IFN-α can induce specific degradation of the nuclear HBV cccDNA without detectable hepatotoxicity. Similar effect can also be achieved by activating the lymphotoxin-β receptor (LTβR), a receptor engaged by members of the TNF cytokine superfamily, which up-regulated APOBEC3A and APOBEC3B cytidine deaminases in HBV-infected cells. This appeared to be mediated through HBV

core protein and its interaction with nuclear cccDNA, resulting in cytidine deamination, apurinic/apyrimidinic site formation. Extensive nucleotide changes [guanine (G) to adenine (A)] in cccDNA, a transition that was a hallmark of cytidine deamination, eventually led to cccDNA degradation that prevented HBV reactivation. Remarkably, this deamination did not occur in cellular DNA, which indicates that viral clearance might involve cccDNA-specific mechanisms[26].

Although there were some questions raised on the technical aspects of the study, the major concern with regards to the clinical application of this discovery was safety issue. LTβR agonists had been known to trigger apoptosis, hepatocellular proliferation, inflammation, and hepatocellular carcinoma. Thus safety considerations are likely to preclude regulatory approval in its current form[27].

Scientists have also developed engineered site-specific mutagenic nucleases as a powerful laboratory technique, and are working to apply these methods to the treatment of human diseases. This type of technology includes the zinc-finger nucleases (ZFNs), the transcription activator-like effector nucleases (TALENs) and the CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-associated caspase-9)[28,29]. Potentially it may be used to strategically disrupt the targeted gene related to viruses such as human immunodeficiency virus (HIV) and HBV, with the aim of mutating the intra-nuclear viral DNA reservoir, making it no longer competent for replication[30].

In HBV infection, the target gene is the HBV cccDNA[29]. For viral hepatitis B, these cleavage enzymes are delivered through viral vectors to reach the target HBV minichromosomes inside the hepatocyte nucleus[31]. ZFNs are derived from consecutive alteration of the amino acids on the zinc-finger protein domains[32]. Three days after cotransfection of ZFN pair with a target cccDNA plasmid, 26% of target remained linear while 10% was cleaved and misjoined tail-to-tail, resulting in loss of function in both groups. It was also found that the simultaneous use of multiple pairs of ZFNs not only inactivate the target DNA but also circumvent the viral resistance if combined with existing antivirals[32].

Alternative to ZFNs are TALENs, which can also specifically disrupt or cleave the target episomal DNA and inactivate it. Bloom *et al*[29] successfully engineered mutagenic TALENs that target four HBV-specific sites within the viral genome. TALENs targeting sequences in the S or C open-reading frames (ORFs) can efficiently disrupt the sequences at the intended sites and suppressed HBV replication. The S TALEN caused targeted mutation in about one third of cccDNA molecules following triple transfection of the TALEN-expression plasmids into HepG2.2.15 cell lines that contained stably integrated copies of HBV genome. Markers of viral replication were also inhibited *in vivo* in a murine hydrodynamic injection model of HBV replication. HBV target sites within S and C ORFs of the injected HBV DNA were mutated without evidence of toxicity. Efficacy *in vivo* indicated these engineered nucleases have potential for use in treatment of chronic HBV infection[29]. Other groups of scientists were trying to develop the best possible mathematical model for how to achieve the best outcome with the effective minimal doses of nucleases while taking all parameters, such as vector delivery, intracellular pharmacodynamics and resistance into consideration, which may eventually bring these DNA-editing technology to clinical applications[31].

**HBV cccDNA transcription inhibitor:** Small molecules had been identified that can hinder cccDNA transcription to pgRNA and HBV replication by alteration of epigenetic regulation. By controlling the acetylation or methylation of the histone proteins surrounding the cccDNA, the transcription of the HBV genome can be inhibited. These small molecules include Class Ⅰ, Ⅱ and Ⅲ histone deacetylase inhibitors; p300 and P300/CBP-associated factor histone acetyltransferases inhibitors; hSirt1 activators; JMJD3 histone demethylase inhibitors[33]. All of these are still in the preclinical phase of development. Due to the wide ranging effect of these target enzymes on the expression multiple genes, the safety profiles of these compounds still requires extensive evaluation before clinical use.

As a proof-of-concept, liver regeneration was tested in uPA/SCID chimeric mice, which showed potent reduction of cccDNA levels and viral replication, when there is rapid regeneration of cccDNA-free hepatocytes. However, this regeneration technology requires further investigations in combination with existing antiviral agents, to have a possible chance of curing of chronic HBV infection[34].

### *Inhibiting viral replication by RNA interference*

RNA interference is carried out using complementary double stranded RNA to silence the homologous gene in post-transcriptional mRNA level[35]. In mammalian cells, the silencing is induced by the small interfering RNA (siRNA). In HBV-infected cells, siRNAs had been used to produce sequence-specific and dose-dependent knockdown of HBV surface or polymerase region of viral mRNA. siRNA functions by binding to complementary HBV mRNA and pgRNA, which will be degraded, resulting in no translation/reverse transcription[36]. Animal studies conducted by biotechnology company had demonstrated long-term suppression of HBV RNA, HBsAg, HBeAg and HBV DNA can be achieved by co-injection of ARC-520 (an anti-HBV siRNA) with DPC delivery vehicle into chimpanzees. This was followed by a phase 1 study, showing that all studied doses were safe and well-tolerable by normal volunteers and this result had led to an ongoing phase 2a clinical trial of ARC-520 in combination with entecavir in Hong Kong. This study will evaluate not only the safety and pharmacodynamics of the drug but also its efficacy on the levels of HBsAg, HBeAg and HBV DNA quantity, when given in combination with entecavir[37]. Meanwhile, some researchers suggested that the use of combination of siRNAs may achieve a stronger inhibition on HBV

replication and antigen expression in HepG2.2.15 cell line and in mice models[38,39].

### *New nucleoside analogues*

Like lamivudine and tenofovir, a number of drugs designed to target the reverse transcriptase of HIV are also potential new active therapies for HBV.

**Besifovir (LB80380):** Besifovir (LB80380), a potent acyclic nucleotide phosphonate, has demonstrated excellent preclinical safety profile, being effective against both wild-type and YMDD mutant HBV. In a randomized placebo-controlled Phase Ⅰ/Ⅱ study of besifovir, 29 HBeAg positive patients were treated for 4 wk, and led to maximum HBV DNA reduction of up to 4 $\log_{10}$ copies/mL respectively[40].

A Phase Ⅱb randomized trial of besifovir *vs* entecavir in chronic hepatitis B patients was carried out[41]. One hundred and fourteen patients were randomized to receive besifovir, at 90 mg or 150 mg daily or Entecavir 0.5 mg daily. Mean $\log_{10}$ HBV DNA changes from baseline were -5.84, -5.91 and -6.18 for HBeAg-positive patients, and -4.65, -4.55, and -4.67 respectively for HBeAg-negative patients ($P > 0.05$). Of ninety-four point one percent of patients on besifovir had lowering of serum L-carnitine, which returned to normal with carnitine supplement.

**MIV-210:** 2,3-dideoxy-3-fluoroguanosine (FLG), a fluorinated guanosine analogue, was initially developed for treatment of HIV-infected patients, and is currently being investigate for HBV therapy. In 2006, Jacquard *et al*[42] studied its mode of inhibition and found that it inhibits the priming of the reverse transcription and also a competitive inhibitor of deoxyguanosine triphosphate incorporation resulting in termination of the DNA chain. It is a more potent inhibitor of wild-type DHBV minus-strand DNA synthesis than lamivudine. In Huh7 cells transfected with HBV, the inhibition of wild-type, lamivudine-resistant, adefovir-resistant and adefovir-plus-lamivudine-resistant HBV mutants by FLG were similar.

In woodchucks infected with woodchuck hepatitis virus (WHV), oral administration of MIV-210 at 20 or 60 mg/kg per day led to a rapid virological response, reducing WHV DNA levels by 4.76 $\log_{10}$ and 5.72 $\log_{10}$ respectively[43]. A daily dose of 10 mg/kg was found to decrease serum WHV load by 400-fold after 4 wk of treatment, and a dose at 5 mg/kg per day was sufficient to maintain this antiviral effect. MIV-210 at 20 or 60 g/kg per day led to a 2.0 $\log_{10}$ drop in hepatic content of WHV cccDNA. MIV-210 is currently undergoing Phase Ⅱ trial in South Korea by Daewoong.

**Tenofovir alafenamide (GS-7340):** Tenofovir alafenamide (TAF, or GS-7340), a new oral prodrug of tenofovir, was found to be more stable in plasma compared to tenofovir disoproxil fumarate (TDF). TAF achieved higher levels of tenofovir diphosphate in target cells at lower doses than TDF[44]. In an open-label phase 1b trial, patients were randomized to TAF 8 mg, 25 mg, 40 mg, 120 mg, and TDF 300 g for 2 d. Most adverse reactions were mild to moderate, with the most common being headache, nausea, vomiting and fatigue. There was also minimal decline in creatinine clearance with TAF compared to TDF. At doses of 8 mg to 120 mg of TAF, there were no differences in viral decline[44]. Compared to TDF, TAF does not interact with OAT1 or OAT3 (renal transporters) and hence is unlikely to accumulate in renal proximal tubules[45].

Multiple studies performed on HIV patients had shown that both TAF and TDF were well tolerated, and adverse events were mild to moderate in severity and self-limiting[46,47]. Patients on the TAF regimen had smaller reductions in estimated creatinine clearance, less renal tubular proteinuria and smaller changes in bone mineral density for hip and spine. However, they also had higher increases in HDL, LDL and total cholesterol. Phase 2 studies of TAF on anti-HBV are currently in progress.

### *Interruption of HBV capsid assembly*

The nucleocapsid of HBV is composed of hundreds of core proteins[48] and its structure is important for HBV DNA synthesis and virion assembly[49,50]. Heteroaryl-dihydropyrimidines (HAPs) are inhibitors of nucleocapsid formation or assembly of core particles. This is achieved by misdirecting the process, without primarily affecting the core protein level and viral transcription level. Nonetheless, diminished core proteins level was observed as a consequence of its inhibitory effect on the capsid assembly and viral replication[51].

Bay 41-4109, a member of HAPs is a highly potent non-nucleoside antiviral whose inhibitory effect on the viral replication could be detected within 5 d of drug administration in mice model, while at the same time controlling the spread of infection. Hepatotoxicity was detected only at doses over 100 mg/kg per day[52]. Unexpectedly, studies revealed its effects on the epigenetic state of host genes, which controlled the host innate immunity[53]. However, the drug action ceased after its withdrawal tested[54]. Despite its reversibility, Bay 41-4109 is still a potential novel therapeutic which could be useful for patients infected with resistant HBV strains or those who failed standard therapy[52].

Another member of the HAP with intriguing property is *HAP12* which binds to core proteins on the nuclear minichromosome, causing structural changes and formation of core protein that does not support cccDNA transcription and further production of pgRNA. Therefore, HAP12 is also another potential therapeutic agent that can suppress cccDNA function in addition to its effective inhibition of capsid proteins[55].

Alternative to HAPs is a direct-acting agent against HBV core protein, known as NVR-1221, which is currently in phase Ⅰ a trial with the drug test tested on approximately 40 healthy volunteers[56]. Other similar alternatives with inhibitory effects on capsid proteins

are 2-amino-N-(2,6-dichloropyridine-3-yl) acetamide derivatives[50], sulphamoylbenzamide derivatives[57] and sulfanilamide derivatives[49], with the first derivatives shown to have synergistic inhibitory effect on HBV load if used together with lamivudine[50].

### *Inhibition of HBsAg secretion*

The release of HBsAg is independent of virion release and the antigen itself is postulated to be involved in suppressing the host innate immunity allowing the virus to cause persistent infection in human liver. *REP 9AC*, a nucleic acid polymer compound[58], is a potent HBsAg secretory inhibitor, which can eliminate HBsAg from the human circulation as early as 7 d of administration in a small study. This was followed by reactivation of the suppressed innate immune system of the host to produce anti-HBs antibody by weeks 15 and achieved sustained virological response after stopping the administration[59]. HBV DNA remained undetectable if add on immunotherapy was given. More work is being carried out to better understand the optimal duration of treatment to sustain its effects, tolerability and the best route of administration[58].

### *Novel immunomodulators*

Host immune response can be broadly divided into the innate and adaptive immunity. The adaptive HBV-specific, T cell-mediated immune responses are widely regarded as the most important elements against HBV. As an essential part of host innate immune system, Toll-like receptors are membrane-bound receptors involved in recognition of pathogens thereby activating the expression of several genes that contribute to antiviral immune responses[60]. Their importance in chronic HBV infection is increasingly recognized in recent years.

Toll-like receptor (TLR)-7, expressed on the dendritic cells and B lymphocytes[61], is able to recognize nucleic-acid like structures of viruses[60] and the stimulation of their respective receptors enhance dendritic cells to produce interferon alpha and other cytokines to further activate natural killer and cytotoxic T lymphocytes[61]. It is also found that TLR-7 and a number of other receptors are suppressed in chronic HBV infected patients leading to immune dysfunction against the infection[62], especially in the presence of HBsAg[63]. GS-9620, a potent oral agent containing TLR-7 agonist action, was shown to have ability to reduce HBsAg as well as HBV DNA in both serum and liver with even short-term usage in woodchucks and chimpanzees. The immune responses triggered by TLR-7 eliminate HBsAg, HBeAg and hepatitis B core antigen (HBcAg) positive hepatocytes and inhibit viral replication directly[61]. The drug also has high bioavailability and tolerability among healthy volunteers without serious side-effects[64]. The combination of GS-9620 and nucleoside analogues may be able to treat HBV infection effectively without significant systemic side-effects associated with interferon-based therapy[61]. An ongoing research has recently been conducted on the safety and pharmacodynamics of GS-9620 in chronic hepatitis B patients[65].

### *Therapeutic vaccine*

GS-4774 is a yeast-based vaccine expressing a recombinant X, large S and core antigens of HBV. Its action includes activation of dendritic cells after phagocytosis, stimulation of $CD4^+$ and $CD8^+$ T cells and reduction of regulatory T cells level. Phase 1 studies of GS-4774 in normal healthy adults provided satisfactory results showing high tolerability while achieving satisfactory immune responses to the administered recombinant antigens and peptides. Further study to further evaluate its usage in chronic hepatitis B patients is being conducted recently[66].

DV-601 is another therapeutic vaccine, containing recombinant HBV surface and core antigens, whose action is to trigger immune responses and to produce antibodies relating to HBV-specific cytotoxic T lymphocyte and B cell. The vaccine was given intramuscularly to chronic HBV infected patients, 8 HBeAg positive treatment-naïve and 6 HBeAg negative entecavir-treated subjects. The vaccine was found to be well-tolerable with only mild adverse effects that resolved without treatment. All patients were found to achieve the desired lymphoproliferative response and HBsAg, HBeAg and viral load were found to be reduced satisfactorily. Anti-HBs antibody and anti-HBe antibody were detected in those receiving the higher dose of DV-601[67].

A combination therapeutic vaccine containing HBsAg and HBcAg (known as NASVAC) was developed at CIGB, Cuba. *NASVAC* is delivered through nasal spray and in phase Ⅰ trial was found to be safe and tolerable. All subjects developed anti-HBc antibody at days 30 after immunization, with 75% developing anti-HBs antibody of more than or equals 10 IU/L at a maximum at days 90[68]. The phase Ⅲ trial to evaluate the therapeutic effects on lowering HBV DNA and other clinically important parameters is presently being conducted.

Synthetic HBV core antigen vaccine, firstly invented in 2012 by Inovia researchers aimed at reducing the risk of liver cancer, was reported to be a highly potent immune-therapeutic vaccine. This vaccine was designed to eliminate the HBV infected hepatocytes, without causing liver damage, through strong HBcAg-specific killer T-cell and antibody responses whose action will add on to existing host T-cells responses in the liver[69].

## HOW CLOSE ARE WE TO CLINICAL APPLICATION?

Most of the new antiviral agents discussed above remains in the early preclinical phase (laboratory and animal study) (summarized in Table 1). A few of these, driven by established biotechnology and pharmaceutical companies, had made it to phase-Ⅰ/ⅡA clinical trials. Gaining momentum and safety profiles from the HIV trials, the newer nucleoside analogues are likely to be the first group of drugs to obtain formal approval for

**Table 1 Novel anti-hepatitis B virus compounds in development**

| Therapeutic agents | Mechanism | Manufacturer | Status |
|---|---|---|---|
| Viral entry inhibitors | | | |
| Myrcludex B | Entry inhibition | Myr_GmbH, Germany | Phase ⅡA, Russia |
| cccDNA Transcription inhibitors | | | |
| CC-0975, CC-0346 (DSS) | Induces deproteinization of rcDNA | | Preclinical |
| LTβR | Induces cccDNA cytidine deamination | | Preclinical |
| Small molecules | cccDNA transcription inhibitor | | Preclinical |
| DNA editing technology | | | |
| ZFNs | cccDNA inactivation | | Preclinical |
| TALENs | cccDNA inactivation | | Preclinical |
| RNAi gene silencer | | | |
| ARC 520 | RNAi gene silencer | Arrowhead Research Pasadena, CA | Phase ⅡA |
| New nucleoside analogues | | | |
| Besifovir (LB80380) | Inhibits viral DNA polymerase | LG Life Sciences, South Korea | Phase Ⅱ |
| MIV-210 | Inhibits viral DNA polymerase | Medivir/Daewoong, South Korea | Phase Ⅱ |
| Tenofovir alafenamide (GS-7340) | Prodrug of tenofovir | Gilead Foster City, CA | Phase Ⅲ |
| Interruption of HBV capsid assembly | | | |
| Heteroaryldihydropyrimidine (Bay 41-4109) | Inhibits viral nucleocapsid | AiCuris, Germany | Phase Ⅰ |
| Heteroaryldihydropyrimidine (HAP12) | Inhibits capsid assembly | | Preclinical |
| NVR-1221 | Capsid inhibitor | Novira Therapeutics, Doylestown, PA | Phase ⅠA |
| HBsAg release inhibitor | | | |
| REP 9AC | HBsAg release inhibitor | REPLICor Inc. Montreal, Quebec | Phase Ⅰ |
| HBsAg-3 shRNA | HBsAg expression inhibitor | | Preclinical |
| Immunomodulator | | | |
| GS-9620 | TLR-7 agonist | Gilead Foster City, United States | Phase Ⅰ |
| CYT 107 (interleukin-7) | Immunomodulator | Cytheris, Paris, France | Phase Ⅰ/ⅡA |
| Therapeutic vaccination | | | |
| GS-4774 | HBV X, surface and core antigens | Gilead Sciences with Globe Immune Louisville, CO | Phase Ⅱ |
| DV601 | HBV surface and core antigens | Dynavax, Berkeley, CA | Phase ⅠB |
| NASVAC | HBV surface and core antigens | CIGB, Cuba | Phase Ⅰ |
| HBV core Ag vaccine | T-cell mediated therapeutic vaccine | Emergent Europe, United Kingdom | Phase Ⅰ |

Modified from hepatitis b foundation drug watch[70]. NASVAC: Nasal Vaccine Candidate; rcDNA: Relaxed circular DNA; RNAi: RNA interference; shRNA: Short/small hairpin RNA; cccDNA: Covalently closed circular DNA; DSS: Distributed sulphonamide; LTβR: LymphoToxin beta receptor; TALEN: Transcription activator-like effector nuclease; ZFN: Zinc finger nuclease; HBsAg: Hepatitis B virus antigen; HBV: Hepatitis B virus; TLR-7: Toll-like receptor-7.

clinical use. Being in the same class as the existing therapy, they are unlikely to be game changers, but they may add to the arsenal of reverse transcriptase inhibitors to tackle the drug-resistant HBV strains that may emerge with long-term use of current agents.

However for the new classes of anti-HBV compounds that truly aims to cure CHB, the development is still in their infancy and major breakthrough is required to deliver the compound to the target (infected hepatocyte nucleus for the cccDNA inhibitors) or overcome established immune tolerance (new immunomodulators), at acceptable safety profiles.

## CONCLUSION

We discussed the new anti-HBV compounds and new targets that can be broadly divided into two main categories: (1) therapies that target the virus either directly or by targeting host factors required for viral replication; and (2) therapies targeting the host innate or adaptive immune response.

To achieve the goal of curing CHB, any new approaches based on targeting the virus, will need to establish that target inhibition ultimately translates into significantly increased cccDNA depletion and HBsAg loss. For example, simply blocking viral replication, even by a mechanism other than nucleoside/nucleotides, is unlikely to cure infection unless the cccDNA reservoir is removed. Directly targeting cccDNA fundamentally tackles the issue of viral persistence but such new technologies as sequence-targeting nucleases and modulation of epigenetic regulators face challenges including drug delivery to target cells and risk of off-target effects. Approaches such as blocking antigen secretion, directly targeting viral RNA and blocking the HBV entry receptor are more technically feasible in the near term, but likely will not clear the cccDNA reservoir on their own. Our growing understanding of the immune defects in CHB is enabling the development of new immunotherapy. TLR-7 agonist represents one of such promising immunotherapeutic approaches. There remains the concern of safety in activating the immune system.

Although many potential new approaches to treat CHB have been identified, therapeutic translation has been challenging and relatively few drug candidates have emerged and entered clinical trials. If the success story of developing effective therapies leading to the ability to cure nearly all hepatitis C infection is any

guide, the search for a functional cure for CHB is definitely not an illusive dream, and it is likely to come in the form of combination therapies of current effective antiviral agents with one or more new anti-HBV agents that either eliminate HBV cccDNA or overcoming specific immune pathways that will result in immunoclearance of the virus.

## REFERENCES


1 **World Health Organization**. Hepatitis B. [Updated 2014 July]. Available from: URL: http://www.who.int/mediacentre/factsheets/fs204/en

2 **Uhl P**, Fricker G, Haberkorn U, Mier W. Current status in the therapy of liver diseases. *Int J Mol Sci* 2014; **15**: 7500-7512 [PMID: 24786290 DOI: 10.3390/ijms15057500]

3 **Grimm D**, Thimme R, Blum HE. HBV life cycle and novel drug targets. *Hepatol Int* 2011; **5**: 644-653 [PMID: 21484123 DOI: 10.1007/s12072-011-9261-3]

4 **Perrillo RP**. Overview of treatment of hepatitis B: key approaches and clinical challenges. *Semin Liver Dis* 2004; **24** Suppl 1: 23-29 [PMID: 15192798 DOI: 10.1055/s-2004-828675]

5 **Liaw YF**. HBeAg seroconversion as an important end point in the treatment of chronic hepatitis B. *Hepatol Int* 2009; **3**: 425-433 [PMID: 19669245 DOI: 10.1007/s12072-009-9140-3]

6 **Spangenberg HC**, Thimme R, Blum HE. Tracking cccDNA in chronic HBV infection. *Hepatology* 2004; **39**: 1736-1738 [PMID: 15185317 DOI: 10.1002/hep.20272]

7 **Cao ZH**, Ma LN, Zhang HW, Liu YL, Chen XY. Extended treatment with peginterferon α-2a in combination with lamivudine or adefovir for 96 weeks yields high rates of HBeAg and HBsAg seroconversion. *J Dig Dis* 2013; **14**: 446-450 [PMID: 23615131 DOI: 10.1111/1751-2980.12065]

8 **Ning Q**, Han M, Sun Y, Jiang J, Tan D, Hou J, Tang H, Sheng J, Zhao M. Switching from entecavir to PegIFN alfa-2a in patients with HBeAg-positive chronic hepatitis B: a randomised open-label trial (OSST trial). *J Hepatol* 2014; **61**: 777-784 [PMID: 24915612 DOI: 10.1016/j.jhep.2014.05.044]

9 **Seeger C**, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000; **64**: 51-68 [PMID: 10704474 DOI: 10.1128/MMBR.64.1.51-68.2000]

10 **Jilbert AR**, Freiman JS, Gowans EJ, Holmes M, Cossart YE, Burrell CJ. Duck hepatitis B virus DNA in liver, spleen, and pancreas: analysis by in situ and Southern blot hybridization. *Virology* 1987; **158**: 330-338 [PMID: 3590623 DOI: 10.1016/0042-6822(87)90205-4]

11 **Block TM**, Guo H, Guo JT. Molecular virology of hepatitis B virus for clinicians. *Clin Liver Dis* 2007; **11**: 685-706, vii [PMID: 17981225 DOI: 10.1016/j.cld.2007.08.002]

12 **Schmitt S**, Glebe D, Alving K, Tolle TK, Linder M, Geyer H, Linder D, Peter-Katalinic J, Gerlich WH, Geyer R. Analysis of the pre-S2 N- and O-linked glycans of the M surface protein from human hepatitis B virus. *J Biol Chem* 1999; **274**: 11945-11957 [PMID: 10207016]

13 **Yan H**, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J, Li W. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife* 2012; **1**: e00049 [PMID: 23150796 DOI: 10.7554/eLife.00049]

14 **Nassal M**. Hepatitis B viruses: reverse transcription a different way. *Virus Res* 2008; **134**: 235-249 [PMID: 18339439 DOI: 10.1016/j.virusres.2007.12.024]

15 **Newbold JE**, Xin H, Tencza M, Sherman G, Dean J, Bowden S, Locarnini S. The covalently closed duplex form of the hepadnavirus genome exists in situ as a heterogeneous population of viral minichromosomes. *J Virol* 1995; **69**: 3350-3357 [PMID: 7745682]

16 **Perlman DH**, Berg EA, O'connor PB, Costello CE, Hu J. Reverse transcription-associated dephosphorylation of hepadnavirus nucleocapsids. *Proc Natl Acad Sci USA* 2005; **102**: 9020-9025 [PMID: 15951426 DOI: 10.1073/pnas.0502138102]

17 **Wu TT**, Coates L, Aldrich CE, Summers J, Mason WS. In hepatocytes infected with duck hepatitis B virus, the template for viral RNA synthesis is amplified by an intracellular pathway. *Virology* 1990; **175**: 255-261 [PMID: 2155510 DOI: 10.1016/0042-6822(90)90206-7]

18 **Chan HL**, Thompson A, Martinot-Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, Tillmann HL, Kao JH, Jia JD, Wedemeyer H, Locarnini S, Janssen HL, Marcellin P. Hepatitis B surface antigen quantification: why and how to use it in 2011 - a core group report. *J Hepatol* 2011; **55**: 1121-1131 [PMID: 21718667 DOI: 10.1016/j.jhep.2011.06.006]

19 **Urban S**, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. *Gastroenterology* 2014; **147**: 48-64 [PMID: 24768844 DOI: 10.1053/j.gastro.2014.04.030]

20 **Volz T**, Allweiss L, Ben MBarek M, Warlich M, Lohse AW, Pollok JM, Alexandrov A, Urban S, Petersen J, Lütgehetmann M, Dandri M. The entry inhibitor Myrcludex-B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. *J Hepatol* 2013; **58**: 861-867 [PMID: 23246506 DOI: 10.1016/j.jhep.2012.12.008]

21 **Allweiss L**, Volz T, Giersch K, Petersen J, Lohse AW, Lutgehetmann M, Dandri M. Proliferation of hepatitis B virus infected human hepatocytes in humanized mice treated with the entry inhibitor Myrcludex-B induces strong cccDNA reduction and maintenance of cccDNA-free hepatocytes. *Z Gasteroenterol* 2014; **52**: 5-36 [DOI: 10.1055/s-0033-1361045]

22 **Haefeli WE**, BlankA, Mikus G, Mier W, Alexandrov A, Urban S. Successful first administration of Myrcludex B, a first-in-class HEPATITIS B and D Virus entry inhibitor, in humans. *Hepatology* 2012; **56** Suppl: 369

23 **Petersen J**, Lutgehetmann M, Volz T and Dandri M. What is the Role of cccDNA in Chronic HBV Infection? Impact on HBV Therapy. *Hepatology Rev* 2007; **4**: 9-13

24 **Cai D**, Mills C, Yu W, Yan R, Aldrich CE, Saputelli JR, Mason WS, Xu X, Guo JT, Block TM, Cuconati A, Guo H. Identification of disubstituted sulfonamide compounds as specific inhibitors of hepatitis B virus covalently closed circular DNA formation. *Antimicrob Agents Chemother* 2012; **56**: 4277-4288 [PMID: 22644022 DOI: 10.1128/AAC.00473-12]

25 **Lucifora J**, Reisinger F, Xia Y, Bester R, Zhang K, Sprinzl M, Protzer U, Heikenwalder M. Lymphotoxin beta receptor activation leads to degradation of HBV cccDNA from infected hepatocytes. *J Hepatol* 2013; **58** Suppl 1: S27 [DOI: 10.1016/S0168-8278(13)60061-6]

26 **Lucifora J**, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, Sprinzl MF, Koppensteiner H, Makowska Z, Volz T, Remouchamps C, Chou WM, Thasler WE, Hüser N, Durantel D, Liang TJ, Münk C, Heim MH, Browning JL, Dejardin E, Dandri M, Schindler M, Heikenwalder M, Protzer U. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science* 2014; **343**: 1221-1228 [PMID: 24557838 DOI: 10.1126/science.1243462]

27 **Xia Y**, Lucifora J, Reisinger F, Heikenwalder M, Protzer U. Virology. Response to Comment on "Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA". *Science* 2014; **344**: 1237 [PMID: 24926011 DOI: 10.1126/science.1254083]

28 **Mao R**, Nie H, Cai D, Zhang J, Liu H, Yan R, Cuconati A, Block TM, Guo JT, Guo H. Inhibition of hepatitis B virus replication by the host zinc finger antiviral protein. *PLoS Pathog* 2013; **9**: e1003494 [PMID: 23853601 DOI: 10.1371/journal.ppat.1003494]

29 **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]

30 **Manjunath N**, Yi G, Dang Y, Shankar P. Newer gene editing technologies toward HIV gene therapy. *Viruses* 2013; **5**: 2748-2766 [PMID: 24284874 DOI: 10.3390/v5112748]

31 **Schiffer JT**, Swan DA, Stone D, Jerome KR. Predictors of hepatitis B cure using gene therapy to deliver DNA cleavage enzymes: a mathematical modeling approach. *PLoS Comput Biol* 2013; **9**:

e1003131 [PMID: 23861664 DOI: 10.1371/journal.pcbi.1003131]
32 **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]
33 **Belloni L**, Pollicino T, De Nicola F, Guerrieri F, Raffa G, Fanciulli M, Raimondo G, Levrero M. Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. *Proc Natl Acad Sci USA* 2009; **106**: 19975-19979 [PMID: 19906987 DOI: 10.1073/pnas.0908365106]
34 **Allweiss L**, Volz T, Giersch K, Lohse AW, Petersen J, Lutgehetmann M, Dandri M. Proliferation of hepatitis B virus infected human hepatocytes induces suppression of viral replication and rapid cccDNA decrease in humanized mice. *J Hepatol* 2013; **58**: S45-S61 [DOI: 10.1016/S0168-8278(13)60129-4]
35 **Hammond SM**, Caudy AA, Hannon GJ. Post-transcriptional gene silencing by double-stranded RNA. *Nat Rev Genet* 2001; **2**: 110-119 [PMID: 11253050 DOI: 10.1038/35052556]
36 **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]
37 **Corporation AR**. Arrowhead Begins Dosing in Phase 2a Trial of RNAi Therapeutic ARC-520 in Chronic Hepatitis B Patients. 2014. Available from: URL: http://www.arrowheadresearch.com/press-releases/arrowhead-begins-dosing-phase-2a-trial-rnai-therapeutic-arc-520-chronic-hepatitis-b
38 **Li GQ**, Yu DM, Lu J, Chen SL, Zhao JY, Wang YC. Study of the efficacy of combination therapy of SiRNAs in HepG2.2.15 cells. *Hepatogastroenterology* 2011; **58**: 570-574 [PMID: 21661433]
39 **Li G**, Fu L, Jiang J, Ping Y, Huang Y, Wang Y. siRNA combinations mediate greater suppression of hepatitis B virus replication in mice. *Cell Biochem Biophys* 2014; **69**: 641-647 [PMID: 24549857 DOI: 10.1007/s12013-014-9846-2]
40 **Yuen MF**, Kim J, Kim CR, Ngai V, Yuen JC, Min C, Kang HM, Shin BS, Yoo SD, Lai CL. A randomized placebo-controlled, dose-finding study of oral LB80380 in HBeAg-positive patients with chronic hepatitis B. *Antivir Ther* 2006; **11**: 977-983 [PMID: 17302367]
41 **Lai CL**, Ahn SH, Lee KS, Um SH, Cho M, Yoon SK, Lee JW, Park NH, Kweon YO, Sohn JH, Lee J, Kim JA, Han KH, Yuen MF. Phase IIb multicentred randomised trial of besifovir (LB80380) versus entecavir in Asian patients with chronic hepatitis B. *Gut* 2014; **63**: 996-1004 [PMID: 23979965 DOI: 10.1136/gutjnl-2013-305138]
42 **Jacquard AC**, Brunelle MN, Pichoud C, Durantel D, Carrouée-Durantel S, Trepo C, Zoulim F. In vitro characterization of the anti-hepatitis B virus activity and cross-resistance profile of 2',3'-dideoxy-3'-fluoroguanosine. *Antimicrob Agents Chemother* 2006; **50**: 955-961 [PMID: 16495257 DOI: 10.1128/AAC.50.3.955-961.2006]
43 **Michalak TI**, Zhang H, Churchill ND, Larsson T, Johansson NG, Oberg B. Profound antiviral effect of oral administration of MIV-210 on chronic hepadnaviral infection in a woodchuck model of hepatitis B. *Antimicrob Agents Chemother* 2009; **53**: 3803-3814 [PMID: 19564357 DOI: 10.1128/AAC.00263-09]
44 **Agarwal K**, Fung SK, Nguyen TT, Cheng W, Sicard E, Ryder SD, Flaherty JF, Lawson E,Zhao S, Subramanian M, McHutchison JG, Gane EJ, Foster GR. Twenty-eight day safety and efficacy of tenofovir alafenamide (TAF) fumarate in chronic hepatis B (CHB) patients. 64th Annual Meeting of the American Association for the Study of Liver Diseases. Washington, DC, 2013. Available from: URL: http://www.natap.org/2013/AASLD/AASLD_91.htm
45 **Bam RA**, Yant SR, Cihlar T. Tenofovir alafenamide is not a substrate for renal organic anion transporters (OATs) and does not exhibit OAT-dependent cytotoxicity. *Antivir Ther* 2014; **19**: 687-692 [PMID: 24699134 DOI: 10.3851/IMP2770]
46 **Ruane PJ**, DeJesus E, Berger D, Markowitz M, Bredeek UF, Callebaut C, Zhong L, Ramanathan S, Rhee MS, Fordyce MW, Yale K. Antiviral activity, safety, and pharmacokinetics/pharmacodynamics of tenofovir alafenamide as 10-day monotherapy in HIV-1-positive adults. *J Acquir Immune Defic Syndr* 2013; **63**: 449-455 [PMID: 23807155 DOI: 10.1097/QAI.0b013e3182965d45]
47 **Sax PE**, Zolopa A, Brar I, Elion R, Ortiz R, Post F, Wang H, Callebaut C, Martin H, Fordyce MW, McCallister S. Tenofovir alafenamide vs. tenofovir disoproxil fumarate in single tablet regimens for initial HIV-1 therapy: a randomized phase 2 study. *J Acquir Immune Defic Syndr* 2014; **67**: 52-58 [PMID: 24872136 DOI: 10.1097/QAI.0000000000000225]
48 **Zlotnick A**, Mukhopadhyay S. Virus assembly, allostery and antivirals. *Trends Microbiol* 2011; **19**: 14-23 [PMID: 21163649 DOI: 10.1016/j.tim.2010.11.003]
49 **Cho MH**, Song JS, Kim HJ, Park SG, Jung G. Structure-based design and biochemical evaluation of sulfanilamide derivatives as hepatitis B virus capsid assembly inhibitors. *J Enzyme Inhib Med Chem* 2013; **28**: 916-925 [PMID: 22803663 DOI: 10.3109/14756366.2012.694879]
50 **Cho MH**, Jeong H, Kim YS, Kim J-W, Jung G. 2-Amino-N-(2,6-dichloropyridin-3-yl)acetamide derivatives as a novel class of HBV capsid assembly inhibitor. *J Viral Hepat* 2014; **12**: 843-852 [DOI: 10.1111/jvh.12214]
51 **Stray SJ**, Zlotnick A. BAY 41-4109 has multiple effects on Hepatitis B virus capsid assembly. *J Mol Recognit* 2006; **19**: 542-548 [PMID: 17006877 DOI: 10.1002/jmr.801]
52 **Brezillon N**, Brunelle MN, Massinet H, Giang E, Lamant C, DaSilva L, Berissi S, Belghiti J, Hannoun L, Puerstinger G, Wimmer E, Neyts J, Hantz O, Soussan P, Morosan S, Kremsdorf D. Antiviral activity of Bay 41-4109 on hepatitis B virus in humanized Alb-uPA/SCID mice. *PLoS One* 2011; **6**: e25096 [PMID: 22162746 DOI: 10.1371/journal.pone.0025096]
53 **Gruffaz M**, Testoni B, Luangsay S, Ait-Goughoulte M, Petit M-A, Ma H, Klumpp K, Javanbakht H, Durantel D, Zoulim F. Hepatitis B core (HBC) protein is a key and very early negative regulator of the interferon response. *J Hepatol* 2013; **58** Suppl 1: S155-S156 [DOI: 10.1016/S0168-8278(13)60380-3]
54 **Deres K**, Schröder CH, Paessens A, Goldmann S, Hacker HJ, Weber O, Krämer T, Niewöhner U, Pleiss U, Stoltefuss J, Graef E, Koletzki D, Masantschek RN, Reimann A, Jaeger R, Gross R, Beckermann B, Schlemmer KH, Haebich D, Rübsamen-Waigmann H. Inhibition of hepatitis B virus replication by drug-induced depletion of nucleocapsids. *Science* 2003; **299**: 893-896 [PMID: 12574631 DOI: 10.1126/science.1077215]
55 **Belloni L**, Li L, Palumbo GA, Chirapu SR, Calvo L, Finn M, Zlonick A, Levrero M. HAPs Hepatitis B virus (HBV) capsid inhibitors affect core protein interaction with the minichromosome and target cccDNA function. *J Hepatol* 2013; **58** (Abstr 371): S153 [DOI: 10.1016/S0168-8278(13)60373-6]
56 **Newswire P**. Novira Therapeutics Begins Phase 1a Clinical Study of NVR-1221 for the Treatment of Chronic Hepatitis B (HBV) Infection. 2014. Available from: URL: http://www.marketwatch.com/story/novira-therapeutics-begins-phase-1a-clinical-study-of-nvr-1221-for-the-treatment-of-chronic-hepatitis-b-hbv-infection-2014-05-08
57 **Campagna MR**, Liu F, Mao R, Mills C, Cai D, Guo F, Zhao X, Ye H, Cuconati A, Guo H, Chang J, Xu X, Block TM, Guo JT. Sulfamoylbenzamide derivatives inhibit the assembly of hepatitis B virus nucleocapsids. *J Virol* 2013; **87**: 6931-6942 [PMID: 23576513 DOI: 10.1128/JVI.00582-13]
58 REPLICor presents clinical efficacy and toxicology results in patients with chronic hepatitis B with short term exposure to immunotherapy after REP 9AC' induced clearance of serum HBsAg. The 15th annual TIDES meeting; 2013 May 14; Boston, USA. Available from: URL: http://replicor.com/replicor-presents-clinical-efficacy-toxicology-results-patients-chronic-hepatitis-b-short-term-exposure-immunotherapy-rep-9ac-induced-clearance-serum-hbsag/
59 **Al-Mahtab M**, Bazinet M, Vaillant A, Sheikh B. REP 9AC is a potent HBsAg release inhibitor which clears serum HBsAg and elicits SVRS in patients with chronic hepatitis B. *J Hepatol* 2011; **54** Suppl 1: S34
60 **Takeda K**, Akira S. Toll-like receptors in innate immunity. *Int Immunol* 2005; **17**: 1-14 [PMID: 15585605 DOI: 10.1093/intimm/

dxh186]
61 **Lanford RE**, Guerra B, Chavez D, Giavedoni L, Hodara VL, Brasky KM, Fosdick A, Frey CR, Zheng J, Wolfgang G, Halcomb RL, Tumas DB. GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. *Gastroenterology* 2013; **144**: 1508-1517, 1517.e1-10 [PMID: 23415804 DOI: 10.1053/j.gastro.2013.02.003]
62 **Momeni M**, Zainodini N, Bidaki R, Hassanshahi G, Daneshvar H, Khaleghinia M, Ebrahim M, Karimi-Googheri M, Askari A, Arababadi MK, Kennedy D. Decreased expression of toll like receptor signaling molecules in chronic HBV infected patients. *Hum Immunol* 2014; **75**: 15-19 [PMID: 24120739 DOI: 10.1016/j.humimm.2013.09.015]
63 **Jiang M**, Broering R, Trippler M, Poggenpohl L, Fiedler M, Gerken G, Lu M, Schlaak JF. Toll-like receptor-mediated immune responses are attenuated in the presence of high levels of hepatitis B virus surface antigen. *J Viral Hepat* 2014; **21**: 860-872 [PMID: 24498958 DOI: 10.1111/jvh.12216]
64 **Lopatin U**, Wolfgang G, Tumas D, Frey CR, Ohmstede C, Hesselgesser J, Kearney B, Moorehead L, Subramanian GM, McHutchison JG. Safety, pharmacokinetics and pharmacodynamics of GS-9620, an oral Toll-like receptor 7 agonist. *Antivir Ther* 2013; **18**: 409-418 [PMID: 23416308 DOI: 10.3851/IMP2548]
65 **Gane EJ**, Sicard E, Gruener D, Lau D, Visvanathan K, Gordon SC, Roberts SK, Kim S, Cheng W, Coffin CS, Fedorak R, Kwo PY, Leggett BA, Lim Y-S, Kim YJ, Pflanz S, Massetto B, McHutchison JG, Freilich B. Safety and Pharmacodynamics of Oral TLR-7 agonist GS-9620 in patients with Chronic Hepatitis B. 2013. Available from: URL: http://liverlearning.aasld.org/aasld/2013/thelivermeeting/35900/doctor.edward.gane.safety.and.pharmacodynamics.of.oral.tlr-7.agonist.gs-9620.html
66 **Gaggar A**, Coeshott C, Apelian D, Rodell T, Armstrong BR, Shen G, Subramanian GM, McHutchison JG. Safety, tolerability and immunogenicity of GS-4774, a hepatitis B virus-specific therapeutic vaccine, in healthy subjects: a randomized study. *Vaccine* 2014; **32**: 4925-4931 [PMID: 25045824 DOI: 10.1016/j.vaccine.2014.07.027]
67 **Spellman M**, Martin JT. Treatment of chronic hepatitis B infection with DV-601, a therapeutic vaccine. *J Hepatol* 2011; **54** Suppl: S302 [DOI: 10.1016/S0168-8278(11)60753-8]
68 **Betancourt AA**, Delgado CA, Estévez ZC, Martínez JC, Ríos GV, Aureoles-Roselló SR, Zaldívar RA, Guzmán MA, Baile NF, Reyes PA, Ruano LO, Fernández AC, Lobaina-Matos Y, Fernández AD, Madrazo AI, Martínez MI, Baños ML, Alvarez NP, Baldo MD, Mestre RE, Pérez MV, Martínez ME, Escobar DA, Guanche MJ, Cáceres LM, Betancourt RS, Rando EH, Nieto GE, González VL, Rubido JC. Phase I clinical trial in healthy adults of a nasal vaccine candidate containing recombinant hepatitis B surface and core antigens. *Int J Infect Dis* 2007; **11**: 394-401 [PMID: 17257877 DOI: 10.1016/j.ijid.2006.09.010]
69 **Inovio Pharmaceuticals Inc**. Inovio Therapeutic Hepatitis B Vaccine Killer T Cells Demonstrate Potential to Clear HBV in Liver. [Cited 2012 Nov 12]. Available from: URL: http://ir.inovio.com/news/news-releases/news-releases-details/2012/Inovio-Therapeutic-Hepatitis-B-Vaccine-Killer-T-Cells-Demonstrate-Potential-to-Clear-HBV-in-Liver/default.aspx
70 **Foundation HB**. HBV Drug Watch: Compounds in Development for Chronic Hepatitis B. [Updated 2014 Nov 15]. Available from: URL: http://www.hepb.org/professionals/hbf_drug_watch.htm

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# Key role of hepatitis B virus mutation in chronic hepatitis B development to hepatocellular carcinoma

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## Abstract

Chronic hepatitis B virus (HBV) infection is a major risk factor for hepatocellular carcinoma (HCC). The HBV mutations, which include point mutation, deletion, insertion and truncation mutation of *HBV* gene in 4 open reading frames (S, C, P, X), are closely associated with HCC pathogenesis. Some mutations accumulated during chronic HBV infection could be regarded as a biomarker to predict the occurrence of HCC. The detection of the mutations in clinical practice could be helpful for defining better preventive and therapeutic strategies and, moreover, predicting the progression of liver disease.

**Key words:** Hepatitis B virus; Mutations; Hepatocellular carcinoma; Carcinogenesis; Chronic hepatitis B



**Core tip:** This mini review fully describes the relationship between hepatitis B virus (HBV) genome mutations in different regions with liver disease progression and development to hepatocellular carcinoma (HCC) according to the recent data of mutations in chronic hepatitis B patients. Accordingly, the HBV mutations, either in the preS or PreC or/and core promoter region, are significantly associated with HCC and could be regarded as a biomarker to predict the occurrence of HCC.

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## INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a medical problem and the main cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) worldwide. The major risk factor of HCC carcinogenesis is chronic HBV infection, especially in China. Other factors,

including products of HBV, HBV integration and mutation and host susceptibility, contribute to HBV-related HCC. At present, carcinogenesis in HBV infection is not well understood[1]. The main contribution of HBV infection development to HCC is progressive stages of liver fibrosis and nonresolving inflammation[2,3]. The *HBV* genome contains four partially overlapping open reading frames (ORFs) (preS/S, preC/C, P and X) which have diverse kinds of patterns of mutations. The mutations, including point mutation, deletion, insertion and truncation in 4 ORFs, are closely associated with HCC pathogenesis and liver disease progression[4,5]. This mini review provides an overview of the recent data regarding development to HCC of HBV mutation in chronic hepatitis B patients.

## *S* REGION GENE MUTATION

The S region of ORFs are composed of three translation start codons (AUG codons) coding the expression of three kinds of proteins: large (L), middle (M) and small (S). PreS1 region is unique for L protein. PreS2 region is the shared sequence with the M protein and the S region is seen in all three kinds of proteins. The L and S proteins are fundamental for virion formation and the M enhances the virion secretion efficiency[6,7]. The S and M proteins are detected as hepatitis B s antigen (HBsAg). The dominant epitopes of HBsAg are the targets of neutralizing B cell responses, located in the "a" determinant (aa 124-147) in the major hydrophilic region[8].

The relationship between *S* gene mutation and HCC were studied focusing on the preS region. HBV *preS* mutations are closely related to an increased risk of HCC. Both the single and combined mutations with the other region gene mutations may be predictive for hepatocarcinogenesis.

Qu *et al*[9] found that T53C mutation, preS2 start codon mutations and preS deletions were related to HCC significantly in a study. The longitudinal study showed that the preS deletion mutations occurred during the long course of liver diseases, but not at the beginning of HBV infection.

Wang *et al*[10] found that preS mutant large HBV surface antigens (LHBs) can initiate endoplasmic reticulum (ER) stress to induce oxidative DNA damage and genomic instability. *PreS* mutant LHBs can up-regulate cyclooxygenase-2 and cyclin A to induce cell cycle progression and proliferation of hepatocytes. Dysplasia of hepatocytes can be induced by *preS* mutants in transgenic mice. In a nested control study, the presence of *preS* mutants was closely associated with development to HCC in HBV carriers.

In an interesting recent study, Su *et al*[11] observed that in a transgenic mice model, *preS2* mutants induced ER stress-dependent and independent pathways, leading to oxidative DNA damage, genomic instability and transforming capabilities. In their study, the combined expression of HBx and *preS2* mutant showed enhanced oncogenic effects in HCC development; however, the concrete role of X protein (*HBx*) and *preS2* mutant protein in HCC carcinogenesis is still to be clarified.

T53C, preS1 deletion, preS2 start codon mutation, C7A, A2962G, C2964A and C3116T in the preS region are significantly related to an increased risk of HCC[5]. Furthermore, the effects of other HBV *preS*/*S* mutations in hepatocarcinogenesis are still limited.

## *PREC/C* REGION GENE MUTATION

Mutations in the core promoter and precore regions of HBV lead to downregulation of hepatitis B e antigen (HBeAg). These mutations are related to chronic hepatitis, cirrhosis and HCC. C ORF of HBV genome encodes core protein and HBeAg[12]. The core shell of HBV is an effective immune stimulator, activating an intense neutralizing immune response to foreign epitopes. Mutations in this region of the HBV genome focus in the region of basal core promoter (BCP) and PreC[13].

T1762/A1764 double mutations in BCP is the most convincing association between HBV mutation and the development of HCC. The relationship between BCP double mutations and HCC were proved in two large prospective cohort studies[14]. Moreover, V1753, T1766, A1768 in BCP and T1653 mutations in box-α of Enhancer Ⅱ have been shown to be associated with the development of HCC in several reports.

Park *et al*[15] analyzed the 8 key mutations (G1613A, C1653T, T1753V, A1762T, G1764A, A1846T, G1896A and G1899A) in 442 serum samples of 310 non-HCC and 132 HCC patients to confirm the combinations with HCC. They reported that the BCP combination mutations of ⩾ 6 mutations that include G1613A + C1653T + A1846T + G1896A and ⩽ 5 mutations with reduced HBeAg production may increase the risk of HCC occurrence compared to only the number of mutations.

In our study, we also found five high frequency mutations (⩾ 10%) in the BCP and preC region. We observed thirteen types of multi-mutations in one fragment, among which, 3 types were common combinations (⩾ 5%). The three most common multi-mutations were A1762T/G1764A (36%), A1762T/G1764A/G1896A (11%) and T1753 (A/C)/A1762T/G1764A/G1896A (8%). The multi-mutations in HBV genomes (⩾ 3) may carry a high risk of liver cirrhosis or HCC. G1896A mutation had an effect on liver disease progression independent of patient age. Additionally, in our study, the results showed that the more viral mutations detected (⩾ 3) and G1776A mutation contribute to HBeAg negativity[16].

Finally, accumulation of mutations, including V1753 and/or A1768 aside from T1762/A1764 in BCP region, were closely associated with HCC among the patients infected with HBV/C1, as shown in a study by Li *et al*[17]. The BCP mutations have an effect on the biological functions of HBx, increasing the risk of HCC. T1653 mutation in the box-α of the core upstream regulatory sequence and V1753 mutation of BCP region in HBV-infected patients has also been reported to increase the risk of HCC[17,18]. However, the mechanism between BCP

mutation and hepatocarcinogenesis is mainly unknown.

Several studies have shown that HBV mutations, including C1653T, T1674C/G, T1753V, A1762T/G1764A and C1766T/T1768A in the enhancer Ⅱ/BCP regions and G1899A, C2002T, A2159G, A2189C and G2203A/T in the precore/core region, are significantly related to an increased risk of HCC[19].

## *P* REGION GENE MUTATION

One of the regions with mutation susceptibility in HBV ORF is the P region, which encodes the polymerase protein (reverse transcriptase). The envelope (*S*) gene is completely overlapped by the polymerase gene, so it is logical to assume that changes in virus encoding related to antiviral resistance in the polymerase may have impact on the envelope gene, showing a close relationship between mutations in the *S* and *P* regions of the HBV genome[20]. Mutations in the HBV *P* gene are frequently associated with drug resistance. Cross-sectional studies on the mutations of this gene are rare. Mutations in this region have not been assumed to be responsible for HCC as frequently as other regions spoken above, but antiviral therapy associated mutations did impact the disease progression on the subject. Several approved antiviral therapeutic agents are available at present, including regular or pegylated interferon and nucleoside/nucleotide analogues (NUCs) such as lamivudine (LAM), adefovir dipivoxial (ADV), entecavir (ETV), telbivudine and tenofovir. The mutation of rtM204V/I with LAM resistance is the most frequent, located at the catalytic YMDD motif. The rtL180M mutation often occurs at the same time. The rtA181T mutation was reported largely in LAM-resistant patients. The rtN236T and rtA181T/V mutations were the most frequent in ADV-resistant patients. ETV resistance is rare in antiviral therapy in naïve patients (1.5% by the fifth year), but if the rtI169T, rtT184A/F/G/I/L/S, rtS202G/I or rtM250V mutation coexists, ETV resistance can occur in the presence of rtM204I/V mutations. The most resistant mutant of telbivudine was rtM204I. Tenofovir has a low risk of drug resistance.

Yeh *et al*[21] in a study on 123 LAM-resistant chronic hepatitis B patients reported that the occurrence of the rtA181T/sW172* mutant in LAM-resistant patients could increase the risk of HCC development in the subsequent courses of antiviral therapy.

In our study, we followed up 131 cases of HBV-infected patients who had taken antiviral therapy. The results showed that the antiviral drug resistance was not significantly associated with the progression of the liver disease. Once resistance happened, there was no difference between chronic hepatitis B (CHB) patients with successful and unsuccessful rescue therapy after 6 years. I126T mutation in the S region may be associated with a poor prognosis for patients with CHB. I269L mutation in the P region and I68T mutation in the S region may be associated with poor response of NUCs treatment. These mutations could be potential new resistant mutations. Whether the mutations mentioned above are related to progression of the liver disease is still to be proved. In our hepatitis B related cirrhosis cohort study, the two year cumulative incidence of HCC in antiviral resistance (DR) patients (30.6%) was significantly higher than that in both complete virologic response patients (4.3%). Of these DR patients especially, the extremely high incidence of HCC was 55.6% in the failed rescue therapy. The rtA181T mutation was closely associated with rescue therapy failure[22]. It is suggested that the P region gene mutation related DR is associated with chronic hepatitis B development to HCC.

## *X* GENE MUTATION

X ORF produces the HBx and, despite the specific function of HBx, is still undefined during HBV replication. Many studies show that HBx is essential for viral replication *in vivo* and *in vitro*. HBV *X* gene, extremely easy to mutate and integrate into hepatocytes, plays a significant role in HBV infection and HCC development. Mutations in the X region can effect viral replication through the BCP and the enhancer Ⅱ. Because the BCP region overlaps with the *X* gene in the concomitant reading frame, the *X* gene at xK130M and xV131I was changed by the A1762T plus G1764A core promoter mutations in the aforementioned study[23].

Wild-type HBx has been proved to activate hypoxia-inducible factor-1α (HIF-1α), which could contribute to HCC development and progression. Liu *et al*[24] sequenced 101 HCC tissues in Hong Kong. In their study, the double mutations K130M/V131I increased the function of HBx as they upregulated the HIF-1α expression and transcriptional activity. Wang *et al*[25] reported that during the infection and replication of HBV, HBx mutates to adjust itself to the hepatocyte and increase the carcinogenesis. COOH-terminal truncated HBX may play a stimulative part in HBV-related HCC development as well as hydrophobic/hydrophilic character changes in some specific amino acid sites.

Besides, Tuteja *et al*[26] reported 222 cases with HBsAg positive patients and they found that T36A and G50R mutations in the *X* gene were associated with HCC. The integration of the viral genome into the host cellular genome was detected in 80%-90% of these cancers. The viral DNA integration also may cause insertional mutagenesis and result in a 3'-terminal truncation of HBX that was deleted at the C-terminal region by 20-40 amino acids. In the HBX sequence, multiple point mutations may be a consequent change with integration. Moreover, it showed that both truncation and point mutations may increase the oncogenic activation processes. It has been found that the C-truncated HBx proteins transform immortalized liver cell lines and interact with the mutant p53 protein p.R249S to change genetic stability and proliferation of non-transformed hepatocytes in

experimental models[27]. Lee *et al*[19] found that a specific HBx mutation may contribute to the development of HCC in chronic hepatitis B patients by activating nuclear factor-kappa B activity. The HBx 5 mutation in genotype C2 HBV was shown to increase the risk of the development of HCC.

HBV *X* gene multi-site mutations were found frequently in the clinical HCC tissues. Wang *et al*[28] analyzed the *HBx* gene sequences of 60 cases of HCC tumor tissues and paratumor tissues from China. The results showed that the most frequent mutations were at amino acid 30, 88, 144 from tumor samples and at amino acid 31, 43, 87, 94 from non-tumor samples. It has been found that HBx-linked mutations, such as at aa L30F/S144A, was 29.5% positive in the tumor tissues.

Among the HCC-associated mutations, combined rather than single mutations are associated with the risk of HCC significantly. In the preS region, the frequencies of combined mutations (haplotypic carriages), including 2964C-3116T-preS2 start codon wildtype-7A, 2964C-3116T-7A-76C and 2964A-3116T-7C-76A/T, are significantly higher in patients with HCC than in those without HCC, and yet the haplotypic carriages with single mutation are inversely associated with HCC. In the preS and EnhⅡ/BCP regions, HCC patients have a more frequent occurrence of a haplotypic carriage with 105C and 2962G than those without HCC. The frequency of 2962G-preS2 start codon wild type-105C-1762T/1764A is 47.9% in HCC and 4.3% in those without HCC.

Accordingly, the HBV mutations, either in the preS or in the core promoter region, are significantly associated with HCC, whereas the wild-type nucleotides in these regions are mostly associated with liver cirrhosis. HBV mutations can be used as indicators for the prediction of end-stage liver diseases, including HCC. Although these mutations and the combinations are specific for HCC to some extent, it will be more practical if they can predict the malignancy in HBV-infected subjects before the occurrence of HCC.

## IN THE FUTURE

Many factors have an effect on the development of HBV-associated HCC, including products of HBV, HBV integration and mutation and host susceptibility. HBV sequences from these individuals demonstrate numerous mutations/deletions and alterations that can result in decreased immune recognition of the virus, thereby affecting the expression and functions of specific genes and contributing to liver disorders. However, the aforementioned studies mostly lacked a series of observation and detection. Additionally, sequencing the HBV genome to find the HCC-related HBV mutations have conflicting results, suggesting that the pathogenesis of development to HCC is a combination. The hepatocarcinogenesis of chronic inflammation, host immunity and environment in chronic hepatitis B patients with different patterns of mutation should be further studied.

## REFERENCES


1 **Chemin I**, Zoulim F. Hepatitis B virus induced hepatocellular carcinoma. *Cancer Lett* 2009; **286**: 52-59 [PMID: 19147276 DOI: 10.1016/j.canlet.2008.12.003]

2 **Brechot C**, Kremsdorf D, Soussan P, Pineau P, Dejean A, Paterlini-Brechot P, Tiollais P. Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC): molecular mechanisms and novel paradigms. *Pathol Biol* (Paris) 2010; **58**: 278-287 [PMID: 20667665 DOI: 10.1016/j.patbio.2010.05.001]

3 **Neuveut C**, Wei Y, Buendia MA. Mechanisms of HBV-related hepatocarcinogenesis. *J Hepatol* 2010; **52**: 594-604 [PMID: 20185200 DOI: 10.1016/j.jhep.2009.10.033]

4 **Xu C**, Zhou W, Wang Y, Qiao L. Hepatitis B virus-induced hepatocellular carcinoma. *Cancer Lett* 2014; **345**: 216-222 [PMID: 23981576 DOI: 10.1016/j.canlet.2013.08.035]

5 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]

6 **Yuan Q**, Ou SH, Chen CR, Ge SX, Pei B, Chen QR, Yan Q, Lin YC, Ni HY, Huang CH, Yeo AE, Shih JW, Zhang J, Xia NS. Molecular characteristics of occult hepatitis B virus from blood donors in southeast China. *J Clin Microbiol* 2010; **48**: 357-362 [PMID: 19940057 DOI: 10.1128/JCM.01781-09]

7 **Hsu CW**, Yeh CT. Emergence of hepatitis B virus S gene mutants in patients experiencing hepatitis B surface antigen seroconversion after peginterferon therapy. *Hepatology* 2011; **54**: 101-108 [PMID: 21503942 DOI: 10.1002/hep.24363]

8 **Huang CH**, Yuan Q, Chen PJ, Zhang YL, Chen CR, Zheng QB, Yeh SH, Yu H, Xue Y, Chen YX, Liu PG, Ge SX, Zhang J, Xia NS. Influence of mutations in hepatitis B virus surface protein on viral antigenicity and phenotype in occult HBV strains from blood donors. *J Hepatol* 2012; **57**: 720-729 [PMID: 22634131 DOI: 10.1016/j.jhep.2012.05.009]

9 **Qu LS**, Liu JX, Liu TT, Shen XZ, Chen TY, Ni ZP, Lu CH. Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in Qidong, China. *PLoS One* 2014; **9**: e98257 [PMID: 24849936 DOI: 10.1371/journal.pone.0098257]

10 **Wang HC**, Huang W, Lai MD, Su IJ. Hepatitis B virus pre-S mutants, endoplasmic reticulum stress and hepatocarcinogenesis. *Cancer Sci* 2006; **97**: 683-688 [PMID: 16863502 DOI: 10.1111/j.1349-7006.2006.00235.x]

11 **Su IJ**, Wang LH, Hsieh WC, Wu HC, Teng CF, Tsai HW, Huang W. The emerging role of hepatitis B virus pre-S2 deletion mutant proteins in HBV tumorigenesis. *J Biomed Sci* 2014; **21**: 98 [PMID: 25316153 DOI: 10.1186/s12929-014-0098-7]

12 **Nguyen DH**, Ludgate L, Hu J. Hepatitis B virus-cell interactions and pathogenesis. *J Cell Physiol* 2008; **216**: 289-294 [PMID: 18302164 DOI: 10.1002/jcp.21416]

13 **Roseman AM**, Borschukova O, Berriman JA, Wynne SA, Pumpens P, Crowther RA. Structures of hepatitis B virus cores presenting a model epitope and their complexes with antibodies. *J Mol Biol* 2012; **423**: 63-78 [PMID: 22750730 DOI: 10.1016/j.jmb.2012.06.032]

14 **Lin CL**, Kao JH. The clinical implications of hepatitis B virus genotype: Recent advances. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 123-130 [PMID: 21199523 DOI: 10.1111/j.1440-1746.2010.06541.x]

15 **Park YM**, Jang JW, Yoo SH, Kim SH, Oh IM, Park SJ, Jang YS, Lee SJ. Combinations of eight key mutations in the X/preC region and genomic activity of hepatitis B virus are associated with hepatocellular carcinoma. *J Viral Hepat* 2014; **21**: 171-177 [PMID: 24344773 DOI: 10.1111/jvh.12134]

16 **Zhang D**, Ma S, Zhang X, Zhao H, Ding H, Zeng C. Prevalent HBV point mutations and mutation combinations at BCP/preC region and their association with liver disease progression. *BMC*

*Infect Dis* 2010; **10**: 271 [PMID: 20846420 DOI: 10.1186/1471-2334-10-271]

17 **Li W**, Chen G, Yu X, Shi Y, Peng M, Wei J. Accumulation of the mutations in basal core promoter of hepatitis B virus subgenotype C1 increase the risk of hepatocellular carcinoma in Southern China. *Int J Clin Exp Pathol* 2013; **6**: 1076-1085 [PMID: 23696925]

18 **Yuen MF**, Tanaka Y, Shinkai N, Poon RT, But DY, Fong DY, Fung J, Wong DK, Yuen JC, Mizokami M, Lai CL. Risk for hepatocellular carcinoma with respect to hepatitis B virus genotypes B/C, specific mutations of enhancer II/core promoter/precore regions and HBV DNA levels. *Gut* 2008; **57**: 98-102 [PMID: 17483190 DOI: 10.1136/gut.2007.119859]

19 **Lee JH**, Han KH, Lee JM, Park JH, Kim HS. Impact of hepatitis B virus (HBV) x gene mutations on hepatocellular carcinoma development in chronic HBV infection. *Clin Vaccine Immunol* 2011; **18**: 914-921 [PMID: 21490166 DOI: 10.1128/CVI.00474-10]

20 **Sheldon J**, Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. *J Antimicrob Chemother* 2008; **61**: 766-768 [PMID: 18218641 DOI: 10.1093/jac/dkn014]

21 **Yeh CT**, Chen T, Hsu CW, Chen YC, Lai MW, Liang KH, Chen TC. Emergence of the rtA181T/sW172* mutant increased the risk of hepatoma occurrence in patients with lamivudine-resistant chronic hepatitis B. *BMC Cancer* 2011; **11**: 398 [PMID: 21933446 DOI: 10.1186/1471-2407-11-398]

22 **Li L**, Liu W, Chen YH, Fan CL, Dong PL, Wei FL, Li B, Chen DX, Ding HG. Antiviral drug resistance increases hepatocellular carcinoma: a prospective decompensated cirrhosis cohort study. *World J Gastroenterol* 2013; **19**: 8373-8381 [PMID: 24363530 DOI: 10.3748/wjg.v19.i45.8373]

23 **Datta S**, Ghosh A, Dasgupta D, Ghosh A, Roychoudhury S, Roy G, Das S, Das K, Gupta S, Basu K, Basu A, Datta S, Chowdhury A, Banerjee S. Novel point and combo-mutations in the genome of hepatitis B virus-genotype D: characterization and impact on liver disease progression to hepatocellular carcinoma. *PLoS One* 2014; **9**: e110012 [PMID: 25333524 DOI: 10.1371/journal.pone.0110012]

24 **Liu LP**, Hu BG, Ye C, Ho RL, Chen GG, Lai PB. HBx mutants differentially affect the activation of hypoxia-inducible factor-1α in hepatocellular carcinoma. *Br J Cancer* 2014; **110**: 1066-1073 [PMID: 24346287 DOI: 10.1038/bjc.2013.787]

25 **Wang D**, Cai H, Yu WB, Yu L. Identification of hepatitis B virus X gene variants between hepatocellular carcinoma tissues and pericarcinoma liver tissues in Eastern China. *Int J Clin Exp Pathol* 2014; **7**: 5988-5996 [PMID: 25337243]

26 **Tuteja A**, Siddiqui AB, Madan K, Goyal R, Shalimar V, Kaur N, Panda SK, Narayanasamy K, Subodh S, Acharya SK. Mutation profiling of the hepatitis B virus strains circulating in North Indian population. *PLoS One* 2014; **9**: e91150 [PMID: 24637457 DOI: 10.1371/journal.pone.0091150]

27 **Gouas DA**, Villar S, Ortiz-Cuaran S, Legros P, Ferro G, Kirk GD, Lesi OA, Mendy M, Bah E, Friesen MD, Groopman J, Chemin I, Hainaut P. TP53 R249S mutation, genetic variations in HBX and risk of hepatocellular carcinoma in The Gambia. *Carcinogenesis* 2012; **33**: 1219-1224 [PMID: 22759751 DOI: 10.1093/carcin/bgs068]

28 **Wang Q**, Zhang T, Ye L, Wang W, Zhang X. Analysis of hepatitis B virus X gene (HBx) mutants in tissues of patients suffered from hepatocellular carcinoma in China. *Cancer Epidemiol* 2012; **36**: 369-374 [PMID: 22178505 DOI: 10.1016/j.canep.2011.11.006]

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ORIGINAL ARTICLE

Retrospective Study

# Management of telaprevir-based triple therapy for hepatitis C virus recurrence post liver transplant

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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at email address: kerstin.herzer@uk-essen.de. The presented data are annonymized without risk of identification.

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## Abstract

**AIM:** To characterize management of telaprevir (TVR)-based triple therapy of hepatitis C virus (HCV) reinfection after liver transplantation (LT).

**METHODS:** We retrospectively analyzed safety and efficacy of telaprevir - based triple therapy in a single center cohort of 19 patients with HCV genotype (GT) 1 recurrence after LT, with respect to factors possibly predicting sustained viral response (SVR) or non-SVR. All patients were treated with TVR, pegylated (PEG) and ribavirine (RBV) for 12 wk followed by a dual phase with PEG/RBV for 12 wk in 7 patients and for 36 wk in 5 patients.

**RESULTS:** In total 11/19 (58%) of patients achieved a sustained response. All (11/11) SVR patients showed a rapid viral response at treatment weeks 4 and 11/14 rapid virological response (RVR) patients achieved SVR. Notably, all (7/7) patients who completed 48 wk of therapy and 80% (4/5) patients who completed

24 wk of therapy achieved SVR24. Treatment failure was significantly ($P$ > 0.049) more frequent in GT1a infection (5/7) compared to GT1b (3/12) infection and was associated with emergence of resistance-associated mutations in the NS3 protease domain. Bilirubin level at baseline is also related to SVR ($P$ > 0.030). None of the patients had to discontinue treatment due to side effects.

**CONCLUSION:** RVR, GT and bilirubin are clearly related to achievement of SVR. Providing a thorough patient selection and monitoring, a full course of TVR-based triple therapy in LT patients is feasible and achieves high SVR rates.

**Key words:** Liver transplantation; Telaprevir; Hepatitis C virus recurrence; Predictors; Hepatitis C virus therapy



**Core tip:** Experiences with telaprevir-based triple therapy in 19 patients with hepatitis C virus recurrence after liver transplantation are analysed and described in detail. We observed a exceptionally high sustained viral response rate and analyzed clinicopathological factors which might contribute to predict which patients rather benefit from this therapy and which do not. While the new generation directly acting antivirals start to be available in some countries, many parts of the world will not have the privilege of these therapeutic options for a long time. Therefore we are eager to share our experiences with telaprevir in liver transplantation patients with the international hepatologist community.

Herzer K, Papadopoulos-Köhn A, Achterfeld A, Canbay A, Piras-Straub K, Paul A, Walker A, Timm J, Gerken C. Management of telaprevir-based triple therapy for hepatitis C virus recurrence post liver transplant. *World J Hepatol* 2015; 7(9): 1287-1296 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i9/1287.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i9.1287

## INTRODUCTION

About 160 million people worldwide are currently affected by a chronic hepatitis C virus (HCV) infection with the deleterious consequences of decompensated cirrhosis and hepatocellular carcinoma (HCC)[1,2]. In western countries, HCV-induced liver cirrhosis and HCC can be therapeutically addressed by liver transplantation (LT). However, reinfection of the liver graft occurs in virtually all patients typically followed by an accelerated course of progressive liver damage. About 3 to 5 years post-LT, 30% of HCV-positive patients develop cirrhosis of the graft with a consecutively unfavorable prognosis[3]. Over the past decade, treatment of HCV-reinfection with pegylated interferon (PEG-IFN) and ribavirin (RBV) was the only treatment option associated with moderate sustained virological response (SVR) rates of only 8%-50% depending on the genotype (GT), defining patients who received liver transplant as a "difficult-to treat" group[4]. In 2011, the first generation of directly acting antivirals (DAAs), the protease inhibitors (PI) telaprevir (TVR) and boceprevir (BOC), were approved for treatment of chronic HCV infection, in combination with PEG-IFN and RBV[5]. The TVR-based triple regimen was reported to achieve SVR rates of up to 75% in treatment naïve GT1 non-LT patients compared to 44% with the previous dual therapy[6]. Unfortunately, in the post-LT setting the triple regimen comprised unforeseen challenges. Severe drug-drug-interactions of the immunosuppressive (IS) agents and the PI which may result in increased toxicity or/ and loss of efficacy of both drugs, potentially resulting in severe allograft rejections[7,8]. Nevertheless, reports from different transplant centers supported the principal feasibility of combining immunosuppressive agents and PIs in the post-LT setting[9,10]. SVR rates of 20%-50% were reported in hard to treat (mostly intensively pretreated) cohorts of patients[11]. In the meantime, new DAA are approved[12]. Thus, a therapeutic hold is observed in the prospect of new treatment options promising no drug-drug interactions and less severe side effects, most importantly because interferon may become dispensable[13]. However, when and whether at all the first generation PIs will be replaced by novel DAAs also depends on economic aspects in a number of countries and potentially the emergence of resistances[14,15].

The aim of our retrospective analysis was to summarize and communicate the rather good experience of our center with treatment course and outcome of a large single center cohort of patients with HCV GT1 reinfection after LT with TVR-based triple therapy. We intend to give a thorough description of our observations and estimations, in order to share our experiences with the community, in particular in those parts of the world were the second generation DAAs are not yet available.

## MATERIALS AND METHODS

### *Patients studied*

Between April 2012 and January 2013, 19 patients with HCV GT1 recurrence after liver transplantation were selected for treatment with TVR-based triple therapy. For demographic parameters and baseline clinical chemistry (Table 1).

Patients were considered eligible for TVR-based triple therapy upon clinical and histological evidence of recurrence of HCV GT1 infection. Before PEG-IFN was administered, all patients underwent an allograft biopsy for evaluation of fibrosis stage according to the METAVIR system and for exclusion of graft rejection. Exclusion criteria for antiviral treatment were evidence of biopsy-proven acute rejection during the past 3 mo or any medical contraindication to treatment with PEG-IFN and RBV that would predict the occurrence of complications during IFN administration, such as

**Table 1 Characteristics of patients at baseline**

| | | |
|---|---|---|
| Demography | | |
| Age, yr | 57 | 41-70 |
| Gender (male/female) | 16/3 | |
| Body mass index (kg/m$^2$) | 26 | 21-33 |
| Race, *n*/% | 19 | 100 |
| Caucasian, *n*/% | 18 | 94.74 |
| Hispanic, *n*/% | 1 | 5.26 |
| Immunosuppressive regimen | | |
| TAC/CSA | 17/2 | |
| MMF (*n*) | 8 | |
| Steroids (*n*) | 0 | |
| Baseline clinical parameters | | |
| Duration of therapy | 24 | 4-48 |
| Ishak fibrosis score (grade) | 2 | 0-4 |
| Inflammation (grade) | 1.5 | 0-2 |
| Fibroscan baseline (kPa) | 13.8 | 4.5-46.4 |
| Time from LT to triple therapy (mo) | 22 | 7-295 |
| Baseline clinical chemistry | | |
| Bilirubin total (mg/dL) | 0.8 | 0.3-3.2 |
| GGT (U/L) | 52 | 13-32 |
| GPT (ALAT), U/L | 41 | 21-159 |
| GOT (ASAT), U/L | 52 | 18-88 |
| AP, U/L | 101 | 53-404 |
| Creatinine (mg/dL) | 1.26 | 0.67-1.89 |
| GFR (MDRD) | 57.3 | 23-133 |
| International normalized ratio | 1 | 0.8-1.2 |
| Hämoglobin (g/dL) | 13.5 | 9-16.8 |
| WBC (/μL) | 3.73 | 1.6-8.3 |
| Platelet count (/μL) | 111 | 62-246 |
| Basline viral characteristics | | |
| HCV GT | | |
| 1a, *n*/% | 7 | 36.8 |
| 1b, *n*/% | 12 | 63.2 |
| VL ($log_{10}$ IU/mL) | 1.95 | 0.13-14.9 |
| Recipient IL-28b polymorphism, *n*/% | | |
| CC | 5 | 26.3 |
| CT | 9 | 47.4 |
| TT | 5 | 26.3 |
| History of any prior PEG-INF/RBV treatment, *n*/% | 11 | 57.89 |
| History of post-LT PEG-INF/RBV treatment, *n*/% | 5 | 26.3 |
| HCC prior to LT (*n*) | 6 | |
| HBV coinfection (*n*) | 0 | |

TAC: Tacrolimus; HCV: Hepatitis C virus; LT: Liver transplantation; HBV: Hepatitis B virus; GFR: Glomerular filtration rate; WBC: White blood count; GT: Genotype; VL: Viral load; IL-28b: Interleukin-28b; PEG-INF: Pegylated-interferon; RBV: Ribavirine; GGT: Gamma-glutamyl transpeptidase; GPT: Glutamat pyruvat transaminase; ALAT: Alanin-aminotransferase; GOT: Glutamat oxalacetat transaminase; ASAT: Aspartate aminotransferase; CSA: Cyclosporine; MMF: Mycophenolate mofetil; AP: Alkaline phosphatase.

platelet count lower than 100000/μL or white blood cell count lower than 2000/μL; clinical signs or laboratory values indicating decompensating liver function; renal insufficiency, with a glomerular filtration rate (GFR) lower than 60 mL/min; and anemia, with a hemoglobin level lower than 10 mg/dL at baseline. Whenever possible, treatment with mycophenolate mofetil and corticosteroids was discontinued before the initiation of antiviral treatment[16].

As limited experience exists in the post-LT setting, patients were thoroughly informed of possible interactions and side effects prior to treatment. Serological and clinical data were collected from the patients' files and retrospectively analyzed. The analysis was conducted in accordance with the Helsinki Declaration of 1975 and approved by the ethics committee of the University Hospital Essen.

### *Antiviral treatment regimen*

Patients were treated with TVR, PEG-IFN and RBV for 12 wk followed by 12 or 36 wk of dual therapy with PEG-IFN/RBV. The RBV dose was administered with 600 mg/d at baseline and only reduced in two cases of acute renal failure. PEG-IFN was initiated with 180 μg/d and reduced in 3 cases due to cytopenia and overall tolerance. TVR was preferred to BOC due to a shorter triple regimen and administered as 1175 mg twice per day. The stopping rule applied was failure to achieve a reduction in HCV viral load (VL) to less than 1000 IU/mL at treatment weeks (TW) 12, a detectable viral load at TW 24 or viral breakthrough (VB) with discontinuation of all antiviral treatment.

For the assessment of efficacy, viral load was monitored in plasma using the ABBOTT Real Time HCV assay (Abbott Molecular, United States; lower limit of detection 12 IU/mL) at baseline and then at week 1, 2, 3, 4, 8, 12, 16, 20, 24, 36 and 48. Genotypes were determined using phylogenetic analyses of the core region. A rapid virological response (RVR) was defined as an undetectable VL at TW 4 of triple therapy. At TW 12, an undetectable viral load was defined as early viral response (EVR). An end of therapy treatment response (EOT) was obtained when the VL remained negative at the time of treatment discontinuation. A SVR24 was defined as negative VL 24 wk after the end of treatment. VB was defined as achieving an undetectable viral load but the subsequent occurrence of a detectable VL over time. In all patients, the whole NS3 region was analyzed by sequencing, and PI resistance mutations were recorded.

### *Resistance analysis of the NS3 protease domain*

The NS3 protease domain was sequenced before therapy and in patients experiencing treatment failure in the first available viremic sample. HCV-RNA was extracted utilizing spin columns and reverse transcribed followed by subsequent amplification of cDNA by nested polymerase chain reaction (PCR). Detailed information on primer sequences and PCR protocols are available upon request. The PCR product was directly sequenced by Sanger technology and obtained sequences were analyzed utilizing the resistance prediction algorithm as implemented in Geno2pheno (HCV) 0.92 (http://hcv.bioinf.mpi-inf.mpg.de).

### *Management of immunosuppression and safety assessments*

Immunosuppressive regimen was left unchanged with tacrolimus (TAC) in 17 and cyclosporine A (CyA) in 2 patients. Mycophenolate mofetil (MMF) as comedication in 8 patients was skipped for the period of therapy in order to avoid myelosuppressive effects. Before starting

triple therapy, patients were on a stable dose of TAC or CyA with stable therapeutic levels. After initiating TVR, TAC dosage was skipped until start of decline of therapeutic level and then administered as 0.1 mg with once or twice daily dosing as described previously[10]. Trough levels of TAC were checked daily for the first 10 d and twice a week for the next two wk and then once a week during the remaining duration of TVR. Once TVR was stopped, TAC was reinstituted with a goal to achieve pre-TVR doses gradually over a period of 5 d with daily trough level checks. The dose of CyA was reduced by 50% upon start of TVR with control of trough levels as described for TAC. The day after stopping TVR, CyA was reinstituted at the dose before TVR-therapy with controls according to TAC. Trough blood concentrations (TBC) ranged from 5 to 7 ng/mL for TAC and from 50 to 80 ng/mL for CyA[16].

Creatinine clearance was estimated using the MDRD formula. Upon decrease of renal function parameters, renal function was immediately stabilized by intensive daily intravenous fluid application and patients were admonished to increase fluid intake from baseline. Erythropoietin (Epoentin®; Hexal) was administered to support the red blood cell count when hemoglobin (HB) levels dropped to below 10 g/dL. Granulocyte colony stimulating factor (G-CSF) (Neupogen®; Amgen Europe BV) was administered to support the neutrophil count when it fell below 1000/μL despite PEG-IFN dose reduction.

Safety and efficacy data were gathered in short intervals during time of treatment. The modalities of treatment, on-treatment surveillance and follow-up were previously described[10].

### *Statistical analysis*

Continuous variables were expressed as medians, means and ranges. The Wilcoxon signed-rank-test was used to compare paired groups. A *P*-value of $< 0.05$ was considered to be significant. Numeric liver values of small groups ($n \leqslant 30$) were compared by Mann-Whitney-*U*-Test. Categorical variables were analysed by $\chi^2$-test with pearson approximation. Statistical analysis were performed using SPSS 19 statistical software (IBM SPSS; Chicago, IL).

## RESULTS

### *Effectiveness*

Patients with stable blood count, liver and renal function as central inclusion criteria were thoroughly selected for therapy. The median time between LT and treatment was 22 mo (7-295). All patients had histologically proven HCV reinfection of the graft. None of the patients had clinical signs of decompensation. None of the patients was suffering from fibrosing cholestatic hepatitis (FCH). Seven patients were infected with GT1a, 12 patients with GT1b. Seventeen patients received tacrolimus as immunosuppressive regimen and 2 patients received cyclosporine A. Eight patients received MMF as concurrent medication which was stopped at the beginning of triple therapy in order to prevent aggravation of myelosuppressive effects of the antiviral substances. Patients' characteristics are shown in Table 1.

Eight patients had to discontinue antiviral therapy early: one because of impaired liver function after 4 wk, three because of VB after TW 8 and TW 12. One patient had a partial response with a viral decline of > 2-log$_{10}$ IU/mL at TW 12 but was stopped when viral load was still detectable at TW 24. One patient had a VB at TW 16 despite RVR and was discontinued. Two patients who had an EVR experienced a VB at TW 14 and TW 24 and were discontinued from therapy. Notably, only one patient had to stop therapy due to side effects, respectably deterioration of liver function, which resolved. All other patients with an unfavourable course of therapy were stopped due to VB or insufficient response (Table 2). Two patients with initial RVR and VB at TW 12 and 16 selected mutations associated with resistance as described below (Table 3).

Five of the 12 remaining patients with RVR and undetectable viral load at TW 24 decided to stop therapy at TW 24. Of these, 4 experienced a sustained response with SVR24 while 1 patient relapsed within 12 wk after the end of treatment (Table 2). Seven patients decided to continue treatment for the full course of 48 wk, all of which achieved SVR24 (Table 2). Notably, all patients with sustained clearance of the virus (4 after a 24 wk-course and 7 after a 48 wk-course) were characterized by RVR (Table 2). In brief, we observed an overall RVR4 in 14/19 patients (73.7%), an EVR in 16/19 patients (84.3 %), an EOT response in 12/19 patients (63.1%) and SVR24 in 11/19 patients (57.9%).

Complete sequence information was obtained for the NS3 protease domain from baseline samples and from the first viremic sample of patients with treatment failure. We were unable to amplify the protease domain from one sample after treatment failure. Of the seven remaining patients, six harbored isolates with substitutions known to be associated with resistance to telaprevir. Four patients with genotype 1a infection and subsequent treatment failure selected the R155K substitution, in two cases combined with a substitution V36M/L. Two patients with genotype 1b infection and subsequent treatment failure selected either an A156F subsititution associated with high-level resistance or a combination of the substitutions V36L and T54S. In one patient infected with genotype 1b resistance-associated substitutions were not detectable by bulk sequencing. Two patients with subsequent treatment failure carried the resistance-associated substitution V36M/L already prior to therapy. Notably, resistance-associated substitutions were not detected at baseline in all patients who achieved SVR. Mutation related to PI resistance were detected in eight patients who all experienced a treatment failure, a VB or a non-response (Table 3).

In order to determine whether successful TVR-based triple therapy influences liver parameters, we

**Table 2 Treatment response**

| | Sustained virological response | | | | | | | | | | | | Treatment failure | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pat. 1 | Pat. 2 | Pat. 3 | Pat. 4 | Pat. 5 | Pat. 6 | Pat. 7 | Pat. 8 | Pat. 9 | Pat. 10 | Pat. 11 | Pat. 12 | Pat. 13 | Pat. 14 | Pat. 15 | Pat. 16 | Pat. 17 | Pat. 18 | Pat. 19 | Pat. 12 |
| HCV GT | 1b | 1b | 1a | 1b | 1b | 1b | 1b | 1b | 1b | 1a | 1b | 1a | 1a | 1a | 1b | 1a | 1b | 1a | 1b | 1a |
| Basline | 2763000 | 4860000 | 1183000 | 5758000 | 1713000 | 1950000 | 441000 | 14900000 | 1920000 | 456000 | 387700 | 10900000 | 11500000 | 1008000 | 2565000 | 1690000 | 1370000 | 135600 | 580400 | 10900000 |
| TW 4 (RVR) | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | 71 | 37 | 34 | < Q | < Q | 15 | 4668 | < Q |
| TW 12 (EVR) | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | 685 | < Q | < Q | 3322000 | < Q | < Q | 6848 | < Q |
| TW 16 | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | 371 | < Q | < Q | ND | 6260 | ND | ND | < Q |
| TW 24 | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | 164 | 466 | 55400 | ND | ND | ND | ND | < Q |
| TW 48 | | | | | < Q | < Q | < Q | < Q | < Q | < Q | < Q | | | | | | | | | |
| 12 wk pt | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | 457200 | 9704 | 1657 | 1564794 | ND | 966400 | 27 | 660300 | 457200 |
| 24 wk pt | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | < Q | 691400 | 4135000 | | 3735000 | ND | 7420000 | 9824 | 3489000 | 691400 |

HCV: Hepatitis C virus; TW: Treatment weeks; GT: Genotype; RVR: Rapid virological response; EVR: Early viral response; pt: Post treatment; Pat: Patient; ND: Not determined.

determined liver stiffness in SVR patients by fibroscan at baseline and at 24 wk post treatment. The fibrosis score improved for all patients significantly ($P < 0.003$) with 14.6 kPA (4.8-46) at baseline to 8.8 kPa (4.5-23.3) at 24 wk post treatment (pt) (Figure 1A). Concerning liver values, alanine aminotransferases (ALT) improved also significantly ($P < 0.005$) from 49 U/L (21-159) to 25 U/L (11-73) (Figure 1B). Aspartate aminotransferase (AST) improved significantly ($P < 0.028$) from 52 IU/L (21-84) to 31.5 IU/L (18-67) (Figure 1C). Bilirubin was not high at baseline in all patients and was stable from 0.6 mg/dL (0.3-1.1) at baseline to 0.6 mg/dL (0.1-0.9) in median 24 wk pt (Figure 1D). Improvement could be observed for all patients described, irrespective of duration of treatment.

With respect to prediction of outcome, we analyzed several clinical and patient characteristics at baseline and compared patients with SVR to patients without SVR (Table 4). Age, body mass index, fibrosis score and time from LT to start of therapy as well as the recipient interleukin-28b polymorphism and previous antiviral treatment did not significantly influence the outcome in our cohort. As well, liver values, platelet count and viral load did not show a significant influence, while however, a lower platelet count and a higher viral load at baseline rather coincides with an unfavorable outcome. However, a low bilirubin at baseline and the HCV GT1b turned out to significantly correlate with SVR.

### *Safety*

The TVR-based triple therapy has been associated with various and severe adverse events. We tend to differentiate between moderate (treatment not compulsory) and severe (treatment compulsory) side effects that might occur during triple phase (TW 1-12) or during the consecutive dual phase (TW 13-24/48) (Figure 2 and Table 5). The most frequent side effects were changes of the blood count with anemia being the most preponderant, affecting almost all patients. Reduction of the hemoglobin level was observed throughout the whole course of therapy in almost all patients. Therapeutic procedures like blood transfusion and erythropoietin injection were necessary in the majority of cases ($n = 8$) during the triple therapy phase between TW 6 and 12 while only 2 patients needed further erythropoietin injections after TW 13. The RBV dose was reduced in only 2 patients due to renal dysfunction, however, in order not to impair efficiency of therapy RBV dose was not adjusted due to HB changes.

Two patients required a reduction of the PEG-IFN dose due to neutropenia and received G-CSF for neutropenia weekly from TW 2 ongoing. One more patient received G-CFS after TW 12. All patients were treated early with G-CSF when white blood count dropped below 1000/μL. Two patients developed flue-like symptoms and recovered under symptomatic therapy and prophylactic antibiotic treatment. Six patients developed a low platelet count below 50/μL without the need for treatment with platelet growth factors (Table 5).

No serious dermatological adverse events occurred. Seven patients developed a slight rash between TW 4 and 8 which disappeared after a few days and did not require therapeutic intervention. All patients developed anorectal pain between TW 2 and 10, nine of which requiring treatment. Most patients complained about gastrointestinal side effects like diarrhea and loss of appetite. Interestingly, all of these side effects peaked between TW 6 and TW 12 and disappeared upon discontinuation of TVR (Table 5).

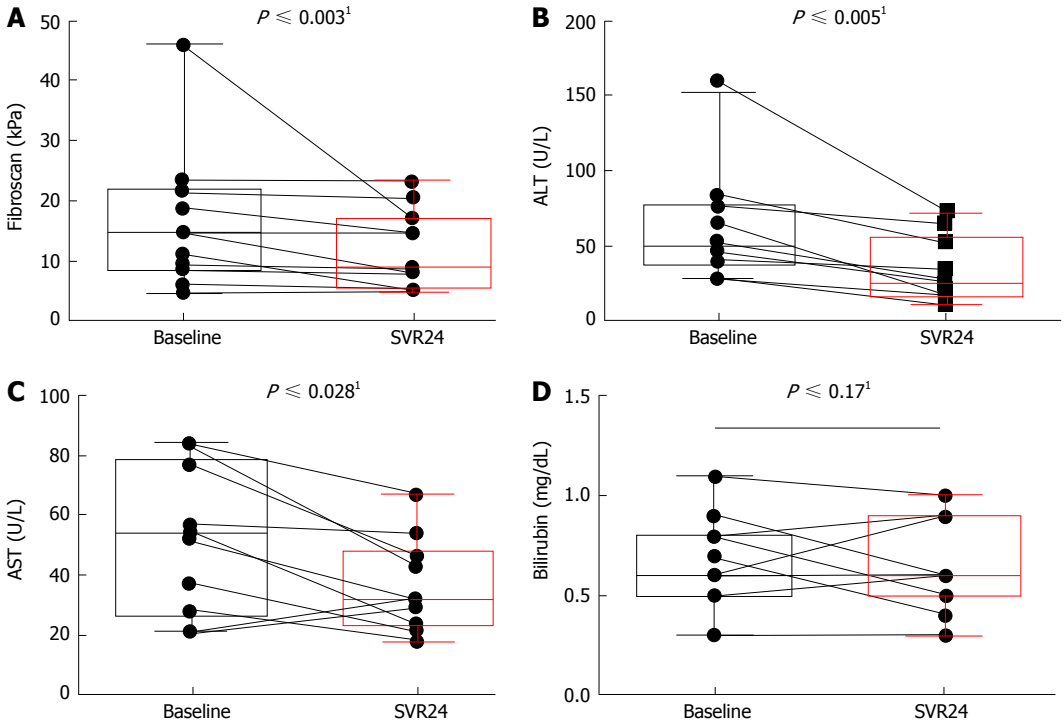


**Figure 1 Development of liver parameters during treatment.** A: Liver stiffness in correlation to fibrosis was determined by fibroscan at baseline and end of treatment. Measurements are calculated in kPa. Student's *t*-test was used to compare categorical characteristics, [a]*P*-value of < 0.01 was considered to be significant; ALT (B), AST (C) and bilirubin (D) were determined at baseline and end of treatment. Student's *t*-test was used to compare categorical characteristics, [a]*P*-value of < 0.01 was considered to be significant. [1]Wilcoxon signed-rank-test, $P \leq 0.05$ set as statistic significant. SVR: Sustained viral response; ALT: Alanine aminotransferases; AST: Aspartate aminotransferase.

**Table 3 NS3 protease domain sequence information**

| HCV GT | Patient | TW | Outcome | Baseline | End of treatment |
|---|---|---|---|---|---|
| 1b | Pat.1 | 24 | SVR | Not detected | NA |
| 1b | Pat.2 | 24 | SVR | Not detected | NA |
| 1a | Pat.3 | 24 | SVR | Not detected | NA |
| 1b | Pat.4 | 24 | SVR | Not detected | NA |
| 1b | Pat.5 | 48 | SVR | Not detected | NA |
| 1b | Pat.6 | 48 | SVR | Not detected | NA |
| 1b | Pat.7 | 48 | SVR | Not detected | NA |
| 1b | Pat.8 | 48 | SVR | Not detected | NA |
| 1b | Pat.9 | 48 | SVR | Not detected | NA |
| 1a | Pat.10 | 48 | SVR | Not detected | NA |
| 1b | Pat.11 | 48 | SVR | Not detected | NA |
| 1a | Pat.12 | 24 | Relapse | Not detected | Not done |
| 1a | Pat.13 | 24 | Non response | Not detected | R155K |
| 1a | Pat.14 | 24 | VB | Not detected | V36M, R155K |
| 1b | Pat.15 | 24 | VB | Not detected | - |
| 1a | Pat.16 | 24 | Relapse | V36L | V36L, R155K |
| 1b | Pat.17 | 16 | VB | V36LV | V36L, T54ST |
| 1a | Pat.18 | 4 | Relapse | Not done | R155K |
| 1b | Pat.19 | 8 | VB | Not detected | A156F |

Resistance mutations before and after treatment in association with outcome and genotype. HCV: Hepatitis C virus; GT: Genotype; TW: Treatment week; VB: Viral breakthrough; SVR: Sustained viral response (undetectable HCV RNA 24 wk after end of antiviral therapy); NA: Not applied.

Two patients had to be hospitalized due to diarrhea and weight loss during the triple phase. Symptoms improved considerably after stop of TVR and the patients could be dismissed at TW 14. One patient had to be hospitalized for an acute flare-up of chronic kidney disease at TW 2 and recovered after rehydration. Half of the patients experienced a reduction of their renal function with a nadir of the GFR between TW 8 and 12 which was stabilized by the intense recommendation to increase fluid intake and daily intravenous fluid application in 3 cases in the outpatient clinic. No severe impairment of renal function could be observed after the triple phase (TW 13-48). Median GFR decreased hardly in patients receiving 24 wk of therapy with - 4 mL/min from baseline to end of treatment, median GFR yet increased in patients receiving 48 wk of therapy by 13 mL/min (Table 5). HB levels decreased in line with deteriorating renal function.

In general, adverse events including moderate and severe adverse events were more frequent during the first 12 wk of therapy. After discontinuation of TVR, adverse events declined substantially to moderate disorders during the second half of the 48 wk course (Figure 2). This pattern encourages us to rather apply TVR instead of BOC in LT patients, as the period of intense monitoring and potential complications due to side effects can be shortened to 12 wk. However, we observe IFN-induced psychiatric side effects and depressive disorders as major problem severely compromising the motivation of the patients. Depressive disorder, weakness and loss of appetite together with weight loss were the most preponderant problems between TW 12 and TW 48, most likely related to IFN.

In our cohort, only one patient had to stop therapy because of deteriorating liver function. None of the

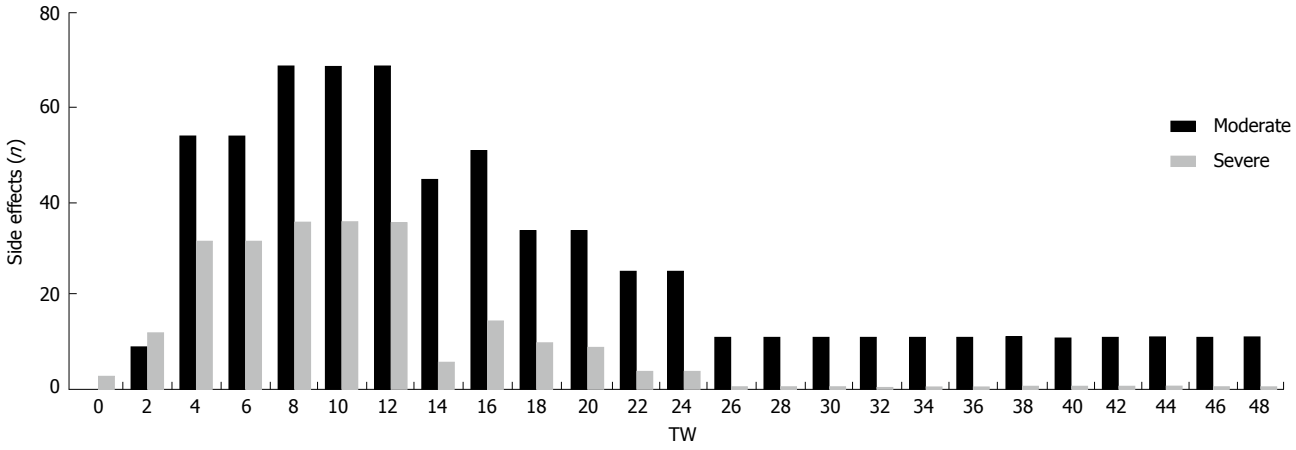


**Figure 2 Safety and adverse events during triple therapy after liver transplantation.** Cumulative analysis of moderate and severe side effects related to the treatment period. TW: Treatment week.

**Table 4 Clinical baseline characteristics and their predictive value for sustained viral response**

| | SVR | | Non SVR | | *P* ≤ |
|---|---|---|---|---|---|
| Patients, *n*/% | 11 | 60% | 8 | 42% | |
| Age, yr | 53 | 41-70 | 58 | 53-64 | 0.115[1] |
| Body mass index (kg/m$^2$) | 26 | 22-30 | 26 | 21-33.1 | 0.505[1] |
| Ischak fibrosis score (grade) | | | | | |
| Ⅰ-Ⅱ | 7 | 64% | 6 | 75% | |
| Ⅲ-Ⅳ | 4 | 36% | 2 | 25% | |
| Fibroscan baseline (kPa) | 14.6 | 4.5-23.4 | 11.3 | 5.9-26 | 0.773[1] |
| Time from LT to triple therapy (mo) | 22 | 7-156 | 23 | 8-295 | 0.869[1] |
| Bilirubin total (mg/dL) | 0.6 | 0.3-1.1 | 1 | 0.4-3.2 | 0.030[1] |
| GPT (ALT) (U/L) | 46 | 21-159 | 40 | 24-85 | 0.563[1] |
| GOT (AST) (U/L) | 52 | 18-84 | 53.5 | 22-88 | 0.620[1] |
| Platelet count (/μL) | 143 | 68-246 | 103.5 | 63-236 | 0.302[1] |
| Viral load ($log_{10}$ IU/mL) | 1.9 | 0.39-14.9 | 4.2 | 0.13-13.70 | 0.409[1] |
| HCV GT | | | | | 0.049[2] |
| 1a, *n*/% | 2 | 18% | 4 | 50% | |
| 1b, *n*/% | 9 | 81% | 4 | 50% | |
| Recipient IL-28b polymorphism, *n*/% | | | | | 0.552[2] |
| CC | 4 | 36% | 1 | 12.50% | |
| CT | 3 | 27% | 6 | 75% | |
| TT | 4 | 36% | 1 | 12.50% | |
| History of any prior PEG-INF/RBV treatment, *n*/% | 7 | 63% | 4 | 50% | 0.552[2] |
| History of post-LT PEG-INF/RBV treatment, *n*/% | 2 | 18% | 3 | 37.50% | 0.345[2] |

[1]Pearson's $\chi^2$ test; [2]Mann-Whitney-*U*-test, $P \leq 0.05$ set as statistic significant. HCV: Hepatitis C virus; PEG-INF: Pegylated-interferon; RBV: Ribavirine; SVR: Sustained viral response; LT: Liver transplantation; GT: Genotype; GPT: Glutamat pyruvat transaminase; GOT: Glutamat oxalacetat transaminase; ALT: Alanine aminotransferases; AST: Aspartate aminotransferase.

patients had to stop therapy because of severe side effects and none died. All patients recovered completely from all side effects after discontinuation of treatment. Trough blood concentrations of IS were kept stable using a special dosing regimen as described[16]. Frequent controls of TBC were performed. Thus, none of the patients experienced acute rejection which had been excluded by graft biopsies in all patients after end of treatment.

## DISCUSSION

We report our single center experience with TVR-based triple therapy in a cohort of 19 LT patients with recurrent HCV reinfection, retrospectively analyzing treatment response, SVR rates, adverse events, resistance mutations before and after treatment and clinical and patients characteristics with potential predictive value for SVR.

We observed a substantial rate of sustained viral response in 11 out of 19 patients (58%) in our cohort of 11 pre-treated and 8 treatment-naïve patients. Of note, all (7/7; 100%) of the patients who completed the full course of 48 wk of treatment and 4/5 (80%) of the patients who completed 24 wk of treatment achieved SVR24. While we observe a SVR rate of 58%, from other multi-centric post-LT cohorts, where SVR rates between 20% and 41% were reported[17,18].

**Table 5 Appearance of adverse events in dependence on the course of therapy *n* (%)**

| | Moderate side effects | | Severe side effects | |
|---|---|---|---|---|
| | TW 1-12 | TW 13-48 | TW 1-12 | TW 13-48 |
| Hematological toxicity | 0 | 0 | 0 | 0 |
| Anemia | ⩽ 10 g/dL | ⩽ 10 g/dL | ⩽ 8 g/dL | ⩽ 8 g/dL |
| | 9 (47.4) | 11 (57.9) | 8 (42.1) | 2 (10.5) |
| Low WBC (< 1/μL) | < 3.4/μL | < 3.4/μL | < 1/μL | < 1/μL |
| | 16 (84.2) | 14 (73.7) | 2 (10.5) | 3 (15.8) |
| Low PT (< 50/μL ) | < 50/μL | < 50/μL | < 20/μL | < 20/μL |
| | 3 (15.7) | 6 (31.6) | 0 | 0 |
| Renal failure | 8 (42.1) | 0 | 1 (5.2) | 0 |
| Dermatological toxicity | 0 | 0 | 0 | 0 |
| Rash std. Ⅰ | 7 (36.8) | 0 | 0 | 0 |
| Rash std. Ⅱ | 0 | 0 | 1 (5.2) | 0 |
| Rash std. Ⅲ | 0 | 0 | 0 | 0 |
| Anorectal pain | 9 (47.4) | 0 | 10 (52.6) | 0 |
| Pruritus | 4 (21.0) | 0 | 4 (21.0) | 0 |
| Stomatitis | 3 (15.7) | 0 | 1 (5.2) | 0 |
| Loss of appetite | 5 (26.3) | 0 | 0 | 0 |
| Loss off weight > 10% | 6 (31.6) | 0 | 1 (5.2) | 0 |
| Diarrhoe | 5 (26.3) | 0 | 1 (5.2) | 0 |
| Weakness | 10 (52.6) | 7 (36.8) | 0 | 0 |
| Hospitalisation | 0 | 0 | 3 (10.5) | 2 (15.8 ) |
| Hepatic decompensation | 0 | 0 | 1 (5.2) | 0 |
| Edema | 0 | 0 | 3 (15.8) | 0 |
| Diabetes melitus | 0 | 0 | 3 (15.8) | 3 (15.8) |
| Psychiatric disorders | 0 | 4 (21.0) | 0 | 1 (5.2) |
| Medical induced fever | 0 | 9 (47.4) | 0 | 0 |
| Infection | 0 | 0 | 0 | 2 (10.5) |

WBC: White blood count; TW: Treatment week; PT: Platelets.

Of 8 patients who were discontinued, only one patient had to discontinue because of deterioration of liver function. Seven patients were discontinued due to non-response or viral break through. None of the patients had to discontinue because of adverse events.

RVR4 is considered as a positive predictive factor for SVR[19]. A significant number of our patients (14/19) displayed RVR with non-detectable viral load at treatment-weeks 4, and all patients with SVR achieved RVR. RVR4 has been reported to be an important predictor of SVR[19] and response-guided therapy is well established in non-LT patients[20,21]. This is reflected in our cohort, as all patient achieving SVR24, had a HCV viral load below LLOQ (12 IU/mL) at TW 4. All patients with a detectable HCV viral load at TW 4 had a virological failure later on. These findings underline the exceptional prognostic impact of a rapid viral response in TVR-based triple therapy through all treatment cohorts. These results might suggest a reduction in length of treatment to 24 wk based on prediction by RVR for TVR-based triple therapy after LT. Four out of five patients who decided to stop therapy at TW 4 achieved SVR. Thus, in case of RVR, shortening TVR-based triple therapy to a 24 wk-course in LT patients may be considered to spare IFN-related side effects for the patient and also to increase cost effectiveness.

Clinical parameters (fibrosis score, AST, ALT) improved significantly in all patients achieving SVR. While liver values and platelet count have been described as independent predictors for SVR, these factors differ not with significance between patients with SVR or without SVR in our cohort. However, in our patients a low bilirubin turns out to be favourable together with HCV GT1b.

Notably, there was a significant difference in SVR rates between GT1a and 1b. Although the cohort size is not sufficient to adequately address this question, this reveals a clear trend towards lower SVR rates in patients infected with GT1a. Here, 2 of 7 patients infected with genotype 1a achieved SVR while 9 of 12 patients infected with GT1b were successfully treated. Importantly, treatment-failure in genotype 1a was associated with selection of R155K in all samples tested. This goes in line with previous reports indicating that the barrier to resistance to TVR is lower in GT1a compared to GT1b[22]. Interestingly, two patients, one infected with GT1a and subsequent relapse and one infected with GT1b and subsequent VB, already carried the resistance-associated substitution V36L/M prior to therapy. Resistance-associated substitutions were undetectable in all patients achieving SVR. Although not conclusive, this may suggest that the pre-existence of the resistance-associated substitutions may have contributed to treatment-failure in those two patients. Resistance testing prior to PI treatment in the setting of LT-patients should be considered and the clinical relevance needs further evaluation.

Interactions with IS were a major concern before the treatment of LT patients with PIs. PIs are potent inhibitors of the CYP3A4 enzyme and numerous drug-drug interactions have been described with CNIs[8]. Based on these reports, patients were monitored daily regarding trough blood levels. In addition, we chose a daily low dose application of TAC in order to avoid major variations in trough levels with nephrotoxic potential and risk of rejection as previously described[10]. In our patients, dosage of IS had to be reduced 30-fold for TAC and 2,5 fold for CyA as reported elsewhere[10,16]. Still, our observations confirm that tight monitoring of CNI trough levels is necessary but can be managed. Even daily dosing and trough levels of CNIs are, in our point of view, a hallmark in order to avoid trough peaks with consecutive toxicity. In combination with intensified oral and intravenous fluid supply, stabilization of renal function is a central factor to avoid RBV accumulation with aggravation of anemia. RBV reduction below a certain level should be avoided regarding the consequence of therapeutic efficiency.

Severe adverse events are a major drawback of TVR-triple therapy, being considerably more pronounced in the post-LT group of patients[23]. The most predominant adverse event was anemia in almost all patients, in line with other post-LT experiences. The abundance of erythrocyte concentrates, which were administered to our patients, illustrates this fact impressively. As the baseline hemoglobin level was a predictor for the probability of developing anemia during telaprevir treatment[24], we excluded patients with a HB < 10 g/dL from treatment. It has been reported,

that those patients with a complicated course post-LT before onset of treatment (FCH, recirrhosis, signs of decompensation) developed the most severe adverse events, most importantly viral or bacterial infections. We therefore did not consider patients with decompensated cirrhosis for treatment.

Taken together, our results confirm that TVR-based triple therapy represents a considerable alternative for LT patients with HCV GT1 reinfection in terms of effectiveness. Moreover, our data suggest that 100% response rate after completing a 48 wk course and 80% after completing a 24 wk course can be achieved. A RVR at TW 4 can be confirmed as positive predictor for SVR, and also a low bilirubin at baseline and GT1b are related to a beneficial course and outcome of TVR-based triple therapy. However, conclusions have to be drawn with cautiousness as the sample size is reduced due to a limited number of patients who is eligible for this treatment, analysis has been performed retrospectively and controls are missing for possible confounding factors.

The high rate of treatment failure associated with emergence of resistance mutations in GT1a suggests that GT1b should be preferably selected for TVR-based triple therapy. We recommend daily low dose application of IS and eager stabilization of renal function in order to maintain RBV doses of not less than 600 mg/die. However, severe adverse events are frequent during therapy, therefore, careful selection of patients eligible for TVR-based triple therapy is of eminent importance. To this end, stable liver function and stable blood count at baseline and intensive patient monitoring are recommended. In the prospect of IFN-free DAA-based treatment regimens associated with less harmful side effects, post-LT treatment of HCV with the first generation PIs should be avoided in patients with signs of decompensation, FCH or instable blood count. In order to achieve a maximum benefit together with the least risk, patients should be screened for alternative IFN-free therapy options in those countries where next generation DAAs are available.

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## COMMENTS

### Background

Chronic hepatitis C (HCV) infection is a serious health burden world-wide. Previous therapeutic options were inefficient and accompanied by serious side effects. The introduction of new direct acting antivirals (DAAs), starting with Telaprevir in 2011, improved the efficiency of treatment significantly. However, first generation protease inhibitors must still be combined with interferon (IFN), serious side effects are still common and thorough monitoring of the patients during therapy is still necessary. In particular, patients who received liver transplant (LT) due to HCV cirrhosis and who experienced reinfection of their graft, do have a high medical need for an effective HCV therapy.

### Research frontiers

In 2014, several IFN-free treatment options for HCV were introduced and approved in several countries. Still, real life data are missing for those applications and tremendous costs of the second generation DAAs makes the access difficult for many countries. Patients who received LT due to HCV cirrhosis are appreciated as a special patient population due to additional difficulties concerning the application of HCV therapeutics, like, *e.g.*, drug-drug interactions with immunosuppressants and the risk of graft rejection.

### Innovations and breakthroughs

The authors introduce their experience with a large cohort of patients with recurrent HCV-GT1 infection after LT, who received Telaprevir-based triple therapy for 48 wk or 24 wk. They display characteristics for each course of treatment and discuss predictors for potential shortening of treatment duration without limitation of efficacy.

### Applications

They quite extensive and elaborate description of procedure and experiences will be of interest for centers who might chose to apply a telaprevir-based triple therapy due to lack or availability of next generation DAAs.

### Terminology

TLV, telaprevir: first generation protease inhibitor with direct antiviral efficacy against HCV; 48 wk course of treatment: standard duration of treatment with telaprevir-based triple therapy; predictors of outcome: to define factors that predict the benefit for the patient if undergoing a given treatment with potential severe side effects.

### Peer-review

The study is considered to be of high interest to the field of hepatology due to the concise and thorough work up of a large data set with the described treatment modality.

## REFERENCES


1 **Thomas DL**. Global control of hepatitis C: where challenge meets opportunity. *Nat Med* 2013; **19**: 850-858 [PMID: 23836235 DOI: 10.1038/nm.3184]

2 **Hajarizadeh B**, Grebely J, Dore GJ. Epidemiology and natural history of HCV infection. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 553-562 [PMID: 23817321 DOI: 10.1038/nrgastro.2013.107]

3 **Terrault N**. Liver transplantation in the setting of chronic HCV. *Best Pract Res Clin Gastroenterol* 2012; **26**: 531-548 [PMID: 23199510 DOI: 10.1016/j.bpg.2012.09.010]

4 **Berenguer M**. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. *J Hepatol* 2008; **49**: 274-287 [PMID: 18571272 DOI: 10.1016/j.jhep.2008.05.002]

5 **Marks KM**, Jacobson IM. The first wave: HCV NS3 protease inhibitors telaprevir and boceprevir. *Antivir Ther* 2012; **17**: 1119-1131 [PMID: 23188750 DOI: 10.3851/IMP2424]

6 **Forestier N**, Zeuzem S. Telaprevir for the treatment of hepatitis C. *Expert Opin Pharmacother* 2012; **13**: 593-606 [PMID: 22332992 DOI: 10.1517/14656566.2012.660524]

7 **Werner CR**, Egetemeyr DP, Nadalin S, Königsrainer A, Malek NP, Lauer UM, Berg CP. Treatment of recurrent genotype 1 hepatitis C post-liver transplantation: single center experience with telaprevir-based triple therapy. *Z Gastroenterol* 2014; **52**: 27-34 [PMID: 24420796 DOI: 10.1055/s-0033-1356345]

8 **Charlton M**. Telaprevir, boceprevir, cytochrome P450 and immuno-suppressive agents--a potentially lethal cocktail. *Hepatology* 2011; **54**: 3-5 [PMID: 21710471 DOI: 10.1002/hep.24470]

9 **Werner CR**, Egetemeyr DP, Lauer UM, Nadalin S, Königsrainer A, Malek NP, Berg CP. Telaprevir-based triple therapy in liver transplant patients with hepatitis C virus: a 12-week pilot study providing safety and efficacy data. *Liver Transpl* 2012; **18**: 1464-1470 [PMID: 22941516 DOI: 10.1002/lt.23542]

10 **Herzer K**, Papadopoulos-Köhn A, Timm J, Paul A, Jochum C, Gerken G. [HCV reinfection after liver transplantation - management and first experiences with telaprevir-based triple therapy]. *Dtsch Med Wochenschr* 2013; **138**: 1759-1764 [PMID: 24002874 DOI: 10.1055/s-0033-1349494]

11 **Forestier N**, Zeuzem S. Triple therapy with telaprevir: results in hepatitis C virus-genotype 1 infected relapsers and non-responders. *Liver Int* 2012; **32** Suppl 1: 44-50 [PMID: 22212571 DOI: 10.1111/

j.1478-3231.2011.02720.x]
12 **Pawlotsky JM**. New hepatitis C therapies: the toolbox, strategies, and challenges. *Gastroenterology* 2014; **146**: 1176-1192 [PMID: 24631495 DOI: 10.1053/j.gastro.2014.03.003]
13 **Feld JJ**. The beginning of the end: what is the future of interferon therapy for chronic hepatitis C? *Antiviral Res* 2014; **105**: 32-38 [PMID: 24548815 DOI: 10.1016/j.antiviral.2014.02.005]
14 **Hill A**, Khoo S, Fortunak J, Simmons B, Ford N. Minimum costs for producing hepatitis C direct-acting antivirals for use in large-scale treatment access programs in developing countries. *Clin Infect Dis* 2014; **58**: 928-936 [PMID: 24399087 DOI: 10.1093/cid/ciu012]
15 **Salvatierra K**, Fareleski S, Forcada A, López-Labrador FX. Hepatitis C virus resistance to new specifically-targeted antiviral therapy: A public health perspective. *World J Virol* 2013; **2**: 6-15 [PMID: 24175225 DOI: 10.5501/wjv.v2.i1.6]
16 **Papadopoulos-Köhn A**, Achterfeld A, Paul A, Canbay A, Timm J, Jochum C, Gerken G, Herzer K. Daily Low-dose Tacrolimus Is a Safe and Effective Immunosuppressive Regimen During Telaprevir-based Triple Therapy for Hepatitis C Virus Recurrence After Liver Transplant. *Transplantation* 2015; **99**: 841-847 [PMID: 25208324 DOI: 10.1097/TP.0000000000000399]
17 **Werner CR**, Franz C, Egetemeyr DP, Janke-Maier P, Malek NP, Lauer UM, Berg CP. Efficacy and safety of telaprevir (TVR) triple therapy in a ‚real-life' cohort of 102 patients with HCV genotype 1: interim analysis after 24 weeks of treatment. *J Viral Hepat* 2014; **21**: 333-340 [PMID: 24716636 DOI: 10.1111/jvh.12145]
18 **Coilly A**, Roche B, Dumortier J, Leroy V, Botta-Fridlund D, Radenne S, Pageaux GP, Si-Ahmed SN, Guillaud O, Antonini TM, Haïm-Boukobza S, Roque-Afonso AM, Samuel D, Duclos-Vallée JC. Safety and efficacy of protease inhibitors to treat hepatitis C after liver transplantation: a multicenter experience. *J Hepatol* 2014; **60**: 78-86 [PMID: 23994384 DOI: 10.1016/j.jhep.2013.08.018]
19 **Fried MW**, Hadziyannis SJ, Shiffman ML, Messinger D, Zeuzem S. Rapid virological response is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection. *J Hepatol* 2011; **55**: 69-75 [PMID: 21145856 DOI: 10.1016/j.jhep.2010.10.032]
20 **Sarrazin C**, Schwendy S, Möller B, Dikopoulos N, Buggisch P, Encke J, Teuber G, Goeser T, Thimme R, Klinker H, Boecher WO, Schulte-Frohlinde E, Prinzing R, Herrmann E, Zeuzem S, Berg T. Improved responses to pegylated interferon alfa-2b and ribavirin by individualizing treatment for 24-72 weeks. *Gastroenterology* 2011; **141**: 1656-1664 [PMID: 21784046 DOI: 10.1053/j.gastro.2011.07.019]
21 **European Association for Study of Liver**. 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]
22 **Wyles DL**, Gutierrez JA. Importance of HCV genotype 1 subtypes for drug resistance and response to therapy. *J Viral Hepat* 2014; **21**: 229-240 [PMID: 24597691 DOI: 10.1111/jvh.12230]
23 **Pungpapong S**, Aqel BA, Koning L, Murphy JL, Henry TM, Ryland KL, Yataco ML, Satyanarayana R, Rosser BG, Vargas HE, Charlton MR, Keaveny AP. Multicenter experience using telaprevir or boceprevir with peginterferon and ribavirin to treat hepatitis C genotype 1 after liver transplantation. *Liver Transpl* 2013; **19**: 690-700 [PMID: 23696372 DOI: 10.1002/lt.23669]
24 **Zeuzem S**, DeMasi R, Baldini A, Coate B, Luo D, Mrus J, Witek J. Risk factors predictive of anemia development during telaprevir plus peginterferon/ribavirin therapy in treatment-experienced patients. *J Hepatol* 2014; **60**: 1112-1117 [PMID: 24486089 DOI: 10.1016/j.jhep.2014.01.013]

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