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Peroxisome proliferator-activated receptors as targets to treat non-alcoholic fatty liver disease

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and microsomal omega-oxidation, being markedly decreased by high-fat (HF) intake. PPAR-beta/delta is crucial to the regulation of forkhead box-containing protein O subfamily-1 expression and, hence, the modulation of enzymes that trigger hepatic gluconeogenesis. In addition, PPAR-beta/delta can activate hepatic stellate cells aiming to the hepatic recovery from chronic insult. On the contrary, PPAR-gamma upregulation by HF diets maximizes NAFLD through the induction of lipogenic factors, which are implicated in the fatty acid synthesis. Excessive dietary sugars also upregulate PPAR-gamma, triggering *de novo* lipogenesis and the consequent lipid droplets deposition within hepatocytes. Targeting PPARs to treat NAFLD seems a fruitful approach as PPAR-alpha agonist elicits expressive decrease in hepatic steatosis by increasing mitochondrial beta-oxidation, besides reduced lipogenesis. PPAR-beta/delta ameliorates hepatic insulin resistance by decreasing hepatic gluconeogenesis at postprandial stage. Total PPAR-gamma activation can exert noxious effects by stimulating hepatic lipogenesis. However, partial PPAR-gamma activation leads to benefits, mainly mediated by increased adiponectin expression and decreased insulin resistance. Further studies are necessary aiming at translational approaches useful to treat NAFLD in humans worldwide by targeting PPARs.

Key words: Peroxisome proliferator-activated receptors; Non-alcoholic fatty liver disease; Obesity; Treatment; Insulin resistance; Beta-oxidation; Lipogenesis

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Abstract

Lately, the world has faced tremendous progress in the understanding of non-alcoholic fatty liver disease (NAFLD) pathogenesis due to rising obesity rates. Peroxisome proliferator-activated receptors (PPARs) are transcription factors that modulate the expression of genes involved in lipid metabolism, energy homeostasis and inflammation, being altered in diet-induced obesity. Experimental evidences show that PPAR-alpha is the master regulator of hepatic beta-oxidation (mitochondrial and peroxisomal)

Core tip: Multiple pathways disrupted in obesity and non-alcoholic fatty liver disease (NAFLD) are regulated by genes encoded by peroxisome proliferator-activated receptors (PPARs). Thus, PPARs emerged as potential targets to alleviate NAFLD. The use of PPAR-alpha agonist yields increased mitochondrial beta-oxidation coupled with reduced lipogenesis. Both of them are essential to tackle insulin resistance and hepatic steatosis. PPAR-

beta/delta agonist is still not available as a medicine, but PPAR-beta/delta agonist elicited expressive reduction in hepatic glucose production in murine models. PPAR-gamma agonist is extensively used, and beneficial effects come from partial activation as total PPAR-gamma activation leads to hepatic lipogenesis, being harmful to the liver.

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INTRODUCTION

The current obesity epidemics have resulted in a significant rise in its comorbidities prevalence^[1]. Liver is often significantly affected by obesity and, hence, the non-alcoholic fatty liver disease (NAFLD) is regarded as the hepatic manifestation of metabolic syndrome, showing rising prevalence regardless of economic status or age worldwide^[2]. Despite being a benign process at first, the continuation of the triggering stimuli can lead to harmful conditions such as non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma^[3].

Excessive energy intake concomitant with sedentism are considered essential underpinnings of lipid droplets accumulation^[4]. When the metabolism faces obesity, excessive adipose tissue fat pads elicit low-grade inflammation, which is linked to insulin resistance development^[5,6]. The resulting hyperinsulinemia yields high lipolysis rate in the white adipose tissue coupled with reduced fatty acid oxidation within the hepatocytes^[7]. The balance between fatty acid input and output in the liver is controlled by integrated enzymes that act in the catalysis of hepatic uptake, lipogenesis, oxidation and exportation of fatty acids^[8]. Whenever the hepatic fatty acid synthesis and/or uptake surpass the liver oxidative and/or the exportation capacity, lipid droplets accumulate within the hepatic parenchyma, configuring NAFLD^[9].

Dietary quality has a paramount importance for the hepatic fatty acid metabolism^[10]. Excessive intake of simple carbohydrate such as fructose and sucrose are implicated in high rates of *de novo* lipogenesis (DNL) in the liver, which is defined as the synthesis of fatty acids from a non-lipid source^[11]. In conjunction with a high intake of dietary fats that generates lipotoxicity through the excessive production of ceramides from palmitate and aggravates insulin resistance, the high DNL due to excessive dietary carbohydrate makes a great demand for hepatic oxidation of fatty acids, which exceeds the oxidative capacity of hepatic peroxisomes, mitochondria and microsomes (endoplasmic reticulum). In turn, hepatic lipid metabolic equilibrium is disrupted due to abnormal fat partitioning within hepatocytes^[11-13].

The carbohydrate and lipid intake, as well as the adipokines and insulin levels, exert considerable influence upon key transcription factors that can modulate hepatic lipid metabolism^[14]. Peroxisome proliferator-activated receptors (PPARs) are at the crossroads of NAFLD pathogenesis, once recent evidences point to the modulation of hepatic beta-oxidation and lipogenesis by different PPAR isoforms^[15,16]. Thus, even though weight management and exercise are the most efficient approach to treating NAFLD, adjunctive pharmacological intervention is utterly indicated. PPARs emerge as a target to treat NAFLD by modulating diverse pathways that are impaired by obesity^[17,18]. The role that total or partial PPAR alpha, beta/delta and gamma activation play in lipogenesis and hepatic oxidation as well as in carbohydrate metabolism through the gluconeogenesis, and DNL are relevant targets to fasten the treatment of NAFLD and obesity.

PPAR PHYSIOLOGY IN EXPERIMENTAL MODELS NAFLD

Hepatic metabolic pathways can be disturbed differently according to the nutrient that is excessive in the diet^[19]. Experimental dietary models of NAFLD are influenced by the dietary scheme duration, diet composition, and animal age, all of which directly affect the spectrum of NAFLD pathogenesis^[20].

When there is excessive dietary intake of lipids, hepatic PPAR-alpha expression decreases significantly parallel to an expressive increase of PPAR-gamma^[16,21]. Obese mice fed during 16 wk a high-fat (HF) diet made up of 60% of energy as lipids, predominantly saturated fatty acids from lard, exhibited overweight, insulin resistance and 34.57% of volume density of hepatic steatosis concomitant to a proinflammatory adipokine profile and activation of hepatic stellate cells (HSCs)^[21]. Hepatic PPAR-alpha expression was substantially reduced^[21], agreeing with a reduced number in the numerical density of hepatic mitochondria^[21,22]. PPAR-alpha is related to mitochondrial beta-oxidation of fatty acids, which has got carnitine palmitoyl transferase-1 (CPT-1) as a pivotal enzyme that allows the fatty acid to go through the inner mitochondrial membrane and reach the mitochondrial matrix to be metabolized^[23]. In the absence of a typical PPAR-alpha expression in the liver, the transcription of its target gene CPT-1 is impaired and excessive fatty acids, which are usually stemmed from lipolysis and delivery to the liver of obese individuals, tend to accumulate in the form of triglycerides^[24,25].

A similar dietary scheme (50% of energy as fat for 12 wk) elicited 2.3 fold increase in liver triglycerides, followed by 0.7 fold decrease in PPAR-alpha and a 0.4 fold increase in PPAR-gamma protein expression in the liver. These observations feature a frame that predisposes to NAFLD because PPAR-gamma is linked to lipogenesis and its target gene expression, SREBP-1c, was 0.5 fold increased in HF fed animals^[16]. SREBP-1c

is implicated in the DNL, induced by high insulin levels. Once activated, SREBP-1c activates others lipogenic genes and leads to the conversion of pyruvate into fatty acids. During this process, there is a great production of malonyl co-A, which inhibits CPT-1 and prevents fatty acids from reaching the mitochondrial matrix to be metabolized through mitochondrial beta-oxidation^[26,27]. Alternative pathways, peroxisomal beta-oxidation, and microsomal omega-oxidation are upregulated to try to compensate insufficient mitochondrial oxidative activity. Mitochondrial damage found in NAFLD and increased peroxisomal and microsomal oxidation of fatty acids leads to oxidative stress and the consequent progression to NASH if an adequate intervention is not implemented^[28,29]. The pivotal role that oxidative stress plays in NAFLD progression to NASH was verified through positive immunoreactions for oxidized phosphatidylcholine close to activated stellate cells and in apoptotic hepatocytes in samples of human liver autopsy. Moreover, immunostaining intensity correlated positively with the degree of steatosis^[30].

When the HF diet (49% of energy as lipids) was administered to dams during 8 wk prior to gestation, gestation, and lactation, similar hepatic alterations were detected in the offspring. Pups from HF dams had overweight and glucose intolerance at 3 mo of age, both of which agree with the 1.4 fold increase in hepatic steatosis rate in these animals. PPAR-alpha gene and protein expression were reduced in the liver of offspring of HF dams parallel to increased gene and protein expression of PPAR-gamma. As a result, the hepatic expression of the PPAR-alpha target gene *CPT-1* was decreased, because the expression of the PPAR gamma target genes *SREBP-1c* was elevated^[31]. This pattern of gene and protein expression explains the early NAFLD onset in the offspring of obese dams^[32], albeit with a discrete overweight. Besides the compromised mitochondrial beta-oxidation through the reduced CPT-1 activity and the enhanced DNL, favored by over-expression of SREBP-1c, the offspring presented with expressive reduction in fatty acid translocase (FAT)/CD36 expression, which limits long chain fatty acids (more than 20 carbons) oxidation. FAT/CD36 can shorten the fatty acid chain in order to allow CPT-1 to catalyze its transport to the mitochondrial matrix^[33]. This represents an additive failure in hepatic lipid metabolism due to maternal obesity.

Additionally, hepatic insulin resistance was detected in obese dams offspring through enhanced hepatic expression of glucose 6 phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK)^[31]. Both enzymes are crucial to hepatic gluconeogenesis and are activated by glucagon during the fasting period and inhibited by insulin at the postprandial stage. When insulin resistance occurs, insulin, at high levels, loses its capacity to inhibit gluconeogenesis by the downregulating G6Pase and PEPCK expression^[34,35]. Hence, high hepatic glucose production aggravates insulin resistance. PPAR-beta/delta induces forkhead box-containing protein O

subfamily-1 (FOXO-1), which can regulate G6Pase and PEPCK expression, and might be a viable approach to restoring this pathway^[36]. In addition, PPAR-beta/delta is described to induce HSCs proliferation in fibrogenesis activation *in vitro* and *in vivo*. HSCs constitutively express PPAR-beta/delta and their activation aim at the hepatic recovery from chronic insult. However, the collagen synthesis by HSCs leads to hepatic fibrosis in the long run, being involved in the progression of simple NAFLD to more dangerous types of liver diseases^[37,38].

Excessive intake of sucrose (32% of energy as sucrose) yielded similar effects to liver histology and PPAR expression in C57BL/6 mice. Although animals did not become obese, excessive sucrose supply, elicited 1.3-fold increase in hepatic steatosis coupled with 0.5 fold decrease in PPAR-alpha expression and 0.8 increase in SREBP-1c expression^[19]. Likewise, the intake of 34% of energy as fructose impaired hepatic cytoarchitecture and lipid metabolism in the same mouse model. Even in the absence of significant overweight, mice fed a high-fructose diet presented nearly 55% volume density of hepatic steatosis, which can be accounted for by augmented hepatic expression of PPAR-gamma (0.5 fold) and reduced hepatic expression of PPAR-alpha (-0.25 fold)^[39]. PPAR-gamma increases the transcription of SREBP-1c, leading to higher lipogenesis, and little PPAR-alpha expression favors high hepatic glucose production. There is the interplay between white adipose tissue and liver as a glucose/glycerol cycle that guarantees energy transport (as glucose) from liver to peripheral tissues^[40]. This process relies on PPAR-alpha, being impaired in the fructose-fed animals^[39,40]. In addition, high fructose intake augmented the hepatic expression of PEPCK and GLUT2^[39], indicating high hepatic glucose output, which aggravates insulin resistance and favors the NAFLD progression to NASH^[41].

TARGETING PPARS TO TREAT NAFLD

PPARs encompass a subfamily of a superfamily of nuclear receptors. There are three different isoforms: PPAR-alpha, PPAR-beta/delta, and PPAR-gamma, which are differently expressed in various tissues. They are ligand-dependent transcription factors that regulate the expression of their target genes through specific binding to peroxisome proliferation response elements (PPERs). Each isoform heterodimerize with its retinoid X receptor alpha, beta/delta or gamma and binds to its respective PPARE, forming a structure able to recognize specific DNA sequences (AGGTCA) to activate the transcription of its target genes^[42-44].

Briefly, PPAR-alpha is closely linked to the transcription of genes related to hepatic beta-oxidation, such as CPT-1 and is highly expressed in the liver^[45,46]. Thus, treatment with PPAR-alpha agonist usually yields body mass loss as this isoform is implicated in lipid metabolism pathways^[43]. PPAR-beta/delta is ubiquitously expressed and is crucial to beta-oxidation in skeletal muscle, not in the liver. In the liver, anti-inflammatory

properties by the activation of macrophages and the protection against lipotoxicity are reported. The induction of Stearoyl-CoA desaturase 1 by PPAR-beta/delta activation promotes monounsaturated fatty acids formation instead of saturated fatty acids, decreasing oxidative stress, emerging as a promising approach to the tackle insulin resistance^[47,48]. PPAR-gamma is expressed at low concentrations in the liver (9%-12% of the expression in the white adipose tissue), being related to adipogenesis and insulin-sensitizing effects through the diversion of fatty acids to adipose tissue storage. Patients with NAFLD exhibit abnormal high expression of PPAR-gamma in the liver, which coincides with overexpression of SREBP-1c and the consequent hepatic lipogenesis^[49,50].

Taking into account the above-mentioned PPAR related effects, the use of PPAR agonist to treat NAFLD seems to be a viable strategy. In this regard, the activation of PPAR-alpha by fenofibrate markedly ameliorated the hepatic insulin resistance by the upregulation of enzymes involved with beta-oxidation in fructose-fed mice and the expressive reduction of DNL, albeit with high endoplasmic reticulum stress^[51]. In addition, fenofibrate significantly ameliorated microcirculatory perfusion in a HF mice mouse model of NAFLD, besides the upregulation of genes involved in hepatic lipid oxidation^[52]. These reported effects comply with a significant decrease in hepatic steatosis percentage after the activation of PPAR-alpha by fenofibrate^[51,52]. Activation of PPAR-alpha by fish oil, a nutraceutical, yielded alleviation of hepatic insulin resistance through low G6Pase and PEPCK expression in the liver and reduced steatosis by upregulation of mitochondrial beta-oxidation (high CPT-1 expression) concomitant to reduced lipogenesis (low fatty acid synthase expression)^[53].

In humans, the evaluation of fenofibrate use to treat NAFLD is difficult as it is usually taken with others drugs. It seems that insulin-sensitizing action of fenofibrate is more important to counter hepatic steatosis than its lipid-lowering property. A recent study showed a significant decrease in hepatic transaminases coupled with a marked decrease of hepatocellular ballooning in humans^[54,55].

As far as mice models of NASH are concerned, APOE2 mice fed a western diet showed decreased hepatic macrophage accumulation, which precedes lipid accumulation within hepatocyte, and expressive reduction of lipotoxicity after treatment with fenofibrate. A marked reduction in the expression of proinflammatory genes, great expression of genes implicated in β -oxidation and the suppression of procollagen type 1 expression underlie these findings^[56,57].

Total PPAR-gamma activation by rosiglitazone counters insulin resistance, but do not manage to reduce NAFLD in HF mouse models. It can be argued that full activation of PPAR-gamma favors the transcription of lipogenic transcription factors, such as SREBP-1c, and even though animals benefit from anti-inflammatory effects of high adiponectin levels, the upregulation of lipogenesis

results in obesity, increased hepatic triglycerides and the maintenance of NAFLD^[16,58]. In contrast, mice with NASH benefit from the use of rosiglitazone as it inhibited cell proliferation and diminished collagen expression in hepatic stellate cells *in vivo* and *in vitro*^[59,60]. In addition, the increase in insulin sensitivity due to enhanced adiponectin transcription and reduced levels of tumor necrosis factor (TNF)-alpha is crucial to the treatment of NASH with rosiglitazone in murine models^[61,62].

Humans with NASH also benefit from the regular use of rosiglitazone. The randomized placebo-controlled Fatty Liver Improvement With Rosiglitazone Therapy (FLIRT) trial revealed that 47% of the patients with histologically proved NASH had marked reduction (> 30%) in steatosis score after one year of treatment. Moreover, 38% of the patients achieved normalization of alanine aminotransferase (ALT) values, albeit with no significant improvement of NASH histological features such as hepatocyte ballooning, fibrosis and lobular inflammation/necrosis. In agreement with experimental background, rosiglitazone significantly increased insulin sensitivity and adiponectin levels and the former was correlated with the reduction in the percentage of steatosis in patients of FLIRT trial^[63].

When the same set of patients were revisited two years later (FLIRT 2 extension trial), the long-term efficacy of rosiglitazone was attested by the maintenance of reduced ALT levels and reduced HOMA-IR and insulin levels. However, once again, no beneficial effect on liver histology was perceived. It can be argued that even though rosiglitazone exhibited an antisteatogenic effect during the first year and normalized insulin sensitivity and ALT levels, these effects were not enough to tackle NASH features and additional treatments are encouraged^[64]. In agreement to the FLIRT trial, the Pioglitazone vs Vitamin E vs Placebo for the Treatment of Nondiabetic Patients with nonalcoholic steatohepatitis trial showed that vitamin E and pioglitazone were able to reduce ALT levels, increase insulin sensitivity, decrease hepatic steatosis and ameliorate lobular inflammation, but without significant improvement in hepatic fibrosis and hepatocyte ballooning. The significant weight gain after pioglitazone treatment was an adverse effect^[65].

Recently, the partial activation of PPAR-gamma coupled with the selective activation of PPAR-alpha in the liver by telmisartan (also an AT1 receptor blocker) elicited positive effects to hepatic cytoarchitecture and ultrastructure in mice fed a HF diet^[21,66]. Animals showed normal volume density of hepatic steatosis when compared to the untreated group, followed by reduced SREBP-1c expression and insulinemia parallel to greater mitochondrial numerical density revealed by transmission electron microscopy^[21]. The same drug was also able to counter steatohepatitis in a murine model through the suppression of macrophage infiltration within hepatocytes, induction of high adiponectin levels and reduction of adipocyte size^[66]. In humans, telmisartan showed beneficial effects when compared to losartan (pure AT1 receptor blocker) in the management of

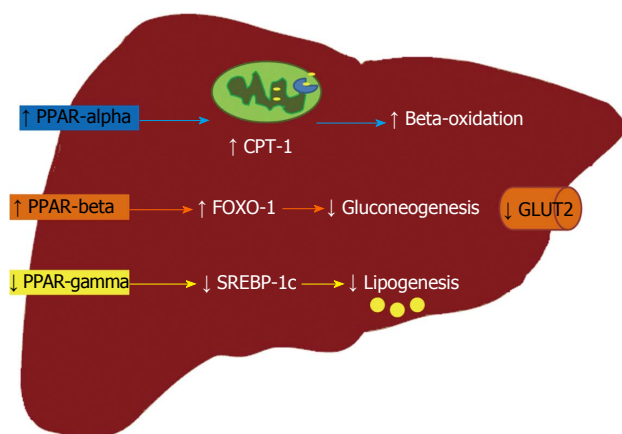


Figure 1 Effects of each peroxisome proliferator-activated receptor isoform in the treatment of non-alcoholic fatty liver disease. PPAR-alpha activation leads to the transcription of CPT-1, a target gene that is crucial to beta-oxidation as it allows the fatty acid to reach the mitochondrial matrix; PPAR-beta/delta activation is involved with FOXO-1 transcription, which reduces the hepatic expression of enzymes involved in gluconeogenesis. Thus, GLUT2 and hepatic glucose production are also significantly reduced; conversely, the partial activation of PPAR-gamma or, even, its reduced expression is linked to diminished lipogenesis. All these events are efficient to tackle NAFLD. PPAR: Peroxisome proliferator-activated receptor; CPT-1: Carnitine palmitoyl transferase-1; FOXO-1: Forkhead box-containing protein O subfamily-1; GLUT2: Glucose transporter 2; SREBP-1c: Sterol regulatory element-binding protein-1c.

NAFLD, highlighting the importance of PPAR activation to treat NAFLD. Telmisartan also yields decreased expression of Nuclear factor κ B target genes, such as TNF-alpha and interleukin-6 in diet-induced obese mice, which coupled with increased adiponectin prevent these animals from NASH onset^[21,67]. In resemblance with telmisartan, ragaglitazar, a dual PPAR-alpha/PPAR-gamma agonist, tackled hepatic insulin resistance, hepatic steatosis and overweight in a mouse model of metabolic syndrome, whereas total PPAR-gamma agonist rosiglitazone elicited visceral adiposity and hepatomegaly^[68].

Pan-PPAR activation by bezafibrate triggered beneficial effects in offspring from obese dams derived from PPAR-alpha activation (increased CPT-1 expression in the liver); PPAR-beta/delta activation (reduced gluconeogenesis due to low hepatic G6Pase and PEPCK expression caused by downregulation of FOXO-1 gene); and PPAR-gamma activation (high FAT/CD36 liver expression, causing greater hepatic lipid oxidation in conjunction with the high CPT-1 expression)^[31]. Bezafibrate and GW501516, a PPAR-delta agonist, inhibited NASH development in mice fed a methionine choline-deficient diet. Both treatments elicited greater expression of genes related to beta-oxidation and lipid transportation in hepatocytes concomitant with reduced levels of genes linked to inflammation^[69]. An overview of the effects of PPAR activation upon the pathways involved with NAFLD pathogenesis is shown in Figure 1.

PPAR transcriptional activity can be influenced by several kinases as they are phosphoproteins^[70]. Mitogen-activated protein kinase (MAPK) activation leads to phosphorylation of PPAR-alpha and PPAR-gamma isoforms. Phosphorylated PPAR-alpha exhibits

higher transcription activity, whereas PPAR-gamma shows reduced transcriptional potential after phosphorylation. This knowledge is utterly important when it comes to the attempt to obtain new drugs with huge effectiveness. PPAR-alpha and gamma activate MAPK, while MAPK activation leads to PPAR-alpha and gamma phosphorylation and modulation, configuring an interplay between these two pathways. So, it can be argued that PPAR-alpha beneficial effects are maximized by the interplay with MAPK, which result in favored beta-oxidation in the liver^[71]. On the other hand, the reduced transcriptional potential of PPAR-gamma due to MAPK activation might also be beneficial provided that insulin-sensitizing effects of PPAR-gamma are more expressive when there is partial activation of this isoform^[72].

CONCLUSION

It is widely understood that PPARs are critically involved in the regulation of hepatic beta-oxidation and lipogenesis pathways, besides influencing hepatic carbohydrate metabolism. These observations prompted the attempt to treat NAFLD by targeting PPARs. Targeting PPAR-alpha has been proved a promising therapeutic approach to control NAFLD through the upregulation of *beta*-oxidation genes and the inhibition of DNL and gluconeogenesis enzymes. On the other hand, total PPAR-gamma activation shows deleterious effects upon liver histology and physiology based on the increased hepatic lipogenesis. However, partial PPAR-gamma activation as well as dual or pan-PPAR activation shows beneficial effects upon liver structure and functioning. In this way, PPAR modulation by partial activation or selective activation is a promising field of study as it possibilities the reduction of side effects that may stem from total agonism of the receptor. In addition, the role that PPAR-beta/delta has upon liver metabolism remains to be completely unraveled as there is not a PPAR-beta/delta agonist available to the population. Evidences related to this isoform come from experimental studies using selective agonists that are not commercialized or pan-PPAR agonist, which challenges the identification of each isoform properties. Further studies are necessary aiming at translational approaches useful to treat this prevalent metabolic disease in humans worldwide by targeting PPARs.

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Hepatocellular carcinoma: From diagnosis to treatment

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Surveillance with ultrasonography detects early stage disease and improves survival rates. Many treatment options exist for individuals with HCC and are determined by stage of presentation. Liver transplantation is offered to patients who are within the Milan criteria and are not candidates for hepatic resection. In patients with advanced stage disease, sorafenib shows some survival benefit.

Key words: Hepatocellular carcinoma; Hepatitis C virus; Liver transplantation; Tumor ablation; Sorafenib

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Core tip: Hepatocellular carcinoma (HCC) is a rising cause of cancer related mortality and viral causes of cirrhosis appear to be a major cause. Surveillance helps to detect early stage disease and treatment options are determined by stage of presentation. Three potentially curative options are radiofrequency ablation, liver transplantation and tumor resection. Emerging therapies such as drug-eluting beads-transarterial chemoembolization or sorafenib will continue to advance treatment options in HCC. The following will provide a concise review of HCC from prevention to treatment.

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Abstract

Hepatocellular carcinoma (HCC) is the sixth most prevalent malignancy worldwide and is a rising cause of cancer related mortality. Risk factors for HCC are well documented and effective surveillance and early diagnosis allow for curative therapies. The majority of HCC appears to be caused by cirrhosis from chronic hepatitis B and hepatitis C virus. Preventive strategies include vaccination programs and anti-viral treatments.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and is the leading cause of mortality in patients with cirrhosis^[1]. An estimated half million new cases are diagnosed each year world-wide with disease burden highest in developing countries (85% of all cases)^[2,3]. The average age of diagnosis is 65 years with

a shift in the last decade toward diagnosis at an earlier age^[4]. This trend is especially seen in developing countries and has implications for treatment. Rates of HCC are two to four times higher in men compared to women^[5]. Over the past 20 years there has been a 3 fold increase in the number of new HCC cases in the United States (estimated 33190 in 2014)^[2,6,7]. The rising incidence of HCC in Western countries appears to correlate with the increasing prevalence of hepatitis C virus (HCV). Currently, the incidence of HCC continues to rise and the 5 year survival rate remains low^[7]. Monotherapy agents targeting HCV have made curative therapy in chronic infection possible and may eventually translate into lower rates of HCC. One may presume that despite the high cost of the monotherapy agents, there will be a profound impact on the downstream costs and related complications from chronic HCV and HCC.

Risk factors for HCC are well documented and effective surveillance with early diagnosis allows for curative measures.

RISK FACTORS

Cirrhosis is the most important risk factor for developing HCC and is present in 80% to 90% of individuals^[8]. The annual incidence of liver cancer in patients with cirrhosis is 1% to 6 %^[8]. Although there exists wide regional variations in distribution and etiology of HCC, chronic hepatitis B virus (HBV) and HCV infection represent the majority of HCC cases worldwide^[9]. The highest incidence of HBV is in eastern Asia and sub-Saharan Africa where it accounts for the majority of cases (greater than 50%)^[10]. Viral load, duration of infection and rate of replication are related to the incidence of HCC^[11,12]. Further, a risk association between HBV and HCC is present in endemic areas where the pattern of transmission is from mother to newborn. Several mechanisms for HBV progression to HCC are proposed. Viral integration into liver cells may cause chromosomal instability and alteration of normal cellular replication resulting in HCC^[13,14]. Further, inflammatory and/or necrotic changes from HBV may alter hepatocyte genetic expression or directly induce malignancy^[15].

On the other hand, HCC cases in North America, Europe and Japan are highest among HCV infected patients. Annual incidence of HCC is 1% to 4% in patients with HCV related cirrhosis^[16,17]. Compared to HCV negative patients, individuals with chronic HCV infection have a 17 times higher risk of developing HCC. In the United States, it is estimated that the incidence of HCV will continue to rise in the following decades^[18,19]. It is hypothesized that the primary mechanism for HCC in HCV patients is inflammatory hepatocyte damage from oxidative stress, promoting cirrhosis^[20].

Alcohol related liver disease and non-alcoholic fatty liver disease increase the risk of HCC alone or in combination with HBV/HCV. Further, obesity and diabetes are independent risk factors for the development of HCC^[21-23]. In patients with chronic viral hepatitis, obesity

may synergistically increase the risk of HCC by 100 fold^[24]. It has also been elicited that patients with a higher BMI often have a higher rate of mortality^[25]. In addition, the number of metabolic syndrome components in a given patient appears to correlate with an increased risk of HCC^[26]. As rates of patients diagnosed with metabolic syndrome rise around the world, even a small contribution to the development of HCC would have a devastating impact.

Finally, a number of less common risk factors for HCC include hereditary hemochromatosis, autoimmune hepatitis, glycogen storage diseases, primary biliary cirrhosis, alpha₁-antitrypsin deficiency, and Wilson's disease.

PREVENTION

Studies for preventive strategies have centered on viral causes of HCC and minimal data exists on risk reduction for other etiologies. Although vaccination and anti-viral treatment remain the primary means of prevention, counseling patients on dietary modifications, weight loss and tobacco/alcohol cessation remain important steps to address.

The HBV vaccine is effective at preventing HCC and vaccination programs have lowered rates of related malignancy^[27]. Over a 10-year period, the Taiwan universal vaccination program reduced the annual incidence of HCC from 0.70 to 0.36 per 100000 children. Thus, one would suspect that initiation of universal vaccination programs in children would have an overall reduction in HCC disease burden in adults. For adults with chronic HBV infection, vaccinations have no role in preventing HCC. Rather, one must focus on anti-viral treatment. Treatment with interferon alpha (IFN- α) reduced the risk of HCC by 6.4% in a meta-analysis of seven studies^[28]. Further analysis revealed that the protective effects of IFN- α were limited to patients with cirrhosis^[29]. Other treatment options include nucleoside/nucleotide analog treatments and most published data is on lamivudine or adefovir. Treatment with these agents appear to effectively suppress viral replication and decrease the risk of developing HCC^[30-32].

Antiviral treatment for HCV may also reduce the risk of HCC. In several studies, treatment by IFN with sustained viral response correlated with a decreased risk of HCC compared to non-responders or no treatment^[33,34]. Newer treatment options for HCV with improved viral response rates may effectively reduce progression to HCC.

SURVEILLANCE

Practice guidelines recommend standardized surveillance programs for HCC with decision analysis models showing that surveillance improves survival and is cost effective if the annual rate of HCC exceeds 1.5% in a given population^[35,36]. Diagnosis at an early stage of HCC confers a survival benefit compared to patients diagnosed with advanced disease^[37]. Curative treatment

options such as liver transplantation available in early stage disease likely contribute to this survival benefit.

Hepatic ultrasound and alpha-fetoprotein (AFP) have historically played a prominent role in HCC surveillance. A randomized controlled trial of 18861 participants assessed the effect of screening on HCC mortality. All study participants had HBV and were divided into 2 groups: patients who underwent screening with ultrasound every 6 mo and AFP compared with no surveillance. Surveillance was associated with a 37% reduction in HCC mortality, despite sub-optimal adherence to surveillance (< 60%)^[38].

For over 40 years AFP has been used in the detection of HCC with variable sensitivity (39% to 65%), specificity (76% to 94%) and positive predictive value (9% to 50%)^[39-43]. Results from several studies have challenged the utility of AFP in screening. A randomized controlled trial of 5581 HBV patients showed that AFP bi-annual screening improved detection rates of HCC but earlier detection did not translate to decreased mortality^[44]. Concurrent AFP and ultrasound testing increased false positive rates and led to unnecessary diagnostic testing. Further, data suggest that for lesions less than 2 cm in diameter, AFP will rarely be elevated^[41,45,46]. An inherent disadvantage of AFP is that it can be elevated in chronic hepatitis even without HCC, resulting in low specificity. Current AASLD guidelines do not recommend AFP for screening or diagnostic purposes. Research into novel biomarkers for early HCC detection continue. As more sensitive assays such as AFP-L3 are developed, the role of serology for surveillance maybe re-analyzed^[47].

The ideal modality for HCC screening remains an area of controversy. Although the recommended method of surveillance is liver ultrasonography, diagnosis by this modality remains operator and equipment dependent (sensitivity of 65% and specificity of 90%)^[45]. Older studies have shown ultrasonography to be equivalent to computed tomography (CT) in detecting hepatic lesions^[48,49]. But more recently, research into CT and magnetic resonance imaging (MRI) for HCC screening have yielded promising results in lesions greater than 2 cm^[50]. Prospective trials are needed before CT or MRI can replace ultrasonography as the primary screening method for HCC. Specifically cost effectiveness, cumulative radiation exposure and mortality benefit will need to be addressed.

The 6 mo interval length for screening is based on tumor doubling time and is not dictated by risk factors for HCC. A shorter 3 mo interval increased small nodule detection without affecting survival rates^[51], while longer periods between screening (12 mo) showed an increased rate of advanced tumors^[52]. Once a lesion has been detected, the size of the lesion determines the next step. Hepatic nodules less than 1 cm should be followed with repeat ultrasonography every 3 mo. If the lesion is stable over 2 years then a return to routine 6 mo surveillance is acceptable^[53]. Liver lesions exceeding 1 cm warrant further evaluation as described below.

DIAGNOSIS

Definitive diagnosis *via* non-invasive testing includes four-phase multidetector CT (unenhanced, arterial, venous and delayed) or dynamic contrast enhanced MRI. The presence of arterial hyper-enhancement with a venous or delayed phase washout of contrast medium, confirms a diagnosis of HCC^[35]. While MRI provides superior contrast resolution compared to CT, metallic implants, respiratory artifact, significant ascites, cost and availability all limit its use. Patients with atypical features for HCC either on CT or MRI should undergo the other imaging modality or lesion biopsy. Individuals with discordant CT/MRI findings or hepatic lesions without cirrhosis should also receive a liver biopsy. The imaging modalities above are valid for patients with cirrhosis or chronic HBV without cirrhosis. Contrast enhanced ultrasonography should not be used for diagnostic purposes as it lacks specificity for HCC^[16]. Unfortunately, biopsies also carry a high false negative rate (up to 30%) - attributed to inadequate sampling^[54]. Despite a negative biopsy, surveillance of the lesion at 3 to 6 mo intervals for changes characteristic for HCC or for lesion enlargement should be completed^[16]. Lesions less than 1 cm are difficult to assess even with the combination of imaging and biopsy (Figure 1).

TREATMENT

Several treatment options exist for patients with HCC and can be categorized as curative or palliative. The three potentially curative options are radiofrequency ablation, liver transplantation, or tumor resection. Given the heterogeneity of HCC and complexity of treatment options patients are optimally managed by a multi-disciplinary team. The best therapy is determined based on the stage of presentation. The barcelona clinic liver cancer staging system, developed in 1999, is a common means to assess prognosis and select appropriate therapy for HCC^[55]. In general, surgical resection or liver transplantation is the first line treatment option for early stage HCC; whereas asymptomatic patients with intermediate stage disease benefit from chemoembolization. Patients with end stage HCC or extensive extrahepatic disease often have a less than 3 mo rate of survival. In these individuals, pain and symptom control to improve quality of life should be the primary focus^[35].

Other staging systems such as Cancer of Liver Italian Program, Okuda stage, French staging system have been validated to a lesser extent. Biomarkers such as vascular endothelial growth factors may have prognostic value in the future^[56].

Resection

Surgical resection is the therapy of choice in early stage HCC without cirrhosis or in the absence of portal hypertension. Selection criteria have been refined

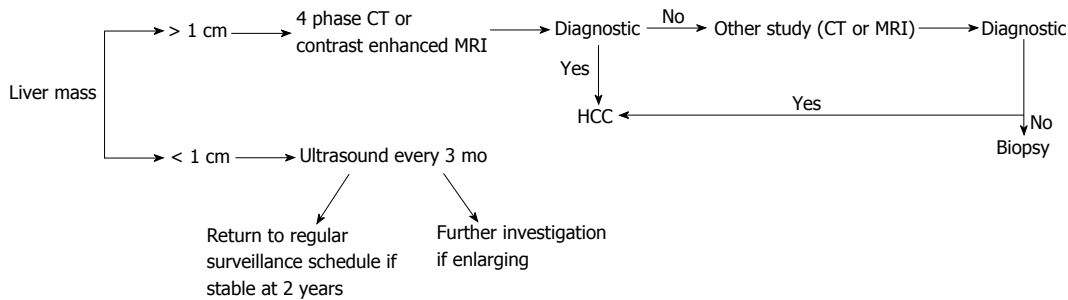


Figure 1 Diagnostic algorithm for hepatocellular carcinoma. Reproduced from Bruix J. *Hepatology*. 2011. CT: Computed tomography; MRI: Magnetic resonance imaging; HCC: Hepatocellular carcinoma.

over the years and include individuals with a tumor size less than 3 cm in diameter, normal bilirubin and absence of portal hypertension. In patients without cirrhosis, a 60% to 75% five year survival rate can be achieved^[57,58]. Hepatic function evaluated by Model for End Stage Liver Disease (MELD) or Child-Pugh correlate with survival following resection. As expected, patients in Child-Pugh A classification have an improved survival rate following resection compared with those in class B or C^[59,60]. In the United States only 5% of individuals will qualify for resection, while in Asia younger age of presentation allows 40% of patients to qualify for surgical resection^[61]. Laproscopic liver resection accounts for 10%-20% of procedures in the United States and minimize postoperative morbidity compared to open resection. Patients with multiple intra-hepatic tumors are not ideal candidates for resection as this often represents intrahepatic metastasis^[62]. Although technically feasible in some patients, multiple hepatic lesion resection must be reviewed on a case by case basis^[63,64]. Further, vascular invasion significantly reduces the five year survival rate from around 50% to 10%^[65]. Individuals with a MELD score greater than 9 have a high mortality rate after resection and alternative therapies should be considered^[66].

Unfortunately, hepatic resection does not alter the course of underlying cirrhosis. At 2 years, 43% to 65% of patients will have a recurrent tumor and by 5 years post-resection 70% will have recurrent HCC^[67,68]. Pre-operative predictors of recurrent free survival include: Child-Pugh class, hepatic function, degree of fibrosis, total serum bilirubin, platelet count, portal hypertension, micro/macrosopic vascular invasion and tumor burden (number and size)^[69]. A case by case selection for patients with cirrhosis is essential to limit complications and mortality. Operative mortality ranges from 4% to 4.7% for resection with the majority of deaths likely in patients with underlying cirrhosis and large tumor burden^[70]. As newer treatment options for HCV are developed, treatment of underlying cirrhosis after resection may alter/delay the development of recurrent HCC.

Liver transplantation

Liver transplantation offers a potential cure of HCC as

it treats the malignancy and the underlying cirrhosis. Given the scarcity of livers available for transplantation, one must carefully select patients to optimize outcomes.

Patients with HCC complicated by cirrhosis and/or portal hypertension should be evaluated for liver transplantation as it carries the lowest rate of tumor recurrence. Traditionally 3 scoring criteria are utilized to determine eligibility [Milan Criteria, University of California San Francisco (UCSF)] and prioritize patients for transplant MELD. The Milan Criteria considers patients eligible for liver transplantation if they present with a single nodule less than 5 cm in diameter or 3 nodules with each less than 3 cm, without evidence of distant metastasis or vascular invasion. With the initial trial showing a 4 year survival rate of 75% and results verified in further studies, organ allocation societies including united network for organ sharing have adopted this criteria^[71-73]. Recurrent free survival for patients meeting Milan criteria is 90% with a 4 year overall survival rate of 85%^[71]. In contrast patients exceeding criteria parameters have a respective 59% and 50% rate of survival^[71]. The UCSF criteria proposed in 2001 expands the eligibility requirements set forth by the Milan criteria to include more patients with HCC. This criteria included individuals with a single tumor less than 6.5 cm or those with 3 nodules less than 4.5 cm (total diameter of no more than 8 cm). Experience with the UCSF criteria has shown similar survival rates compared to the Milan criteria^[74,75]. Unfortunately, the paucity of organs available for transplant remains a major obstacle.

Liver allocation is prioritized by the MELD score. All HCC patients have an adjusted MELD score of 22 with increases at each 3 mo interval. Prioritized allocation with MELD score adjustment has increased the number of HCC patients undergoing liver transplantation.

Tumor ablation

Chemical (ethanol, acetic acid) or thermal ablation [radiofrequency ablation (RFA), microwave, laser, cryoablation] are also used to treat HCC. Historically, percutaneous ethanol injection (PEI) had been used to induce cellular dehydration/necrosis in small HCC tumors. RFA has largely replaced PEI as studies have shown higher rates of complete response with fewer number of treatment sessions^[76-78]. RFA is superior to

PEI in large and small lesions, although the benefit of using RFA is more pronounced in tumors larger than 2 cm in diameter^[79]. Combination RFA and PEI for high risk lesions is an area of ongoing research with promising results^[80].

Radiofrequency ablation

In cases of early stage HCC where surgical resection or liver transplantation are not feasible, RFA is a minimally invasive approach to local ablation. Therapeutic effects are a result of thermal tumor necrosis, parenchymal and protein destruction^[81]. Overall complication rates for RFA are low and are minimized when performed by an experienced physician^[82]. Efficacy of RFA is limited by tumor size and location, with a less than fifty percent rate of ablation in tumors larger than 5 cm^[83]. RFA is also discouraged in large lesions as the risk of side effects may outweigh benefits^[81]. Further, therapy near large vessels may not achieve adequate temperature for coagulative necrosis^[84]. Tumors adjacent to intestine or large bile ducts may also preclude RFA.

Rate of recurrence for RFA is higher compared to surgical resection. For large and small tumors, RFA was associated with a significantly lower survival rate compared to surgical resection^[85,86]. Thus investigating RFA as a bridge to surgical intervention is logically area of research. Several retrospective studies have shown that pre-transplant RFA delays tumor progression and extends time on the liver transplant list^[87-90]. As a major limitation remains the number of organs available for transplant it remains unclear whether the extended time on the liver transplantation list will translate into improved clinical outcomes. Currently guidelines from AASLD support the use of RFA as a bridge to liver transplantation (level II evidence), although the exact role of bridging therapies has not been defined^[35].

Transarterial chemoembolization

Blood supply to HCC tumors are mainly from the hepatic artery. Transarterial chemoembolization (TACE) is the selective occlusion of the blood supply to the tumor with synergistic local distribution of chemotherapy and radioactive substances. The hypervascularity of HCC allows for this targeted therapy, minimizing side effects. The choice of chemotherapeutic agent is not standardized and may include agents such as doxorubicin, cisplatin or epirubicin.

For patients who are not candidates for liver transplantation or resection with tumors too large for local ablation, TACE is effective salvage therapy. Other criteria for treatment include: preserved liver function and no evidence of extrahepatic metastasis or vascular invasion. Approximately 35%-40% of patients will achieve a 25% decrease in tumor size with response rates as high as 60% when surrogate markers for response are utilized^[91-93]. A meta-analysis of six randomized controlled trials showed that patients who underwent TACE had a 2-year improved survival rate compared to those who

only had supportive therapy^[93]. Interestingly, a meta-analysis of nine trials did not show a significant difference in survival based on chemotherapeutic agent used in TACE treatments^[94]. Growing literature supports the efficacy of TACE for HCC down-staging and bridging. The first study to use TACE prior to liver transplantation was published in 1997 and showed successful down-staging of tumors greater than 3 cm with a significant improvement in 5-year survival compared to no TACE^[95]. More recent studies show that 22% to 70% of patients were successfully downstaged with a 2-year post-transplant survival rate of 81%, and among advanced stage HCC (III/IV) patients a median survival of 20 mo^[96-101]. Based on response to therapy, repeat TACE treatments can be scheduled. More intense therapies may be associated with increased risk of acute hepatic decompensation and should be weighed against the potential gains from therapy^[91]. Transarterial radioembolization (TARE), a method of delivering internal radiation to the neoplasm using Yttrium 90, represents an alternative to TACE in intermediate stage HCC^[102]. This modality of treatment is indicated in patients with portal vein thrombosis where conventional TACE is contraindicated. Survival and response rates for TARE were comparable to TACE while a low side effect profile allows for treatment to be completed in the outpatient setting^[103,104].

Novel modalities such as drug-eluting beads-TACE (DEB-TACE) are being investigated in the non-transplant and as neo-adjuvant therapy in patients awaiting transplant. The drug-eluting beads appear to enhance medication delivery and reduce side effects by gradually releasing chemotherapy agents. The PRECISION trial compared non-transplant HCC patients who received DEB-TACE vs TACE. Sub-group analysis revealed a significantly lower hepatic/cardiac toxicity profile in the DEB-TACE group^[105]. A small retrospective analysis in transplant patients also showed that DEB-TACE had improved rates of response with minimal adverse effects compared to embolization alone^[106].

CHEMOTHERAPY

Systemic therapies for the management of patients with HCC continue to be researched. Cytologic agents such as tamoxifen, doxorubicin, everolimus and thalidomide have shown marginal success. Targeted molecular therapies such as bevacizumab, brivanib, erlotinib may be alternatives to conventional cytologic agents. To date, sorafenib is the only systemic therapy effective for treating advanced stage HCC. Sorafenib is an oral tyrosine kinase inhibitor with anti-angiogenic activity, and now is the standard of care in treating individuals with advanced stage HCC and Child's A cirrhosis^[107,108]. Patients with minimal tumor related symptoms, vascular invasion and extrahepatic spread are considered ideal for treatment. Clinical experience has shown significant delay in tumor proliferation and angiogenesis with sorafenib therapy. Those with decompensated cirrhosis or those with a less than 3 mo

life expectancy should not receive sorafenib. Adverse events include diarrhea, hand foot skin reaction, and fatigue and dose reduction achieves tolerance in most patients.

The Sorafenib HCC Assessment Randomized Protocol was a multi-center double-blinded controlled phase III trial that demonstrated a 31% decrease in risk of death with a median 3 mo delay in radiologic progression of disease in patients prescribed sorafenib^[108]. Further, the Global Investigation of Therapeutic Decisions in HCC which included a heterogeneous population of unresectable HCC patients showed that sorafenib was generally well tolerated in the clinical setting^[109]. The role of sorafenib in treating early stage HCC and as neo-adjuvant therapy prior to liver transplantation is evolving. In pre-transplant patients, sorafenib combined with TACE may inhibit angiogenesis and induce tumor necrosis^[110]. Other targeted molecular therapies beyond sorafenib continue to be researched and may represent second line agents for patients that fail or are unable to tolerate sorafenib.

CONCLUSION

HCC is a common cause of malignancy world-wide. Emphasis should be placed on surveillance and early diagnosis. Treatment of HCC has changed significantly over the past few decades with curative options such as liver transplantation, hepatic resection and radiofrequency ablation now available. Further, novel therapies such as DEB-TACE or sorafenib will continue to be areas of research. Despite these advances, there remains much to be learned about HCC. Research into effective prevention and factors that may mitigate malignant transformation should be further explored.

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Current and future antiviral drug therapies of hepatitis B chronic infection

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Abstract

Despite significant improvement in the management of chronic hepatitis B virus (HBV) it remains a public health problem, affecting more than 350 million people worldwide. The natural course of the infection is dynamic and involves a complex interplay between the virus and the host's immune system. Currently the approved therapeutic regimens include pegylated-interferon (IFN)- α and monotherapy with five nucleos(t)ide analogues (NAs). Both antiviral treatments are not capable to eliminate the virus and do not establish long-

term control of infection after treatment withdrawal. IFN therapy is of finite duration and associates with low response rates, liver decompensating and numerous side effects. NAs are well-tolerated therapies but have a high risk of drug resistance development that limits their prolonged use. The imperative for the development of new approaches for the treatment of chronic HBV infection is a challenging issue that cannot be over-sided. Research efforts are focusing on the identification and evaluation of various viral replication inhibitors that target viral replication and a number of immunomodulators that aim to restore the HBV specific immune hyporesponsiveness without inducing liver damage. This review brings together our current knowledge on the available treatment and discusses potential therapeutic approaches in the battle against chronic HBV infection.

Key words: Nucleos(t)ide analogues; Interferon- α ; Drug resistance; Immunotherapy; Hepatitis B therapy

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Core tip: Despite significant improvement in the management of chronic hepatitis B virus (HBV) it remains a public health problem. Current therapeutic regimens include pegylated-interferon (IFN)- α and nucleos(t)ide analogues (NAs). Both treatments do not eradicate the virus and have numerous limitations. IFN therapy is of finite duration and has low response rates while long-term NA therapies have a high risk of drug resistance. The development of new therapeutic approaches is imperative. This review brings together current treatments and the ongoing research efforts on evaluating potential therapeutic strategies that target the suppression of HBV replication the restoration of the weak immune responses against HBV.

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INTRODUCTION

Hepatitis B virus (HBV) is a highly transmissible pathogen infecting humans for more than 1500 years^[1]. Despite the availability of a prophylactic vaccine today HBV continues to pose one of the most serious and prevalent health problems, accounting for over 1 million deaths annually^[2]. HBV is a non-cytopathic virus that can cause a wide spectrum of disease manifestations, ranging from asymptomatic infection to acute self-limiting or fulminant hepatitis, or chronic infection with variable disease activity. Chronic HBV infection (CHB) results in persistent hepatic inflammation and progressive fibrosis that may ultimately lead to hepatic decompensation, cirrhosis, hepatocellular carcinoma (HCC) and liver-related death.

HBV is the prototype of the *Hepadnaviridae* family and has evolved a distinctive and successful strategy for replication, which allows its indefinite persistence in the liver of the infected host. Upon infection of the hepatocyte, the HBV virion is uncoated in the cytosol and the genome translocates to the nucleus. There, its relaxed circular, partially double stranded DNA is converted into a covalently closed circular DNA (cccDNA) molecule, following completion of the shorter positive-strand and repair of the nick in the negative strand. The cccDNA exists as a stable non-integrated minichromosome and forms the template for the synthesis of four co-terminal mRNA transcripts by the action of host RNA polymerase II^[3,4]. One of the transcripts, termed pre-genomic RNA (pgRNA), is the template for genome replication and encodes for the core and polymerase proteins. Translation of the transcripts occurs in the cytoplasm and the encapsidation pgRNA into core particles follows^[5]. The slightly longer precore mRNA is translated to produce a precore protein that is further proteolytically processed into HBV e antigen (HBeAg). Inside the core particle, the viral polymerase directs the synthesis of the minus DNA strand of the genome by reverse transcription of the pgRNA template, which then serves as the template for plus DNA strand synthesis. Mature core particles containing DNA genomes are then enveloped and released or cycled back to the nucleus to replenish the cccDNA pool to perpetuate chronic infection^[3].

The main goal of therapeutic intervention is to achieve a sustained suppression of HBV replication and to improve the quality of life and survival of chronic carriers by preventing progression to cirrhosis, HCC and death. So far, eradication of the virus is impossible and current antiviral treatment aims to reduce liver failure and HCC and to increase survival. The success of antiviral therapy is determined by the HBV surface antigen (HBsAg) and HBeAg serological status, as well

as the levels of HBsAg and HBV DNA during the course of therapy. HBsAg seroconversion associates with a remission activity and improved long-term outcome^[2]. However, HBsAg clearance is achieved in only 10% of the patients and even in these cases both antiviral options are unable to prevent the replenishment of the cccDNA pool from genomic HBV DNA recycled from the cytoplasm, or to reach efficient clearance of cccDNA-containing hepatocytes^[6,7]. This explains the rapid rebound in serum HBV DNA after cessation of antiviral treatment.

Currently there are two therapeutic strategies approved for CHB treatment: five nucleos(t)ide analogues (NAs), which inhibit HBV replication, and the immune-based therapy that includes standard and pegylated interferon- α (IFN- α). Both antiviral treatments are not capable to eliminate the virus and to efficiently control the infection. IFN therapy is of finite duration and associates with low response rates, liver decompensation and numerous side effects, while NAs are long-term, well tolerated therapies but have a high risk of drug resistance development that limits their prolonged use.

This review focuses on current therapies for CHB infection and discusses the development of therapeutic agents that may ultimately lead to the definite eradication of the HBV and cccDNA pool as well as potential immunomodulators that can enhance the host immune responses against HBV that can efficiently control the infection without inducing liver damage.

NATURAL HISTORY OF CHB

The natural history of CHB infection consists five distinct phases of varying duration that are not necessarily sequential and are defined as: immune-tolerant, immune reactive HBeAg-positive, inactive HBV carrier, HBeAg-negative CHB and HBsAg inactive phase^[8,9]. The course of the infection is dynamic and is a result of the complex interactions between the virus, hepatocytes and host immune responses. The periodic activation of the host immune system against the infected hepatocytes is an unsuccessful attempt to eradicate the virus that only leads to disease exacerbations and the development of fibrosis, cirrhosis and HCC^[10]. The progression of HBV-induced liver diseases depends on the geographical area, the presence of HBsAg and HBeAg mutations and viremia levels^[11]. Generally, patients with CHB have a 15%-40% risk to develop cirrhosis and 15% risk to develop compensated cirrhosis, while 60% of the compensated cirrhosis patients risk death^[12].

Control of HBV infection involves the elimination of the infected hepatocytes by cytolytic and non-cytolytic mechanisms. The immune system of the host is capable to eliminate the infection as evidenced by the fact that more than 95% of adults spontaneously resolve the infection and that bone marrow transplantation recipients can resolve CHB infection^[13,14]. In acute infection viral clearance is succeeded by the development of a robust,

polyclonal and multi-specific, HBV-specific cytotoxic T lymphocytes (CTLs) response to multiple epitopes of the viral nucleocapsid, envelope and polymerase. Furthermore, recovery from acute infection occurs by the non-cytolytic viral eradication mediated by HBV-specific CTLs since in the cases of spontaneous viral clearance only a part of the hepatocytes is being destroyed^[15]. Elimination of HBV has been long considered to be T-cell dependent, however, natural killer (NK) cells are now known to be involved early in infection and B cells in the presentation to CD4⁺ T cells and the production of neutralizing antibodies^[16,17].

The complexity of the processes involved in self-limiting infection and natural history of the infection implies the requirement for a combination of therapeutic options. A synergistic approach of boosting the immune response of the host along with an effective viral load suppression is needed to succeed sustained viral clearance and complete eradication of the cccDNA pool in chronic infection.

CURRENT ANTIVIRAL THERAPY

In view of the natural history of CHB infection it is clear that chronic patients constitute a highly heterogeneous population and therefore require different management strategies. To optimise therapy for individual patient, several factors need to be considered related both to the patient, including age, sex, genetic polymorphisms, lifestyle factors, stage of liver disease and co-infections and to viral characteristics such as viremia, HBeAg-positivity, HBV genotype and viral genome heterogeneity. Furthermore the dosage duration, timing, efficacy, side effects, drug resistance and combination of antiviral agents need to be individually optimised. Unfortunately, current available treatment options require long term use and such attempts are expensive and carry a high risk for the development of breakthrough drug resistance.

NAS

Antiviral therapies for CHB using NAs have become standard treatment modalities. Current NA agents approved for treatment of CHB infection, include lamivudine, adefovir, entecavir, telbivudine, and tenofovir. Administration with NAs leads to a strong and long-term control of virus amplification by interfering with the viral replication cycle. Viral suppression can be reached in up to 95% of the patients^[18]. The critical weak point of NA therapy is that it requires life-long administration, has modest effects on HBsAg levels and carries the risk of the development of drug resistance^[2]. In addition, in HBeAg-positive patients the rate of seroconversion is as low as 20%-25% following one year of treatment^[7]. The major adverse effects of long-term administration include nephrotoxicity and myopathy^[19].

NAs are chemically synthesised drugs that com-

petitively inhibit the DNA dependent and reverse transcriptase activity of viral polymerase and therefore inhibiting the reverse transcription of the pgRNA to the first strand of viral DNA. They are mimicking natural nucleotides and during viral replication they are being incorporated into newly synthesised HBV DNA causing chain termination. Moreover, NAs inhibit the synthesis of the HBV negative-DNA strand by reverse transcription and the synthesis of the positive-strand. They reduce significantly the cccDNA pool of infected hepatocytes by inhibiting the recycling of the nucleocapsids that contain viral genomes back to the nucleus but they cannot prevent the initial cccDNA formation in newly infected cells^[20]. NAs are, therefore, efficient in blocking the synthesis of new virions and in reducing HBV DNA serum concentrations to undetectable levels but after cessation of treatment viral reactivation does occur due to the persistence of cccDNA. Experiments in woodchuck animals suggest that the effectiveness of NAs in reducing the cccDNA pool may depend on the cell cycle phase of the hepatocytes^[21].

Development of antiviral resistance

During long-term therapy with NAs, HBV develops resistance to the drug administered. The resistance rates are higher with earlier generation NAs such as lamivudine, telbivudine, and adefovir. Although entecavir and tenofovir are associated with low risk of resistance for treatment to naive patients, it is still challenging to manage pre-existing antiviral resistance because of the risk of cross-resistance^[22]. Emergence of drug resistant variants is commonly accompanied by acute exacerbation of liver disease and in some cases by hepatic decompensation and hence sequential monotherapy with low barrier drugs poses a serious problem^[23,24].

The development of antiviral resistance depends on the interaction of viral, drug and patient factors. HBV replicates through the reverse transcription of an RNA intermediate. This step in the replication cycle is particularly prone to errors as the host RNA polymerase II has an inherent low copying fidelity, and the viral polymerase/reverse transcriptase lacks proof-reading activity^[25]. Considering that HBV is 3.2 kb in size and viral production rate in CHB infection can reach rates as high as 10¹¹ virions per day, it has been estimated that 10⁷ base pairing errors are produced daily in a chronic patient^[26]. Although many of these mutations would be deleterious to the virus, some are advantageous, either by offering a replication advantage, or by facilitating immune escape and therefore predispose to the rise of antiviral resistant mutations^[27]. Under the selection pressure exerted by antiviral drugs or immunological responses, the viral mutants that show maximum resistance to the treatment and high replication capacity are selected as primary drug resistance mutants over the wild type quasispecies^[28]. The hepatocyte turn over rate is greatly increased in the inflammatory liver and, therefore, the drug resistance variants rapidly spread in

uninfected hepatocytes, occupying the new replication space and becoming the dominant viral quasiespecies^[26].

Lamivudine

Lamivudine is a moderate strength deoxycytidine nucleotide analog but due to its relatively low cost and being the first NA approved, it has a pharmacoeconomic advantage and has been widely used worldwide. Lamivudine inhibits the viral polymerase/reverse transcriptase and is equally effective against the wild-type virus and precore/core mutant variants^[29,30]. It is a well-tolerated drug and has been shown to be effective even in patients with severe viral exacerbations and with hepatic failure^[31,32]. Long-term lamivudine therapy results in up to 50% HBeAg seroconversions and maintains low levels of HBV DNA and alanine aminotransferases (ALT) in both HBeAg-positive and HBeAg-negative CHB patients^[33,34]. However, the development of resistant mutations occurs in 20% after a year and as much as 70% following five years of treatment^[35]. The most common mutation that confers resistance to lamivudine is the M204V/I/S mutation and involves a single amino acid substitution within the highly conserved YMDD motif at the catalytic centre of the polymerase^[36]. Lamivudine mutations affect the ability of the dNTP-binding pocket to accommodate the drug, which in turn leads to a reduction in the affinity of lamivudine for the reverse transcriptase domain^[36].

Telbivudine

Telbivudine is a thymidine NA that once administrated is easily phosphorylated to its active triphosphate form^[37]. It is structurally similar to lamivudine and has similar resistance profile, is well tolerated and has no dose-limiting side effects^[38]. The overall rate of drug resistance development is 22% in HBeAg-positive patients and 9% in HBeAg-negative carriers^[39]. Although it is more potent than lamivudine and adenovir, it is cross-resistant with lamivudine and has a considerable risk of drug resistance development^[40].

Entecavir

Entecavir is a guanosine NA and inhibits polymerase/reverse transcriptase by competing with the natural substrate deoxyguanosine triphosphate. It inhibits both the wild type and lamivudine-resistant HBV variants, has a high rate of HBV DNA suppression, low drug resistance, low incidence of adverse reactions, and also been shown to improve liver function in patients with decompensation cirrhosis^[41]. In clinical trials entecavir was found to be superior to lamivudine in NA-naïve and lamivudine refractory HBeAg-positive or HBeAg-negative patients. After five years of therapy in NA-naïve patients the risk of entecavir resistance is low but in lamivudine pre-treated patients, entecavir resistance associates with breakthrough in 50% of the patients^[42].

Adefovir dipivoxil

Adefovir, an acyclic NA, is a potent inhibitor of viral

replication of both the wild type and lamivudine resistance HBV^[43]. In addition to acting as a DNA chain terminator it has been reported to induce NK cell activity and to induce endogenous IFN production^[44]. The main resistance mutations are located in the palm subdomain of polymerase. Following five year treatment, approximately 30% of the patients develop drug resistance^[45]. When adenovir is administered in combination with lamivudine to patients with pre-existing lamivudine resistance, cross-resistance does occur^[46].

Tenofovir disoproxil

Tenofovir, another acyclic NA, is a methyl derivative of adenovir and exhibits anti-viral activity in lamivudine resistance HBV. It has been shown to have an additive suppression effect on viral replication when administered in combination with lamivudine, entecavir or telbivudine^[47,48].

INTERFERON-BASED THERAPY

Recombinant and lymphoblastoid IFN- α , have been introduced as therapeutic regimens in CHB liver disease since the early 1980s. Conventional IFN- α or Pegylated IFN- α (Peg-IFN- α) induces direct antiviral activity by stimulating the host antiviral immune response and mediating divergent effects on viral replication. Peg-IFN- α has replaced conventional IFN- α treatment as it allows the administration of weekly injections compared to three times schedules of conventional IFN- α , while maintaining similar antiviral efficacy. Peg-IFN- α includes two preparations, Peg-IFN- α and Peg-IFN- α , 2 α , that are heterogenous and contain multiple monopegylated isomers.

The response rate of IFN treatment in children is similar to that of adults, being about 30%-40% in those with high ALT levels, but this effectiveness drops to 10% in those with normal levels^[49,50]. Nevertheless response rates can change at the end of the therapy because virological relapses commonly occur^[51]. Sustained responses have been reported to be about 18%-25% at the end of IFN treatment and in relapsed patients that have been pre-treated with IFN^[51,52]. Following IFN treatment factors associated with response to treatment include high ALT levels, low HBV DNA, older age and the absence of previous IFN therapy. Patients with the best outcomes are those with genotype A and high ALT or low HBV DNA, and those with genotypes B or C with both high ALT and HBV DNA levels^[53]. Poor responses correlate with the duration of chronicity, the presence of precore mutations, male sex and human immunodeficiency virus (HIV) co-infection. The main advantages of IFN treatment are finite duration, absence of resistance, a higher rate of HBsAg clearance and HBeAg seroconversion (particularly among genotype A and HBeAg-positive patients), improvement of survival rates and a reduction of HCC occurrence^[54]. However, the adverse effects of IFN include flu like symptoms, fatigue, bone marrow suppression and exacerbation of autoimmune illnesses and, therefore,

Table 1 Potential antiviral drugs for the future treatment of chronic hepatitis B virus

Potential antiviral agents	Mechanisms of action
NAs: MIV-210, elvucitabine, valtorcitabine and clevudine	Inhibition of HBV replication
Lipopeptides: Myrcludex-B	Prevention of viral entry
Disubstituted-sulfonamides: CCC-0975 and CCC-0346	Blockage of the <i>de novo</i> cccDNA synthesis
LTR	Destabilization cccDNA minichromosome
Zinc finger nucleases	Disruption of sequences within viral proteins
Epigenetic regulators	Repression of cccDNA transcriptional activity
Small interfering RNA	Silencing of HBV protein gene expression
Phenylpropenamides: AT-61 and AT-130	Prevention of RNA encapsidation
Heteroaryldihydropyrimidines: BAY41-4109	Nucleocapsid destabilization
Synthetic TLR-7 agonists	Inhibition of HBV replication <i>via</i> pDC activation
IL8 inhibitors	Increase the potency of IFN- α treatment
REP 9AC amphipathic polymers	Inhibition of subviral particles
Inhibitors of PD-1 and TIM3 receptors	Restoration of T cell function
Immunization with DC pulsed with HBV antigens	Induction of viral specific CTLs
Therapeutic vaccines containing viral peptides	Induction HBV-specific responses
Cytokines: IL12, IL2, IFN γ and TNF- α	Restoration of HBV specific T cell activity
Thymosin alpha polypeptide	Induction of T cell function and NK cytotoxicity

cccDNA: Covalently closed circular DNA; CTLs: Cytotoxic T lymphocytes; DC: Dendritic cells; HBV: Hepatitis B virus; MIV: Lagociclovir valactate; NK: Natural killer; pDC: Plasmacytoid DCs; PD-1: Programmed cell death 1; TLR: Toll like receptor; TIM3: T-cell immunoglobulin domain mucin domain-containing molecule-3; IFN: Interferon; TNF: Tumor necrosis factor; NAs: Nucleos(t)ide analogues; LTR: Lymphotoxin receptor; IL: Interleukin.

patients should be closely monitored^[55]. Treatment with IFN- α has been shown to modulate the epigenetic repression of cccDNA activity and its potential role in antiviral treatment is discussed later.

COMBINATION THERAPEUTIC STRATEGIES

Current antiviral monotherapies are not able to eradicate the HBV from the liver, have restricted efficacy, high cost and lead to drug resistance. So far, combination therapy with a number of NAs or with IFN, were not superior in comparison to monotherapy^[56-58]. However, a synergistic antiviral effect may confer an additional benefit^[59,60]. Combining low barrier resistance drugs, such as lamivudine and adenofir, with or without IFN can increase barrier resistance but does not improve viral suppression and HBsAg clearance as compared to monotherapy with new-generation NAs, like entecavir or tenofovir^[61,62]. However, in the absence of alternative antiviral agents, a combination of NAs has been shown to be efficient in patients with partial responses or viral resistance patterns^[63].

Considering the shortcomings of antiviral therapies it is imperative to identify novel drug targets to develop new combination therapies that can achieve the clearance of HBV DNA and cccDNA as well as the restoration of immune defence mechanisms. Research on HBV led to the discovery of number of compounds that could potentially complement NAs or IFN therapies (Table 1) and are being further discussed.

HBV LIFE CYCLE INHIBITORS

HBV DNA polymerase

In addition to the approved NAs, there are several novel

drugs developed to inhibit reverse transcription. Among them, lagociclovir valactate (MIV-210) is a prodrug with high oral bioavailability in humans and is a potent inhibitor of the replication of the wild type, lamivudine-resistant, adenovir-resistant, and lamivudine-adenovir cross resistant mutant HBV genomes^[64]. Other new NAs that show potent inhibition of HBV replication *in vitro*, include elvucitabine, valtorcitabine and clevudine.

Viral entry

Myristoylated preS-peptide (Myrcludex-B) is a lipopeptide derived from the pre-S1 domain of the HBV envelope. It can prevent viral spread from infected hepatocytes *in vivo* and reduces the amplification of cccDNA in newly infected hepatocytes^[65]. Petersen *et al*^[66] demonstrated that it is capable to prevent HBV infection in hepatic cell culture and humanized mice as well as the establishment of hepatitis D virus infection.

Synthesis of cccDNA

Elimination of cccDNA is a prerequisite for a successful therapy and represents a challenging and important antiviral target. Two small molecules that have been reported to specifically target cccDNA synthesis are structurally related disubstituted-sulfonamides and can potentially be used as drugs to block the *de novo* synthesis of cccDNA^[67]. Considering the long nuclear half-life of cccDNA and its dependence on host factors for its activity, eliminating established cccDNA appears to be bigger challenge but evidence suggests that it is not invulnerable to therapy. HBV cccDNA has been shown to be destabilized *in vitro* with inflammatory cytokines and IFN- α by non cytolytic mechanisms while is also eradicated when the infected hepatocytes are being eliminated by host immune mechanisms^[68]. Interestingly, a recent study has shown that high doses

of IFN- α and lymphotoxin receptor (LTR) induced the expression of APOBEC3A or 3B resulting in the non-cytopathic reduction of cccDNA in HepaRG cell and primary human hepatocytes^[69]. Another target of cccDNA is to identify compounds able to interfere with the regulation of its transcriptional activity. A new approach is the generation of zinc finger nucleases (ZFNs) that target sequences within viral proteins such as polymerase, core and X genes^[70]. Delivery of HBV-specific ZFNs in cell culture systems was shown to be achieved successfully by vectors and resulted in the efficient disruption of the target genes by the generation of site-specific mutations. However, the delivery of such targeted proteins in chronic patients remains a therapeutically challenge.

Epigenetic control of cccDNA

Epigenetic mechanisms refer to heritable changes in chromatin organization and gene expression independent of the underlying DNA sequence and have been shown to play a key role in HBV replication. Interfering with the epigenetic regulation of cccDNA minichromosome is another promising therapeutic approach. Viral replication and cccDNA transcriptional activity have been shown to be regulated by the acetylation status of cccDNA-bound H3/H4 histones as well as by the recruitment of cellular acetyltransferases and histone deacetylases onto cccDNA in cell culture and primary human hepatocytes^[71,72]. Experiments in humanized mice and cell culture demonstrated that treatment with IFN- α induces cccDNA-bound histone hypoacetylation and the active recruitment of transcriptional corepressors onto cccDNA^[73]. IFN- α administration was also shown to reduce binding of STAT1 and STAT2 transcription factors to active cccDNA. Identifying, the molecular mechanisms by which IFN- α mediates epigenetic repression of cccDNA transcriptional activity can lead to the development of novel therapeutics. In CHB patient, viral and host DNA methylation density varies significantly has been identified as a host defence mechanism to suppress viral gene expression and replication. Furthermore, an up regulation of DNA methyltransferases has been reported in CHB livers that facilitates the methylation of cccDNA and viral genomes affecting protein production and viral replication^[74,75]. It has been reported that host DNA methylation is the main mechanism to inactivate relevant genes in HCC^[76]. These findings suggest a potential role of methylation in the future treatment of CHB infection.

Small interfering RNAs

RNA interference (RNAi) is an evolutionary conserved process by which double-stranded RNA induces sequence-specific silencing of homologous genes. RNAi-based therapeutics act in a fundamentally different manner than other therapies. They have the potential to specifically knock down the expression of HBV proteins, including HBsAg and pgRNA, thus reducing viral replication. Experiments in transgenic mice showed

that delivery of potent small interfering RNAs (RNAi) resulted in the long and sustainable repression of viral RNA, proteins and HBV DNA levels^[77]. However, the use of RNAi still remains a therapeutic challenge due to the lack of a safe and effective delivery system to patients.

Nucleocapsid assembly and stability

There are a number of studies aiming at the development of agents that inhibit nucleocapsid assembly or stability. A few non-nucleocapsid molecules have been shown to inhibit the replication of both the wild type virus and of drug resistant variants^[78]. These include compounds that belong either to the family of phenylpropenamide (AT-61 and AT-130) and have been reported to prevent RNA encapsidation or to the family of heteroaryldihydropyrimidines (BAY41-4109) that can destabilize nucleocapsids^[55,79]. In addition to their impact on replication cycle, these agents can inhibit cccDNA intracellular amplification by inhibiting nucleocapsid recycling to the nucleus in woodchuck animal model^[80].

IMMUNOMODULATORS

Besides interfering with the viral life cycle, other therapeutic approaches aim to the restoration and duration of the immune responses against HBV. An increasing number of studies have been reporting a number of potential immunomodulators that can be effective in CHB treatment (Table 1).

Innate responses

The important role of the innate immunity in controlling HBV infection has gained significant ground the last years and several studies have focused on the development of compounds that can manipulate NK cell immunity. In CHB infection, NK exert potent antiviral activities either directly by the lysis of infected hepatocytes or indirectly by modulating viral specific T cells while they also contribute to the pathogenesis of liver injury^[81]. Furthermore, it has been proposed that HBV inhibits the innate system *via* the suppression of toll like receptor (TLR) induced antiviral signalling^[82]. TLR7 and TLR9 ligands or agonists have been shown to inhibit viral replication by the production of vast amounts of type I and III IFNs *via* the activation of plasmacytoid dendritic cells (pDCs)^[83]. Experiments in chimpanzee and woodchucks have shown that a synthetic TLR-7 agonist reduced serum and liver viremia as well as HBsAg and increased the expression of IFN- α and interferon stimulated genes^[55]. This compound has reached Phase I clinical trials^[84]. Treatment with entecavir has been reported to restore TLR2 expression in infected cells while administration of TLR2 ligand repressed HBV replication^[85]. These findings suggest that a combination of TLRs agonists with NAs could provide a promising therapeutic approach. Another compound that is being evaluated for its antiviral capacity is the REP 9AC Replicor, which is a nucleic acid-based amphipathic

polymer. It has been shown to facilitate innate responses *via* the inhibition of subviral particles from infected hepatocytes^[86].

Interleukin-8 (IL8) chemokine is an important mediator of innate immunity and T cell function. In patients undergoing HBV reactivation, serum IL8 levels have been shown to parallel viremia levels^[16,87]. Specific inhibition of IL8 has been shown to increase the potency of IFN- α treatment in HBV transfected hepatic cell lines and the addition of recombinant IL8 was reported to rescue almost completely viral replication following IFN- α treatment^[87]. The development of an IL8 blockage strategy combined with IFN- α treatment can be another encouraging future therapeutic approach.

Viral specific T cell responses

CHB infection is characterized by the hyporesponsiveness of HBV-specific CD4⁺ T cell and CTL that is considered to be caused from the presence of large quantities of virions and viral particles in the tolerogenic environment of the liver, particularly in childhood. The dysfunction of viral specific T cells has been associated with defects in co-stimulatory pathways. The negative regulation of T cell function associates with defects in co-stimulatory pathways and in particular with the increased expression of inhibitory receptors programmed cell death 1 (PD-1) and its ligand 1, T-cell immunoglobulin domain, mucin domain-containing molecule-3 (TIM3) and CD244 as well as the impairment of DCs and the increased frequencies of T regulatory cells (Tregs)^[55,88,89]. Restoration of T cell function could, at least partially, be achieved by the blockage of the negative regulatory pathways including inhibitors of such receptors, *e.g.*, anti-PD-1 mAb, and anti-apoptotic drugs that block TIM3^[13]. Another potential therapeutic strategy is to activate DC function, by DC-based immunotherapy. Immunization of DCs pulsed with HBV antigens has been shown to induce viral specific CTLs responses, to overcome tolerance against HBV and to reactivate B cell responses in transgenic mice^[90]. Tregs that significantly contribute to T cell tolerance in CHB were reported to reduce the response to treatment in IFN- α non-responders whereas administration of entecavir reduced their frequencies and function^[89,91]. Expansion of HBV core antigen (HBcAg)-specific CTLs is shown to be essential in HBV replication control and leads to the activation of endogenous DC and HBsAg-specific CTLs without inducing liver damage^[90]. Therefore the suppression of Tregs and HBcAg can also be considered as potential approaches in immunotherapy.

Another adjuvant of potential benefit is CpG DNA, a synthetic oligonucleotide that preferentially stimulates Th1 responses, with the production of IL12 and IFN- γ ^[92]. Immunization of transgenic animals with HBsAg vaccine supplemented with CpG DNA led to clearance of serum HBsAg and the development of anti-HBs, with concurrent down-regulation of HBV mRNA production in the liver. Adoptive transfer experiments of T cells from such animals showed that they were able to partially control transgene expression in the liver and to clear

HBsAg without an antibody requirement^[92]. A CpG-containing HBsAg vaccine was shown to overcome hyporesponsiveness normally seen in immunized orangutans^[93]. Similarly, it was shown that cytokines from peripheral blood mononuclear cells from HBV-negative individuals stimulated with CpG ODN strongly inhibited HBV viral replication, HBsAg and HBeAg production from infected HepaRG and HepG2 cells^[94].

Therapeutic vaccination

Therapeutic vaccination is another approach that can be used in attempts to achieve long-term antiviral treatment. An effective vaccine should both induce a strong antigen-specific immune response and the subsequent deployment of immune response to HBV in the liver. Currently, the vaccines that are being evaluated in CHB patients and experimental animals include recombinant proteins, specific peptides, DNA vaccine or DNA delivered by viral vectors. Clinical trials using vaccines containing HBcAg and HBsAg peptides showed a reduction of HBV replication that were not accompanied by HBsAg clearance^[95,96]. However, a recent vaccine formulation that comprised HBsAg and HBcAg particles and was delivered together with a saponin based ISCOMATRIX adjuvant in transgenic mice induced the activation HBsAg- and HBcAg-specific CTLs and the high production of their antibody^[97].

Cytokines and thymosin

Several cytokines are involved in the defective immune responses and can be used as adjuvant compounds to break the immune tolerance in CHB infection. Among them, IL12 has been reported to restore viral specific-T cell hyporesponsiveness and to down-regulate PD-1 inhibitory receptor^[98]. Combined therapy of lamivudine and recombinant IL2 was shown to increase HBV-specific T cell activity and to induce HBeAg seroconversion^[99,100]. Treatment with lamivudine combined with IFN- γ and tumor necrosis factor- α was shown to induce a stronger inhibition of cccDNA and in the efficient suppression of viral replication without the development of cytotoxicity^[101]. Thymosin alpha 1 (Ta1) is a synthetic polypeptide that has immunomodulating activity and has been shown to promote T cell activity, IFN- γ and IL12 production as well as NK-induced cytotoxicity^[102]. Treatment with Ta1 has been demonstrated to reduce significantly viral replication in chronic patients and woodchuck animals^[103,104]. Long-term combination therapy of lamivudine and Ta1, but not with peg-IFN- α , was found to be superior to monotherapy and correlated with HBeAg seroconversion^[27,105]. The conflicting results on the benefits of Ta1 in combination therapy suggest that more clinical studies are required to further evaluate this compound.

CONCLUSION

Although antiviral therapy of CHB infection has improved dramatically during the last decades an effective treatment is still not available and CHB remains a serious

clinical problem worldwide. Current available antiviral options suppress viral replication and improve patient survival but they do not eradicate the virus and the cccDNA pool resulting in viral reactivation after cessation of treatment and in the development of liver disease progression. The goal of new therapeutic strategies is to eliminate or control HBV and to allow access to therapy in poor high-endemicity areas, where the consequences of HBV infection are more severe. Experience with the treatment of HIV and HCV has proven that combination therapy with compounds targeting multiple steps in the replication cycle would be more efficient than monotherapy. Research efforts focus on the identification of novel compounds that inhibit viral entry, nucleocapsid assembly, reverse transcription and cccDNA formation and stability. Besides interfering with the viral life cycle, an increasing number of studies have reported several promising immunomodulators that aim to restore the HBV specific T cell hyporesponsiveness and to boost the innate immune arm of the host, while blocking potential pathways of liver damage. The development of such agents would help to improve existing therapeutic regimens and provide new opportunities for more efficient combination therapies. New strategies should be clinically evaluated by large-scale trials or by the use of relevant experimental models. Because access to chimpanzees is restricted, human HBV replication is being now being studied in humanized mice. Even if these mouse models are useful in validating novel antiviral compounds have the critical weak point of an immune-deficient host that doesn't reflect the situation of human liver environment.

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Advanced hepatocellular carcinoma and sorafenib: Diagnosis, indications, clinical and radiological follow-up

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treatment, a multi-kinase inhibitor with anti-proliferative and anti-angiogenic effect. Trans-arterial Radio Embolization also represents a promising new approach to intermediate/advanced HCC. Post-marketing clinical studies showed that only a portion of patients actually benefits from sorafenib treatment, and an even smaller percentage of patients treated shows partial/complete response on follow-up examinations, up against relevant costs and an incidence of drug related adverse effects. Although the treatment with sorafenib has shown a significant increase in mean overall survival in different studies, only a part of patients actually shows real benefits, while the incidence of drug related significant adverse effects and the economic costs are relatively high. Moreover, only a small percentage of patients also shows a response in terms of lesion dimensions reduction. Being able to properly differentiate patients who are responding to the therapy from non-responders as early as possible is then still difficult and could be a pivotal challenge for the future; in fact it could spare several patients a therapy often difficult to bear, directing them to other second line treatments (many of which are at the moment still under investigation). For this reason, some supplemental criteria to be added to the standard modified Response Evaluation Criteria in Solid Tumors evaluation are being searched for. In particular, finding some parameters (cellular density, perfusion grade and enhancement rate) able to predict the sensitivity of the lesions to anti-angiogenic agents could help in stratifying patients in terms of treatment responsiveness before the beginning of the therapy itself, or in the first weeks of sorafenib treatment. This would bring a strongly desirable help in clinical managements of these patients.

Abstract

Advanced stage hepatocellular carcinoma (HCC) is a category of disease defined by radiological, clinical and hepatic function parameters, comprehending a wide range of patients with different general conditions. The main therapeutic option is represented by sorafenib

Key words: Modified Response Evaluation Criteria in Solid Tumors; Diffusion weighted imaging; Barcelona clinic liver cancer; Advanced hepatocellular carcinoma; Sorafenib; Advanced hepatocellular carcinoma second line therapies; Perfusion weighted imaging; Response evaluation; Hepatocellular carcinoma follow-up; Response

Evaluation Criteria in Solid Tumors

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Core tip: Advanced stage hepatocellular carcinoma comprehends a wide range of patients with different general conditions. The main therapeutic option is represented by sorafenib. Although the treatment has shown a significant increase in mean overall survival, only a part of patients actually shows benefits. Differentiating responder from non-responder patients is a pivotal challenge for the future. In particular, finding parameters quantitatively describing perfusion grade, and then able to predict the sensitivity of the lesions to anti-angiogenic agents could help stratifying patients in terms of responsiveness before the beginning of the therapy itself. This would bring a great help in management of these patients.

Colagrande S, Regini F, Taliani GG, Nardi C, Inghilesi AL. Advanced hepatocellular carcinoma and sorafenib: Diagnosis, indications, clinical and radiological follow-up. *World J Hepatol* 2015; 7(8): 1041-1053 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i8/1041.htm> DOI: <http://dx.doi.org/10.4254/wjgh.v7.i8.1041>

INTRODUCTION

Hepatocellular carcinoma (HCC) represents the fifth most prevalent tumor worldwide and the third cause of cancer related death^[1]. The feasibility of treatments and the linked prognosis largely vary because of the tumor characteristics that present wide variability in terms of local and extra-hepatic burden. Moreover, the differences in molecular features and aggressiveness of the tumor significantly influence the natural history of the disease. Finally, the management of HCC is also complicated, in the majority of patients, by its development on a background of a cirrhotic liver, that can compromise the viability of the appropriate treatment^[2].

Advanced HCC represents a major problem, as a considerable portion of HCC is diagnosed at this stage despite the wide use of ultrasound for surveillance in patients with increased risk^[3]. This stage of disease is related to a poor prognosis and is reported to be associated with a survival rate of about 25% at 1 year^[4,5]. Unfortunately, patients with advanced HCC are not suitable for curative therapeutic strategies like surgery, loco-regional treatments or orthotopic liver transplant. Moreover, HCC has a significant resistance to classic radio- or chemotherapy, that represent the standard of care in the majority of advanced tumors. Although the setting changed with the introduction of the multi-kinase inhibitor named sorafenib in 2008 for the treatment of advanced HCC, relevant issues in the management of this disease are still open. In particular,

this therapy owns a wide variability in the prolongation of the survival of these patients. Furthermore, sorafenib therapy has some significant side effects and is very expensive.

On this background, the aim of this review is to remind the main problems related to diagnosis, staging and treatment allocation in case of advanced HCC, the principal indications of sorafenib, how to evaluate and to predict the response to treatment and when a second line therapy is suitable.

DIAGNOSIS, STAGING AND TREATMENT ALLOCATION

The development of radiological techniques has radically changed the approach to the diagnosis of HCC in the past decade. According to the American HCC guidelines, in 2005 a diagnosis of HCC without biopsy could be made in presence of a mass > 1 cm showing characteristic arterial enhancement, observed in two different imaging modalities, either biphasic computed tomography (CT) or magnetic resonance (MR)^[6]. In the following years the diagnostic accuracy of a single tomographic contrasted technique has been largely validated. The last American guidelines published in 2011 made possible the diagnosis of HCC in a cirrhotic patient when a nodule > 1 cm shows arterial enhancement and portal/delayed phase "washout", with the use of a single tomographic exam (CT or MR)^[7]. Future guidelines may probably include the use of organ-specific contrast agents (CA), that have shown a high sensitivity in the detection of new HCC lesions and of post-surgical disease recurrence as well as a good potential in hypo-vascular HCC diagnosis^[8-10]. This additional radiological advancement, which has been included in Japanese guidelines^[11] and is currently used in clinical practice, might further reduce the diagnostic role of liver biopsy in HCC in the next years.

Many staging systems have been developed for HCC, and so far there is no international consensus for the use of a favored one. The Barcelona Clinic Liver Cancer (BCLC) is the staging system most widely endorsed in HCC evaluation^[12]. It was developed in 1999 and refined during the following years^[3,5,6,13,14]. Considering different parameters such as the tumor burden, the hepatic function and the presence of disease-related systemic symptoms, the BCLC individuates five different stages of disease and suggests the appropriate first line therapeutic strategy. Moreover, it considers the impact of treatment on overall survival (OS), linking the stage with the prognosis^[3].

According to BCLC, advanced stage (BCLC-C) is defined by the presence of unresectable HCC with extra-hepatic spread (metastases or lymph nodes involvement) and/or vascular invasion (portal or segmental invasion) and/or systemic symptoms, defined by an Eastern Cooperative Oncology Group^[15] performance status 1 or 2, with a liver function defined by a Child Pugh^[16] stage not greater than B. It is easy to understand

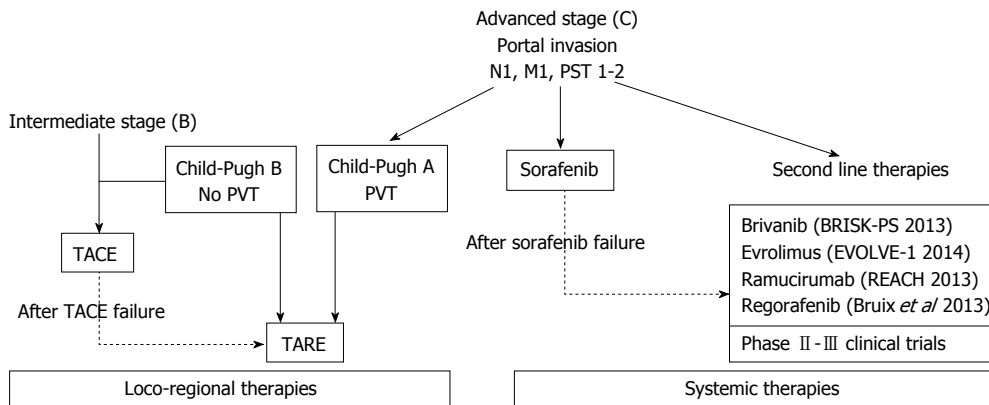


Figure 1 Main therapeutic options for advanced hepatocellular carcinoma treatment. TACE: Trans-arterial chemo-embolization; TARE: Trans-arterial radio embolization; PST: Performance status; PVT: Portal vein thrombosis.

how advanced stage HCC includes a heterogeneous population of patients, with different prognosis. For instance, the grade of liver function is significantly related to prognosis: patients with a Child Pugh B class have a shorter median survival (5 mo) than patients with more preserved liver function (7 mo)^[5,17]. This stage of disease has been considered untreatable until 2008, when sorafenib has proven his efficacy in prolonging the survival of these patients in two different large studies^[17,18]. Since then, sorafenib has become the suggested therapy for advanced HCC in the BCLC algorithm (Figure 1).

Despite its wide use, the definition of advanced HCC by the BCLC and the allocation of sorafenib show some minor flaws.

The first one is represented by the treatment of intermediate HCC, a stage of disease that includes a heterogeneous group of clinical presentations. Trans-arterial chemo-embolization (TACE)^[3,5] is the recommended primary therapy for this stage, but some authors suggest its use also in selected BCLC-C patients with a better liver function^[19,20]. Conversely some others consider TACE not safe in patients with so advanced disease and recommend this treatment only in patients with Child-Pugh A cirrhosis and segmental portal vein thrombosis^[21]. Besides, the BCLC does not lead to a clear therapeutic indication for patients who cannot afford or have failed TACE. This problem has been partially solved through the introduction of the concept of "treatment stage migration": if patients are not candidates for first-line therapy as per stage, they can be shifted to the treatment option for a more progressed BCLC stage^[3,5]. Translated in clinical practice, sorafenib should be administered also in intermediate HCC patients who can't afford or have failed the treatment with TACE. At the same time TACE may be considered a suitable alternative for advanced stage HCC patients who are not compliant with oral therapy or could not have access to sorafenib^[22]. In the last years even the combined use of sorafenib and TACE for intermediate and/or advanced HCC has been evaluated in different studies^[23-25]. However, data published so far about safety and efficacy

of this therapeutic regimen is controversial and a precise validation is still needed.

The second problem is related to the notion that BCLC defines as "advanced HCC" any patient presenting an Eastern Cooperative Oncology Group performance status of 1-2. In clinical practice, it means that patients could be excluded from potentially curative treatments if they are "restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work"^[15]. In our judgment, this approach could seriously limit the clinical benefit in this particular kind of patients. It should be stressed that every therapeutic choice, especially in this kind of patients, deserves a multidisciplinary approach, as every disease represents an unique case.

A relatively new promising therapeutic option for intermediate/advanced HCC is represented by trans-arterial radio embolization (TARE). Differently from TACE, its main effect is not related to a mechanic obstruction of the arteries that feed the tumor: by the use of yttrium-loaded glass or resin particles a localized beta radiation of the mass can be obtained^[26] (Table 1). Although there are some absolute contraindications, represented by a tumor burden over 75% of liver parenchyma and lung or gastrointestinal uncorrectable shunts^[26] (that may lead to development of a radiation induced pneumonia), TARE has emerged as a safe treatment option and showed survival rates similar to TACE and sorafenib in studies published so far^[27,28]. In particular this therapeutic option may be considered an interesting alternative to TACE, especially in patients with portal vein thrombosis^[29]. However, data from randomized control trials are needed in order to confirm the therapeutic role of TARE for HCC in clinical practice.

SORAFENIB TREATMENT

Sorafenib still represents the only approved therapy for advanced HCC^[5]. It is a multi-kinase inhibitor with anti-angiogenic and anti-proliferative effect. It acts by inhibiting the serine-threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of vascular

Table 1 Main loco-regional therapies in advanced hepatocellular carcinoma treatment

Loco-regional therapies
TACE is the most common used loco-regional treatment in patients with unresectable HCC, without macrovascular invasion or extrahepatic spreads (BCLC stage B)
The use of TACE in advanced HCC is controversial: some authors affirm its better efficacy in term of survival benefit, than the best supportive care in HCC with extrahepatic spreads and macrovascular invasion. Some other ones recommend to be careful and suggest its use only in selected patients with Child A cirrhosis and segmental portal vein thrombosis
TACE can be a valid alternative for advanced HCC patients who are not compliant with oral therapies or have severe side effects or could not have access to sorafenib because of health authorities or high cost
In advanced HCC, TARE shows survival rates similar to sorafenib and TACE, especially in patients with portal vein thrombosis
TARE contraindication: important arterial shunt to gastrointestinal tract or lung, any contraindication to catheterization

TACE: Trans-arterial chemo-embolization; TARE: Trans-arterial Radio Embolization; HCC: Hepatocellular carcinoma; BCLC: Barcelona clinic liver cancer.

endothelial growth factor receptors 1, 2, and 3 and platelet-derived growth factor receptor β ^[30-32]. Sorafenib, according to technical schedule, can be prescribed in patients with preserved liver function (Child-Pugh A) and it should be orally administered at 800 mg/die (400 mg twice a day). The therapy should be carried on until disease progression or unacceptable adverse effects (AE) occur^[33]. Fatigue, diarrhea, hand-foot syndrome, bleeding, arterial hypertension and hepatic toxicity (represented by the elevation of transaminase and/or bilirubin) are some of the most frequent AE observed during treatment, and can compromise the quality of life during a therapy that in any case is palliative^[34,35]. Sorafenib treatment cost varies from about 2600 to 5300€ per month, depending on the dose (400 mg/die vs 800 mg/die), with a mean cost about 4079 United States dollars per month^[36].

Although sorafenib is the only drug which has indication for advanced HCC, only a few patients obtain a real benefit from this therapy. In general, the outcome and the extent of therapy is also linked to liver function: Child B patients have lower survival than Child A ones^[37].

In the two largest studies published so far, "SHARP" and "Asia-Pacific", the main objective tumor response ratio according to Response Evaluation Criteria in Solid Tumors (RECIST) was only 2%-3% in the sorafenib group patients, and a stable disease was observed in 34%-43% of patients, with an OS only three months longer than placebo group^[17,18]. In fact, in the first of these phase III studies conducted comparing sorafenib (at 800 mg/die) and placebo with a double blind fashion on a total of more than 600 patients with advanced HCC^[17], this drug showed a significant improvement in terms of OS (median OS 10.7 mo vs 7.9 mo of the placebo control group) and of time to progression, but the number of partial responses in the treatment group

was low (7 out of 299)^[17,38,39].

The increase in median OS was confirmed also in the second of the two abovementioned studies, conducted in China, Taiwan and South Korea on 226 advanced patients: mean OS was 6.5 mo in the treatment group against a 4.2 mo in the placebo arm^[18]. Unfortunately the development of AE can reduce the compliance to therapy and worsen patient prognosis: in the SHARP study the incidence of AE was 70%-85% (vs 43%-60% in the placebo groups) but severe effects were observed in 9.4%-14.6% of patients^[17]. The median duration of treatment was 5.3 mo (range, 0.2 to 16.1) and 176 of the patients in the sorafenib arm discontinued the study because of AE^[17]. In both studies the most common significant AE causing a drug dose reduction (from 800 to 400 mg/die) were Hand-Foot Syndrome (10%-11% of patients) and diarrhea (5%-7%)^[18].

Recent studies suggest that a dose reduced regimen of 400 mg/die could be equally effective in prolonging OS^[40]. This data should advise the use of a "softer" regimen in patients who are more likely to develop AE during sorafenib treatment (*e.g.*, Child B7, elder patients). In those cases sorafenib could be started at reduced dose, *e.g.*, 400 mg/die, and "ramped up" to 600 or 800 mg/die if the patient shows a good profile of tolerability. Post-marketing clinical studies showed that only a portion of patients actually benefits from sorafenib treatment (Figure 2), and an even smaller percentage of patients treated shows partial/complete response on follow-up examinations (Figure 3), up against relevant costs and an incidence of drug related AE probably higher (24%-28% of severe AE) than reported in the SHARP and Asia Pacific studies^[17,35,41].

Because of the problems related to the poor effectiveness of sorafenib and because of its cost, many studies tried to compare sorafenib to other commonly used treatments for unresectable HCC. Although, according to BCLC, TACE has no indication for advanced HCC, a study comparing this two different therapeutic options reported similar benefits from TACE and sorafenib in advanced stage HCC^[42].

Association therapy of TACE and sorafenib has been investigated in some recent works that showed good results in term of safety and efficacy in BCLC-B patients^[24,43], but its therapeutic role in BCLC-C patients is still unclear. In fact, most of the studies have shown that association therapy may improve time to progression, but it does not seem to improve OS if compared to TACE alone^[44-47]. Conversely, Bai *et al.*^[48] have found some benefits in terms of OS, in patients treated with sorafenib plus TACE. This combination finds its theoretical physiological basis on the anti-angiogenic effect of the drug, in contrast with the physiological release of angiogenic factors consequent to the arterial iatrogenic obstruction^[30]. Nevertheless the results about this kind of treatment are still uncertain^[44].

In a recent study sorafenib has also been compared to TARE: median OS was similar in the two groups^[49].

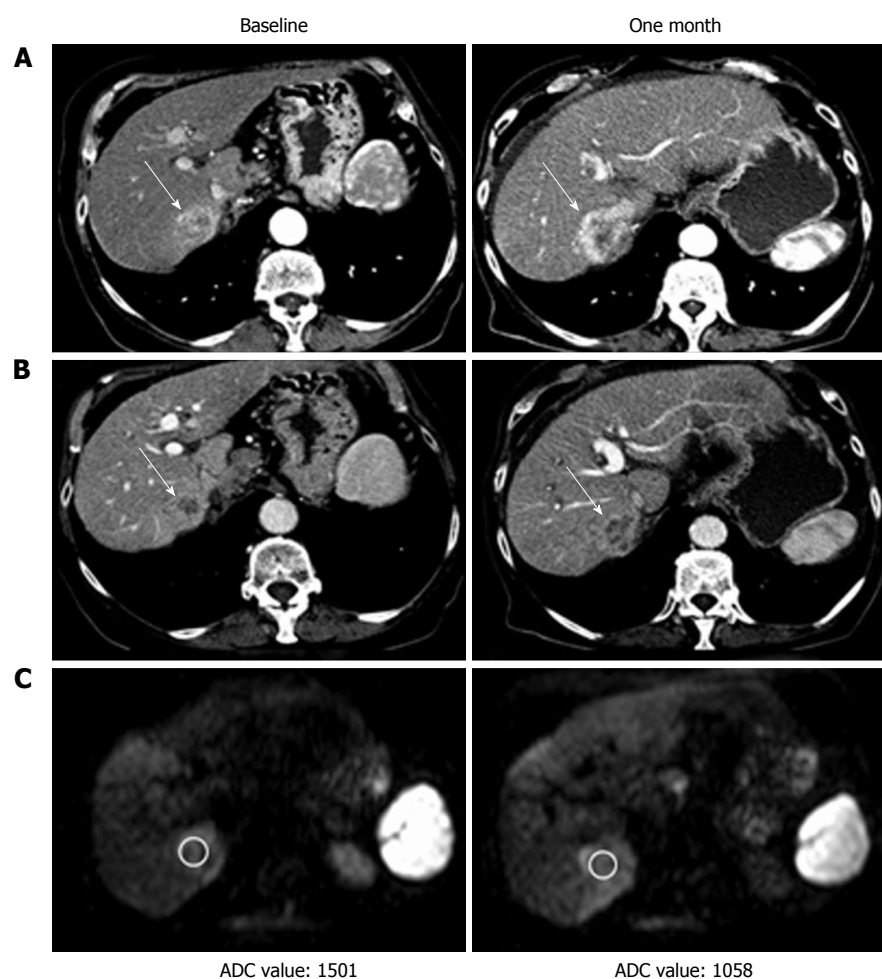


Figure 2 Computed tomography and magnetic resonance imaging examination at baseline and one month after the start of sorafenib therapy of patient showing progressive disease. A: Arterial phase computed tomography (CT); B: Venous phase CT; C: Magnetic resonance imaging diffusion weighted imaging. ADC: Apparent diffusion coefficient.

The extension of portal invasion resulted to be an important prognostic factor for the good result of TARE since patients with partial portal invasion of a branch of the vein had better prognosis than those who had disease extended to the main trunk^[50]. The association of TARE and sorafenib has been investigated and showed good results in terms of safety, although data about OS with this combined therapy are still being investigated^[51]. The physiological basis to combine these two therapies is that sorafenib seems to decrease the risk to develop a new lesion or distant metastasis, while TARE is more efficient in controlling primary hepatic lesion.

Ravaioli *et al.*^[52] reported two cases of advanced disease HCC that became suitable to liver transplantation after TARE treatment. TARE ability to downstage tumor has also been reported by other authors^[53].

Over against its apparent simplicity, the treatment with sorafenib owns relevant open issues that can make the management problematic for the clinician. In fact, to reach a real benefit for the patients and to obtain a proper allocation of the money resources, it is crucial to identify a suitable method to evaluate response and hopefully early predictors of response and survival.

BIOCHEMICAL RESPONSE EVALUATION PARAMETERS

According to reported data we deduce that sorafenib therapy does not improve the prognosis in all advanced HCC patients and a part of responders have not such an important benefit to justify an expensive and rich in terms of AE therapy. Therefore, one of the primary objectives is to identify some biomarkers that may predict the efficacy of sorafenib treatment and may help the clinicians to select possible responder patients.

To clarify this point, many studies have focused on serum anti-angiogenic factors concentration; in particular, in the SHARP study, Llovet *et al.*^[17] found that low baseline concentration of vascular endothelial growth factor-A (VEGF-A) and high baseline concentration of Ang-2 correlated with a better OS in both arms of the study (sorafenib and placebo group). These data suggest that VEGF-A and Ang-2 are independent prognostic factors, but they have not a straight correlation with sorafenib therapy efficacy^[54]. Similar results were shown in another study on patients treated with sorafenib and metronomic tegafur/uracil^[55]. The possible role of some cytokines

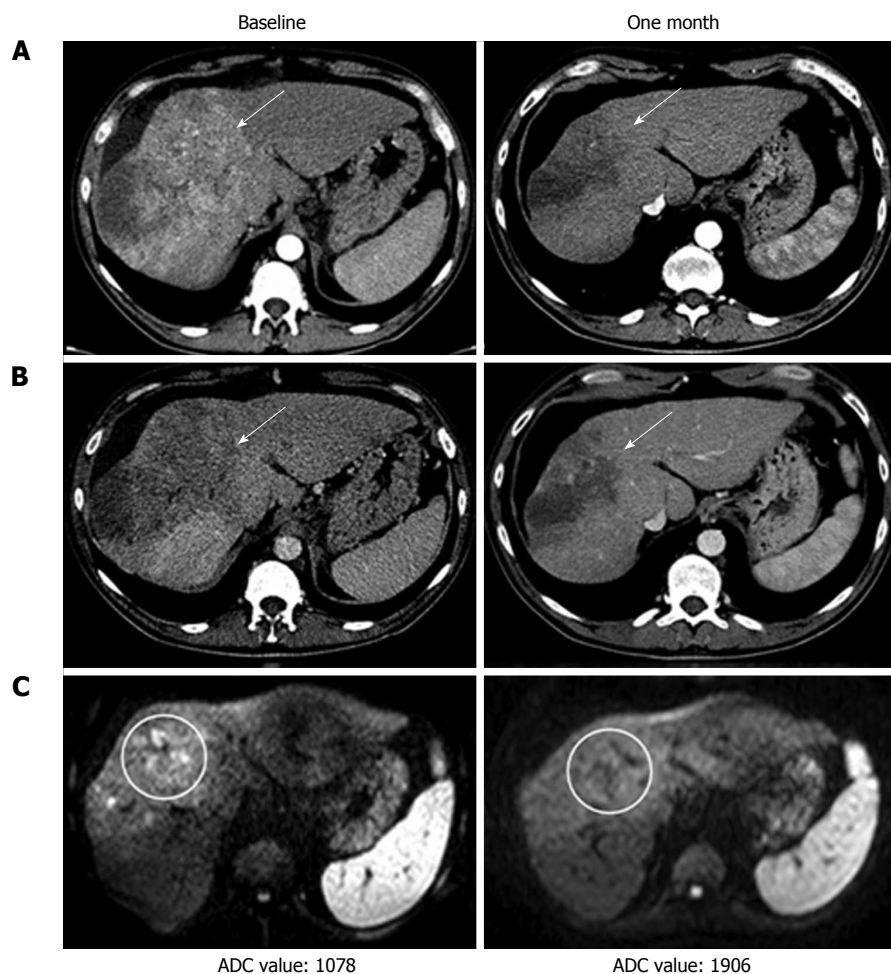


Figure 3 Computed tomography and magnetic resonance imaging examination at baseline and one month after the start of sorafenib therapy of a patient showing partial response. A: Arterial phase computed tomography (CT); B: Venous phase CT; C: Magnetic resonance imaging diffusion weighted imaging. ADC: Apparent diffusion coefficient.

[interleukin 6 (IL-6)/IL-8] as predictive biomarker of sorafenib treatment efficacy has also been evaluated, but no significant results have been found^[56]. Some interesting, but preliminary results have been found using insulin-like growth factor-1 (IGF-1) baseline serum concentration: high IGF-1 blood levels seem to correlate with a better OS during anti-angiogenic therapy^[57]. In the last years, great interest was devoted on serum alpha-fetoprotein (AFP) levels in HCC patients during systemic therapy: high basal levels of AFP generally correlate with a poor prognosis, both in intermediate and advanced HCC^[54]. Personeni *et al.*^[58] analyzed a cohort of 85 patients treated with sorafenib and individuated a significant association between the decrease of > 20% in AFP in the first 8 wk and OS. Similar results have been found in other studies^[59,60]. An important problem in the use of AFP as a biomarker is the difficulty in establishing a reference of percentage decrease (relatively to baseline values) as a cut-off to assess a response to therapy; in fact, an accepted worldwide threshold has not been defined, and the choice of this cut-off differed in the various studies, usually between 20% and 50%. Moreover, measuring the early change in AFP level seems

to be a valid predictive factor only for patients who have higher baseline AFP serum level. For this reason, some authors suggest that only patients with pre-treatment AFP level > 200 microg/L are suitable for this analysis^[61]. Despite the key role of AFP in diagnosis and follow-up of HCC, the effectiveness in outcome prediction during anti-angiogenic treatment is not clear yet, and needs to be evaluated in future.

In general, countless field-practice studies have analyzed the possible role of other biochemical and clinical parameters in early evaluation of response to sorafenib^[40,62-67], *i.e.*, aspartate transaminase, alkaline phosphatase basal and on-going levels, as well as the development of AE such as hand-foot syndrome or diarrhea have been related to a significantly prolonged OS, that represents the ultimate goal of treatment in patients with advanced HCC.

IMAGING RESPONSE EVALUATION PARAMETERS

Evaluation by imaging is another important tool and is usually performed every 2-3 mo during sorafenib

treatment^[68] by dynamic imaging (CT or MR contrast enhanced scan), applying the modified RECIST (mRECIST)^[69].

The introduction of the mRECIST radically changed the approach to treatment response evaluation. While RECIST 1.1 is principally based on lesion dimensions without any consideration for tumoral vitality, mRECIST introduced the evaluation of the actual vital part of the lesions, which is the one that shows contrast enhancement at CT or MR.

Although the efficacy of mRECIST in tumor response evaluation in comparison with old RECIST 1.1 during sorafenib treatment has been recently confirmed by different studies^[70,71], these criteria, based on vital lesions size measurements in time, still have some limitations. In fact, since sorafenib mainly operates through an anti-angiogenic effect, considering only the diameter of the vital portion is inadequate for a proper response evaluation. Some other parameters, able to quantitatively assess intralesional vitality or vascularization, are necessary to integrate mRECIST in order to make tumour response evaluation more reliable. It is proven that not all tumour progressions at imaging translate into a decreased OS and some improvements in prognosis have been shown in absence of tumour burden reduction^[17,72]. This means that, even considering the increase in median OS, only a part of patients actually shows appreciable benefits, and those whose life expectancy is increased by the treatment are difficult to individuate since they rarely show a decrease in terms of lesion size/conspicuity. In other terms, the response does not correlate, at least initially, with a change in lesion dimension, but more probably it brings some intralesional decrease in cellularity and/or vascularization changes^[30,72,73].

In this direction, the analysis of new radiological parameters in evaluation of response to sorafenib has shown promising results, and many attempts to evaluate different tumoral characteristics, such as intralesional perfusion and cellular density, have been performed so far.

Perfusion weighted imaging (PWI) is a relatively new MR/CT technique for qualitative and quantitative evaluation of the delivery of blood to biological tissues^[74]. The importance of local changes in blood flow, angiogenesis and capillary permeability in cancer progression and treatment motivate the researchers' increasing interest in PWI. The primary mechanisms for the cancer lesions enhancement are the filling of the vasculature with CA enhanced blood, and the diffusion of this CA from the blood into the extravascular-extracellular interstitial spaces; these phenomena are increased by tumoral angiogenesis. An increase in blood flow leads to a more rapid CA filling of the vessels, with faster changes in signal intensity/density while a greater blood or extravascular-extracellular volume will increase the fraction of the voxel to be filled with CA^[74,75]. In tumoral lesions the level of peak enhancement and the rate of passage of the extravasated CA back to the vessels, with a

return of signal intensity/density to its baseline values, is altered. In order to use image signal intensities to track and analyze enhancement dynamics, in PWI it is necessary to form a temporally resolved series of images (multiple acquisitions on the same area) that tracks the signal/density changes in different times after the CA administration, in analogy to tracer studies in nuclear medicine^[75,76]. CT PWI parameters evaluation have shown significant changes during sorafenib treatment, in particular with a reduction in intralesional mean transit time as possible consequence of the anti-angiogenic effect of the drug^[68,76].

Simple parameters, which indirectly correlate with intralesional vascularization have also been elaborated: Ronot has recently presented that in follow-up during sorafenib treatment the use of CHOI criteria, based on intralesional density on arterial phase CT acquisition, has shown promising potentials in terms of tumor response evaluation, comparable to those of mRECIST, although with minor reproducibility^[71].

Studies on perfusion changes during therapy were also developed in ultrasonography. Contrast enhanced ultrasound, a technique which is now available in a large number of centers, and that can be repeated more than once in the first weeks from the beginning of therapy has shown, despite some major limits (such as operator dependency and partial liver volume exploration) some promising results in early response evaluation during sorafenib treatment, since it is able to evidence changes in target lesions enhancement during treatment^[77].

The role of MR diffusion weighted imaging (DWI) in response assessment has been evaluated as well with controversial results^[78-80]. This MR technique is based on water diffusion, which is the inconsistent and random microscopic motion of molecules caused by thermal energy, also known as Brownian motion. Even the more basic DWI principles description is beyond the aim of this review. It is sufficient to know that DWI indirectly describes the cellular density and the architectural changes of a tissue^[81,82]. In fact, if within a tissue or a tumor several cells and many architectural barriers are present (as fibrosis, edema, any type of disorders or derangements), water molecules have difficulties in free movements and so "diffusion" is low (and, in general, signal intensity increases). On the contrary, if the cellular density is low and environment homogeneous, water molecules freely move, "diffusion" is easy and in general signal intensity decreases^[81]. DWI technique could then be able to show some intralesional changes that are not evident on standard CT/MR scans. As regards to early assessment by DWI, in general, some studies conducted on different tumoral lesions have shown that apparent diffusion coefficient (ADC) changes in the first few weeks of treatment may precede dimensional reduction since, early after the start of treatment, changes in cellularity and necrosis may occur^[83-85]. Conversely to what has been observed in solid cancers during chemotherapy treatment^[86], Schraml *et al.*^[79] found an unexpected decrease in

Table 2 Main systemic therapies in advanced hepatocellular carcinoma treatment**Systemic therapies**

The only drug approved for the treatment of advanced HCC. Patients treated with sorafenib have longer OS than placebo group in the two largest studies

The efficacy of this treatment is linked to liver function: Child B patients have much lower survival than Child A ones (5.5 mo *vs* 11.3 mo). Child C patients have very poor prognosis and seem not to be suitable for sorafenib therapy (1.6 mo)

Patient treated with sorafenib has longer survival than those treated with sunitinib. No difference in OS has been found comparing sorafenib treatment to brivanib

Some combination therapies have been proposed, but none of these has shown superiority compared to sorafenib alone

At now there is no therapeutic plan approved as second line in advanced HCC pretreated with sorafenib

Some drugs as capecitabine, brivanib, sunitinib, everolimus have been tested in monotherapy, moreover some combination therapies as erlotinib with sorafenib, and gemcitabine with oxaliplatin have been evaluated as second line options, but all of them have not given significant results

Many studies are still in progress and some interesting, but preliminary results have been obtained in patients with high expression of c-met in treatment with brivanib

HCC: Hepatocellular carcinoma; OS: Overall survival.

HCC mean intralesional ADC values in the first 3-4 wk of sorafenib therapy (maybe due to some micro-hemorrhagic intralesional injury), with a subsequent increase at 3 mo evaluation^[79].

Also in case of DWI, the main limitation remains the large variability of data (both in different acquisitions and in different centers and scanners), which reduces the reproducibility of this technique^[87,88]. However it has also been demonstrated that timing of imaging is relevant: changes in ADC could precede changes in tumor size but may even disappear after a certain time because of repair mechanisms such as edema decrease and necrosis organization^[89,90]. The early changes in intralesional ADC described by Schraml *et al.*^[79] in advanced HCC could be expression of some intralesional temporarily changes, preceding an eventual dimensional reduction and expressing a possible sensitivity to sorafenib action^[79].

Until now, none of the aforementioned radiological technique has been positively tested in a large number of patients, but the good results obtained so far are suggestive for a possible integration of some of these parameters to standard follow-up and response evaluation.

Even more important would be the prediction of the response based on pre-treatment examinations. This continues to be controversial. From a general point of view, tumors with necrotic areas, often surrounded by hypoxic but viable cells, were shown being less sensitive to ionizing radiation^[91], more prone to aggressive behavior and probably less sensitive to cytotoxic agents^[92]. In case of HCC, on the contrary to what reported for other solid tumors, higher ADC values on DWI baseline

images could be related to a minor cellular density and a higher vascularization, and this could be somehow an index of treatment sensitivity (particularly in case of anti-angiogenic drug such as sorafenib itself), while low levels of intralesional ADC could correlate with a worse prognosis a poor response to treatment, as shown by some studies, since they could be expression of a poorly vascular lesion with high cellular density^[80]. In these terms also a CT/MR pretreatment evaluation could give some additive information about tumor cellular density and vascularization, and maybe help stratifying patients in terms of anti-angiogenic therapies sensitivity.

Data available in this field are still limited and controversial, but more researches will certainly be made, as being able to identify patients with high probability of response before or shortly after the start of the therapy is strongly desirable.

Even if the first encouraging results will be confirmed in a larger scale, the addition of CT/MR perfusion parameters evaluation to a routinely liver study and then the quantitative evaluation on a per patient basis is not possible yet. The main problems related to perfusion studies are some technical difficulties and the acceptable, but suboptimal reproducibility of these parameters, particularly with MR; while the greatest limitation in DWI use is the mentioned large standard deviation of the measurements and then the low reproducibility^[93,94].

SORAFENIB FAILURE AND SECOND-LINE THERAPIES

As already mentioned, no other systemic treatment other than sorafenib have, so far, shown the capability to improve the OS in patients with advanced HCC.

Despite the results in terms of survival during treatment, only a very small percentage of patients actually shows benefits in terms of radiological staging^[17,18], so it is still discussed whether sorafenib treatment should actually be prolonged also in case of tumor progression at first follow-up examinations^[56,95]. Anyway, even in case of evident benefits from the treatment, most of the patients experience a loss of efficacy of the drug during time^[96].

There is then a strong request from clinicians for an established second line therapy to propose to patients when sorafenib cannot be administered or has to be interrupted due to AE or loss of efficacy (Table 2).

Metronomic capecitabine has been largely used as second line treatment in patients showing progressive disease after sorafenib treatment mainly because of its high tolerability^[97].

In the randomized controlled trial that compared brivanib *vs* placebo as second-line therapy after sorafenib failure^[98], the improvement of time to progression observed in brivanib arm did not translated in an increased OS^[72]. An interesting phase III trial comparing sunitinib with sorafenib has shown similar results in terms of time to progression between the two drugs, but

with worse results for sunitinib in terms of survival^[99]. The use of brivanib and the combination of erlotinib with sorafenib have also been tested but failed in phase III trials^[72,100-102].

From ongoing studies, the most promising results come from the observation of a significantly better outcome in patient with high expression of c-met treated with tivantinib^[103]. From these data, a large phase III trial in second line is currently ongoing.

Although, it has been demonstrated that HCC patients who respond to TACE usually have poor response to a subsequent sorafenib treatment^[104], as we mentioned above the possible role of the synchronous use of both therapies is also being investigated^[105].

CONCLUSION

Advanced stage HCC is a category of disease defined by clinical, functional and radiological parameters, comprehending a wide range of patients with different general conditions, but with poor prognosis and life expectancy.

Since 2008 the main option for this stage of disease is represented by systemic treatment with sorafenib, that mainly shows an anti-angiogenic effect.

Although the treatment has shown an increase in OS in different studies, only a part of patients actually shows some benefits with a little percentage of partial response, while the incidence of drug related significant AE and the economic costs are high.

Being able to properly differentiate responder from non-responder patients as early as possible is then a pivotal challenge and could spare several patients a therapy often difficult to bear, directing them to some other second line treatment, at now under investigation.

For this reason, some supplemental parameters as biochemical and radiological prognostic factors are being searched for. In particular, finding some parameters quantitatively describing perfusion grade, and then able to predict the sensitivity of the lesions to anti-angiogenic agents could help in stratifying patients in terms of treatment responsiveness before the beginning of the therapy itself.

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Percutaneous microwave ablation vs radiofrequency ablation in the treatment of hepatocellular carcinoma

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steatohepatitis and liver autoimmunity. Surgical resection and orthotopic liver transplantation have curative potential, but fewer than 20% of patients are suitable candidates. Interventional treatments are offered to the vast majority of patients. Radiofrequency (RFA) and microwave ablation (MWA) are among the therapeutic modalities, with similar indications which include the presence of up to three lesions, smaller than 3 cm in size, and the absence of extrahepatic disease. The therapeutic effect of both methods relies on thermal injury, but MWA uses an electromagnetic field as opposed to electrical current used in RFA. Unlike MWA, the effect of RFA is partially limited by the heat-sink effect and increased impedance of the ablated tissue. Compared with RFA, MWA attains a more predictable ablation zone, permits simultaneous treatment of multiple lesions, and achieves larger coagulation volumes in a shorter procedural time. Major complications of both methods are comparable and infrequent (approximately 2%-3%), and they include haemorrhage, infection/abscess, visceral organ injury, liver failure, and pneumothorax. RFA may incur the additional complication of skin burns. Nevertheless, there is no compelling evidence for differences in clinical outcomes, including local recurrence rates and survival.

Key words: Microwave; Radiofrequency; Ablation; Hepatocellular carcinoma; Percutaneous

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Core tip: Hepatocellular carcinoma (HCC) is a common neoplasia with high morbidity and mortality. Nowadays, technologic progress has led to several diagnostic and therapeutic challenges regarding HCC, including the optimal use of percutaneous ablation methods, defining their indications and assessing the survival impact. Both radiofrequency and microwave ablation are widely used with their respective advantages and may both offer palliation or cure in the context of a multifaceted treatment approach.

Abstract

Hepatocellular cancer ranks fifth among cancers and is related to chronic viral hepatitis, alcohol abuse,

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EPIDEMIOLOGY OF HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is the most common primary liver neoplasia and a protean disease with a poor prognosis. Its incidence is estimated to range from 500000 to 1000000 cases annually, ranking it fifth across cancers worldwide^[1] and third as cause of death from neoplasia^[2]. HCC is more prevalent in Asia due to hepatitis B virus (HBV) infection endemicity and among males aged between 30 and 50 years^[3]. According to National Comprehensive Cancer Network, patients at risk for HCC are those with cirrhosis related to HBV, HCV, alcohol abuse, hereditary haemochromatosis, non-alcoholic fatty liver disease, stage 4 primary biliary cirrhosis, alpha 1 antitrypsin deficiency, or exposure to aflatoxins. The incidence of HCC has increased in the United States from 1.6 to 4.9 cases per 100000^[4], and this increase is expected to continue. Plausible reasons include the effects of the HCV epidemic as well as the rise in Non-Alcoholic Steatohepatitis-associated HCC cases^[4].

DIAGNOSIS AND SURVEILLANCE

Imaging is important at all stages of diagnosis, therapy and follow-up of patients with HCC. The diagnostic modalities used in the diagnosis, treatment planning, management and follow-up of HCC are ultrasonography (US), computed tomography (CT) scanning and magnetic resonance imaging (MRI)^[5]. The European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Disease suggest US as the preferred modality for bi-annual surveillance of patients at high risk of HCC^[6].

The most characteristic imaging findings of HCC on contrast-enhanced CT and MRI studies are arterial enhancement, contrast washout and pseudocapsule bright enhancement on portal, venous and delayed phase^[7]. Heterogeneity, central necrosis and abnormal internal vessels are characteristic findings of large HCCs^[8].

The prognosis and treatment decisions of solid tumours are generally related to tumour stage. However, prognosis for HCC patients also depends on the underlying liver function. Currently, the Barcelona Clinic Liver Cancer (BCLC) staging system^[9] is widely used in clinical practice and in clinical trials. It is a staging system that also assigns treatment based on tumour stage, liver function, performance status, and treatment intent^[10].

Since most HCCs develop in the setting of chronic liver disease, the risk of death involves tumour and non-tumour related factors. An HCC diagnosed as symptomatic disease has a disappointing 5-year survival of 0% to 10%^[11], as opposed to early detection of small HCCs by surveillance which may be amenable to cure. The best case scenario is for a malignant nodule to be found before reaching 2 cm in size. It is crucial to diagnose HCC at an early stage, given that major advances are unlikely to emerge from treating late stage disease.

STANDARD TREATMENT

HCC treatment has a short time window before end-organ liver dysfunction leads to increased complications rate and mortality. In past years, diagnosis of HCC was made at advanced stage, with symptomatic disease and various extent of liver function compromise. As a consequence, no treatment (whether surgical resection or systemic chemotherapy) provided significant curative potential or the substantial capacity to prolong survival and the associated morbidity. Owing to the surveillance guidelines currently in place, early detection is now common, liver function is adequately preserved, symptoms are absent and several treatment options are feasible^[12].

The standard treatment options of HCC consist of surgical resection, orthotopic liver transplantation, ablation, transarterial therapies (chemoembolization or radiotherapy) and chemotherapy and notably targeted molecular therapies.

Therapies with curative potential include hepatectomy, liver transplantation and percutaneous thermal ablation. The remaining options are mostly palliative, with a non-curative intent but with a positive impact on survival. For patients with solitary HCC or early multifocal disease and decompensated cirrhosis, the optimal choice is liver transplantation^[13,14]. The Milan criteria applied in liver transplantation require a solitary lesion < 5 cm or up to three lesions < 3 cm^[15].

Surgical resection may be warranted for patients that either do not have cirrhosis or have cirrhosis with residual liver function, normal bilirubin and hepatic vein pressure gradient < 10 mmHg. Five-year disease-free survival estimates exceeding 50% have been described for resection and liver transplantation^[16,17].

Systemic chemotherapy has limited activity and is outweighed by frequent toxicity and lack of significant survival benefit^[18]. Molecular targeted approaches include sorafenib, a multikinase inhibitor which has prolonged overall survival rates over placebo in a recent study^[1]. Expert opinion is mandatory for the selection of candidates and their assignment to different treatments.

INTERVENTIONAL TREATMENTS

Few patients (less than 20%) are amenable to

resection and transplantation due to difficulties related to size, location and number of tumours, vascular and extrahepatic involvement and functional hepatic reserve due to cirrhosis. The ultimate treatment choice for the remaining 80% is interventional therapies. In patients with early- or intermediate-stage disease, interventional therapies could control disease progression until definitive therapy or increase the patient's eligibility for a curative treatment. In advanced disease, the main aim of treatment is to control symptoms, prolong survival, and improve quality of life^[19]. Available interventional therapies include direct ablation, transarterial embolization or chemoembolization (TACE), drug-eluting beads and transarterial radioembolization.

Ablation involves the use of chemicals or thermal energy delivered directly to the tumour to achieve necrosis. The types of thermal ablation available are hyperthermic [radiofrequency ablation (RFA), microwave ablation (MWA), and laser ablation] and hypothermic (cryoablation).

Percutaneous thermal ablation, either RFA or MWA, is considered the optimal locoregional treatment choice for focal unresectable HCC of early stage, but its use has been proposed for several other clinical scenarios such as the reduction of the tumour burden and as a bridge to transplantation^[20,21].

RFA VS MWA: PRINCIPLES AND APPLICATION

In RFA, an electrical current in the radiofrequency range is delivered through a needle electrode under imaging or surgical guidance, producing heat-based thermal cytotoxicity^[22]. A complete electrical circuit is created and completed through grounding pads attached to the thighs or back. Temperatures range between 60 °C to 100 °C and result in almost instant coagulation necrosis^[23]. These temperatures are observed near the electrode resulting in a small area of necrosis, with the larger portion of the final ablation zone being attributed to thermal conduction into more peripheral areas around the electrode^[24]. Tissue boiling and charring act as electrical insulators and limit the effect of RFA through increased impedance; hence, the important tissue properties for RFA are electrical and thermal conductivity^[24]. Radiofrequency ablation is also moderated by the heat-sink effect, a phenomenon that occurs when thermal energy is dispersed from the target lesion due to blood flow in the vessels adjacent to it^[25]. Consequently, the shape and size of the ablation zone may be unpredictable and the efficacy of RFA may be restricted as multiple sessions are necessary for complete tumour eradication^[26]. In order to attain larger necrosis volumes, numerous innovative electrode modifications are applied, such as expandable electrodes or internally cooled electrodes as well as multiple electrodes. The result is ablation zones of lesions up to

2-5 cm. A margin of 0.5-1.0 cm of healthy liver tissue is mandatory to be ablated in order to secure treatment of the peripheral tumour, including any microscopic extension beyond the radiographically visible margins^[27].

RFA is more effective in HCC than in liver metastases due to the so-called "oven effect". Owing to cirrhosis and its pseudocapsule, the surrounding fibrotic liver of HCC functions as an oven, and higher peak temperatures with prolongation of the duration of cytotoxic temperatures are achieved within the tumour^[28].

MWA uses electromagnetic energy (up to 2 cm surrounding the antenna); in the absence of current flow, the electromagnetic field creates a rapid and homogeneous heating of tissue and subsequently coagulation necrosis. The best heating effect is achieved in tissues with a high content of water and the worst is observed in fat^[24]. Another mechanism of MWA function is ionic polarization with conversion of kinetic energy into heat. A more homogeneous, larger ablation zone that is easily predicted is feasible and the heat-sink effect is attenuated^[29,30]. One reason for the reduced heat-sink effect may be the faster heating and higher temperatures provided by microwave energy. Notably, the ablation heat beyond the microwave field is conducted in a similar way as in RFA with the heat-sink effect still present^[31]. Another consequence of the different production of heat seen with MWA is that the time needed for ablation is less in MWA than that required in RFA.

MWA equipment consists of a generator and a monopolar electrode connected to the generator that is introduced to the lesion through an access needle, applying a coaxial technique^[32]. The devices use frequencies higher than 900 MHz (in the United State 915 MHz and 2.54 GHz). Microwaves of 915 MHz can penetrate more deeply than 2450 MHz microwaves^[33]; thus, the low frequency MWA may theoretically result in larger ablation zones. To prevent skin burns at the insertion site, internal circulation of fluid or carbon dioxide through the needle shaft is applied achieving continuous cooling^[34]. As opposed to RFA, MWA permits the simultaneous treatment of multiple lesions with multiple electrodes that can produce larger ablation volumes. Each microwave application can produce a discrete focus of approximately 1.6 cm of necrosis for 120 s at 60 W^[32]. In contrast to RFA, grounding pads are not needed because the completion of an electrical circuit is not required. Therefore, the presence of metallic materials like surgical clips or a pacemaker does not constitute a contraindication.

These advantages of MWA are also its flaws. The higher thermal efficiency of MWA can easily injure the adjacent critical tissues because the tissue surrounding the antenna may be ablated rapidly. Simultaneous deployment of multiple probes of microwave antennae can significantly increase the diameter of the ablation zone, whereas recession of the coagulation zone for the inter-antenna distance may not entirely cover the

Table 1 Comparison of radiofrequency over microwave ablation methods

RFA	MWA
Electric current	Electromagnetic energy
Grounding pads (risk of burns due to ground pads)	No grounding pads (no risk of burns)
Tissue charring and boiling cause increase of impedance that reduce electrical and thermal conductivity	Rapid and homogeneous heating + ionic polarization
Lower intratumoral temperatures	Higher intratumoral temperatures
More peri-procedural pain	Less peri-procedural pain
Unpredictable ablation zone	More predictable ablation zone
Heat-sink effect	Less susceptible to heat-sink effect
Single lesion can be treated	Simultaneous treatment of multiple lesions
More procedural time	Shorter procedural time
Less ablation volume	Larger ablation volume
Similar complications and complication rate	
Surgical clips or pacemaker are contraindications	Surgical clips or a pacemaker not a contraindication

RFA: Radiofrequency; MWA: Microwave ablation.

large tumour and result in incomplete ablation^[35]. The summary comparison of the two methods is seen in Table 1.

EVOLUTION OF ELECTRODES

Since the most important disadvantage of RFA is that the temperature falls quickly as the distance from the electrode tip increases due to increased tissue impedance. Research has focused to the development of new electrodes that would overcome this limitation^[36]. The evolution in RFA ablation devices and technologies has improved the results of RFA in terms of achieving a larger necrotic burden. Expandable and multitined electrodes were initially introduced which are now widely used and are adequately studied with satisfactory results. Attempts to increase ablated lesion sizes have involved the use of perfused electrodes^[37], expandable-wet electrodes^[38], cooled-wet electrodes^[39,40] and saline-enhanced bipolar single electrodes^[41].

Another technological progress in electrodes is the use of bipolar and multipolar electrodes rather than the monopolar type. In monopolar mode, the current travels outward toward a dispersive pad and the heat is diverted from the ablation site in all directions. A bipolar electrode does not require a grounding pad since both electrodes are located inside one probe and the alternative current circuit is concentrated between the probes within the target lesion only^[42,43]. Additionally, one electrode is thermally shielded by the opposing electrode, an effect that results in active heating of the tissue in its proximity^[44]. The heating effect is trapped between the two electrodes, producing higher temperatures and larger ablation lesions. Haemmerich *et al.*^[45] demonstrated that bipolar modes showed an improved electric potential profile and temperature distribution as compared with the monopolar mode. Multipolar mode is based on simultaneous insertion of multiple, internally cooled bipolar probes^[46]. In bipolar mode, the two parallel probes should be inserted and the lesion must be between them; this is sometimes

technically difficult and can cause probe insertion-related complications. Moreover, there is no way of controlling the heat generated in the vicinity of the probes. Of note, in terms of technical effectiveness, Seror *et al.*^[47] showed that multipolar ablation of small HCC lesions improves the rate of complete necrosis during pathologic examination compared with monopolar techniques.

The introduction of MRI-compatible devices providing real-time control of tissue temperature proved a useful tool and signalled an evolution in ablation techniques^[48]. MRI is the only imaging modality that can provide quantitative and high spatial resolution real-time monitoring of rate-of-change temperature (and hence thermal dose) in the heated area, determining the cut-off point (or endpoint) for the application of power.

Microwave ablation is a highly effective modality, with its most important limitation being the heating of antenna shaft that results in reduced power delivery^[49-52]. Some manufacturers have introduced internal or external water-cooling systems of the antenna, at the expense of increased shaft diameter and complexity^[53,54]. A microwave ablation system has recently been introduced that can provide high power (140 W). It uses a small diameter antenna (17 gauge) as it incorporates a novel gas-cooling mechanism^[55].

CLINICAL STUDIES OF RFA IN HCC

RFA is indicated in patients with early HCC, as staged by BCLC, who are not eligible for surgical treatment due to comorbidities, and in patients who refuse resection or when there is a need to preserve liver function^[56]. The ablation success rate for lesions smaller than 2 cm reaches 90% with a local recurrence rate of 1%^[57]. For this reason, RFA is considered effective for tumours < 3 cm; combined locoregional treatment should be considered for lesions > 3 cm^[58]. RFA combined with TACE is recommended for tumours larger than 3 cm in diameter, but RFA may also be used for four or more nodules where applicable^[59].

The main contraindications of RFA are severe

bleeding diathesis (platelet count less than 50000/ μ L), haemostatic compromise, decompensated ascites, jaundice and presence of metallic devices such as pacemakers. Relative contraindications are lesions near the gastrointestinal tract, biliary system and heart. RFA should also be avoided for tumours within 1 cm proximity to the hepatic portal tract. Major complications include liver failure, bleeding, infection, abscesses, intercostal nerve injury, organ injury, tumour lysis syndrome and pneumothorax^[60]. In a multicentre study of RFA for malignant liver tumours in 2320 patients, the rate of major complications reached 2.2%^[61].

The technical effectiveness of RFA is evaluated with the use of contrast-enhanced CT or MRI. A tumour is considered successfully ablated by the lack of any enhanced regions during the arterial phase and the presence of at least a 0.5 cm margin of apparently normal surrounding hepatic tissue during the portal phase. An incomplete safety margin is shown to be an independent risk factor for local tumour progression on multivariate analysis. Nodular peripheral enhancement is suggestive of tumour viability^[59].

Local recurrence rates of small HCCs after RFA were reported within the range of 1.3%-12% at 1 year, 1.7%-24% at 2 years, and 3.2% at both 5 and 10 years. Factors correlated with local recurrence included larger tumour size (diameter > 2 cm or > 3 cm), tumour without encapsulation, poorly-differentiated HCC, sub-capsular location, an ablative margin of less than 1 cm and a nearby vessel that could induce a heat-sink effect. This increase in local recurrence is presumably due to unexplored peri-tumoral satellite nodules, insufficient safety margin, or incomplete ablation. Owing to underlying advanced liver disease in the presence of HCC, additional new recurrence is very common in patients with HCC^[62].

Complete tumour necrosis in early stage HCC is reported to be 80%-95% and 5%-year survival 33%-57%^[63]. According to some series, percutaneous RFA show 5-year survival rates of 48%-55% in early stage HCC, and 51%-64% in Child-Pugh class A cirrhosis^[64]. Patients with resectable tumours may have prolonged survival over those with non-resectable tumours; this is likely a reflection of the better physiologic state of patients deemed eligible for surgery^[65].

HCC appears most commonly in patients with cirrhosis. Since these patients are not usually considered ideal candidates for surgery, it is difficult to conduct a study comparing RFA against surgery in such patients. Most reports of percutaneous RFA for HCC are single-centre retrospective studies conducted among patients not eligible for resection. Resection remains the gold standard therapy in early stage HCC. The few published studies that compared RFA to resection showed no benefit in survival rates (overall or disease-free): 4-year overall survival of 67.9% for ablation vs 64% for surgery^[66]. Huang *et al*^[67] applied the Milan criteria (no more than one HCC of 5 cm or smaller, or

up to 3 HCCs measuring 3 cm or smaller) and patients were randomized to receive RFA or surgery. Significant differences were reported: 4-year and 5-year survival rates of 66% and 55% respectively for ablation vs 83% and 76% for surgery. Overall, recurrence was more frequent in the group of patients that were ablated. The limitations of this study lay in more patients being lost in follow-up in the surgery group^[23].

CLINICAL STUDIES OF MWA IN HCC

Indications and contraindications for MWA are the same as those for RFA, apart from the size of a lesion that can be ablated; according to most studies, MWA can treat 5-8 cm tumours^[68]. Furthermore, MWA allows simultaneous ablation of multiple tumours or even combined resection and ablation. In a multicentre effort that gathered data for patients treated with MWA for tumours of any origin, the advantages included the short total time of microwave application for each lesion (median: 4 min/lesion) and the fewer microwave applications for each ablated lesion (> 50% had one application and > 75% two applications). Of the 140 patients analysed, 114 (81.4%) patients received microwave alone, and 26 (18.6%) were treated with microwave combined with resection. Forty per cent of patients were treated with microwave for multiple tumours^[31].

Major complications include bile duct stenosis, bleeding, haemothorax or intrahepatic haematoma, peritoneal haemorrhage, liver abscess, colon perforation and tumour seeding^[68]. In another multicentre study, 736 patients with hepatic lesions underwent MWA; the reported rate of major complications was 2.9%. MWA was not proven to increase the risk of damage of vascular structures and/or bleeding. Minor complications included pain, post-ablation syndrome, and asymptomatic pleural effusions, which are usually self-limiting and do not require any further treatment. With the peri-procedural mortality rate being reported to be as low as < 0.01%, the safety of MWA was established^[69].

MWA shares a high rate of local recurrence in HCC with all other ablation modalities. Lee *et al*^[70] studied surgical MWA in tumours of 2-6 cm in diameter. All early postoperative CT imaging showed no residual lesions; however, on follow-up, 42% of patients experienced local tumour progression. As Lee *et al*^[70] noted, high local tumour progression is a drawback of MWA and can be attributed to the use of a large applicator (5 mm in diameter), which increases the risk of tumour puncture and subsequent tumour seeding.

Although MWA is a new method and the cumulative reported experience is limited, there is growing interest in this modality as a treatment choice of HCC that can yield promising survival results^[71,72]. The reported 1-year and 5-year survival estimates were 92.7% and 56.7%, respectively^[73]. A recent multicentre study from China documented that 1007 patients with primary liver cancer treated by MWA achieved 1-year and 5-year

Table 2 Comparison of clinical outcomes across published series of hepatocellular carcinoma patients for microwave ablation over radiofrequency

Ref.	Method	Guidance	Patients	Lesions	Mean age	Time	Size in cm	Complete ablation (%)	Local recurrence (%)	1 yr (%)	2 yr (%)	3 yr (%)	4 yr (%)	5 yr (%)	Median (mo)
Shibata <i>et al</i> ^[50]	MWA	Percutaneous	36	46	62.5	-	< 4	89	17.4	-	-	-	-	-	-
	RFA	Percutaneous	36	48	63.6	-	< 4	96	8.3	-	-	-	-	-	-
Xu <i>et al</i> ^[84]	MWA	Percutaneous	54	112	53.4	-	2.5 ± 1.1	94.6	7.1	-	-	-	-	-	-
	RFA	Percutaneous	43	78	53.4	-	2.6 ± 1.4	89.7	12.8	-	-	-	-	-	-
Simo <i>et al</i> ^[83]	MWA	Laparoscopic, US	13	15	59	8-10 min	2.31	-	-	-	-	-	-	-	7
	RFA	Laparoscopic, US	22	27	59	10-12 min	2.53	-	-	-	-	-	-	-	19
Lu <i>et al</i> ^[78]	MWA	Percutaneous, US	49	98	50.1	5 min	3 (25/49)	94.9	11.8	81.6	61.2	50.5	36.8	-	32.5
	RFA	Percutaneous, US	53	72	54.5	10 min	3 (32/53)	93.1	20.9	71.7	47.2	37.6	24.2	-	27.1
Qian <i>et al</i> ^[63]	MWA	Percutaneous, US	22	22	52	-	4.8	95.5	18	-	-	-	-	-	-
	RFA	Percutaneous, US	20	20	56	-	3.5	95	15	-	-	-	-	-	-
Zhang <i>et al</i> ^[86]	MWA	Percutaneous, US	77	105	54	8 min	< 3 (36), 3.1 to 5 (41)	86.7	10.5	92.2	-	51.7	-	38.5	-
	RFA	Percutaneous, US	78	97	54	6-20 min	< 3 (47), 3.1 to 5 (31)	83.4	11.8	91	-	64.1	-	41.3	-
Abdelaziz <i>et al</i> ^[79]	MWA	Percutaneous	66	-	53.5	-	2.9 ± 0.97	96.1	3.9	96.4	62	-	-	-	-
	RFA	Percutaneous	45	-	56.8	-	2.95 ± 1.03	94.2	13.5	67.6	47.4	-	-	-	-
Ding <i>et al</i> ^[81]	MWA	Percutaneous	85	98	59	10	< 3	98.5	10.9	98.7	92.3	82.7	77.8	-	45.34
	RFA	Percutaneous	113	131	58.6	12	< 3	99	5.2	98	90.7	77.6	77.6	-	52.99
Ohmoto <i>et al</i> ^[80]	MWA	Percutaneous	49	56	64	-	< 2	-	19	89	70	49	39	-	-
	RFA	Percutaneous	34	37	67	-	< 2	-	9	100	83	70	70	-	-

MWA: Microwave ablation; RFA: Radiofrequency; US: Ultrasonography.

survival rates of 91.2%, and 59.8%, respectively^[72,74]. For larger tumours, (> 5 cm) the reported 5-year survival rates ranged from 29% to 68.6%.

COMPARISON OF MWA VS RFA IN HCC

Obvious advantages of MWA over RFA include the capacity to heat charred tissues without increasing impedance and lower intratumoral temperature, as well as the lack of grounding pads that can cause skin burns. In addition, microwaves in animal studies seem to achieve larger ablation zones and faster ablation times^[34], thereby allowing tumours to be treated with fewer applicator insertions compared to RFA^[70]. The effect of MWA on perivascular tumours is also better because of the attenuated heat-sink effect^[75]; consequently, microwave ablation should be preferred for tumours near the hepatic veins and inferior vena cava.

RFA appears to be effective for lesions up to 3 cm distant from the vessels due to the heat-sink effect (efficacy: 97% and 5-year survival: 68%)^[76], while MWA seems to overcome size limitation of RFA treating lesions up to 7 cm, showing a faster ablation procedure, a reduced heat-sink effect and improved convection profile; however, MWA emerges as more appropriate for superficial lesions^[77].

The results concerning local disease control rate of MWA vs RFA are controversial. Early reports show similar rates for local tumour control for MWA and RFA. In a retrospective comparative study of 102 patients, a complete ablation rate of 95% was reported for MWA and 93% for RFA^[78]. On the other hand, Shibata *et al*^[50] performed a randomized trial in 72 patients and reported local control rates of 89% for MWA and 96% for RFA. Overall, the published studies support the comparability of the two methods in terms of overall survival, local recurrence and complication rates (Table 2), with some notable exceptions.

One study^[79] showed MWA to be superior in terms of local recurrence (3.9% vs 13.5% in the RFA group). On the contrary, Ohmoto *et al*^[80] and Ding *et al*^[81] describe fewer recurrences in the RFA group over MWA (9% vs 19% and 5.2% vs 10.9%, respectively). In the latter study, authors ascribe this difference to the fact that larger lesions were ablated (26.7% were > 3 cm in the MWA group as opposed to 15.3% in the RFA group). The size of the tumour lesion is a well-known factor associated with

local recurrence; it seems to have played a role in different results among studies.

Across studies, the survival rates were generally comparable for MWA over RFA groups, having being reported within the range of 68%-100% at 1st year and 24%-78% at 4th year.

EVALUATION OF RESPONSE

As locoregional therapies can increase tumour dimensions due to necrosis or haemorrhage, the role of tumour size quantification in assessing tumour response in this setting is limited^[15]. The modified RECIST and EASL criteria are applied to HCC. The EASL criteria were developed in 2000 for evaluating the HCC response to locoregional therapies. Residual viable tumour tissue is defined as the arterially enhancing tissue within the treated HCC and is measured to assess treatment response. The EASL criteria use bi-dimensional measurements and categorize response in a similar way to the World Health Organization guidelines. On the other hand, modified RECIST were proposed in 2010 which quantified the longest diameter of the enhancing part of HCC, assessed in the arterial phase of CT or MRI and measured to avoid any major areas of intervening necrosis^[82]. However, different liver tumours in the same patient may be treated at different points, and the lack of provision for that fact poses a significant limitation of all current criteria for quantifying liver tumour response to locoregional therapies. More specifically, the same patient may have both treated and untreated tumours. Nevertheless, knowledge of these criteria is necessary as they are part of a common language between radiologists and oncologists.

Evolving imaging biomarkers involve volumetric quantification, diffusion-weighted imaging of lesions and apparent diffusion coefficient values, lesion perfusion, MR spectroscopy and US and MR elastography^[83]. The use of positron emission tomography in the evaluation of treatment response is also increasing.

CONCLUSION

The great progress of oncology over the last few years now permits the treatment of more patients with advanced disease who were previously considered unfit for surgery or indeed any kind of palliative treatment. Locoregional treatments such as RFA and MWA constitute the backbone of interventional treatment in HCC, a malignancy that affects up to a million people per year worldwide. The two methods differ in their mechanism of action (RFA uses current as opposed to MWA that uses electromagnetic energy), with MWA having a more advantageous profile in terms of ablation volume, procedural time and simultaneous treatment of multiple lesions. However, with respect to clinical end-points, there is no solid proof as yet to support the advantage of one over the other. The evolution of devices and instruments coupled with the progress of multidisciplinary patient

management may allow a better stratification that would maximize treatment benefit.

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Risk for hepatocellular carcinoma in the course of chronic hepatitis B virus infection and the protective effect of therapy with nucleos(t)ide analogues

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Abstract

Hepatocellular carcinoma (HCC) is a major health problem worldwide, representing one of the leading causes of death. Chronic hepatitis B virus (HBV) infection (CHB) is the most important etiologic factor of this tumor, accounting for the development of more than 50% of the cases in the world. Primary prevention of

HCC is possible by hepatitis B vaccination conferring protection from HBV infection. However, according to the World Health Organization Hepatitis B Fact sheet N° 204 (update of July 2014) globally there exists a large pool of > 240 million people chronically infected with HBV who are at risk for development of HCC. These individuals represent a target population for secondary prevention both of cirrhosis and of HCC. Since ongoing HBV replication in CHB is linked with the progression of the underlying liver disease to cirrhosis as well as with the development of HCC, effective antiviral treatment in CHB has also been evaluated in terms of secondary prevention of HCC. Currently, most patients with active CHB are subjected to long term treatment with the first line nucleos(t)ide analogues entecavir and tenofovir. These compounds are of high antiviral potency and have a high barrier to HBV resistance compared to lamivudine, adefovir dipivoxil and even telbivudine. Many studies have shown that patients under antiviral treatment, especially those in virological remission, develop less frequently HCC compared to the untreated ones. However, the risk for development of HCC cannot be eliminated. Therefore, surveillance for the development of HCC of patients with chronic hepatitis B must be lifelong or until a time in the future when new treatments will be able to completely eradicate HBV from the liver particularly in the early stages of CHB infection. In this context, the aim of this review is to outline the magnitude of the risk for development of HCC among patients with CHB, in the various phases of the infection and in relation to virus, host and environmental factors as evaluated in the world literature. Moreover, the benefits of antiviral treatment of CHB with nucleos(t)ide analogs, which have changed the natural history of the disease and have reduced but not eliminated the risk of HCC are also reviewed.

Key words: Chronic hepatitis B; Cirrhosis; Hepatocellular carcinoma; Hepatitis B virus; Treatment; Interferon; Lamivudine; Adefovir; Entecavir; Tenofovir; Virological

remission; Nucleos(t)ide analogues

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Core tip: Hepatocellular carcinoma (HCC) represents a major health problem worldwide. It develops on the grounds of chronic liver disease, with chronic hepatitis B virus infection (CHB) being responsible for more than 50% of HCC worldwide. Currently, the vast majority of patients with CHB are being treated with nucleos(t)ide analogues, which have changed the natural history of the disease, reducing at a considerable extent its long-term consequences. However, although the risk of HCC has also been reduced, it has not been eliminated even after HBsAg loss or seroconversion. Therefore, constant surveillance, according to guidelines should never be omitted, unless new more potent treatment options are identified.

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INTRODUCTION

Hepatocellular carcinoma (HCC) represents a major health problem, being one of the leading causes of death worldwide with 782000 new cases diagnosed in 2008 and highest incidence rates being reported in East/Southeast Asia, North and West Africa^[1]. It is the 5th most common tumor in men accounting for 7.5% of the total number of tumors and the 9th most common in women (3.4% of the total number of tumors in them), while it stands as the 2nd commonest cause of death due to cancer in the world (746000 deaths in 2012)^[2]. It has a very bad prognosis with a 14% 5-year survival from diagnosis, being worse than lung, esophagus or stomach cancer and better only in comparison to the 5-year survival of 6% of pancreatic cancer^[3].

HCC usually develops on the grounds of chronic liver disease, particularly cirrhosis mainly due to the hepatitis B virus (HBV) or HCV, with HBV being responsible for more than 50% of the world cases of HCC, reaching 78% in areas of high HCC incidence^[4,5]. Patients with chronic HBV infection (CHB) are at a 100-fold higher risk for developing HCC compared to healthy individuals while HBV cirrhotic patients are at an even higher risk^[5,6].

From an historical point of view it is noteworthy that the link of HCC with chronic HBV infection was first pointed out in 1969 and 1970 in Europe, a geographical area in which HCC was previously thought to be extremely rare to practically non-existent. Up to those

days the etiology of HCC was linked with exposure to aflatoxin which was particularly common in Sub-Saharan Africa, an area of high HCC incidence. Thus, it came out of a surprise when in 1970 2 clinical reports one from England and one from Greece published in *Lancet* pointed out that Australia antigen, a marker of HBV infection, already referred in those days as hepatitis associated antigen, prevailed in patients with cryptogenic chronic liver disease and cirrhosis and that a significant percentage of such patients developed HCC^[7,8]. As stressed by Sherlock^[9] in 1971 up to those early days the link between HBV infection and HCC had been missed by B.S.Blumberg the scientist who had discovered HBsAg. This is obvious by the content of a letter he published in 1969 in *Lancet*^[10] despite his subsequent claims^[11]. However, regardless of the early European data on the possible link between chronic hepatitis B in the development of HCC, it was only in the mid 1970s that the etiological role of chronic HBV infection in HCC was unequivocally established thanks to the large prospective studies of Beasley *et al*^[6] in Taiwan. On the other hand, the early observations in Greece on the possible link between chronic HBV infection and HCC have stimulated further clinical research in the country aiming at the identification of risk factors, determinants and predictors of development of HCC^[8,12-15]. They have also been followed by epidemiological studies with positive results disclosing an association between the prevalence of HBsAg in the various geographical departments of the Country and the incidence of HCC in the same areas.

To sum up, HCC is a major health problem especially in patients with chronic liver disease and mainly CHB with or without cirrhosis, established since the early 70s and therefore, the possible elimination of this risk with oral treatment is considerably important.

RISK FACTORS

The risk for development of HCC in CHB differs significantly between the various areas of the world being highest in Asian and African patients^[16-20]. It is also higher in males than in females with CHB^[21-23], in patients older than 40 years and in cirrhotics compared to non-cirrhotic ones^[18,22,24]. Its incidence increases if the patient has a family history of HCC^[25], if the viral load (HBV-DNA) is high^[21,26,27], if the genotype of the infecting HBV is C^[28,29] and if pre-core or basic core promoter mutations have developed^[30,31]. The risk also increases in patients with heavy alcohol consumption^[32,33], in those co-infected with HDV or/and HCV^[34-37] and in those who consume unsafely stored crops (dietary exposure to aflatoxin)^[38]. In the evaluation of the risk for HCC by variables of activity of HBV replication, a linear association with serum HBV-DNA levels has been proved, while, more recently, high levels of HBsAg (> 1000 IU/mL), even in patients with relatively low viral loads (2000-20000 IU/mL) have been reported to confer medium to high risk for HCC development^[21,39,40]. As far

as baseline viral load is concerned, in a large prospective cohort study of 3653 patients, the cumulative incidence rate of HCC at the end of follow-up ranged from 1.3%, when baseline serum HBV-DNA was less than 300 copies/mL, to 14.89% for a viral load of $\geq 6\log_{10}$ copies/mL^[21].

PATHOGENETIC MECHANISMS IN HCC

Several molecular mechanisms have been implicated in the pathogenesis of HBV-linked HCC. In the 1980s the identification of integration of HBV-DNA into the genome of hepatocytes in patients with HCC, has been implicated in the development of HBV-related HCC with several HCC cases being reported harboring integrated HBV DNA sequences in the malignant hepatocytes while serum and non-malignant liver tissue was negative for any marker of active HBV infection^[15]. The protein HBx and the epigenetic regulation of the minichromosome of covalently closed circular HBV DNA have also been implicated as factors contributing to chromosomal instability, to activation of cancer-related genes and to inactivation of protective genes. They have also been considered to interfere with cellular transcription and signal transduction through various pathways and cellular promoters^[41-43]. Moreover, adaptive immune reactions developing as a consequence of chronic HBV infection result in release of cytokines and of growth factors leading to necrosis of hepatocytes and proliferation of fibroblasts, resulting in the development of fibrosis/cirrhosis. Furthermore, a high turnover of hepatocytes can confer to the host DNA certain mutations that are probably responsible for their malignant transformation^[41-44]. In view of these crucial considerations regarding various factors in chronic active HBV infection with possible involvement in the development of HCC, it is reasonable that several studies have tried to evaluate the effect of antiviral treatment of CHB not only regarding prevention of disease progression to cirrhosis and its decompensation but also in terms of possible prevention from development of HCC and death, although HBV-DNA becomes integrated into the genome of hepatocytes of the patient, from the early phases of HBV infection, probably years before the start of treatment^[45,46].

TREATMENT OF CHB

Currently, the great majority of patients with CHB are treated worldwide with nucleos(t)ide analogues (NAs). Finite courses of treatment with pegylated interferon (IFN) for 1 or 2 years also represent first line therapies both for HBeAg positive and HBeAg-negative patients and should be first applied to all patients eligible for therapy, provided, of course, that there are no contraindications and that IFN is tolerated without major side effects. The aim of such therapies is to achieve sustained virological response and subsequent HBsAg loss representing the closest to cure outcome of chronic HBV infection^[46]. However, the frequency of such an effect, achieved

even on the basis of response guided treatment, does not exceed 20% or at maximum 30%^[47-49]. Thus, it is understandable why currently long-term NA therapy has turned out to be the number one first choice treatment of CHB. Prerequisite for the success of such long-term therapies is the use of compounds of robust antiviral potency and of high barrier to HBV resistance as are the current first line NAs entecavir (ETV) and tenofovir (TDF).

Long-term NA therapies reduce the incidence of unfavorable long-term outcomes of CHB (cirrhosis, decompensation of cirrhosis, liver transplantation) and can even lead to regression of advanced fibrosis/cirrhosis^[50-53]. Thus, long-term therapy with these compounds represents a first-line recommendation of the treatment guidelines of the AASLD, EASL and APASL^[46,54,55]. After all, the ultimate goal of therapy in CHB is to improve the quality of life and the survival of patients by preventing the progression of the underlying liver disease to cirrhosis, to decompensated cirrhosis, end-stage liver disease, to development of HCC and death^[46].

THE EFFECT OF LONG-TERM TREATMENT WITH NAs ON THE DEVELOPMENT OF HCC

Lamivudine and adefovir dipivoxil

Most data is derived from the application of lamivudine (LAM), the first NA used for the treatment of CHB. The story begun with a breakthrough Asian study published ten years ago, showing that a single pill can change the natural history of CHB^[56]. This study is the only randomized controlled clinical trial comparing the efficacy of treatment with lamivudine vs placebo on disease progression in a large number ($n = 651$) of patients with CHB and advanced fibrosis or cirrhosis with 58% of them being HBeAg-positive. Though the study was planned for a maximum of five years, it was terminated prematurely after 32.4 mo, since the difference between treated and untreated control patients became obvious from the first year of therapy and it was considered unethical to continue treating with placebo patients with advanced liver disease. The lamivudine arm showed cumulative development of HCC of 3.9% vs 7.4% in the placebo arm ($P = 0.047$), yet the clinical benefits for disease progression and HCC development were lost when resistance to the drug was developed (11% in patients with resistance vs 5% in placebo treated patients). Moreover, when HCC cases diagnosed in the first year of the trial were excluded, only a marginal difference could be detected ($P = 0.052$). It has, however, been implied, that if the study had continued for longer, then the difference in favor of lamivudine would have been more profound.

Following this initial study, many more were conducted. Most of them have been retrospective with matched historical controls and all of them showed that lamivudine reduced statistically the risk for disease

progression to cirrhosis and for development of HCC^[57-60]. However, different methods of HBV-DNA assay with different limits in its detection were used in these studies, many patients had YMDD mutations and in some studies there was no match with untreated controls for HBV-DNA levels, HBeAg positivity, and duration of follow-up or age. These limitations could have possibly resulted in downgrading of the differences between the treated and control patients^[57-60].

During the last eight years, one meta-analysis^[61] and one systematic review^[62] were published dealing with the crucial debate of whether nucleos(t)ide analogs and mainly lamivudine treatment of chronic hepatitis B significantly reduces the risk of HCC both in cirrhotic and in non-cirrhotic patients^[61,62].

The meta-analysis included 5 studies^[56-60] with 1267 patients treated mostly with LAM and compared them with 1022 untreated ones^[61]. Overall, with the use of LAM the incidence of HCC was reduced by 78% (2.5% vs 11.7%, RR = 0.22, $P < 0.001$). The risk of HCC was significantly reduced in cirrhotics (3.9% vs 22.4%, RR = 0.17, $P = 0.020$), non-cirrhotics (1.8% vs 8%, RR = 0.21, $P < 0.001$), both in patients who developed drug resistance (3.3% vs 6.4%, RR = 0.52, $P = 0.04$) or not: $P = 0.008$ and in HBeAg (+) (1.7% vs 7.9%, $P < 0.001$) patients while in HBeAg (-) patients the difference was not statistically significant (3% vs 10%, $P = 0.06$).

The systematic review included 21 studies with 3881 patients treated mainly with lamivudine [and/or adefovir (ADV)] and 534 untreated ones^[62]. Of the studied patients, 33% were cirrhotics and 49% were HBeAg (+), while sixteen studies included treatment-naïve patients and five included patients with lamivudine resistance. Three studies followed-up both treated and untreated patients^[56-58] and their analysis showed that treated patients had a significantly lower risk for HCC (2.8% vs 6.4%, $P = 0.003$) than untreated ones, while this benefit remained the same whether being in virological remission (2.5% vs 6.4%, $P = 0.015$) or not (2.8% vs 6.4%, $P = 0.016$). Patients under remission had a lower risk for HCC compared to those with breakthrough or without response to treatment (2.3% vs 7.5%, $P < 0.001$). Moreover, if remission was accomplished with the initial treatment, then the risk for HCC was lower compared to the risk in patients who accomplished remission under rescue therapy with ADV (2.3% vs 5.9%, $P = 0.003$). As expected, treatment naïve patients with cirrhosis had higher HCC incidence than non-cirrhotic ones (10.8% vs 0.5%, $P < 0.001$), while the risk for HCC was higher in older (≥ 50 years old) (6% vs 2.8%, $P < 0.001$) and HBeAg (-) patients (5.5% vs 0.5%, $P < 0.001$) than in younger (< 50 years old) and HBeAg (+) ones, respectively. In patients with lamivudine resistance, those with cirrhosis had a higher risk for HCC compared to non-cirrhotic ones (17.6% vs 0%, $P < 0.001$). Rescue treatment with ADV in patients who developed biochemical breakthrough did not appear to reduce the risk for HCC compared to patients who remained untreated without remission (8.8% vs

5.6%, $P = 0.466$).

Therefore, lamivudine treatment with/or without rescue treatment with ADV seems to reduce but not eliminate the risk for HCC. However, a more recent study^[63] revealed that even on-treatment virological remission achieved in cirrhotic patients may not lead to reduction of the incidence of HCC, while another study showed significant reduction in the risk for HCC with on-treatment response^[64]. Nevertheless, it must be taken into account that most studies of lamivudine treatment with/without rescue treatment with ADV conducted in the past suffer from several drawbacks: (1) Usually they are non-randomized trials without pretreatment stratification for age, gender, severity of disease and other HCC predictors; (2) Surrogate endpoints of response, such as HBeAg seroconversion or biochemical responses have been applied and not hard endpoints, such as reversal of cirrhosis or prevention from HCC; (3) Because of their design they were completed while serum HBV-DNA was still detectable in many cases; (4) Probably the length of the follow-up was too short to detect a change in the risk for HCC; and (5) HBV resistance to NAs a factor linked with increased risk for HCC was most frequently encountered in the treatment with lamivudine.

Following lamivudine and ADV, many steps forward were made in the therapy of chronic hepatitis B with the newer NAs ETV and TDF of high potency and high barrier to HBV resistance and several studies have accumulated on the effect of long-term NA therapy in the prevention of development of HCC in the course of chronic HBV infection.

ETV, telbivudine, TDF

There are 3 third generation anti-HBV NAs approved for the treatment of CHB: ETV approved in 2005, telbivudine (LdT) approved in 2007 and TDF approved in 2008. All 3 are highly potent anti-HBV compounds but the barrier of resistance of LdT is low and thus for the time being only therapies with ETV and TDF are considered as first line ones. Since ETV has been licensed and used longer than TDF, especially in Asian countries, there is more information regarding the potential benefit of the former than of the latter in CHB as well as on its comparison to lamivudine. Most studies with these compounds have been conducted in Asiatic populations but significant evidence has now accumulated regarding TDF and ETV also in Western countries.

In a retrospective study from Japan the outcome of 316 patients under ETV treatment was compared to that of an equal number of historical untreated controls and of 182 patients under LAM treatment without rescue therapy upon development of HBV resistance^[65]. The cohort of ETV treated patients had a 63% reduction of HCC incidence compared to untreated ones (cumulative HCC incidence at 5 years 3.7% vs 13.7%, $P < 0.001$), which was most obvious in cirrhotic patients (7% vs 39%, $P < 0.001$), but not in non-cirrhotic ones (2.5% vs 3.6%, $P = 0.44$). Moreover, reduction in the incidence of

HCC under ETV treatment was greater than in the non-rescued LAM group of cirrhotic patients (7% vs 22%, $P = 0.043$), but again not in the non-cirrhotics (2.5% vs 4.9%, $P > 0.05$). Nevertheless, the advantage of ETV over lamivudine could not be proved in two other studies, one again from Japan and the other from Greece as well as in a recent meta-analysis^[66-68].

In the Asian study, 129 naïve patients (22% cirrhotics) under ETV therapy were prospectively followed (median F-UP: 4.25 years) and compared with 127 patients (27% cirrhotics) under LAM treatment^[66]. The cumulative 5-year risk for HCC was 12.4 in both groups, yet patients under LAM therapy who developed resistance (60/127, 47%) had statistically higher HCC risk compared to those without resistance ($P = 0.035$).

Similar to the aforementioned results are those of a multicenter Greek study, published three years later, in which 321 HBeAg (-) patients (86% naïve-14% treatment experienced, 25% cirrhotics) under ETV treatment were followed (median F-UP: 30 mo) and were compared with a known cohort of 818 patients under LAM treatment rescued with ADV upon development of HBV resistance (26% cirrhotics)^[67]. In this study, only a trend towards lower 5-year cumulative HCC incidence in the ETV-group was shown compared to the LAM-group (4.8% vs 5.6%, $P = 0.096$), while in multivariate analysis, HCC development was statistically associated with older age, male gender and presence of cirrhosis but not with the type of initial treatment.

In a most recent study from Asia, 5374 patients either under LAM or ETV treatment (3374 and 2000 patients with median treatment duration 6.1 and 2.6 years respectively from 1999 until 2011) were retrospectively analyzed (median F-UP for the LAM and ETV treated patients: 3.1 and 8.7 years respectively)^[69]. Importantly, ETV-treated cirrhotic patients had statistically lower relative risk for death or liver transplantation compared to LAM-treated ones. However, no difference was found between the two groups regarding the risk for development of HCC.

Conflicting is the information derived from a large retrospective nationwide cohort study conducted in Taiwan with 21595 patients treated for at least 90 d with LAM, ETV or LdT^[70]. The results were compared with those of 21595 controls treated with only an hepatoprotective agent. Treated patients had a significantly lower 7-year incidence of HCC compared to controls (7.32% vs 22.7%, $P < 0.001$) and the difference was more obvious in young patients without cirrhosis as well as in those without diabetes mellitus.

Moreover, in a roll-over study of registration trials of TDF in HBeAg (+) and HBeAg (-) patients, using the REACH-B scoring system for HCC development, a 55% reduction in HCC risk was shown among 641 patients who completed 6 years of treatment and in cirrhotic patients after the 5th year of treatment, while no difference could be detected in the non-cirrhotic ones^[71].

Yet, while a statistical significant difference in the risk for development of HCC between treated and untreated

patients has been clearly documented in many Asiatic studies, this has not been the case with studies in Caucasian population, in which the difference in the risk for HCC between treated and untreated patients has been only marginal^[63,67,72-74].

Thus, in agreement with the results of the Greek studies^[63,67], an Italian one of long-term treatment with ETV showed an annual development of HCC of 0.8% in non-cirrhotic patients and of 2.6% in the cirrhotic ones. These rates are not statistically different from those in untreated historical controls^[16,72].

Moreover, similar are the results in European multicenter studies published this year. A total of 744 patients from 11 European centers were included in the first of the studies (42% Caucasian, 29% Asian, 77% naïve, 22% cirrhotic)^[73]. They were all treated with ETV and after a median follow-up of 167 wk, 14 patients developed HCC with 64% of them being cirrhotics. The 5-year cumulative HCC incidence was 2.1% for non-cirrhotic patients and 10.9% for cirrhotic ones ($P < 0.001$), with HCC incidence being higher in older patients and those with lower baseline platelet counts.

In another large European multicenter, retrospective cohort study 1666 CHB patients [85% HBeAg (-), 67% with CHB, 29% cirrhotics and 3% with decompensated cirrhosis], from 7 centers treated with ETV or TDF were followed-up for a median period of 39 mo^[74]. HCC developed in 71 (4.3%) of the 1666 patients with an incidence rate of 1.37 new HCC cases per 100 patients per year. The cumulative probability of HCC was 1.3% at the 1st year, 3.4% at the 3rd year and 8.7% at the 5th year after the onset of treatment. Again these findings are not different from those on the risk of HCC development among published untreated or lamivudine treated cohorts of patients^[16,56,60,62]. Virological remission was achieved in 92% of the patients and it was not found to be significantly associated with the probability of HCC development. In the multivariate analysis, the factors positively associated with development of HCC were age, severity of liver disease and platelet count at the start of treatment^[74]. The summary of the mentioned studies and meta-analyses is outlined in Table 1.

Furthermore, the last two studies evaluated the recently developed scoring systems (GAG-HCC, CU-HCC and REACH-B scores) in population of Caucasian patients^[73,74]. These scores, based on characteristics of the virus and of the patients, were validated and used to predict HCC development in treated Asian patients^[75-78], Table 2. In both studies, the predictability of these scoring systems was poor to modest for the overall Caucasian population of patients, showing that a considerable proportion of individuals, particularly Caucasians, who will develop HCC, cannot be identified by these scoring systems. Hence, their clinical utility especially in Caucasians remains debatable.

The topic of the risk for development of HCC in chronic hepatitis B patients and its possible reduction by antiviral treatment has been widely covered in the

Table 1 Summary of studies and meta-analyses evaluating the effect of antiviral therapy on the incidence of hepatocellular carcinoma in chronic hepatitis B patients

Ref.	Total no. of patients, txr/untrx	Type of study	NUC used	F-up (median) txr/untrx	Cumulative incidence of HCC % txr/% untrx	RR or SIR (95%CI)	P value
Liaw <i>et al</i> ^[56]	436/215	RCT, prospective	LAM vs placebo	2.7 yr	3.9/7.4	RR: 0.49	0.047
Yuen <i>et al</i> ^[57]	142/104	Prospective	LAM	8.2 yr	0.7/2.4	RR: 0.20 (CI: 0.03-2.76)	0.005
Matsumoto <i>et al</i> ^[58]	657/2138	Retrospective	LAM vs placebo	2.7/5.3 yr	1.1/13.3	RR: 0.08 (CI: 0.03-0.22)	< 0.001
Eun <i>et al</i> ^[59]	872/699	Retrospective	LAM vs placebo	5.1 ± 2.7/6.1 ± 4.3 yr	Annual incidence: 0.95 (VR with LAM)/4.1 (controls)/2.18 (R)	RR: 0.14 (0.006-0.34)	0.005
Papatheodoridis <i>et al</i> ^[60]	201/195	Retrospective	LAM, LAM + ADV	3.8 ± 1.4 yr	2.48/7.7	RR: 0.32 (0.12-0.87)	(CI:R), 0.123 (non-CIR)
Papatheodoridis <i>et al</i> ^[61]	818	Retrospective	LAM, ADV (add-on), switch to ADV or ETV upon R	4.7 yr	6% (2.5%/yr)	RR: 1 (VR)	0.01
Kurokawa <i>et al</i> ^[64]	293	Retrospective	LAM	67.6 ± 27.4 mo	3 (CHB)/30 (CIR)	RR: 2.04 (0.49-8.3) (no VR)	0.322
Hosaka <i>et al</i> ^[65]	316 (ETV)/316 (untrx)/182 (LAM)	Retrospective	LAM, ETV	5.4 yr	ETV/untrx 3.7/13.7	HCC reduction by 63% with ETV	< 0.001
Kobashi <i>et al</i> ^[66]	ETV/LAM 129/127	Prospective	ETV, LAM	4.25 yr	12.4	HR: 0.37 (0.15-0.91)	No difference ETV vs LAM
Papatheodoridis <i>et al</i> ^[67]	ETV/LAM 321/818	Retrospective	ETV, LAM	30 mo	ETV/LAM 4.8/5.6	ETV/LAM	0.096
Lim <i>et al</i> ^[68]	LAM/ETV 3374/2000	Retrospective	LAM, ETV	LAM: 3.1 yr ETV: 8.7 yr	137/2000 (6.85)/234/3374 (6.9)	HR: 1.01 (CI: 0.8-1.24) CI: 6.77-7.87/22.1-23.3	0.95
Wu <i>et al</i> ^[70]	21595/21595	Retrospective	LAM, ETV, LdT vs controls	7 yr	7.32/22.7		< 0.001
Kim <i>et al</i> ^[71]	641	Prospective roll-over	TDF	5.52 yr	56% reduction in CIR after the 5 th year	SIR: 0.55 (0.32-0.94) at 5.52 yr	
Lampertico <i>et al</i> ^[72]	418	Retrospective/prospective	ETV	58 mo	4 (CHB)/13/(CIR)		CIR/non-CIR < 0.001
Arends <i>et al</i> ^[73]	744	Prospective	ETV	167 wk	Cumulative (5-yr) 2.1 (non-CIR)/10.9 (CIR)		No difference from untrx published cohorts
Papatheodoridis <i>et al</i> ^[74]	1666	Retrospective	ETV, TDF	39 mo	1.3(1 st year), 3.4 (3 ^d year), 8.7 (5 th year)	RR: 0.22 (0.10-0.50)	< 0.001
Sung <i>et al</i> ^[61]	5 studies 1267/1022	Meta-analysis	LAM		HBsAg (-): RR: 0.25 (0.06-1.06)	NS	
Papatheodoridis <i>et al</i> ^[62]	21 studies 3881/534	Systematic review	LAM		2.8/6.4		0.003
Singal <i>et al</i> ^[68]	49 studies 10025/3571	Meta-analysis	LAM, ADV, ETV, LdT, TDF		Pooled HCC incidence rate: 1.3 (1.1-1.6)/100 person-years	LAM vs untrx RR: 0.48 (0.38-0.61)	< 0.001 No difference between NA

Trx: Treated; Untrx: Untreated; RR: Relative risk; SIR: Standardized incidence ratio; LAM: Lamivudine; VR: Virological remission; R: Resistance; CIR: Cirrhosis; ADV: Adefovir; CHB: Chronic hepatitis B; ETV: Entecavir; HR: Hazard ratio; LdT: Telbivudine; TDF: Tenofovir; NS: Non-significant; HCC: Hepatocellular carcinoma; NA: Nucleoside analogues; RCT: Randomized controlled trial.

Table 2 Risk scoring system for hepatocellular carcinoma in chronic hepatitis B

CU-HCC score ^[76]		GAG-HCC score ^[75]		REACH-B score ^[77]	
Variable	Points	Variable	Points	Variable	Points
Age		Age		Age	
> 50 yr	3	Per year	1	Per 5 years over 30	1
Albumin		Gender		Gender	
< 3.5 g/dL	20	Male	16	Male	2
Bilirubin		BCP mutations		ALT (IU/L)	
> 1.1 mg/dL	1.5	Present	19	15-44	1
Cirrhosis		Cirrhosis		≥ 45	2
Presence	15	Presence	30	HBeAg (+)	2
HBV-DNA		HBV-DNA		HBV-DNA	
4-6 log10	1	Per log10	3	< 4log10	0
> 6 log10	4			4 - < 5log10	3
				5 - < 6log10	5
				≥ 6log10	4
Risk category		Risk category		Risk category	
Low	< 5	Low	< 101	A 17 point risk scale	
Intermediate	5-20	High	≥ 101		
High	> 20				

CU-HCC: Chinese University-Hepatocellular Carcinoma Score; GAG-HCC: Guide with Age, Gender, HBV-DNA, Core Promoter Mutations and Cirrhosis; REACH-B: Risk Estimation for Hepatocellular Carcinoma in Chronic Hepatitis B; BCP: Basic core promoter; ALT: Alanine transaminase.

last AASLD meeting of 2014 in Boston with several oral presentations and posters^[79-88]. From an overall analysis of these studies it can be deduced that antiviral therapy is associated with reduction of the risk for development of HCC. However, the risk still remains high, particularly in males of older age and in patients with cirrhosis. Therefore continuous surveillance is imperative in all CHB patients regardless of the outcome of anti-HBV therapy even if HBsAg has been cleared and anti-HBs have developed. Irrespective of virological remission induced by antiviral treatment, CHB patients and especially those with the highest risk - men > 50 and cirrhotics - should continue to be surveilled, according to the existing recommendations^[45]. Moreover, CHB patients whether with or without cirrhosis, who experience HBsAg loss with or without seroconversion to anti-HBs, continue to remain at risk for HCC and therefore, their surveillance should also be continued^[89,90].

Moreover, loss of HBsAg at the age ≥ 50 years was found to be an independent predictor of development of HCC.

CONCLUSION

In view of the above pooled data from studies of more than ten years, it is reasonable to conclude that treatment of chronic hepatitis B with oral antiviral agents, especially the first line ones ETV and TDF, definitely prolongs survival and changes the natural history of the disease, with significant reduction of the incidence of cirrhosis, decompensation of cirrhosis, and end-stage liver disease leading to death or liver transplantation^[52,53,69,91,92]. Yet, the potential benefits of antiviral treatment in the reduction of the risk for development of HCC have not been very impressive. A

reduction but not elimination in its incidence has been documented even in patients who achieved loss of HBsAg. This has an impact also in the waiting lists of liver transplantation. Thus, in the United States patients enlisted for transplantation for complications of CHB, a 42% relative reduction of end-stage liver disease and a concomitant 72% relative increase of HCC are recorded. To a significant extent, these changes have been secondary to antiviral treatment^[93]. Furthermore, in Europe the percentage of HBV cirrhotics transplanted for consequences of viral hepatitis has been reduced from 24% to 16% of the total^[92].

The self-contradictory finding that antiviral treatment in CHB can prevent clinical decompensation while it does not seem to affect considerably the development of HCC, is due on the one hand to the prolongation of survival without clinical consequences of hepatic decompensation, and on the other hand to the ongoing extended exposure of the patients to the harmful effects of integrated HBV sequences.

Hopefully, in the years to come, new anti-HBV therapies may manage to timely and completely eradicate HBV from the host genome and therefore may also manage to eliminate the risk for development of HCC in CHB^[94].

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Autoantibodies in chronic hepatitis C: A clinical perspective

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disease may be present or may be precipitated by IFN-based HCV treatment. In this paper, we review the prevalence of autoantibodies in individuals with hepatitis C, the clinical significance of these autoantibodies, and the approach recommended for such situations.

Key words: Hepatitis C; Autoimmunity; Antibodies; Antinuclear; Hepatitis; Autoimmune; Thyroid diseases; Hashimoto disease; Thyroglobulin; Celiac disease; Transglutaminases; Diarrhea; Interferon-alpha

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Core tip: We review the prevalence of Non-organ-specific autoantibodies, thyroid autoantibodies, and gluten-related seromarkers and their significance in predicting autoimmune diseases in individuals with hepatitis C. Autoantibodies' importance for treatment choice and possible complications due to their presence during interferon-based treatment are appraised.

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Abstract

Non-organ-specific autoantibodies and thyroid autoantibodies have been frequently found in chronic carriers of hepatitis C virus (HCV). With respect to endomysial antibodies and tissue transglutaminase, it is controversial whether the prevalence of gluten-related seromarkers is higher in patients with HCV. In such cases, in addition to acknowledging any currently existing autoimmune disease, recognizing the risk of the patient developing an autoimmune disease during interferon (IFN)-based treatment must be a principle concern. From a clinical point-of-view, the presence of autoantibodies arouses suspicion that an autoimmune

INTRODUCTION

It is estimated that 2%-3% of the world's population is infected with the hepatitis C virus (HCV)^[1]. HCV causes chronic hepatitis, cirrhosis, and hepatocellular carcinoma^[2]. HCV has been implicated both in the triggering of autoimmune diseases and in the development of autoantibodies^[3,4]. HCV might be involved in the breaking of tolerance to self-antigens and thus in triggering autoreactivity. A number of extrahepatic manifestations have been described in association with

chronic HCV infections, most of which can be mediated by immunological mechanisms, rather than being related to the infection of extrahepatic tissues^[3,4].

Until recently, the association of pegylated interferon-alfa (IFN) with ribavirin was the gold-standard treatment for hepatitis C^[5,6]. IFN may induce autoimmune disorders or worsen pre-existing autoimmune disorders^[7-14]. Therefore, it is advisable to screen autoantibodies prior to treatment; the diagnosis of an autoimmune disease may be a relative contraindication to IFN-based therapy^[6,15].

Non-organ-specific autoantibodies (NOSA), particularly smooth muscle antibodies (SMA) and antinuclear antibodies (ANA), among others, have been frequently found in chronic HCV carriers^[16-25]. In such cases, the principal concern is to discriminate between autoimmune hepatitis (AIH) and viral liver disease; this knowledge will influence treatment choices^[13,18,24,26].

A high prevalence of thyroid dysfunction and anti-thyroid antibodies in patients with HCV infection has been described in the literature^[27-29]. Furthermore, a major and common adverse effect of HCV IFN-based treatment is the development of thyroid disease during therapy. A broad spectrum of autoimmune thyroid diseases have been reported, including Graves' disease, thyroiditis, and frank primary hypothyroidism^[10,11,30-34].

With respect to the presence of organ-specific antibodies, although it has been postulated that HCV can induce immunologic intolerance to gluten in susceptible individuals, whether the prevalence of celiac disease (CD), or the levels of endomysial antibodies (EmA) and tissue transglutaminase (tTG) antibodies, are higher in patients with hepatitis C, remains controversial^[19,35-40].

From a clinical point-of-view, the presence of autoantibodies arouses suspicion that an autoimmune disease may be present or may be precipitated by IFN-based hepatitis C treatment. Here we review the prevalence of autoantibodies in individuals with hepatitis C, the clinical significance of these autoantibodies, and the approach recommended for such situations.

NOSA

NOSA were first described in autoimmune disorders^[41], and are now frequently found in chronic HCV carriers. Their prevalence varies according to country, as does the titer considered as a cut-off point for positivity (Table 1). The autoantibody most commonly found in chronic hepatitis C is SMA, which exhibits a large variation in its prevalence, ranging between 4% and 78%^[16-21,23-26,33,42-44]. ANA, a marker for autoimmune liver disease and other inflammatory conditions, has been detected in 4%-54% of patients with chronic HCV infection in several studies^[13,16-21,23-26,33,42,44-47]. Among NOSA, anti-liver kidney microsome-1 (LKM1) is less frequent, with a prevalence of between 0% and 13%^[3,5,7,9,11,13,18,19,29,33,36,40,44]. The major concern regarding the presence of NOSA is the overlap with AIH in HCV-infected patients^[21,26,48,49]. In AIH, the detection of NOSA, although not pathognomonic, remains the hallmark for

diagnosis^[50]. However, most individuals with hepatitis C and NOSA do not meet the diagnostic criteria for AIH^[41,49]. Although the actual prevalence of AIH in this group is unknown, it is estimated that only a minority present overlap^[49]. AIH is treated with glucocorticoids and an immuno-suppressor such as azathioprine^[50]. As a rule, such treatment is not recommended for patients with chronic HCV infections, as it generally increases the viremia levels^[51]. Whereas IFN-based therapy is typically not recommended for patients with AIH, because the immune stimulation produced by such treatment may lead to exacerbation of disease activity^[52-54]. Thus, a careful distinction needs to be drawn between chronic HCV infection and AIH.

It has been suggested that the management of patients with a possible HCV-AIH overlap syndrome must start with the determination of the predominating entity, thus enabling the selection of the appropriate form of therapy^[55]. Although no single histological feature is pathognomonic of either HCV or AIH, distinct composite histological patterns have been described for each entity. Patients with AIH are more likely to have severe lobular necrosis and inflammation, piecemeal necrosis, multinucleated hepatocytes, and broad areas of parenchymal collapse. Whereas patients with HCV are more likely to have bile duct damage, bile duct loss, steatosis, and lymphoid cell follicles within portal tracts^[48,56]. However, a histological pattern demonstrating intense interface hepatitis has been reported in HCV patients^[26,57,58]. In this pattern, a rosette formation of periportal hepatocytes may not always be considered suggestive of autoimmune injury, since it reflects hepatic regeneration activity as a consequence of greater necroinflammatory activity, and can be observed in other etiologies of liver diseases^[26,48,56,59].

In the past, at a time when the treatment of choice for hepatitis C was being defined in the literature, when NOSA and histological features of AIH were present, many scientists administered corticosteroids (and sometimes azathioprine) as a first-line treatment of HCV-AIH overlap syndrome^[60-64]. In such cases, biochemical and histologic improvement were achieved despite an apparent increase in the degree of viremia^[60]. Whether these patients should be further treated with IFN while they were in biochemical remission and receiving steroids was already under debate at this time.

Today, despite much research, the real relevance of the presence of NOSA in individuals with chronic HCV infection remains a matter of discussion.

Several authors have described higher serum levels of liver tests in HCV patients who test positive for NOSA^[16,19,21,65,66], probably reflecting the severity of the underlying liver lesions^[20,25,44]. It has been proposed that ANA could be helpful in predicting a more rapid progression of fibrosis^[45]. Nevertheless, previous reports have failed to demonstrate significant histological differences between NOSA-positive and NOSA-negative patients^[17,19,46,47,65-67].

Table 1 Prevalence of non-organ specific autoantibodies in patients with chronic hepatitis C

Antibody	%	n	Titer	Country	Year	Ref.
SMA	78	25/40	-	Taiwan	2001	Peng <i>et al</i> ^[16]
	74.5	76/102	> 1:80	Greece	2007	Gatselis <i>et al</i> ^[23]
	66.2	43/65	> 1:20	Germany	1995	Clifford <i>et al</i> ^[17]
	55	34/62	> 1:20	United States	1993	Fried <i>et al</i> ^[18]
	27.3	137/502	> 1:40	Italy multicenter	2004	Stroffolini <i>et al</i> ^[19]
	26	9/35	> 1:40	India	2012	Daschakraborty <i>et al</i> ^[24]
	26.7	12/45	> 1:40	Brazil	2013	Marconcini <i>et al</i> ^[20]
	20	59/290	> 1:40	Italy	1997	Cassani <i>et al</i> ^[21]
	17.8	62/348	> 1:40	Italy	2005	Muratori <i>et al</i> ^[33]
	15	28/186	> 1:80	France	2009	Chrétien <i>et al</i> ^[25]
	12.7	36/283	> 1:40	Italy	2003	Squadrito <i>et al</i> ^[44]
	9.6	7/52	> 1:40	Iran	2006	Daryani <i>et al</i> ^[67]
	5.4	5/92	-	Brazil	2010	Badiani <i>et al</i> ^[26]
	4.3	6/138	> 1:40	Greece	2007	Rigopoulou <i>et al</i> ^[43]
ANA	54	55/102	> 1:80	Greece	2007	Gatselis <i>et al</i> ^[23]
	32	60/186	> 1:80	France	2009	Chrétien <i>et al</i> ^[25]
	22.9	11/48	> 1:50	Taiwan	2001	Peng <i>et al</i> ^[16]
	21	13/62	> 1:80	United States	1993	Fried <i>et al</i> ^[18]
	20	7/35	> 1:80	India	2012	Daschakraborty <i>et al</i> ^[24]
	19.9	79/502	> 1:40	Italy multicenter	2004	Stroffolini <i>et al</i> ^[19]
	14	13/92	> 1:80	Germany	1995	Clifford <i>et al</i> ^[17]
	12	11/92	> 1:80	Brazil	2010	Badiani <i>et al</i> ^[26]
	11.5	6/52	> 1:40	Iran	2006	Daryani <i>et al</i> ^[67]
	9.4	22/234	> 1:80	Brazil	2009	Narciso-Schiavon <i>et al</i> ^[46]
	9	26/290	> 1:40	Italy	1997	Cassani <i>et al</i> ^[21]
	7.8	50/645	> 1:40	Europe multicenter	2004	Yee <i>et al</i> ^[47]
	7.7	22/283	> 1:40	Italy	2003	Squadrito <i>et al</i> ^[44]
	7.6	5/66	> 1:40	Brazil	2013	Marconcini <i>et al</i> ^[20]
	6	21/348	> 1:40	Italy	2005	Muratori <i>et al</i> ^[33]
	5.8	14/243	> 1:80	Taiwan	2012	Hsieh <i>et al</i> ^[45]
	3.6	5/138	> 1:40	Greece	2007	Rigopoulou <i>et al</i> ^[43]
Anti-LKM1	13	18/138	> 1:40	Greece	2007	Rigopoulou <i>et al</i> ^[43]
	8	28/348	> 1:80	Italy	2005	Muratori <i>et al</i> ^[33]
	6.8	3/44	> 1:40	Brazil	2013	Marconcini <i>et al</i> ^[20]
	6	18/290	> 1:40	Italy	1997	Cassani <i>et al</i> ^[21]
	3	3/102	> 1:40	Greece	2007	Gatselis <i>et al</i> ^[23]
	2.2	11/502	> 1:40	Italy multicenter	2004	Stroffolini <i>et al</i> ^[19]
	2	1/41	> 1:10	Germany	1995	Clifford <i>et al</i> ^[17]
	1.9	1/52	-	Iran	2006	Daryani <i>et al</i> ^[67]
	0.7	2/283	> 1:40	Italy	2003	Squadrito <i>et al</i> ^[44]
	0.5	1/186	> 1:40	France	2009	Chrétien <i>et al</i> ^[25]
	0	0/35	> 1:80	India	2012	Daschakraborty <i>et al</i> ^[24]
	0	0/92	-	Brazil	2010	Badiani <i>et al</i> ^[26]
	0	0/62	-	United States	1993	Fried <i>et al</i> ^[18]
	0	0/24	> 1:10	United States	1992	Czaja <i>et al</i> ^[22]

SMA: Smooth muscle antibody; ANA: Antinuclear antibody; LKM1: Anti-liver kidney microsome-1.

In terms of antiviral treatment outcome, a negative correlation between the efficacy of anti-viral treatment for HCV and the presence of NOSA^[23,45,66,68,69] has been demonstrated, particularly for non-1 genotypes^[65]. Conversely, baseline ANA status was not a consistent predictor factor of non-response in the majority of earlier studies^[19,21,46,47,65,67,70,71]. Nowadays, IFN-based therapy is considered to be effective and safe in NOSA-positive chronic hepatitis C patients for whom the major diagnosis of probable autoimmune hepatitis has been ruled out^[45,72]. Alanine aminotransferase (ALT) flares have been reported during IFN treatment in NOSA-positive individuals^[45,69]. Some cases may remit with the suspension of the drug and there have been reports of AIH being triggered by IFN, with subsequent of

immunosuppression^[69]. Autoimmune thrombocytopenic purpura is another possible complication in patients with high titers of ANA that have been exposed to IFN-based treatment^[73].

NOSA titers may increase during treatment^[23,65,68,74], or might also fade/become negative in some cases^[23,65,68,69]; moreover, patients that were NOSA-negative prior to treatment may develop autoantibodies during treatment^[23,65,69,74]. The increase of NOSA titers during IFN-based treatment has been correlated to poor sustained virological response (SVR) rates^[23]. A careful monitoring of liver biochemistry and NOSA levels is recommended during treatment^[33,45,68]. Autoantibodies should be screened every 3 mo, with monthly monitoring of ALT. High titers of autoantibodies

Table 2 The influence of non-organ specific autoantibodies in interferon based treatment outcome in patients with chronic hepatitis C

Ref.	Country	n	Treatment	NOSA evaluated in the study	Titers of NOSA increased during treatment	Development of NOSA during treatment	Autoimmune disease triggered during treatment	Influence on SVR ²
Lopes <i>et al</i> ^[74]	Brazil	21	IFN-α	ANA, SMA, AMA, etc.	↑	ANA	N	N
Cassani <i>et al</i> ^[21]	Italy	144	IFN-α	ANA, SMA	N/A	N/A	N/A	N
Muratori <i>et al</i> ^[69]	Italy	22 ¹	IFN-α	ANA, SMA, LKMI	N/A	ANA, SMA	2 ALT flare 7-10 xULN	Y
Wasmuth <i>et al</i> ^[66]	Germany	48	IFN-α + RBV	ANA, SMA, LKMI, AMA, ANCA	N/A	N/A	N/A	Y
Yee <i>et al</i> ^[47]	Europe multicenter	258	IFN-α	ANA	N/A	N/A	N/A	N
Stroffolini <i>et al</i> ^[69]	Italy	502	IFN-α + RBV	ANA, SMA, LKMI, AMA	N/A	N/A	N	N
Muratori <i>et al</i> ^[65]	Italy	143	IFN-α + RBV	ANA, SMA, LKMI	↑	Y	N	N
Gatselis <i>et al</i> ^[68]	Greece	57	IFN-α + RBV	ANA, SMA, LKMI, AMA, ANCA, etc.	↑	ANA, LKMI, ANCA	N	Y
Gatselis <i>et al</i> ^[23]	Greece	102	IFN-α/PEG + RBV	ANA, SMA, LKMI, AMA, ANCA, etc.	↑	Y	N	Y
Daryani <i>et al</i> ^[67]	Iran	52	IFN-α + RBV	ANA, SMA, LKMI, AMA	N/A	N/A	N/A	N
Narciso-Schiavon <i>et al</i> ^[66]	Brazil	234	IFN-α + RBV	ANA	N/A	N/A	N/A	N
Bai <i>et al</i> ^[76]	China	46	IFN-α	ANA, LKMI	N/A	N/A	N	N
Hsieh <i>et al</i> ^[45]	Taiwan	243	PEG + RBV	ANA, SMA, LKMI, AMA, ANCA	N/A	N/A	ALT flare	Y
Mauss <i>et al</i> ^[77]	Germany	12369	PEG + RBV	ANA, SMA, LKMI, AMA	N/A	N/A	N	N
Khairy <i>et al</i> ^[78]	Egypt	3673	PEG + RBV	ANA	N/A	N/A	N	N

¹Children; ²Higher sustained virological response rates in NOSA negative group. NOSA: Non-organ specific autoantibodies; IFN-α: Interferon alpha; SVR: Sustained virological response; ANA: Antinuclear antibody; SMA: Smooth muscle antibody; xULN: Times the upper limit of normality; RBV: Ribavirin; AMA: Anti-mitochondrial antibodies; LKMI: Liver kidney microsomal type 1 antibody; ANCA: Anti-neutrophil cytoplasmic antibody; PEG: Pegylated interferon alpha; ↑: Increase; N: No; Y: Yes; N/A: Not available; ALT: Alanine aminotransferase.

during treatment, with normal ALT, should be monitored, but without great concern. ALT exacerbations should be interpreted with caution, especially if the titers of autoantibodies are high, as they may (or may not) reflect autoimmunity. Differential diagnosis in these cases include drug hepatotoxicity (by IFN or some other drug the individual may have taken during treatment) or another viral infection, among others. Table 2 details the influence of NOSA on interferon-based treatment outcome in several studies.

Considering the above, IFN-free regimens^[75] are the logical choice for patients with high titers of NOSA and histological findings that suggest HCV-AIH overlap syndrome, despite the fact that no clinical trials have specifically evaluated this issue.

THYROID AUTOANTIBODIES

Autoimmune thyroid diseases (AITD) are a group of disorders characterized by loss of immunological self-tolerance, whose most common forms include Graves' disease and Hashimoto's thyroiditis^[79,80]. AITD are characterized by the presence of thyroid autoantibodies (TAAB), such as thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb), and thyroid stimulating hormone (TSH) receptor antibodies (TRAb)^[12,80-83].

Hashimoto's thyroiditis is the most common clinical manifestation of AITD. The disease manifests itself through subclinical hypothyroidism (elevated TSH levels, normal free thyroxine (FT4) levels), or clinically apparent hypothyroidism (elevated TSH, low FT4). Goiter occurs in some patients. The disease is diagnosed on the basis of hypothyroidism symptoms and the presence of TPOAb and/or TGAb^[12]. Graves' disease is an autoantibody-mediated autoimmune disease characterized by thyrotoxicosis. Graves' disease is caused by direct stimulation of the thyroid epithelial cells by TRAb^[84]. Physical examination shows hyperthyroidism symptoms and goiter. Graves' ophthalmopathy may be apparent. Laboratory tests show a characteristic decrease in TSH levels, an increase in FT4 and free triiodothyronine (FT3) levels, and the presence of TRAb^[12]. The ability of TRAb to provide differential diagnoses of overt hyperthyroidism is excellent, with a sensitivity and specificity above 90%^[84].

Thyroid autoimmunity is a common characteristic of HCV infection^[85,86]. A high prevalence of TAAB in chronic HCV carriers has been reported over the years, varying

Table 3 Prevalence of serum thyroid autoantibodies in patients with chronic hepatitis C

Autoantibody	%	n	Positive values (U/mL)	Country	Year	Ref.
TAAb	25	132/630	> 150	Italy	2004	Antonelli <i>et al</i> ^[87]
	14	9/66	> 50/100	France	1992	Pateron <i>et al</i> ^[159]
	12.5	9/76	-	France	1993	Tran <i>et al</i> ^[89]
	9.4	42/449	≥ 100	Taiwan	2012	Huang <i>et al</i> ^[88]
	9.7	7/72	-	Italy	2002	Carella <i>et al</i> ^[90]
	7	5/71	≥ 60	Greece	2011	Vasiliadis <i>et al</i> ^[10]
	6.7	14/207	-	Spain	1996	Marazuela <i>et al</i> ^[91]
	5.6	4/71	≥ 100	Italy	2006	Floreani <i>et al</i> ^[29]
	4.5	5/111	≥ 100	United Kingdom	1997	Metcalfe <i>et al</i> ^[92]
	30.8	60/195	≥ 50	China	2011	Yang <i>et al</i> ^[28]
TPOAb	21	132/630	> 150	Italy	2004	Antonelli <i>et al</i> ^[87]
	20	26/134	> 150	Spain	1998	Fernandez-Soto <i>et al</i> ^[95]
	16.3	51/312	> 35	China	2013	Shao <i>et al</i> ^[34]
	15	30/200	> 18	Greece	1997	Deutsch <i>et al</i> ^[94]
	14	9/66	> 50/100	France	1992	Pateron <i>et al</i> ^[159]
	10	3/32	> 100	Italy	1996	Roti <i>et al</i> ^[97]
	7.4	4/54	-	Brazil	2013	Marconcini <i>et al</i> ^[20]
	6.7	13/192	> 100	Spain	1995	Boadas <i>et al</i> ^[93]
	6.5	29/449	≥ 100	Taiwan	2012	Huang <i>et al</i> ^[88]
	5.4	9/168	-	France	2005	Moncoucy <i>et al</i> ^[96]
TGAAb	3.5	9/254	> 60	Norway	2002	Dalgard <i>et al</i> ^[102]
	30.8	60/195	≥ 40	China	2011	Yang <i>et al</i> ^[28]
	17	108/630	> 150	Italy	2004	Antonelli <i>et al</i> ^[87]
	13.3	44/312	> 35	China	2013	Shao <i>et al</i> ^[34]
	11	15/134	> 200	Spain	1998	Fernandez-Soto <i>et al</i> ^[95]
	10	13/130	-	Taiwan	1999	Huang <i>et al</i> ^[98]
	8	13/162	-	France	2005	Moncoucy <i>et al</i> ^[96]
	7.6	5/66	≥ 50	France	1992	Pateron <i>et al</i> ^[159]
	5.8	13/449	≥ 100	Taiwan	2012	Huang <i>et al</i> ^[88]
	0	0/48	-	Brazil	2013	Marconcini <i>et al</i> ^[20]

TAAb: Thyroid autoantibodies; TPOAb: Anti thyroperoxidase; TGAAb: Antithyroglobulin antibody.

from 4.5%-25%^[10,27,29,87-92]. The prevalences of TPOAb and TGAAb vary from 5.4%-30%^[20,27,28,34,87,88,93-97] and 0%-30.7%^[20,27,28,34,88,95,96,98], respectively (Table 3). Such a remarkable variation may be attributable to the different methods used, and/or to the different geography, race, age, and sex of the populations targeted in these reported studies^[99]. Environmental cofactors such as iodine intake or other infectious agents could also play an important role in the development of autoimmune thyroid disorders^[100]. TAAb are more frequent among women^[29,93-96,101], and their prevalence increases with age^[101].

The presence of TAAb does not always reflect the presence of AITD; many individuals may be asymptomatic with normal levels of thyroid hormones. The presence of TAAb may indicate subclinical thyroid disease and an increased risk of developing clinical thyroid disease^[87,101]. The prevalence of thyroid dysfunction in individuals with chronic hepatitis C varies from 3.6%-23%^[33,87,90,93-96,98,102]. Several possible explanations exist for these wide variations in the incidence of reported TAAb in IFN-treated patients, including the various assays used to test for TAAb, the cut-offs used to define serum positivity, and the variability in ethnicity of the patients studied^[80].

No relationship has been observed between serum concentrations of TSH or thyroid hormone and autoantibody titers^[100]. Nonetheless, the high prevalence of

AITD (*i.e.*, Hashimoto's thyroiditis, atrophic autoimmune thyroiditis, and Graves' disease) in patients with chronic HCV infections is often associated with humoral thyroid autoimmunity (TAAb serum levels above normal values)^[87,94,95,98].

A major concern about the presence of TAAb, besides the current existence of AITD, is to recognize the risk of the patient developing thyroid disease during IFN-based treatment^[27]. It has been long known that pretreatment-reactive TAAb represent a high risk for overt thyroid dysfunction during IFN-based therapy^[27]. The pegylated form of IFN seems to have the same effects as standard IFN^[103]. IFN dose and duration do not influence the development of IFN-induced thyroiditis^[102,104], nor do they affect virological response^[102]. Although some authors do not agree^[90,98], several studies have shown that IFN-based treatments of hepatitis C can either induce the production of TAAb, or cause a significant increase in TAAb levels, in individuals who were positive for TAAb prior to IFN therapy. Seropositivity for LKM1 may also predispose patients receiving IFN therapy for hepatitis C to develop AITD^[33]. The rate of development of TAAb secondary to IFN therapy varies from 1.9%-40.0%^[27,90,91,93,94,97,104-110]. Besides immunomediated thyroid dysfunction, it is noteworthy that TAAb are not detected in approximately 50% of patients with thyroid function disorders during IFN therapy. This finding indicates the direct toxic effect of IFN on thyroid cells,

without the participation of immunological factors^[12,96]. There are two recognized clinical forms of non-auto-immune thyroiditis: destructive thyroiditis^[97,109] and non-autoimmune hypothyroidism^[91,97,108], which will not be addressed here since they are beyond the scope of this article.

IFN-induced thyroiditis is a major clinical problem for patients who receive IFN therapy, with complications such as thyrotoxicosis being especially severe^[97]. Symptoms of thyroid dysfunction can easily be mistaken for adverse effects of the HCV therapy, and could remain undiagnosed if patients do not undergo routine periodic screening of TSH and fT4 levels^[111]. The reversibility of AITD after IFN withdrawal is controversial. Initially, the thyroid disorders induced by IFN were described as reversible^[110]. Later, it was demonstrated that in more than one third of treated patients, hypothyroidism may persist^[94-96]. Although it has been demonstrated that Graves' thyrotoxicosis may not be reversible with IFN withdrawal^[108], in a recent cohort of 18 hepatitis C patients who developed thyroiditis during INF-based treatment, all cases recovered^[112]. Late-onset thyroid dysfunction has also been observed after discontinuation of IFN-based treatment (6-mo post-treatment)^[94,95]. Perhaps monitoring for thyroid disease could be safely ceased at the 6-mo follow-up, coinciding with the SVR review^[112].

Finally, it has been reported that IFN-based therapy does not aggravate previous existing thyroid disease^[94], although some patients treated with thyroid medication before IFN treatment may require increased doses during therapy, and decreased doses after IFN therapy has been completed^[107]. When hypothyroidism occurs, thyroxin therapy should be initiated promptly^[100]. Hashimoto's thyroiditis is rarely the reason for premature termination of therapy with IFN^[12]. While in cases of symptomatic thyrotoxicosis, withholding IFN therapy should be considered only after consulting with an endocrinologist^[108]. If thyrotoxicosis is suspected, and TRAb is negative, patients should undergo a thyroid scan to check for diffusely increased uptake^[80]. Patients with destructive thyroiditis should be closely monitored for the development of hypothyroidism, which typically follows the hyperthyroid phase within a few weeks^[80].

Regardless of symptoms, all patients should be screened for TAAb (TPOAb, TGAAb, TRAb) and thyroid function (serum TSH, fT4) prior to starting IFN therapy^[80]. In patients with TAAb positivity, the choice of an IFN-based therapy must be made cautiously, taking into account the potential benefit of IFN treatment and the high risk of thyroid disease. IFN-free regimens^[30] are likely to be more suitable in such cases. In patients without TAAb, thyroid function and the presence of TAAb must be systematically tested (every 2-3 mo) during IFN therapy, particularly in women^[80,94,95,99,113].

CD ANTIBODIES

CD is a chronic, small-intestinal, immune-mediated

enteropathy precipitated by exposure to dietary gluten in genetically predisposed people^[114]. CD is now considered to be a multisystemic disorder, rather than a sole gastrointestinal process^[115]. CD is triggered by the ingestion of gluten, the protein component of wheat, rye, and barley^[116,117]. Such exposure results in a variable degree of intestinal damage^[118]. Since many patients have minor but chronic symptoms long before the full-blown malabsorption pattern develops, it may be readily possible to identify these patients at an earlier stage of the disease process by accurate screening blood tests: *e.g.*, IgA anti-EmA, IgA anti-tTG, and the more recent test for deamidated gliadin peptides (DGP)^[119-121]. Positive serology with normal histology, formerly called latent CD^[122], is now defined as potential CD^[114]. Positive serology and characteristic morphological changes in the small intestinal biopsy, in the absence of clinical signs and symptoms, was previously classified as silent^[122], but is now defined as asymptomatic CD^[114]. Once a diagnosis of celiac sprue has been established, the conventional treatment is a gluten-free diet^[122]. Adherence to a gluten-free diet and mucosal healing may not only relieve symptoms and improve the patient's quality of life, but also prevent or ameliorate CD-associated complications, such as intestinal lymphoma and the emergence of other autoimmune diseases^[115,121,123].

Gliadins, the alcohol-soluble fraction of gluten, elicit a strong humoral response in CD, which originates in the submucosa^[120]. Anti-gliadin antibodies (AGA), which have been used for decades, have moderate sensitivity but are far less specific than tests for IgA antiendomysial antibodies^[124,125]. Thus, AGA is no longer recommended for the primary detection of CD^[126,127]. Endomysium is a connective tissue protein found in the collagenous matrix of human tissue. The test to detect EmA is based on the immunofluorescence findings of reticular staining when EmA binds to the endomysium. Although highly specific when positive, EmA will be absent in individuals with CD with IgA deficiency^[120]. Selective IgA deficiency affects approximately 2%-5% of patients diagnosed with CD^[128]. tTG is a cytosolic protein that is released by the injured epithelium and serves as a cross-linker of various extracellular matrix proteins, including gliadin^[120]. IgA and IgG enzyme-linked immunosorbent assay tests are available with high sensitivity and specificity for the diagnosis of CD. Optimal results were achieved by combining a positive EmA test result and a positive IgA-tTG test result, with a sensitivity of 0.81 and a specificity of 0.99^[119]. In patients with a high-probability CD and IgA deficiency, DGP IgG-based testing is advocated^[126].

Liver involvement in CD has been widely described in case reports and case series. CD is at least twice as common in cirrhotic patients than in the general population^[129]. Some individual present abnormal liver tests, by the diagnosis of CD, that regularize with a gluten-free diet^[130-135]. CD has been described in association with autoimmune liver diseases^[136-140], and also with HCV^[36,40,139,141-143]. In the

Table 4 Prevalence of celiac disease autoantibodies in patients with chronic hepatitis C

Autoantibody	%	<i>n</i>	Country	Year	Ref.
AGA	32	82/359	United States	2001	Fine <i>et al</i> ^[142]
	11	11/104	Sweden	1997	Sjöberg <i>et al</i> ^[141]
	6.3	37/583	France multicenter	2007	Thevenot <i>et al</i> ^[145]
EmA/tTG	3.5	7/195	Italy	2004	Durante-Mangoni <i>et al</i> ^[14]
	2.0	5/244	Italy	2007	Ruggeri <i>et al</i> ^[36]
	1.2	3/259	United States	2001	Fine <i>et al</i> ^[142]
	0	0/210	Italy	2012	Gravina <i>et al</i> ^[35]
	5.8	3/52	Brazil	2013	Marconcini <i>et al</i> ^[20]
EmA	0.2	1/623	France multicenter	2007	Thevenot <i>et al</i> ^[145]
	0	0/195	United States	2008	Hernandez <i>et al</i> ^[40]
	1	2/195	United States	2008	Hernandez <i>et al</i> ^[40]
tTG	0	0/34	Brazil	2013	Marconcini <i>et al</i> ^[20]
	0	0/41	France multicenter	2007	Thevenot <i>et al</i> ^[145]

AGA: Antigliadin antibody; EmA: Anti-endomysial antibody; tTG: Tissue transglutaminase; EmA: Endomysial antibodies.

presence of intestinal inflammation, liver disease may be driven by lymphocytes generated in the intestine, which enter the portal circulation and trigger hepatic inflammation upon reactivation. This enterohepatic pathway is facilitated by the aberrant expression of adhesion molecules and chemokines that, under normal conditions, are restricted to either the gut or liver^[144].

Few studies have evaluated the prevalence of celiac antibodies in the HCV population. AGA prevalence varies between 6.3% and 32%^[141,142,145], while EmA prevalence varies between 0% and 5.8%^[20,40,145], and tTG antibodies have been reported in between 0% and 1% of patients with HCV^[20,40,145] (Table 4). Among patients with chronic liver disease, AGA positivity generally occurs at an increased frequency and may represent non-specific immune activation^[141,142,145]. Therefore, in the presence of liver disease, AGA testing is not useful in screening for CD. Whereas the EmA test seems to be highly specific for CD^[141].

A French multi-center study failed to demonstrate an association between HCV and CD, perhaps due to the low prevalence of CD in that country^[145]. Similarly, Hernandez *et al*^[40] did not find evidence for a higher prevalence of HCV among individuals with CD and *vice versa*^[40]. Silano *et al*^[146] identified a low prevalence (0.91%) of reactive anti-HCV in individuals with CD. In a recent Italian study, CD serologic screening was negative in all HCV patients; the prevalence of HCV infection among celiac patients was 1.54%, comparable to that reported in the Southern Italy population^[35]. Given these findings, there is little evidence to support the role of screening HCV patients for CD^[40,146]. Even if there is no association between the two diseases (and this question is yet to be definitively answered), the main concern is that patients may present severe cases of overt CD during HCV treatment, leading to IFN discontinuation. It is not clear whether the development of CD during IFN-based therapy is due to the general increased risk of developing autoimmunity or is specifically related to the role of IFN in promoting T helper cell type 1 responses in the small intestine in

CD^[147,148].

The activation of CD during IFN treatment has been reported in some cases. Patients may experience severe diarrhea with weight loss during IFN treatment^[9,149-154], as well as dermatitis herpetiformis^[9,155], hypoferritinemia^[154,156,157], and refractory anemia that persist after treatment has stopped^[149,152,154]. Treatment interruption has been reported^[149,150,152]. However, early diagnosis of CD enables prompt management with a gluten-free diet, which can permit the completion of IFN-based treatment^[151]. Some individuals with a previous diagnosis of CD while following a gluten-free diet may experience symptoms such as diarrhea during IFN treatment, while other individuals may experience no symptoms^[39]. Late onset CD has also been observed after discontinuation of IFN-based treatment (various months post treatment)^[8,37]. Intestinal diffuse large B cell lymphoma has been reported in an IFN-experienced elder non-adherent to a gluten-free diet^[38].

Durante-Mangoni *et al*^[14] retrospectively evaluated 534 hepatitis C patients during IFN treatment. Prior to treatment, tTG were detected in 1.3% of hepatitis C patients and in 0.4% of controls (not significant). Eighty-six percent of patients with tTG showed activation of CD while receiving IFN-based treatment. Overall, 1.3% of IFN-treated patients had discontinued treatment of a CD-like condition.

Although IFN-based therapy *per se* can cause diarrhea in up to 10% of patients^[127], it is important to exclude other causes (mainly infectious and autoimmunity) prior to attributing the symptoms to IFN therapy^[154]. Given the difficulty in determining the cause of new symptoms while on IFN-based therapy, baseline screening for celiac-associated antibodies prior to the commencement of therapy is likely to be beneficial in guiding further investigations and disease management in patients who develop symptoms that may be attributable to CD during therapy^[14,143,149-152,155,156,158]. For patients with positive antibodies, IFN-free therapies should be considered. If IFN-based therapy is the first choice, a gluten-free diet must be started

preemptively^[14,153], considering the risk of developing overt CD.

In conclusion, autoantibodies are extremely important in the follow-up of chronically infected HCV individuals, in determining the choice of treatment, and in IFN-based treatment management. Positive autoantibodies require careful consideration of IFN-free regimens. If IFN-free regimens are not available, NOSA and TAAb must be tested every 2-3 mo and physicians should be aware of the risk of the onset of an autoimmune disease.

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Clinical relevance of hepatitis B virus variants

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problem with more than 240 million people chronically infected worldwide, who are at risk for end-stage liver disease and hepatocellular carcinoma. There are an estimated 600000 deaths annually from complications of HBV-related liver disease. Antiviral therapy with nucleos/tide analogs (NA) targeting the HBV polymerase (P) can inhibit disease progression by long-term suppression of HBV replication. However, treatment may fail with first generation NA therapy due to the emergence of drug-resistant mutants, as well as incomplete medication adherence. The HBV replicates *via* an error-prone reverse transcriptase leading to quasispecies. Due to overlapping open reading frames mutations within the HBV P can cause concomitant changes in the HBV surface gene (S) and vice versa. HBV quasispecies diversity is associated with response to antiviral therapy, disease severity and long-term clinical outcomes. Specific mutants have been associated with antiviral drug resistance, immune escape, liver fibrosis development and tumorigenesis. An understanding of HBV variants and their clinical relevance may be important for monitoring chronic hepatitis B disease progression and treatment response. In this review, we will discuss HBV molecular virology, mechanism of variant development, and their potential clinical impact.

Key words: Molecular virology; Genetic heterogeneity; Quasispecies; Drug resistance; Immune escape; Viral lymphotropism; Hepatitis B virus

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Core tip: The hepatitis B virus (HBV) has significant genomic diversity and some HBV variants are associated with antiviral therapy response, vaccine escape, diagnostic failure, liver fibrosis progression and hepatocellular carcinoma development. Understanding HBV molecular epidemiology as well as the clinical and pathological relevance of HBV variants during different disease phases may enable more accurate risk-stratification of individual patients at risk for serious sequelae of chronic hepatitis B infection.

Abstract

The hepatitis B virus (HBV) is a global public health

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EPIDEMIOLOGY OF CHRONIC HEPATITIS B

Chronic hepatitis B virus (HBV) infection (CHB) is a serious global public health problem. There are an estimated 600000 deaths annually from complications of HBV-related liver disease. For over 3 decades, there has been a safe and effective HBV vaccine consisting of recombinant HBV surface (S) (*i.e.*, envelope) protein that has reduced infection rates in countries with widespread immunization programs^[1]. The HBV is transmitted parenterally by contact with blood or body fluids of an infected person. In highly endemic areas, such as China, the incidence of HBV infection is greater than 8%, and is often acquired at birth or in early childhood from exposure to HBV infected mothers or family members. About 90% of unvaccinated infants born to mothers with CHB will become chronic carriers, and the risk of CHB is up to 30% in children infected at 1-4 years of age^[2]. Despite implementation of widespread childhood vaccination programs, the incidence and mortality of HBV-related cirrhosis and hepatocellular carcinoma (HCC) continues to increase due to the enormous burden of chronically infected carriers worldwide.

NATURAL HISTORY OF CHB INFECTION

The HBV is a non-cytopathic virus and liver cell injury is due to a host immune mediated antiviral response to an infected cell. CHB is a dynamic disease, and the interplay between the virus and the host immune system influences disease course. In clinical practice, CHB is divided into four disease phases: immune tolerant, immune clearance, inactive, and reactivation phase^[3]. The immune tolerant phase is characterized by persistently normal serum alanine aminotransferase (ALT) levels, high HBV DNA levels and presence of HBV e antigen (HBeAg), but with no evidence of liver injury. The immune clearance phase is characterized by presence of HBeAg, persistently high ALT and HBV DNA levels with some degree of liver inflammation. HBeAg seroconversion may occur at the late stage of the immune clearance phase. Thereafter, patients are likely to progress to the immune inactive phase characterized by normal ALT level, low/undetectable HBV DNA (< 2000 IU/mL or < 10⁴ virus copies/mL), absence of HBeAg and presence of anti-HBe, as well as no/minimal histological injury. HBV reactivation can occur in some and is characterized by rebound viremia, presence of anti-HBe, elevated ALT levels and liver inflammation. This so-called "reactivation phase" may also occur due to the presence of preC/basal core promoter (BCP) mutations that abolish or downregulate

HBeAg production leading to HBeAg negative CHB. There is recent data challenging the classification of these clinical phases. Immunological characterization of apparent immune-tolerant HBV-infected adolescents did not reveal any tolerogenic T-cell pattern^[4]. Further, histologically active disease has been reported in CHB children considered to be immune tolerant^[5,6]. Finally, analysis of HBV quasispecies (QS) in children with an immune tolerant clinical profile showed significant HBV diversity, which may be due to immune selective pressure^[7].

In general antiviral therapy for CHB is recommended in patients with advanced liver disease (*i.e.*, cirrhosis) or prolonged immune active disease flares due to the risk of liver fibrosis progression. The currently approved anti-HBV therapies include interferon [*i.e.*, pegylated-interferon (Peg-IFN)], which has non-specific antiviral and immunomodulatory effects and nucleos(tide) analogs (NA) targeting the HBV polymerase/reverse transcriptase (P/RT) region. There are five currently available NAs: lamivudine (LMV), telbivudine (LdT), entecavir (ETV), adefovir (ADF) and tenofovir (TDF). The second generation NA's (*i.e.*, TDF and ETV) are potent with a high genetic barrier to resistance and persistently suppress HBV replication. These drugs have a low reported risk of drug resistance or treatment failure despite years of sustained therapy^[8,9]. In contrast older generation NA has an increased risk of treatment failure with long-term use due to drug resistance (Table 1)^[10]. NA are very effective at reducing liver disease risk but must be used for prolonged periods as they do not offer a cure for CHB.

OVERVIEW OF HBV REPLICATION AND TISSUE TROPISM

The HBV is the prototype member of the *Hepadnaviridae* family which includes various avian and mammalian viruses sharing similar genome structure and organism tropisms^[11]. It is a small DNA virus with approximately 3.2 Kb partially double stranded relaxed circular DNA (rcDNA) genome within a nucleocapsid surrounded by a lipid envelope. The full-length virus negative-strand has a approximately 7-9 nucleotide redundancy and the complementary positive-strand is approximately 50%-70% full genome length. The HBV genome consists of 4 overlapping open reading frame (ORF) encoding the polymerase gene (*P*), pre-S1/pre-S2/S gene (*preS1/preS2/S*), precore/core gene (*preC/C*) and X gene. Viral entry occurs after binding of the viral pre-S1 protein to its specific functional receptor, the recently identified sodium taurocholate cotransporting polypeptide^[12]. The intact virion or "Dane particle" uncoats in the cytoplasm and the rcDNA genome is transported into the nucleus and repaired to covalently closed circular DNA (cccDNA) by host and viral polymerases. The presence of cccDNA indicates successful establishment of HBV

Table 1 Summary of clinically relevant hepatitis B virus variants

Location	Amino acid or nucleotide substitution (associated overlapping gene mutation)	Clinical impact
P (RT-A)	rtI169T (sF161L)	ETV resistance
P (RT-B)	rtL180M (sE164D)	ETV resistance
P (RT-B)	rtA181T/V	LMV, LdT, ADF/TDF resistance
P (RT-B)	rtT184S/A/I/L/G/CM	ETV resistance
P (RT-C)	rtS202C/G/I	ETV resistance
P (RT-C)	rtM204V/I	LAM resistance
P (RT-C)	rtM204I (sW196S)	LdT resistance
P (RT-C)	rtM204V (sI195M)	ETV resistance
P (RT-D)	rtN236T	ADF/TDF resistance
P (RT-E)	rtM250I/V	ETV resistance
P (RT-A)	rtL80V/I	Poor antiviral response to ADF with prior LMV resistant variants
P (RT-B)	rtF166L (sF158Y)	LMV-associated, compensatory
P (RT-B)	rtV173L (sE164D)	Compensatory mutation associated with LMV resistance (enhanced replication)
P (RT-B)	rtA194T	TDF resistance
S ("a" determinant)	sG145R (rtW153Q)	Antibody-associated escape mutation; reduced HBsAg level; restore LMV resistant HBV replication
S ("a" determinant)	sD144E/G145R (rtG153E)	Antibody-associated escape mutation
S ("a" determinant)	sP120T (rtT128N)	Reduced HBsAg level
EnhII	C1653T	HCC development (genotype C)
BCP	T1753V	HCC development (genotype B)
BCP	A1762T/G1764A	HBeAg production reduced by 50%; HBeAg seroconversion; escape anti-HBe immunity
Pre-C	G1896A	HBeAg seroconversion; escape anti-HBe immunity; more severe course of disease; HCC development
S	W172* (rtA181T)	Cirrhosis and HCC development
Pre-S1/Pre-S2	Pre-S1/pre-S2 deletion (pre-S2 start codon and/or deletions in the 5'-terminal half of the pre-S2 region and pre-S1 3'-terminal half of the pre-S1 region)	More common in genotype C; progressive liver diseases; HCC development
Pre-S	Pre-S1 promoter mutation Pre-S2 promoter mutation	HCC development
X	K130M + V131I (double)	HCC development
X	V5M/L + K130M + V131I (triple)	HCC development

HCC: Hepatocellular carcinoma; BCP: Basal core promoter; HBeAg: HBV e antigen; HBV: Hepatitis B virus; ADF: Adefovir; TDF: Tenofovir; LMV: Lamivudine; HBsAg: Hepatitis B surface (S) antigen; LdT: Telbivudine; ETV: Entecavir; RT: Reverse transcriptase.

infection^[13]. The cccDNA is transcribed to a 3.5 Kb pregenomic (pg)-RNA molecule with a unique stem-loop epsilon structure located at its 5' end. Thus, HBV cccDNA is the "master" template for HBV negative-strand synthesis *via* reverse transcription, as well as hepatitis B core antigen or nucleocapsid protein and P/RT translation^[14]. Additionally, the cccDNA is the template for four subgenomic messenger RNAs (mRNAs), which are translated into soluble or secreted HBeAg (from 3.5 kb precore mRNA), subviral S or envelope particles (2.4 kb and 2.1 kb mRNA) and X (0.8 kb mRNA). The HBV pgRNA is transported to the cytoplasm and binding of the viral polymerase to its 5' end epsilon structure initiates encapsidation by HBV core particles^[15]. Following encapsidation, the pg-RNA is reverse-transcribed and is gradually degraded by viral polymerase ribonuclease H (RNase H). The positive-strand DNA is then synthesized from the newly transcribed negative-strand DNA template^[11,16]. Once the relaxed circular (rc) HBV genome synthesis is complete, the nucleocapsid interacts with envelope protein in the endoplasmic reticulum to form mature virions and they

are secreted from the host cell. Alternatively, The rcDNA genome within the nucleocapsid core particles may also recycle to the cell nucleus to replenish the nuclear cccDNA pool. In summary, the HBV is a DNA virus but utilizes reverse transcription of an RNA intermediate to replicate its genome similar to retroviruses. This error-prone replication strategy combined with high viral replication rate (approximately 10^{12} virus/d) leads to significant viral variability or QS. The HBV genomic mutation rate occurring at each nucleotide of the HBV genome is estimated at approximately 10^{-5} base/site per cycle^[13]. The long half-life of hepatocytes and cccDNA template play an important role in archiving spontaneously occurring and antiviral drug-associated mutants^[17].

Although the HBV is predominantly a hepatotropic virus, there is increasing evidence documenting that the immune (lymphoid) system is also an important site for maintaining viral persistence^[18]. In the closely related woodchuck animal model of HBV, woodchuck hepatitis virus (WHV) infection can be completely restricted to the lymphoid system and WHV invasion of lymphoid cells

is related to the viral load^[19,20]. In human studies, HBV genomes are detectable in peripheral blood mononuclear cells (PBMC) from chronically infected patients despite long-term suppressive anti-HBV NA therapy^[21], in patients after resolution of acute hepatitis B with HBV surface antigen (HBsAg) clearance^[22,23], and in circulating transplacental PBMC from HBV positive mothers possibly leading to *in utero* infection of the neonate^[24]. HBV antigens, mRNA, cccDNA and integrated forms have been detected in PBMC and extrahepatic tissues such as, bone marrow cells, spleen, and lymphoblastoid cell lines^[25,26]. Additionally, upregulation of HBV replication in PBMC occurs following *ex-vivo* mitogen stimulation and the release of viral particles capable of further infection and replication from these HBV infected PBMC^[27]. HBV genomes and viral proteins have been detected within a variety of immune cell subpopulations and, in some reports the virus appears to specifically target B cells and monocytes^[28-31].

OVERVIEW OF HBV GENOTYPES

There are nine major HBV genotypes (A-I) worldwide, which are identified by greater than 7.5% divergence across the HBV full genome between each genotype^[32]. There is also a tenth putative genotype "J" isolated from a Japanese individual^[33]. In addition to HBV genotypes, at least 35 subgenotypes (*i.e.*, within genotype A, B, C, D, F, H, but not in genotype E, G) have been identified. The HBV genotypes/subgenotypes are ethnically and geographically distributed. For instance, genotype B and C are prevalent in Asia, while genotype A and D are most frequently seen in Europe, the Mediterranean region and the Middle East^[34]. Certain genotypes may exhibit different mutations. The common HBV pre-core (pre-C) mutation more frequently exists in genotype B, C, and D than in genotype A^[35,36]; genotype C tends to carry more mutations compare to genotype B^[37]. In addition, genotypes are also linked to the natural history of CHB leading to distinct clinical outcomes and responses to therapy^[38-40]. For instance, the cumulative rate of spontaneous HBeAg seroconversion with genotype B is higher than patients with genotype C infection^[41,42]. Others report genotype-specific differences in NA response, resistance to older generation NA (*i.e.*, LMV or ADF) and, durability of HBeAg seroconversion (138.) Whilst this has less clinical relevance with the newer potent NA (*i.e.*, TDF and ETV), alternative therapy endpoints such as HBsAg loss and HCC potential may be identified. The role of genotypes in CHB management has been extensively reviewed^[43-45]. In summary, clinically relevant features of HBV genotypes include: the rate and durability of HBeAg loss/seroconversion (A and D > B and C), the risk of developing aggressive HBeAg (-) CHB (C and D > A), spontaneous HBeAg loss (B > C), cirrhosis (C), HCC (C in Asians, F in Alaska Natives), and response to antivirals (A and B > C and D).

OVERVIEW OF HBV QUASISPECIES AND CLINICALLY RELEVANT HBV VARIANTS

HBV quasispecies

The HBV replicates *via* an error-prone RT leading to non-identical but a genetically closely related variants pool, which is known as QS. Both the wildtype and HBV QS are archived in the hepatocytes reservoir. In the process of Darwinian evolution, QS that survive selective pressure (*i.e.*, host immune response and/or NA therapy) may predominate. Thus, the HBV QS diversity may reflect host humoral response. It was reported that less HBV variants were found in patients in the immune tolerant phase compared to the immune active phase^[46]. Recent studies have found that HBeAg seroconversion was associated with dynamic changes in the HBV QS pool years before viral load drop, hence HBeAg seroconversion may be a slow process rather than a sudden immunological event^[47]. In other studies, NA-associated HBV mutations were commonly found in CHB patients as minor populations even before the initiation of antiviral therapy^[48]. It has been reported that NA treatment experienced patients, even without carrying a specific drug resistant mutation (*i.e.*, LMV-R), still demonstrate a high possibility to develop cross-resistance to a related drug^[49]. Thus, it is possible that LMV-R mutations may pre-exist as a minor HBV QS strain. Further, HBV QS diversity and/or complexity 4 wk after initiation of antiviral therapy has been associated with response to treatment^[50,51]. Due to the sensitivity of direct sequencing assays, some minor variants may not be detected, especially when the mutation proportion is less than 20%^[52,53]. However, clonal sequencing and next generation sequencing assays can overcome these limitations and detect even minor QS variants.

HBV variants have been shown to be relevant to disease progression, development of HCC, reliability of diagnostic assay detection, vaccine failures and response to antiviral therapy^[54,55]. We will summarize how specific mutations can impact the major functions of the 4 HBV gene products, highlighting variants associated with liver disease development (Table 1).

HBV preS/S variants (immune escape, diagnostic assay detection, and occult HBV infection)

The HBV envelope protein is encoded by *preS1/preS2/S* gene in a frame-shift manner generating three different envelope proteins: large (L), middle (M) and small (S). Detection of either the secreted or virion associated HBsAg for greater than 6 mo in serum confirms chronic infection. The HBsAg pre-S1 is involved in attachment to host cell receptor and neutralizing antibody binding. The antibodies predominantly target the hydrophilic region of major HBsAg protein, known as the "a-determinant", located at amino acid position 99-170. Thus, "a-determinant" mutations may affect

HBsAg antigenicity, leading to vaccine escape, false-negative results by diagnostic HBsAg detection assays, and hepatitis B immunoglobulin treatment failures^[56].

The transmission of HBV vaccine escape variants to susceptible individuals may have significant public health care implications^[57]. The sG145R point mutation is the most widely reported "vaccine escape" mutant, which can infect anti-HBs positive individuals by reduced anti-HBs binding. The sG145R mutant is stable and can be transmitted horizontally in presence of high titer anti-HBs^[58]. Furthermore, G145R mutant along with an insertion between 122 and 123 in the "a" determinant was reported in patients with fulminant reactivation of hepatitis B^[59]. In addition to sG145R, the K141E, T131I variant, and insertion of three amino acids between 123 and 124 can significantly affect the structure of HBsAg^[60]. More recently, other a-determinant substitutions were reported in association with vaccine escape (*i.e.*, T116N, P120S/E, I/T126A/N/I/S, Q129H/R, M133L, K141E, P142S and D144A/E). Although vaccine-escape mutations appear to be more common in endemic areas with universal immunization programs, to date these mutants have not caused any negative effect on global immunization programs since they appear to develop slowly^[61].

Due to the overlapping ORF of the HBV S gene and P gene, NA targeting the HBV RT/P gene and induced antiviral mutations may lead to corresponding S gene mutation (and vice-versa), or so called antiviral-drug-associated S gene mutations (ADASM)^[62]. The ADASM may influence clinical outcome by altering envelope protein antigenicity, viral fitness and oncogenic potential. For example, the S gene premature stop codon at position 172 (W172*), with a 55 amino acids missing at 3'-terminus, might result from the rA181T mutation in the overlapping P gene. The W172* was shown to be associated with liver cirrhosis and HCC^[63].

Occult HBV infection (OBI) is characterized by negative HBsAg in serum but with persistent HBV DNA in liver. According to the Taormina consensus conference definition, OBI is usually due to the presence of low-level replication competent virus in which viral HBsAg cannot be detected by standard commercial assays^[64]. The viral DNA is only detectable in liver, serum, as well as PBMC but the viral load is usually very low (< 200 virus copies/mL). However, HBsAg negativity with ongoing moderate to high-level viral replication may be due to infection with HBsAg mutants that produce a modified HBsAg that cannot be detected by current commercial assays. Further, based on our groups studies it is speculated that during OBI, the HBV preferentially infects PBMC (compared to liver), especially at very low viral load suggesting a specific selective mechanism involved in the course of OBI infection of the host immune system^[21,65,66].

HBV preS1/preS2 deletion mutations

The preS gene represents the highest heterogeneity

of the HBV genome^[67]. The preS region mediates virus binding with hepatocytes, and interaction with B cells and T cells indicating that it plays an important role in the host immune response against HBV infection^[68-71]. Thus immune pressure from vaccination as well as immunotherapy may induce the preS region mutation. Previous researchers have reported that the preS gene mutation can affect immune response, virus expression, synthesis and secretion^[71-74]. It was found that preS deletion mutants often exist in CHB, especially in patients with HBV genotype C infection^[75]. The preS deletion mutant strongly correlates with liver disease progression, possibly due to defective secretion, accumulation of HBsAg in the hepatocyte endoplasmic reticulum (ER), leading to ER-induced cell stress. The cell cytotoxicity can contribute to oncogenesis^[76]. It was suggested that the preS deletion mutation together with another S point mutation is correlated with coexistence of HBsAg and anti-HBs, indicating specific immune selection pressure^[77]. Additionally, the preS deletion mutation has been associated with the occurrence of HCC in several studies, which reported a 52%-62% incidence of preS deletion in patients who developed HCC^[71,76,78-80]. The HBV genome can also integrate into human chromosome and play an oncogenic role. For instance, preS2/S genes were found with a 3' end truncation from integrated HBV DNA in HCC tissue. The truncated proteins may have transcriptional/transactivation potential leading to HCC development^[81,82].

HBV P variants and drug-resistant mutations

The HBV P has 4 functional domains: a priming region, a spacer region, a catalytic region that plays a RNA-dependent RNA polymerase/DNA polymerase function, and a carboxy terminal region that has ribonuclease H activity. There are 7 domains in P/RT region: A-G. The YMDD (tyrosine, methionine, aspartate, aspartate) motif locates in catalytic site in the domain C. It is highly conserved in all genotypes and plays an essential catalytic role in HBV replication. Thus, YMDD mutations, such as YVDD (rtM204V, methionine to valine mutation) and YIDD (rtM204I, methionine to isoleucine mutation) mutations could lead to antiviral resistance and defective viral replication. As noted, NAs inhibit the HBV P/RT and both plus and minus strand HBV DNA synthesis. The NAs have a similar structure to natural nucleotides with a modified sugar ring or base group that competes with the natural nucleotides in binding to the HBV P, leading to chain termination. Compared to IFN, NAs are more commonly used due to their more favorable side effect profile. However they require prolonged treatment as they have minimal effect on the cccDNA pool. The molecular mechanism of drug-resistance is specific to the NA sugar ring structure. To date, four major drug resistance pathways have been identified^[83]: (1) L-nucleosides pathway which is characterized by rtM204V/I mutation resulting in resistance to LAM and

LdT; (2) acyclic/alkyl phosphonate sugar pathway which is identified by presence of rtN236T substitution leading to resistance to ADF and reduced susceptibility of TDF; (3) the pathway which is shared by both L-nucleosides (LMV, LdT, reduced sensitivity to TDF) and ADF by emergence of rtA181T/V; and (4) the D-cyclopentante pathway which is characterized by presence of rtL180M and rtM204V/I mutations plus at least one substitution in one of the rtT184, rt202 and rtM250 amino acid (aa) positions. LMV has the worst resistance profile with an annual resistance rate of 15%-25% and > 80% after 5 years treatment^[84]. The *rtM204V/I* mutant, which is located at position 204 of YMDD motif, can result in LMV and LdT resistance and is often accompanied with compensatory mutations (*i.e.*, rtL80V/I, rtI169T, rtV173L, rtL180M, rtT184S/G, rtS202I and rtQ215S)^[85]. The compensatory mutations are able to restore HBV replication activity to near wild type levels. In addition, YMDD variants were also found in patients without prior NA exposure^[86]. In recent study, the spontaneous YMDD variants were reported more frequently occurred in HCC patients with HBV genotype C, which might be the cause of greater oncogenesis of genotype C compare to genotype B^[87]. Thus, it is important to monitor YMDD mutations in patients on NA therapy in order to adjust treatment regimen in time. The resistance rate to ADF is approximately 30% after 5 years treatment but may be higher in patients with pre-existing NAs-associated mutations^[88]. Two primary mutations induced by ADF and TDF (rtA181T and rtN236T) belong to the acyclic/alkyl phosphonates pathway. The rtA194T variant has been reported to be associated with partial TDF resistance, and confer reduced HBV replication *in vitro*^[89]. In clinical practice, however, TDF resistance and virological breakthrough has not been reported in patients after more than six years of treatment^[8]. Similarly, rtP177G and rtF249A have also been shown to impact HBV replication and enhance resistance to TDF both *in vitro* and *in vivo*^[90]. ETV also has a very high genetic barrier to the development of drug-resistant mutations; the rate of resistance occurrence is 1.2% after 5 years in treatment naïve patients^[91]. The resistance to D-cyclopentante group (ETV) occurs only when at least three mutations are present: rtL180M + rtM204V and either rtT184G/S or rtS202I/G or rtM250V^[17]. However, due to cross-resistance, the presence of LMV-resistant mutations can lead to ETV resistance and treatment failure. Of note the rtA181T/V mutation in domain B of HBV P, was reported to confer resistance to both L-nucleosides and acyclic/alkyl phosphonates^[92]. Further, the rtA181T also encodes a stop codon at aa172 in the overlapping S region (sW172*) in a frame-shift manner, which leads to truncated S protein production. The rtA181T/sW172* mutation can cause defective secretion of HBV S and may play an oncogenic role leading to HCC by transactivation of cellular promoters^[93].

Due to the overlapping ORF of HBV P/S gene, HBV P drug-resistance variants selected by NAs may lead

to HBsAg amino acid change and altered antigenicity. Conversely, immune pressure on HBsAg is able to introduce variants that correspond with primary or compensatory drug-resistant mutations in the P gene^[94], as noted above.

PreC/BCP mutations (HCC associated)

The HBV *preC/C* gene encodes both the HBV precore and core protein with distinct start codon (*i.e.*, preC initiates from the first start codon while core protein from the second). The preC protein encodes soluble HBeAg. It has an additional 29 aa at the N-terminus end, which serves as a signal to transport the pre-core protein to the cellular ER, the first 16 aa is cleaved, and the viral protein secreted from the cell as a soluble HBeAg antigen. HBeAg is believed to play an important role in immune tolerance and viral persistence. The HBeAg-negative CHB phase with active hepatitis occurs in association with a precore and BCP region variant^[95,96]. The most prevalent mutation in preC region is G1896A, which generates a premature stop codon at aa 28 in the sequence of HBeAg, which affects the trafficking of the precore to the ER and subsequent HBeAg secretion. This mutation is significantly associated with HBV genotypes harboring a T nucleotide (genotypes B, D, E and part of genotypes C and F) rather than C nucleotide at position 1858^[95]. This is because this variant affects the stability of the pregenomic epsilon structure, and the pregenomic encapsidation signal. The preC mutation is more often observed in genotype D HBV infection (65%) compared to HBV genotype A infections (9%). It was found that the preC deletion mutation is often associated with more severe liver disease, but has also been found in inactive HBV carriers. In addition, the preC and BCP mutations are also related to response to IFN therapy: *e.g.*, the G1896A mutation was showed to be associated with poor response to IFN therapy independent of HBeAg status^[97] while the presence of less mutations in BCP region are associated with a better treatment response^[98].

The HBV BCP is located upstream of the *preC* gene, hence mutations that occur in the BCP region can downregulate preC mRNA transcription and inhibit HBeAg synthesis. The A1762T/G1764A double mutation in the BCP region, leads to preC mRNA reduction resulting in HBeAg seroconversion and a approximately 50% reduction of HBeAg levels^[99,100]. Similar to preC mutations, BCP mutations also show genotype specific prevalence, and are more often seen in HBV genotype C and D infections^[101]. One study demonstrated a significant temporal correlation between the relative increase in mutant concentration and HBeAg seroconversion. In HBeAg-negative hepatitis patients, viral load is usually several log lower compared to HBeAg-positive patients but the HBV replication capacity may be partially restored by BCP mutant, especially if accompanied with any of 3 additional BCP mutations (T1753C, C1766T, T1768A). The increased HBV replication may be associated with

disease progression^[102]. The preC stop codon mutation and BCP mutations often appear together. Recent studies demonstrated that the combination of BCP and preC mutations and preS1, preS2 deletion mutants could lead to more severe liver disease including fulminant hepatitis and HCC. It is now believed that the development of HBV-induced HCC involves various factors in the interaction between HBV and the host. Multiple HBV mutations existing in different regions were shown to play an important role in HBV associated oncogenesis. For example, the BCP A1762T/G1764A double mutations and preC mutations are prone to HCC generation compared to patients with wild type HBV infection^[37,103]. A recent meta-analysis concluded that HBV carriers, especially Asians, were significantly more likely to develop to HCC and severe liver disease with the presence of G1896A mutations. The other mutations in preC and BCP regions, such as G1899A, T1753V and C1653T are also associated with an increased risk of HCC development^[104].

HBV X variants

The HBV X is the smallest gene, with an N-terminal negative regulatory/anti-apoptotic domain and a C-terminal transactivation/pro-apoptotic domain. The HBV X protein (HBx) is an unique regulatory viral protein since it does not bind to either viral or host DNA, however, it is able to activate transcription of viral and cellular genes by direct or indirect interaction with a variety of targets^[105]. Thus, it is required for HBV persistence. Additionally, it can modulate various cellular functions, including active humoral and cellular immune responses which may ultimately result in HBV-associated hepatocarcinogenesis^[106]. It was demonstrated that the HBx was a nuclear coactivator or could stimulate signal transduction by several pathways, such as nuclear factor- κ B (NF- κ B) signaling pathway. The NF- κ B pathway was reported stimulated by HBx though direct acting on NF- κ B itself, stimulating phosphorylation of NF- κ B or interaction with upstream signal transduction pathway^[107,108]. NF- κ B is necessary for cell growth and viability; recent study showed the activation of NF- κ B could prevent apoptosis. Thus, the HBx-induced NF- κ B pathway activation may promote the survival of infected and mutated cells that favors the hepatocarcinogenesis^[109,110]. Several X gene mutants and deletions have been reported in HCC patients. For instance, the existence of HBx130 + HBx131 double mutation and HBx5 + HBx130 + HBx131 triple mutation showed a significant risk for HCC development^[111]. This was suggested due to the increasing activity of NF- κ B by double HBx mutation and increased cell burden of triple HBx mutation and its potential influence on structure and NF- κ B activity^[111]. The HBV DNA integrates into host cellular chromosomes often with 3'-end deletion that may play an important role in HBV oncogenesis. Integrated HBV X gene sequences were found in liver tissue of most CHB patients and approximately 86% of HBV-related HCC patients^[112].

CONCLUSION

The HBV has significant genomic diversity and some HBV variants are associated with antiviral therapy response, vaccine escape, diagnostic failure, liver fibrosis progression and HCC development. Understanding HBV molecular epidemiology as well as the clinical and pathological relevance of HBV variants during different disease phases may enable more accurate risk-stratification of individual patients at risk for serious sequelae of chronic hepatitis B infection.

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Role of antiviral therapy in the natural history of hepatitis B virus-related chronic liver disease

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interactions among HBV, hepatocytes, and the host immune system. Natural history studies of chronic hepatitis B (CHB) infection have shown an association between active viral replication and adverse clinical outcomes such as cirrhosis and hepatocellular carcinoma. The goal of therapy for CHB is to improve quality of life and survival by preventing progression of the disease to cirrhosis, decompensation, end-stage liver disease, hepatocellular carcinoma (HCC) and death. This goal can be achieved if HBV replication is suppressed in a sustained manner. The accompanying reduction in histological activity of CHB lessens the risk of cirrhosis and of HCC, particularly in non-cirrhotic patients. However, CHB infection cannot be completely eradicated, due to the persistence of covalently closed circular DNA in the nucleus of infected hepatocytes, which may explain HBV reactivation. Moreover, the integration of the HBV genome into the host genome may favour oncogenesis, development of HCC and may also contribute to HBV reactivation.

Key words: Hepatocellular carcinoma; Nucleos(t)ide analogues; Liver fibrosis; Pegylated interferon; Cirrhosis

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Core tip: The goal of therapy for chronic hepatitis B is to improve quality of life and survival by preventing progression of the disease to cirrhosis, decompensation, end-stage liver disease, hepatocellular carcinoma and death. Current therapeutic options do not eradicate hepatitis B virus (HBV) infection, since HBV remains either integrated in the host genome or in the nuclei of hepatocytes as covalently closed circular DNA, a fact that may favour oncogenesis towards the development of hepatocellular carcinoma, and explains HBV reactivation. It is mandatory for clinicians to start viral suppression in patients with active chronic liver disease, particularly in

Abstract

Hepatitis B virus (HBV) infection is a dynamic state of

patients who have already developed advanced hepatic disease.

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INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most serious health problems worldwide. It has been estimated that almost one third of world's population has serological evidence of past or actual exposure to HBV^[1,2] and 350-400 million people are chronically infected^[1,3,4]. More than 780000 people die every year due to the consequences of hepatitis B^[5].

The natural history of HBV infection and of the ensuing liver disease is variable and complex. HBV infection is a dynamic state of interactions among HBV, hepatocytes, and the host's immune system. The resultant hepatic necro-inflammatory response to injury, reflected by alanine aminotransferase elevation or hepatitis activity, may stimulate, during the immune clearance phase, new fibrogenesis that may even lead to progressive fibrosis, causing architectural distortion and cirrhosis. This process may culminate in end-stage liver disease with portal hypertension and may also lead to the development of hepatocellular carcinoma (HCC)^[6]. Approximately 15% to 40% of infected patients who develop chronic hepatitis B are expected to progress to cirrhosis and eventually to end-stage liver failure^[4,7]. These data have been confirmed in Italy as well, where the HBe-negative/anti-HBe-positive type of chronic B hepatitis (CHB) is predominant, and the 5-year incidence of cirrhosis has been estimated to be 38%^[4].

Although it has been generally held true that advanced fibrosis, once present, is static and irreversible, evidence is accumulating to suggest that fibrogenesis is a dynamic process, amenable to arrest or possibly even reversal with removal of the inciting agent^[8]. Analogous to the improvement observed with continued abstinence in alcoholic liver disease, with immunosuppression in chronic autoimmune hepatitis, with weight loss in steatohepatitis, and with clearance of hepatitis C virus with interferon and ribavirin, suppression of HBV replication and loss of hepatitis B e antigen (HBeAg) or hepatitis B surface antigen (HBsAg) with antiviral therapy may prevent progressive fibrosis and decompensation.

Recently, concerns have been raised regarding the long-term benefit of HBeAg seroconversion for such patients. Although some observational studies suggest that most Asian patients experience some clinical benefit after HBeAg seroconversion^[9,10], this is

still an incomplete marker of immune control. HBeAg seroconversion associated with incomplete viral suppression may result in the emergence of precore mutant hepatitis B, with its expected chronic sequelae.

It has been demonstrated that active replication of HBV constitutes the principal trigger for immune clearance, which, in turn, has an impact on clinical outcome^[11,12]. Therefore, treatment is primarily aimed at eliminating or permanently suppressing HBV, reducing the activity of hepatitis and slowing down or limiting the progression of hepatic injury. Ultimately, the goals of therapy are prevention or reduction of the risk of developing hepatic decompensation, cirrhosis or HCC, and prolonging survival, through the achievement of sustained viral response and clearance of HBsAg^[6].

Several pharmacologic agents including standard interferon (IFN), lamivudine (LAM), pegylated IFN (PEG-IFN), adefovir dipivoxil (ADV), telbivudine (LdT), entecavir (ETV), and tenofovir disoproxil fumarate (TDF) are capable of fulfilling the goals of therapy and have been established as treatment of chronic HBV infection^[1].

Moreover, both short- and long-term outcomes of patients with chronic HBV infection are improved, and this will be the focus of this review. Indeed, in this review we will address the following issues: (1) How antiviral therapy may influence fibrosis progression and resolution; (2) The role of the antiviral therapy in patients with decompensated liver disease; and (3) The role of the antiviral therapy in reducing the risk of HCC.

ANTIVIRAL THERAPY, FIBROSIS PROGRESSION AND RESOLUTION

Injury, may it be chronic or acute, elicits a cellular- and cytokine-mediated healing response aimed at limiting or encapsulating injury, which results in fibrosis or scarring of the liver. Damage caused by infections, drugs, metabolic disorders or immunological alterations, and which is maintained in time, promotes the accumulation of significant fibrosis^[12].

Hepatic fibrosis is mainly stimulated by hepatic necro-inflammatory activity, and several studies have shown that prolonged antiviral therapy is associated with improvement in liver histology and even reversal of cirrhosis in CHB infection.

Patients who respond to interferon therapy have substantially fewer life-threatening liver complications than non-responders^[13], although the evidence of the effect of this therapy on the incidence of hepatocellular carcinoma is less conclusive^[14-16]. However, the use of interferon is restricted by costs, side effects, and among patients with advanced liver disease or cirrhosis, due to the risk of liver failure correlated with hepatitis flares. These limitations do not apply to oral nucleos(t)ide analogues-(NUCs), such as Lam and/or ADV, agents that have been used for decades, and the more recent agents ETV, Ldt or TDF. These drugs can produce marked viral

suppression, reduction of hepatic necro-inflammatory activity, histologic improvement of liver fibrosis, and amelioration of liver function, even in patients with decompensation. One of the first pieces of evidence in favor of this statement was established when a reduced risk of liver complications was demonstrated in patients affected by CHB with advanced fibrosis or cirrhosis who were treated with LAM. The magnitude of protection conferred by LAM was substantial, with a reduction of approximately 50% in disease progression during a median period of 32 mo of treatment^[17]. Dienstag *et al.*^[18] confirmed these data showing histological improvement and a reduction in fibrosis score to non-cirrhotic levels in more than 70% of patients treated with LAM with pre-treatment cirrhosis; the proportion was similar regardless of the presence of a tyrosine-methionine-aspartate-aspartate variant. Also noteworthy was the fact that only 2% of non-cirrhotic patients progressed to cirrhosis over this 3.5-year. Sampling error, however, could have perhaps contributed to the observed regression of cirrhosis in these patients, although this is unlikely the case for all patients.

Significant improvement in liver histology was also observed following long-term treatment with ADV. The median change in Knodell necro-inflammation score from the time patients were started on ADV was of 4.5 points at 192 wk and of 5.0 points at 240 wk, and the median change in Ishak fibrosis score was of 1.0 point for both groups. After 48 wk of treatment with adefovir dipivoxil, treatment with adefovir dipivoxil resulted in an increase in the proportion of patients who had improvement of at least 1 point according to Ishak from 35% after 48 wk to 55% and 71% after 192 and 240 wk of treatment, respectively. Of twelve patients with pre-treatment bridging fibrosis or cirrhosis, seven (58%) demonstrated an improvement of at least 2 points in their Ishak fibrosis scores, while a 4-point histologic improvement was observed in 3 of 4 patients with cirrhosis^[19]. In another study comparing post- and pre-treatment biopsies in patients treated with ADV, significant improvement of hepatic necro-inflammation and fibrosis was observed^[20].

Although LAM and ADV have been associated with reversal of fibrosis and cirrhosis, their long-term efficacy has been limited by the emergence of antiviral resistance^[18,19]. After treatment with lamivudine for 3 years, 72% of patients with cirrhosis show histologic improvement and a reduction in fibrosis score to non-cirrhotic levels. However, in the same study, 65% of the cohort (41 of 63 patients) developed resistance, including one patient with cirrhosis, who also experienced progression of liver disease at follow-up. Virologic resistance emerged in 20% of the patients treated for 5 years with ADV^[18].

For this reason, high genetic barrier treatment regimens have been adopted during the last years, such as ETV, LdT and tumor necrosis factor. Nucleoside-naïve, HBeAg(+) and HBeAg(-) patients with cirrhosis/advanced fibrosis at baseline (Ishak fibrosis score, \geq

4) and at least 3 years of ETV treatment demonstrate durable suppression of HBV replication, improvement in liver histology, and reversal of fibrosis/cirrhosis^[21]. After a median exposure of approximately 6 years to ETV therapy, histological improvement was observed, with a reduction or stability of necroinflammatory score in 96% and reduction of fibrosis in 88% of patients. Most patients (75%) in the cohort who had a F4 baseline HAI score achieved a F3 score by the time of long-term biopsy. No evidence of virological rebound or genotypic resistance to entecavir was observed in this study^[22].

In a multicentre study, Marcellin *et al.*^[23] analyzed the long-term efficacy and safety of TDF as well as sequential histological data obtained for 5 years in 348 patients, 96 of whom had been diagnosed with cirrhosis at baseline. HBV DNA was undetectable in almost all patients treated with TDF, associated with prevention of fibrosis progression in 96% of the patients overall, and with cirrhosis regression in 74% of patients. Furthermore, a high genetic barrier was demonstrated for TDF, with no evidence for emergence of resistant variants during 5 years of treatment. Although this study provides solid evidence for fibrosis regression, some experts believe that once established, parenchymal destruction and disrupted blood flow in cirrhosis are irreversible. A study showed that hepatic venous pressure gradient (HVPG) was reduced in 18 of 19 cirrhosis patients treated with LAM for 12 mo, whereas portal pressure was reduced at least 20% or below 12 mmHg in 10 of 13 patients in whom baseline HVPG was ≥ 12 mmHg, suggesting that vascular changes in cirrhosis are reversible in patients with virological and biochemical response^[24]. Thus, it seems fairly clear that aside from abatement of HCC incidence demonstrated in the study by Marcellin *et al.*^[23] cirrhosis is to some degree reversible in patients with sustained HBV suppression and annulled hepatitis activity with NUC treatment.

ANTIVIRAL THERAPY AND DECOMPENSATED LIVER DISEASE

Ascites, hepatic encephalopathy, jaundice, and variceal bleeding represent decompensation milestones in the natural history of an individual cirrhotic patient^[25]. In HBV-related cirrhosis, the reported yearly rate of decompensation is 2%-5%^[26], and this event can present as part of an acute hepatitis flare or in a more insidious manner^[27,28].

Decompensation entails an ominous prognosis, as the 5-year survival rate drops from 84% in compensated cirrhosis to 14%-35% once decompensation has ensued^[29,30]. A bulk of evidence indicates that the risk of disease progression is closely linked to a patient's serum HBV DNA level^[30-34]. Indeed, a study analyzing 161 patients followed for a median of 6.6 years showed that the risk of hepatic decompensation was 4 times higher in HBV DNA positive patients (13%-18%) vs in

HBeAg negative/HBV DNA negative patients (4%, $P = 0.04$)^[34]. Persistent HBeAg seropositivity was shown to be significantly ($P = 0.035$) associated with the probability of decompensation in a study analyzing 93 patients with newly developed cirrhosis, and patients in whom HBeAg was persistently positive had a 6 times higher risk of decompensation compared to HBeAg seronegative subjects at entry, during a mean follow-up period of 102 mo^[35].

Although it is recommended to commence antiviral treatment as soon as CHB is diagnosed, IFN use, even at low doses, increases the risk of bacterial infections and may provoke an episode of hepatic decompensation. In the era of NUCs, interferon is contraindicated in this patient population^[1]. Patients with decompensated cirrhosis may show slow clinical improvement over a period of 3-6 mo under NUCs, after which transplantation may be avoided. In such cases, life-long treatment is recommended^[1]. In contrast, some patients with advanced hepatic disease reflected by a high Child-Turcotte-Pugh (CTP) or model of end stage liver disease (MELD) score, may have progressed beyond the point of no return, and may not benefit from medical therapy, thus requiring liver transplantation^[36]. In that situation, treatment with NUCs which induces HBV DNA undetectability at transplantation will decrease the risk of HBV recurrence in the graft^[37].

LAM has been demonstrated to enact an effective suppression of HBV DNA replication and to significantly ameliorate liver function in decompensated CHB^[38,39]. A major drawback of LAM, however, lies in its frequent association with resistant mutants and therefore elevated drug resistance rates^[40]. However, the choice of the most adequate antiviral agent at a later disease stage often becomes remarkably difficult, due to the relentless and rapid progression of disease and poor liver function. In the last years, researchers have tried to identify the risk factors for developing decompensation or early signs of non-response to therapy. Post-treatment response was comparatively poor for cases with a cut-off of CTP > 10, MELD > 20, HBV DNA > 7.4 log and total bilirubin > 3.7 mg/dL ($P < 0.05$). Srivastava *et al.*^[41] showed that a MELD score > 20 was the most potent predictor of mortality among all the factors considered, and that these patients should be considered for orthotopic liver transplantation. In the same paper, the clinical efficacy of antiviral therapy with TDF was proven and showed a rescue activity, achieving more than 90% survival at one year and > 80% survival at 2 years in decompensated Child C cirrhosis^[41].

A further issue to discuss regarding antiviral therapy in decompensated cirrhosis due to HBV is the possible occurrence of adverse events. Whereas the safety of LAM has been established, with no reported serious adverse events (SAE), ADV has been reportedly associated with SAE in 4% of patients, including 2% of treated patients with hypophosphatemia in a study analyzing 226 treated patients^[42]. In a pooled

analysis of two studies, SAE affected 6% of patients treated with ETV^[43]. Similar frequency of SAE with ADV and ETV were reported in one prospective study, whereas EDF and ETV were associated with similar SAE rates (4% vs 0%, $P = 0.89$) and (7% vs 9%, $P = 0.72$), respectively^[43,44]. Under ADV treatment, 9% (5%-17%) of patients developed renal insufficiency (defined as an increase in serum creatinine by 0.5 mg/dL over the baseline) occurred in, while this complication was present in 10% (6%-17%) of patients treated with ETV. No cases of renal insufficiency are, on the contrary, reported with LAM. Moreover, renal function improvement (expressed as an increase in estimated glomerular filtration rate from baseline) was significantly greater in patients treated with LdT with respect to patients on LAM therapy (3.3 ± 3.3 mL vs 4.3 ± 3.1 mL, $P = 0.02$), according to a prospective randomised controlled trial on LdT and LAM^[45]. The frequency of renal insufficiency at 1-year after starting antiviral treatment was reportedly similar between ETV- and TDF-treated patients (5% vs 9%, $P = 0.53$) in a different study^[44].

ANTIVIRAL THERAPY AND THE RISK OF HCC

The third cause of malignancy-related death in the world, HCC commonly arises in patients with pre-existing cirrhosis or chronic liver disease^[46]. Seventy-eight percent of HCCs are related to CHB and chronic hepatitis C infections, occurring approximately in the ratio of 7 to 3, respectively^[47]. Different etiologies of liver disease are associated with a greater or lesser risk of HCC, and CHB is the principal underlying cause worldwide^[48].

The intricate mechanisms by which the action of the established carcinogen HBV triggers the onset of HCC have not yet been well established. Presumably, the integration of HBV's DNA into the host genome, alongside the direct effect of viral proteins on the hepatocyte are both key components of the direct carcinogenic effect of HBV on hepatocytes. However, possibly the paramount driver of HCC development is the inflammation elicited by HBV, leading to the establishment of cirrhosis, which is almost invariably present in patients with HCC^[49].

Not strangely, marked geographical differences in HCC incidence coincide with the prevalence of CHB. While in Scandinavia, the United States, and Canada, incidence is approximately less than 5 cases per 100000, incidence rates peak in central and southeast Asia, with incidence rates that vary from 29 to 99 per 100000^[50]. Apart from the solid epidemiological association between the incidence of HCC and the prevalence of CHB, numerous other observations point towards an etiologic link between CHB and the development of HCC^[51]. The prevalence of HBsAg is high amongst patients with HCC and HBsAg carriers

have a 98-fold increased relative risk for developing HCC with respect to HBsAg-negative subjects. Further strengthening the evidence in favor of this correlation are the fact that integrated HBV-DNA has been found within neoplastic HCC cells, the recognition that HBV vaccination has been followed by a decrease in HCC incidence, and the observation of an elevated risk of HCC development in animal models of CHB^[52].

Preventing disease progression and HCC development in HBV-infected patients is mandatory^[53]. As already mentioned, the current therapeutic options for patients with CHB infection may be summarized into treatment with standard or PEG-IFN, a drug with antiviral, immunomodulatory and perhaps antitumoral activities, as well as treatment with oral NUC^[1]. In terms of prevention of HCC, IFN therapy, which stimulates immunological check of viral replication, might theoretically represent an advantage regarding prevention of HCC. On the contrary, NUC therapy is likely to represent an advantage over IFN if the direct carcinogenic effect of HBV DNA levels occupies a more preponderant role. Numerous studies and meta-analyses have analyzed the impact of IFN on HCC incidence in patients with CHB, concluding that probably a reduction in the overall incidence of HCC can be obtained with the use of IFN, and that this reduction is more important in patients who maintain a sustained viral response. These effects have been more clearly demonstrated in Asian studies vs European studies, probably due to the fact that HCC incidence is higher in Asia^[15,54-56]. The effect of oral antiviral therapy on HCC incidence, however, has not been clarified.

It has been shown that long-term NUC therapy with initial lamivudine monotherapy is not effective in abolishing the risk of developing HCC risk in HBeAg-negative patients with CHB, particularly in subjects with cirrhosis at baseline. Established independent risk factors for HCC development in CHB patients even after NUC treatment include older age and male gender^[57]. Although induction and maintenance of virological suppression appears not to significantly diminish overall incidence of HCC, virological remission on-therapy might be protective in HBeAg-negative patients with CHB but no cirrhosis. On the contrary, as patients with established cirrhosis still stand a high risk of HCC even with an effective antiviral therapy, strict surveillance is warranted^[57].

The reduction of the risk of developing HCC is largely dependent upon an agent's capacity to maintain virological remission. In fact, patients in whom a virologic breakthrough is observed, the risk of developing HCC is increased, notwithstanding subsequent suppression of viral replication with rescue therapy. This observation constitutes another element against the use of lamivudine as the first-line treatment of choice, due to its association with high resistance rates during long-term treatment^[58].

In a recent randomized controlled trial, Huang *et al.*^[59] showed that in patients with hepatitis B-related HCC treated with adefovir, antiviral therapy leads to

a reduction of late HCC recurrence and significantly improves overall survival after hepatic resection, as opposed to no treatment at all.

In a recent update of the HEPNET Greece cohort study, the authors compared ETV with LAM, showing a lower HCC incidence (of 0.3%, 1.2%, 2.8% vs 0.7%, 3.8%, 5.6% at 1, 3, and 5 years, respectively; $P = 0.024$) in the first group. However, in the multivariable Cox regression analysis, the HCC risk was independently associated with older age ($P < 0.001$), male gender ($P = 0.011$) and cirrhosis ($P = 0.025$), but not with the initial antiviral agent^[60].

Finally, two recent papers showed that antiviral treatment with ETV did not completely eliminate the risk of developing HCC in patients with cirrhosis^[61,62]. These data were confirmed in a recent, large, real-life multicenter United States-based observational cohort study, in which antiviral therapy was associated with a significant decrease in the risk of HCC in patients with chronic HBV infection, but did not eliminate it^[47].

In conclusion, it is clear that only with treatments that can completely eradicate the virus from the liver will we be truly able to eliminate the risk of HCC development in patients with HBV-related liver disease.

CONCLUSION

Treatment for CHB infection aims to maximize viral suppression with the objective of controlling liver fibrosis and preventing progression to clinical complications associated with hepatic decompensation and hepatocellular carcinoma. Since necroinflammatory activity is the main stimulator of hepatic fibrosis^[12] amidst the intricate pathways leading to HCC development in chronic viral hepatitis^[63], it is conceivable that the fibrogenic process will be arrested or even down-graded along with the subsidence of hepatitis activity subsequent to HBV suppression^[64]. On the other hand, maintaining undetectable levels of HBV DNA may also increase the rate of HBeAg and HBsAg seroconversion, which are the desired endpoints of CHB therapy^[65].

Notwithstanding the solid evidence of viral replication blockade with approved antivirals, the demonstration of advantages in terms of long-term outcomes is more difficult. This is due to the fact that clinical complications develop over decades, and clinical trials with necessarily lengthy follow-up periods are difficult, if not impossible, to perform. In decompensated HBV patients, the waxing frequency of resistance to LAM, ADV and LdT monotherapy, render these three drugs less appropriate. Antiviral therapy using newer NUCs with lower resistance rates such as ETV or TDF could suppress HBV replication, improve liver function in patients with compensated or decompensated cirrhosis, delay or obviate the need for liver transplantation in some patients, and reduce the risk of HBV reactivation.

Finally, current therapeutic options do not eradicate HBV infection and in spite of adequate treatment, the virus remains indefinitely in a latent state, representing

a continuous threat of reactivation and of oncogenic potential leading to HCC development. It is nevertheless mandatory for clinicians to start viral suppression in patients with active chronic liver disease, in particular with those that have already developed advanced hepatic disease, with the aim of avoiding future complications and hopefully reversing at least some degree of hepatic damage.

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Prevention of hepatocellular carcinoma by correction of metabolic abnormalities: Role of statins and metformin

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activated protein kinase kinase (MEK)/extracellular-signal-regulated kinase (ERK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and Wnt/ β -catenin signaling. Metformin (through 5'-adenosine monophosphate-activated protein kinase pathway activation) and statins (through 3-hydroxy-3-methylglutaryl coenzyme A inhibition) show anti-tumoral properties modifying several steps of RAS/RAF/MEK/ERK, PI3K/AKT/mTOR and Wnt/ β -catenin signaling cascades. On the other hand, metformin and statins have been found to reduce the risk of hepatocellular carcinoma up to 50% and 60%, respectively. Furthermore, both drugs have shown a dose-dependent protective effect. However, information about chemopreventive role of metformin and statins is mainly obtained of observational studies, which could not take into account some bias. In conclusion, given the rising of incidence of hepatocellular carcinoma and the important morbidity and mortality rates associated with this cancer, looking for chemopreventive strategies is an essential task. Randomized controlled trials are needed to determine the definite role of metformin and statins on the prevention of hepatocellular carcinoma.

Key words: Hepatocellular carcinoma; Metformin; Metabolic syndrome; Mammalian target of rapamycin; Statin

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Abstract

Hepatocellular carcinoma is the third leading cause of cancer-related deaths in the world. It is associated with an important mortality rate and the incidence is increasing. Patients showing metabolic syndrome seem to have higher incidence and mortality rates from hepatocellular carcinoma than healthy subjects, especially those with type 2 diabetes mellitus and obesity. Thus, metformin and statins, both to treat features of metabolic syndrome, have been proposed to decrease the risk of hepatocellular carcinoma. Otherwise, liver cancer is the result of a complex process which impairs several signaling cascades, such as RAS/RAF/mitogen-

Core tip: Hepatocellular carcinoma is the result of a complex process which impairs several pathways, such as RAS/RAF/mitogen-activated protein kinase kinase/extracellular-signal-regulated kinase, phosphatidylinositol-4,5-bisphosphate 3-kinase/AKT/mammalian target of rapamycin and Wnt/ β -catenin signaling. Patients showing metabolic syndrome seem to have higher incidence and mortality rates from hepatocellular carcinoma than healthy subjects, especially those with type 2 diabetes mellitus and obesity. Thus, metformin and statins,

both to treat features of metabolic syndrome, have been proposed to decrease the risk of hepatocellular carcinoma. Metformin (by decreasing hyperglycemia state through 5'-adenosine monophosphate-activated protein kinase pathway activation) and statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) show anti-tumoral properties modifying several steps of the crucial signaling cascades.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths in the world^[1]. In recent years, a significant increase in HCC incidence and mortality rates has been observed in Western countries. Given that primary liver cancer shows a poor prognosis due to its infiltrating and malignancy power, we should closely assess those risk factors that could be preventable. Although the main risk factors for HCC are hepatitis C virus (HCV), hepatitis B virus (HBV) and chronic alcohol abuse, many individuals who have been exposed to these factors never develop HCC, while 15%-50% of cases occur among those without exposure, suggesting that further risk factors could be responsible for the increased incidence of HCC^[2].

Patients showing metabolic syndrome seem to have higher incidence and mortality rates from HCC than healthy subjects, especially those with type 2 diabetes mellitus (T2DM) and obesity^[3]. T2DM is an emerging risk factor of many chronic liver diseases, such as chronic hepatitis, non-alcoholic fatty liver disease and cirrhosis. Furthermore, DM has been proposed as a risk factor for HCC^[4]. On the other hand, previous studies have demonstrated that cirrhosis and HCV increase the susceptibility to diabetes mellitus^[5]. Nevertheless, exact pathophysiological mechanisms of these significant associations are still unclear. Otherwise, metformin and statins, both to treat features of metabolic syndrome, have been proposed to decrease the risk of HCC^[6]. Therefore, in this review, we aim to evaluate the role of some of possible intermediary mechanisms that could be associated with the onset and progression of HCC development, as well as the impact of metformin and statins on the appearance of the liver tumor.

CELL SURVIVAL, PROLIFERATION AND DIFFERENTIATION SIGNALING PATHWAYS

HCC is a kind of tumor based on inflammation. As a

result, there are an incessant cell injury, necrosis and regeneration that, ultimately, lead to activate mutations in key genes (especially, oncogenes and tumor suppressor genes)^[7]. This complex process results in impairment of several signaling cascades. In this review, we focus on RAS/RAF/mitogen-activated protein kinase kinase (MEK)/extracellular-signal-regulated kinase (ERK) (cell proliferation signaling pathway), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) (cell survival signaling pathway) and Wnt/ β -catenin (cell differentiation signaling pathway) signaling cascades (Figure 1). Furthermore, we revise the main pathways of DM associated with HCC.

The RAF/MEK/ERK *via* is one of the most powerful pathway that regulates crucial cellular processes^[8]. It is triggered by growth factors [epidermal growth factor (EGF), platelet-derived growth factor, Vascular endothelial growth factor, and insulin-growth factor (IGF)] and activating mutations of major oncogenic proteins, being RAS the key molecular signal regulator^[9]. Importantly, RAS also plays a regulatory role in other signaling pathways, especially the PI3K/AKT/mTOR pathway. RAS cascade is one of the main targets of sorafenib, the only currently effective therapy for advanced HCC^[10].

Activation of the PI3K-AKT signalling pathway is promoted by binding of growth factors (especially, IGF and EGF) to their receptors, resulting in disruption of the mTOR pathway^[11]. PI3K/AKT/mTOR axis has linked to angiogenesis and survival^[12]. Therefore, mTOR has emerged as an exciting target for cancer therapy. The mTOR complex comprises two forms: (1) mTOR complex 1 (mTORC1), closely implicated in protein translation; and (2) mTORC2, which is the primary responsible for the phosphorylation of AKT and could be necessary to sustain the oncogenic phenotype related to loss of Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase (PTEN)^[13]. PTEN negatively regulates the PI3K-AKT signaling pathway and has been associated with tumor grade, advanced disease stage and reduced overall survival in patients with HCC^[14]. In 40%-50% of HCC, dysregulated expression of effectors of mTOR has been observed^[15]. On the other hand, mTORC1 activation shows prognostic implications in terms of patient tumor recurrence after surgery^[16].

Wnt/ β -catenin signaling pathway has a close relationship with cancer^[17]. It consists of a large number of proteins that interact with each other. Mutations in β -catenin, which activate the Wnt signalling pathway, occur in one-third of HCCs^[18]. Wnt pathway regulates the expression of many genes (*c-Myc*, *c-Jun* and *cyclin D1*) *via* interaction with Frizzled receptors^[19]. In particular, the MYC proto-oncogene family contributes carcinogenesis by unrestricted cell proliferation and inhibiting cell differentiation^[20]. Accumulation of β -catenin induces transcription of several genes related to cell differentiation and proliferation. In fact, studies have shown that the expression of β -catenin was higher in HCC than in non-tumor tissues^[21], and Wnt-1 is a survival factor for HCC cells^[22]. On the other hand, mTOR regulates the expression level of β -catenin^[23].

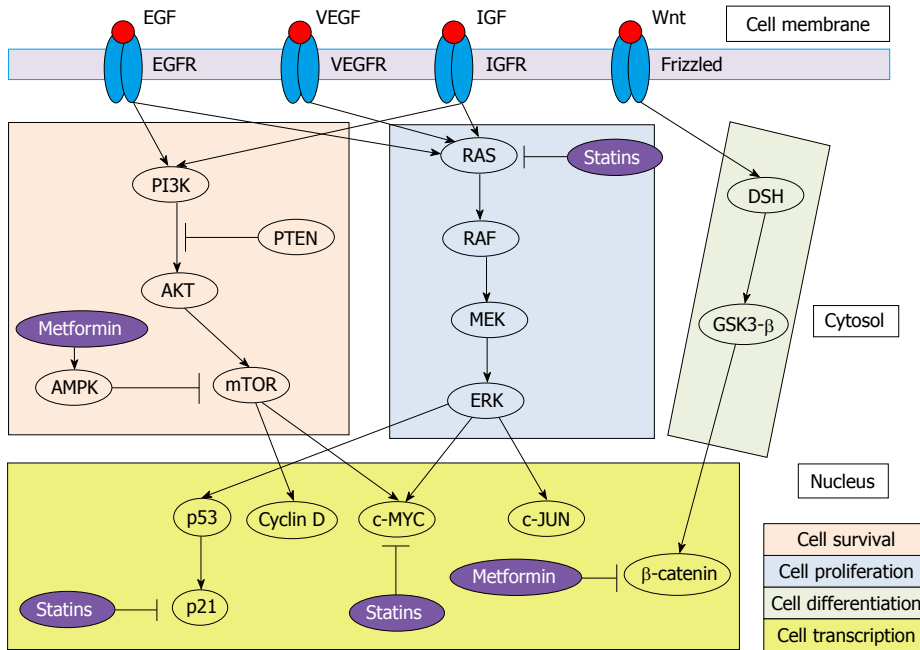


Figure 1 Pathogenic pathways of hepatocellular carcinoma and targets for metformin and statins. PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN: Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase; AMPK: Adenosine monophosphate-activated protein kinase; mTOR: Mammalian target of rapamycin; MEK: Mitogen-activated protein kinase kinase; ERK: Extracellular-signal-regulated kinase; GSK3-β: Glycogen synthase kinase 3 beta; EGF: Epidermal growth factor; VEGF: Vascular EGF; IGF: Insulin-growth factor; EGFR: EGF receptor; VEGFR: VEGF receptor; IGFR: IGF receptor.

Thus, this pathway is critical for tissue and liver regeneration.

On the other hand, patients showing features of metabolic syndrome may have higher incidence of HCC and mortality rates than those without it^[24]. In fact, DM and obesity increase the risk of appearance of HCC. Therefore, one hypothesis for this fact could be that patients with features of metabolic syndrome have more aggressive tumor characteristics, such as increased vascular invasion and metastasis. DM has been proposed as an independent risk factor for HCC^[25,26]. Mechanisms proposed for diabetes-induced liver cancer include: (1) hyperinsulinemia state, caused by insulin resistance, increases levels of IGF-1, which is one of the most powerful activators of cellular proliferation. This fact leads to elevated binding and consequently downstream signaling through the RAF/MEK/ERK and PI3K/AKT/mTOR pathways^[27]; (2) insulin activates the intrinsic tyrosine kinase of insulin receptor, by phosphorylation of insulin-receptor substrate-1. This latter, together with IGF-1, are overexpressed in tumor cells, generating inhibition of apoptosis^[28]; (3) insulin resistance leads to increase the releasing of multiple proinflammatory cytokines, including tumor necrosis factor alpha (TNFα) and interleukin 6, which promote the development of hepatic steatosis, inflammation and subsequent HCC^[29]; and (4) reactive oxygen species are also produced, impairing mitochondrial respiration and causing oxidative damage to the mitochondrial genome by activation of the apoptosis cascade^[30].

ANTITUMORAL EFFECTS OF METFORMIN AND STATINS

Metformin is an insulin-sensitizer drug frequently used in the first-line oral treatment of T2DM patients. Antioxidant, anti-inflammatory, growth inhibitory and antiangiogenic effects of metformin have been associated to reduce the risk of some solid tumors, such as prostate, colorectal, breast and pancreas^[31]. Metformin mainly works by decreasing hyperglycemia state through 5'-adenosine monophosphate-activated protein kinase (AMPK) pathway activation. Proposed anti-tumoral mechanisms of metformin include: (1) activated AMPK has growth inhibition effects on human cancer cell lines, *via* inhibition of mTOR^[32]; (2) metformin has demonstrated to limit cell growth through cell cycle G₀/G₁ arrest in hepatoma cell lines, by inhibiting cyclin D1 expression^[33]; (3) it can also inhibit carcinogenesis by downregulating c-Myc and upregulation miR-33a, which require activation of AMPK^[34]; (4) metformin is able to modulate the expression of cytokines, such as TNFα, and oxidative stress^[35]; (5) metformin, through AMPK, decreased β-catenin protein levels leading to suppression of Wnt/β-catenin signaling^[36]; and (6) metformin is taken up in hepatocytes by the organic cation transporter-1 (OCT-1), which is an essential step for the glucose-lowering effect^[37,38]. Interestingly, OCT-1 and OCT-3 expression has been found downregulated in HCC patients and associated with impaired prognosis^[39].

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. Additionally to the effect on cholesterol biosynthesis, statins also have antineoplastic properties. Antitumoral effects of statins are related to the following mechanisms: (1) they can effectively downregulate the RAF/MEK/ERK pathway, contributing to the apoptotic response^[40]; (2) statins limit the degradation of the cyclin-dependent kinase inhibitors p21 and p27. These molecules show growth-inhibitory effects; (3) HMG-CoA reductase is a crucial regulator of MYC phosphorylation and activation. Consequently, inhibition of HMG-CoA reductase prevents from both c-Myc phosphorylation and activation^[41]; and (4) anti-inflammatory and antioxidant effects of statins may be partly mediated by the PI3K/AKT pathway^[42], causing a decline in toll-like receptor 4 expression on blood monocytes and TNF α plasma concentration^[43].

CHEMOPREVENTIVE ROLE OF METFORMIN AND STATINS

Metformin use seems to decrease the risk of HCC in diabetic patients in several observational studies. Hassan *et al.*^[44] compared 420 diabetic patients with 1104 healthy controls [DM was related to HCC (OR = 4.2; $P < 0.05$)]. They analyzed different treatments, showing metformin and thiazolidinediones (TZD) as protective agents (OR = 0.3; $P < 0.05$) and sulphonylureas (OR = 7.1; $P < 0.05$) and insulin therapy (OR = 1.9; $P < 0.05$) as negative factors^[44]. Donadon *et al.*^[45] obtained similar results, assessing 610 patients with HCC, 618 cirrhotic patients without HCC and 1696 healthy controls. Metformin was shown as protective therapy (OR = 0.33; $P < 0.05$), opposite to sulphonylureas and insulin exogenous (OR = 3.06; $P < 0.05$). Nkontchou *et al.*^[46] observed prospectively a reduced incidence of HCC in diabetic HCV-related cirrhotic patients treated with metformin (HR = 0.19; $P < 0.05$). Lai *et al.*^[47] confirmed that T2DM was associated with HCC and that the HCC risk reduction was greater for diabetics taking metformin than those taking TZD (51% vs 44% reduction). Recently, Chen *et al.*^[48] concluded that metformin use was related to lower risk of HCC in diabetic patients in a dose-dependent manner. Similar results have been reported in meta-analysis. Zhang *et al.*^[49] included three cohort studies and four case-control studies, concluding that metformin treatment was associated with reduced risk of HCC in diabetic patients. Singh *et al.*^[50] performed a systematic review and a meta-analysis to evaluate the effect of antidiabetic therapy on the risk of HCC, including ten studies reporting 22650 cases of HCC in 334307 patients with T2DM. Meta-analysis showed a 50% of reduction in HCC incidence with metformin use, a 62% and a 161% increase in HCC incidence with sulphonylurea and insulin use, respectively, while TZD did not modify the risk of developing.

Statins may decrease the risk of HCC in patients with other underlying liver diseases, according to

observational studies. Tsan *et al.*^[51] reported a dose-dependent association between statin use and decreased risk of HCC development in patients with HCV (HR = 0.33; $P < 0.05$) taking higher daily doses of statins. The same group performed a similar study in HBV patients, and they observed a risk reduced up to 66% in patients which received more than one year cumulative treatment compared to those never treated. Furthermore, they observed that the reduction in HCC risk was a class effect^[52]. A recent meta-analysis evaluated 4298 cases of HCC in 1459417 patients. Authors found a 37% overall reduction in HCC risk with the use of statins. Interestingly, the risk reduction was higher in Asian people (OR = 0.52; $P < 0.05$), although this effect was also present in Western populations (OR = 0.67; $P < 0.05$), maybe due to interactions between statins and HBV^[53]. Furthermore, statins have been associated with decreased HCC recurrence after resection^[54]. In contrast to observational studies, randomized controlled trials have failed to show such association. In a post-hoc analysis from the Cholesterol Treatment Trialists' collaboration, there was no difference in the risk of appearance of HCC regardless the consumption of statins^[55]. However, randomized controlled trials were performed for cardiovascular endpoints, showing limitations: (1) patients enrolled were at low risk for development of HCC, limiting the power to detect a significant difference to development of HCC; (2) the follow-up was shorter than expected to evaluate the developing of HCC; and (3) statin nonusers in these groups had a elevated risk of cardiovascular mortality^[56].

Information about chemopreventive role of metformin and statins is mainly obtained of observational studies. However, best level of evidence comes from randomized clinical trials, so the current available data of these drugs show a lack of randomization necessary to control cofounders^[57]. The heterogeneity of the studies, the lack of randomization and the increased risk of reporting bias should indicate caution. A main concern about metformin use is the safety profile in patients with advanced liver disease, as metformin has been associated with serious adverse effects. However, there are studies in which well-compensated cirrhotic patients have taken metformin without adverse effects, beyond an increased prevalence of diarrhea^[58]. In addition, there is a concern about the safety of using metformin and statins in cirrhotic patients (who show the highest risk of HCC), which could introduce a selection bias at the moment of indicating the treatment. On the other hand, most of studies do not take into account to adjust for concomitant medications. Thus, the protective effect of metformin or statins could be enhanced by the other one, as they are relatively common in patients with metabolic syndrome. Interestingly, etiology of cirrhosis could influence on the antitumoral effect of these drugs, especially the closely relationship between HCV infection and metabolic syndrome^[59]. In fact, HCV directly affects the host lipid metabolism, favoring its own replication^[60], so inhibitors of lipid synthesis, such as statins, could decrease viral replication. Lastly, the

influence of environmental, like aflatoxin, or genetic factors, like PNPLA3^[61], could impact and mask the conclusions.

CONCLUSION

In this review, we have summarized the intermediary mechanisms responsible for the association between some features of metabolic syndrome and HCC development. Given the rising of incidence of HCC, especially in the Western countries, and the important morbidity and mortality rates associated with this cancer, looking for chemopreventive strategies is an essential task. Identifying who will benefit, optimal duration of treatment and relevant biomarkers will be crucial to design the appropriate strategy. Non-etiology-specific medications, such as statins and metformin, are cheap, have a favorable safety profile and could have metabolic effects in additional organs. However, further studies are needed to establish the definitive role of metformin and statins on the prevention of HCC. Randomized clinical trials, controlling comedications and genetic factors, are required for this purpose. Therefore, prevention through surveillance of risk populations is the best current option in day-to-day clinical practice to improve the prognostic of patients with HCC.

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Adrenal insufficiency in patients with decompensated cirrhosis

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well. Both stable cirrhotics and liver transplant patients (early and later after transplantation) have been reported to present AI. The mechanisms leading to reduced cortisol production in cirrhotics are the combination of low cholesterol levels (the primary source of cortisol), the increased cytokines production that overstimulate and exhaust HPA axis and the destruction of adrenal glands due to coagulopathy. AI has been recorded in 10%-82% cirrhotics depending on the test used to evaluate adrenal function and in 9%-83% stable cirrhotics. The similarity of those proportions support the assumption that AI is an endogenous characteristic of liver disease. However, the lack of a gold standard method for AI assessment and the limitation of precise thresholds in cirrhotics make difficult the recording of the real prevalence of AI. This review aims to summarize the present data over AI in stable, critically ill cirrhotics and liver transplant recipients. Moreover, it provides information about the current knowledge in the used diagnostic tools and the possible effectiveness of corticosteroids administration in critically ill cirrhotics with AI.

Key words: Critically ill; Cirrhosis; Adrenal insufficiency; Corticosteroid

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Core tip: Adrenal insufficiency is present in both critically ill and stable cirrhotics and in liver transplant recipients early or later after transplantation. Due to certain difficulties in determining cortisol levels and lack of gold standard method, the incidence of adrenal failure varies and depends on each test used for assessment of adrenal function. Corticosteroid administration has not been elucidated whether it leads to beneficial outcome in critically ill cirrhotics.

Abstract

Adrenal reserve depletion and overstimulation of the hypothalamus-pituitary-adrenal (HPA) axis are causes for adrenal insufficiency (AI) in critically ill individuals. Cirrhosis is a predisposing condition for AI in cirrhotics as

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INTRODUCTION

Cirrhosis is characterized by hyperdynamic circulatory failure, low arterial pressure, peripheral vasodilation and increased production of cytokines^[1,2]. Although, the adrenal insufficiency (AI) among critically ill cirrhotics was firstly described in 1960 by Peterson *et al*^[3], there is still an increased interest in it during the last decade. Initially, Marik *et al*^[4] used the term "hepato-adrenal syndrome" to describe the AI found in the critically ill cirrhotic patients correlated with increased mortality. Nowadays, it is established that AI is found in critically ill cirrhotic patients with or without sepsis^[5,6], in those with stable cirrhosis^[7-9] and in liver transplant recipients^[4,10]. There may be a deficient response of adrenal glands to the increased stress stimulation of hypothalamus-pituitary-adrenal (HPA) axis in critically ill patients named initially relative AI (RAI)^[11-16], replaced later on by the term critical illness related corticosteroid insufficiency (CIRCI)^[17]. This review aims to summarize the published data regarding AI in cirrhotics and in liver transplant recipients, additionally focusing in the diagnostic tools and the possible effectiveness of corticosteroids administration.

PATHOPHYSIOLOGY

AI has been described in all stages cirrhotic patients, critically ill and stable, implying that adrenal failure is a feature of liver dysfunction *per se*^[7-9]. However, the exact mechanism leading to AI in cirrhotic population is not yet clear. It is known that cholesterol is an important substrate for steroidogenesis and adrenal glands synthesize cortisol whenever is necessary^[18,19]. One main characteristic of cirrhotic patients is the low levels of total cholesterol, high density lipoprotein (HDL) and low density lipoprotein, which are correlated with the severity of liver disease^[20,21]. Thus, in cirrhosis, the adrenal glands cannot synthesize the adequate quantities of cortisol especially under stress conditions leading to "adrenal exhaustion syndrome" ending to AI^[22,23]. In addition, cirrhosis is characterized by the increased circulating pro-inflammatory cytokines, like tumor necrosis factor (TNF)- α , interleukin-6 (IL-6), IL-1 and endotoxin- like lipopolysaccharide^[24-27], which affect negatively the feedback of HPA axis. TNF- α reduces the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary gland, *via* completion with corticotrophin receptor and contributes to glucocorticoid deficiency^[28,29] and the pro-inflammatory cytokines contribute to the decreased levels of HDL cholesterol *via* inhibition of apolipoprotein- A1 synthesis resulting in limited delivery to adrenal glands^[30,31]. Finally, prolonged prothrombin time, a common finding in cirrhotic patients, could rarely lead to adrenal hemorrhage and impaired cortisol production^[23].

ASSESSMENT OF HPA FUNCTIONALITY

Total cortisol consists of free and binding forms^[32]. Only 10% of circulating cortisol is free and bioactive^[33]. The rest is mainly bound with corticosteroid-binding globulin (CBG) and less with albumin. In cirrhotic patients, hypoalbuminemia is positively correlated with the severity of liver disease leading to decrease of total cortisol and increase of the free bioactive fraction. Thus, the common methods for assessing adrenal function, based on total cortisol, may lead to overestimation of AI in patients with cirrhosis. In this case, the optimal method would be the direct evaluation of free cortisol, but its measurement is difficult in daily clinical practice. Indirectly free cortisol can be calculated by Coolens equation based on total cortisol and CBG^[34]. Salivary cortisol has been used as a surrogate marker of free cortisol but present limitations in cirrhotics^[7,35-37] including the high incidence of oral candidiasis, gums bleeding and parotitis especially in alcoholics^[38]. Finally, free cortisol index (FCI = total cortisol/CBG ratio) reflecting serum free cortisol levels has been used^[39]. FCI > 12 is indicative of normal adrenal function. However, it should be mentioned that none of these formulae/indexes takes into account albumin levels.

Basal serum cortisol and ACTH

A basal standard total cortisol level < 138 nmol/L between 8.00-9.00 am indicates AI, while basal total cortisol > 415 nmol/L makes the diagnosis of AI unlikely. Primary AI is indicated by ACTH > 22 pmol/L, while normal values of ACTH could not rule out secondary AI.

Short synacthen test

Tetracosactide (Synacthen) and cosyntropin (Cortrosyn) are the analogues used for Short synacthen test (SST). Plasma cortisol is monitored at 0, 30 and 60 min after intravenous (*iv*) or intramuscular injection of 250 μ g corticotrophin (Synacthen). If poststimulation cortisol exceeds 550 nmol/L, primary AI is excluded^[40]. SST uses supraphysiological doses of corticotrophin and is preferred in critically ill patients^[41]. In this patient group, AI is defined either by random total cortisol < 276 nmol/L or by delta cortisol < 250 nmol/L (CIRCI criteria)^[42]. Delta cortisol is the difference between basal cortisol and cortisol measured 60 min after *iv* injection of corticotrophin analogue^[43].

Low dose SST

Plasma cortisol is measured 30 min after stimulation with 1 μ g corticotropin given *iv*. If peak cortisol exceeds 500 nmol/L, adrenal function is normal. This test seems to be more sensitive than SST and evaluates better the stable cirrhotic patients^[41].

Corticotrophin-releasing hormone test

This is a test with high cost in which both cortisol and ACTH are measured at 0, 15, 30, 45, 60, 90 and 120 min after injection of 1 μ g/kg corticotrophin-releasing

hormone given intravenously. High ACTH levels after stimulation suggest primary AI, while a more blunted response indicates a possible secondary AI^[43].

Insulin-induced hypoglycemia test

It is considered the gold-standard to evaluate both the HPA axis growth hormone sufficiency, but it is not commonly used due to its contraindications, particularly in elderly people, those with cardiovascular disease and seizure disorders^[44]. A dose of 0.15 IU/kg regular insulin is given *iv* causing symptomatic hypoglycemia or blood glucose levels < 40 mg/dL, while cortisol levels are measured at 15, 30, 45, 60 and 90 min after stimulation. Failure of cortisol to exceed 500-550 nmol/L suggests AI.

Metyrapone test

This is the sensitive alternative test for ACTH reserve evaluation. Its utility is restricted by the limited availability of this compound in many countries. Metyrapone reduces cortisol production *via* blockage of 11 β hydroxylase, the enzyme that catalyzes the conversion of 11-deoxycortisol to cortisol. Thirty milligram per kilogram metyrapone are administered at 11:00 pm and ACTH, plasma cortisol and 11-deoxycortisol are measured in the next morning. Values of 11-deoxycortisol < 202 nmol/L in combination with rising levels of ACTH indicate primary AI, while neither 11-deoxycortisol nor ACTH rising indicates pituitary or hypothalamus impairment^[45,46].

Serum free and salivary cortisol

The thresholds concentrations of serum free cortisol that indicate AI in critically ill patients are < 50 nmol/L at baseline and < 86 nmol/L after SST^[47]. AI is indicated when basal values of salivary cortisol are < 1.8 ng/mL or salivary cortisol after SST is < 12.7 ng/mL, or an increment of < 3 ng/mL^[7,36].

ADRENAL FAILURE AND LIVER DISEASE-CURRENT EVIDENCE

The percentage of AI in cirrhotic patients varies among different studies and depends on the methodology and criteria used to estimate adrenal function^[5,7,8,23]. The classification of trials according to critical illness, stability of cirrhosis, and whether or not researchers included liver transplant population makes the evaluation of existing data more straight forward. The relevant studies were extracted conducting research in the following databases until August 2014: PubMed/MEDLINE, gms, gms meetings and Scopus using the term "cirrhosis and AI". Moreover we included the related posters and oral announcements of the European (EASL) and American (AASLD) liver meetings of 2013 and 2014.

Critically ill cirrhotic patients

The data regarding the prevalence of AI in critically ill cirrhotic patients are summarized in Table 1. Marik *et*

al^[41] were the first who evaluated AI in 340 critically ill cirrhotic patients using LDSST. For highly stressed patients the applied cut offs were random total cortisol < 552 nmol/L and for stressed patients the cut offs were either random cortisol < 414 nmol/L or a 30 min post synachten level of cortisol < 552 nmol/L. AI was reported in 72% critically ill cirrhotics overall; 33% presented with acute liver failure; 66% with chronic liver failure (CLF), while 62% were short term liver transplant recipients and 92% long term recipients. HDL was the only predictive factor for the AI prevalence. The same authors reported 54% AI in a similar group of patients applying the aforementioned criteria^[23]. Another study came from Thevenot *et al*^[7] who prospectively evaluated 30 septic cirrhotic patients. AI was found in 3 (10%), by using serum total cortisol < 510.4 nmol/L 60 min after SST. Salivary cortisol was also assessed. It was found to be significantly correlated with serum free cortisol ($P < 0.0001$) which was very high in patients with Child Pugh score C. The authors concluded that salivary cortisol was the most suitable marker adrenal function evaluation in patients with cirrhosis in the absence of serum-free cortisol availability. In another study including 75 cirrhotic patients with sepsis^[48], a higher proportion (76%) had AI compared to the study of Thevenot *et al*^[7]. The discrepancy between these two studies could be explained by the different criteria used to determine AI (in the latter study, AI was defined as delta cortisol < 250 nmol/L) (Table 1).

In a prospective study conducted in United Kingdom from 2007 to 2009^[49], 56 patients with ALF and 36 with acute on CLF (ACLF) underwent SST for adrenal function assessment. All were critically ill patients under vasopressor administration secondary to cardiovascular instability. According to CIRCI criteria, AI was found in 58% ACLF patients and it was related with HDL levels and with worse outcome. Triantos *et al*^[5] conducted an observational prospective trial evaluating the presence of AI (using both SST and LDSST) in 20 critically ill patients with cirrhosis and variceal bleeding. This group was compared with 14 healthy individuals and 60 patients with stable cirrhosis. According to SST, AI was found in similar proportion (30%) in critically ill and stable patients, while according to LDSST (peak cortisol level < 690 nmol/L or delta cortisol < 250 nmol/L for critically ill cirrhotics and peak cortisol < 414 nmol/L for stable cirrhotics) AI was found in 60% critically ill patients vs 48% stable cirrhotics. Moreover, the hypothesis that CIRCI occur both in septic and non-septic cirrhotics was confirmed in two more studies^[50,51]. AI (by using the SST) was found in 38% septic cirrhotics with severe variceal bleeding and in 73.5% non-septic critically ill cirrhotics.

In the study of du Cheyron *et al*^[6], AI was retrieved in 31 (62%) of 50 critically ill cirrhotics (according to the thresholds of 414 nmol/L for baseline cortisol and 250 nmol/L for delta cortisol, if baseline cortisol values were between 414 and 938 nmol/L). Using the same criteria, AI was found in 10 (77%) out of 14^[52] and 17 (68%)

Table 1 Characteristics and outcomes of the included studies in critically ill cirrhotics

Ref.	Study design; study period; country	No. of patients; type of liver disease	Adrenal failure	Other observations	Definition of adrenal failure
Etogo-Asse <i>et al</i> ^[40]	Prospective, observational; 2007-2009; United Kingdom	163 patients; 89 ALF and 74 AOCLF-56 ALF and 36 AOCLF underwent SST	AOCLF: 21/36 58% ALF: 27/56 48%	Among those with AI 17/32 (47%) with HDL < 0.1 mmol/L vs 2/17 (12%) with HDL > 0.6 mmol/L had increment < 250 nmol/L HDL was lower in non survivors both in AOFLD and ALF	SST to those required vasopressor administration or cardiovascular instability CIRCI: Basal cortisol < 275 nmol/L or delta cortisol < 250 nmol/L
Triantos <i>et al</i> ^[5]	Prospective, observational; NR; NR	20 patients; cirrhosis and variceal bleeding vs 74 controls (14 healthy and 60 stable cirrhosis)	SST: 6/20 30% LDSST: 6/10 60% Healthy (SST and LDSST): 0/14 0% Stable (LDSST): 24/50 48% Stable (SST): 3/10 30% 3/30 10%	AI wasn't associated with outcome Those with AI and variceal bleeding had higher baseline and peak level of cortisol with stable cirrhotic, but similar delta cortisol With SST for albumin > 2.5 mg/dL, AI: 4/16 (25%) with variceal bleeding vs 1/8 (12.5%) in cirrhosis control With LDSST, for albumin > 2.5 mg/dL, AI: 6/10 (80%) with variceal bleeding vs 16/39 (41%) in cirrhosis control Significant correlation between salivary and serum free cortisol ($P < 0.0001$) Serum total cortisol were significantly lower in Child-Pugh score C than B or A, in contrary with free cortisol which had a non significant rise	SST AI: Peak cortisol < 500 nmol/L in non-stressed patients and delta cortisol of < 250 nmol/L or a random total cortisol < 276 nmol/L in stressed patients LDSST AI: Peak cortisol < 500 nmol/L in non-stressed patients and peak cortisol level of < 690 nmol/L or a delta cortisol < 250 nmol/L in stressed patients
Thevenot <i>et al</i> ^[7]	Prospective; 2008-2009; France	30 patients; septic cirrhotic			SST-AI: Post-SST SC < 510.4 nmol/L Salivary cortisol was also calculated
Arabi <i>et al</i> ^[48]	Randomized double blind; 2004-2007; Saudi Arabi	75 patients; septic shock and cirrhosis in ICU	57/75 76%		SST RAI: Delta cortisol < 250 nmol/L
du Cheyron <i>et al</i> ^[6]	Prospective; 2003-2005; France	50 patients; decompensated cirrhosis in ICU (critical ill with acute on chronic liver disease)	31/50 62%		SST AI: Baseline cortisol value < 414 nmol/L, or delta cortisol < 250 nmol/L with a baseline value between 414 and 938 nmol/L
Thierry <i>et al</i> ^[52]	Prospective; March to December 2005; France	34 patients; septic shock, 14 with and 20 without cirrhosis	Cirrhotic: 11/14 77% Non cirrhotic: 10/20 50%		SST baseline cortisol < 414 nmol/L and/or delta cortisol < 250 nmol/L
Fernández <i>et al</i> ^[53]	Prospective and retrospective; group 1 2004-2006, group 2 2001-2004	Group 1: 25 patients; cirrhosis and septic shock Group 2: 50 patients; no assessment of adrenal function	17/25 68%		SST RAI: (1) Baseline cortisol concentration < 414 nmol/L or (2) delta cortisol < 250 nmol/L in patients with baseline cortisol concentration < 966 nmol/L
Tsai <i>et al</i> ^[54]	2004-2005; Taiwan	101; cirrhosis and severe sepsis required ICU	52/101 51.4% Hemodynamically unstable: 43/54 79.61% Stable: 9/47 19.14%	ICU mortality: 71.4% vs 26.5% Hospital mortality: 80.7% vs 36.7% (AI vs normal) Correlation with the severity of liver disease	SST AI: Baseline value < 414 nmol/L, or delta cortisol < 250 nmol/L with a baseline value between 414 and 938 nmol/L

Marik <i>et al</i> ^[23]	Retrospective; NR; United States	221 patients; LTICU	At admission: 120/221 54% In 3 d: 16/101 16%	Low HDL could predict the development of AI	LDSST AI: (1) a random (stress) cortisol < 552 nmol/L in patients with hypoxemic respiratory failure, hypotension or requiring vasopressor agents and (2) a random level < 414 nmol/L or a 30-min post-low-dose cosyntropin stimulation test level of < 552 nmol/L in non-highly stressed patients LDSST AI: (1) a random (stress) cortisol < 552 nmol/L in patients with hypoxemic respiratory failure, hypotension or requiring vasopressor agents and (2) a random level < 414 nmol/L or a 30-min post-low-dose cosyntropin stimulation test level of < 552 nmol/L in non highly stressed patients
Marik <i>et al</i> ^[4]	Retrospective; 2002-2004; United States	340 patients; ALD, CLD, post OLT recently and remote LT	Overall: 245/340 72% ALD: 8/24 33% CLD: 97/146 66% Remote LT: 31/51 61% Recent LT: 109/119 92% Among those treated with vasopressors: 125/166 75% 73.5%	Low HDL could predict the development of AI	LDSST AI: (1) a random (stress) cortisol < 552 nmol/L in patients with hypoxemic respiratory failure, hypotension or requiring vasopressor agents and (2) a random level < 414 nmol/L or a 30-min post-low-dose cosyntropin stimulation test level of < 552 nmol/L in non highly stressed patients
Nair <i>et al</i> ^[51]	India	Critical ill cirrhotic in ICU, without sepsis		AI is not associated with severity of liver disease, CRP or etiology of cirrhosis	SST RAI: random basal TC ≤ 276 nmol/L or delta cortisol ≤ 250 nmol/L
Saffioti <i>et al</i> ^[51]	2009-2013	80; cirrhotic pre-LT	18/80 22.5%	Patients with AI had higher MELD (19 vs 15; <i>P</i> = 0.003), pre-LT INR, bilirubin and potassium, and lower AI: At least 2 of the following: baseline sodium and haemoglobin levels	SST cortisol < 148 nmol/L, peak cortisol < 550 nmol/L, delta cortisol < 250 nmol/L
Graupera <i>et al</i> ^[50]	Spain	37; cirrhotic with severe variceal bleeding	14/37 38%	6 wk survival 64% without and 31% with RAI No differences in overall survival	SST RAI: Baseline serum cortisol < 414 nmol/L or delta cortisol < 250 nmol/L

NR: Not reported; AI: Adrenal insufficiency; HDL: High density lipoprotein; SC: Serum cortisol; ICU: Intensive care unit; ALF: Acute liver failure; AOCLF: Acute on chronic liver failure; SST: Short synacthen test; LDSST: Low dose short synacthen test; NR: Not reported; CIRC: Critical illness related adrenal insufficiency; RAI: Relative adrenal insufficiency; LTICU: Liver transplant intensive care unit; OLT: Orthotopic liver transplantation; CLD: Chronic liver disease; ALD: Acute liver disease; LT: Liver transplantation; MELD: Model for end-stage liver disease; CRP: C-reactive protein; INR: International normalized ratio.

out of 25^[53] cirrhotics with sepsis. In the latter study, AI was related with the severity of liver disease (AI was found in 76% of in patients with Child-Pugh class C vs 25% of patients with Child-Pugh class B, *P* = 0.08), a finding which was confirmed by the trial of Tsai *et al*^[54] evaluating 101 cirrhotics with sepsis as well. The common finding in the last four studies was the strong association between AI and outcome concluding that glucocorticoid supplementation could lessen the mortality.

Summarizing the above data, SST was the commonly used test for adrenal function assessment. LDSST or both tests were used in three studies^[4,5,23]. All studies reported values of total cortisol. The presence of AI ranged from 10% to 77% according to SST and 54% to 72% according to LDSST. This wide variation in the AI prevalence could be explained by the variant thresholds used. When more strict criteria for the AI diagnosis were applied, AI prevalence appeared low around 22.5%^[55]. DSST overestimated AI, when performed in stressed patients and was more reliable for the detection of subclinical AI in stable cirrhotics. This assumption was confirmed

in the study of Triantos *et al.*^[5] in which both tests were applied. With the exception of sepsis, variceal bleeding was the complication of the underlying cirrhosis in two studies^[50,56]. In the latter group of cirrhotics with variceal bleeding, AI was diagnosed in 30%-38% with SST and in 60% with LDSST. It was LDSST again, which overestimated the prevalence of AI. Furthermore, the fact that Marik detected AI in non-adrenal insufficient cirrhotics three days after the first evaluation, indicated that adrenal function is a dynamic process and critically ill cirrhotics should be re-assessed^[23]. The common study endpoints were that low HDL levels predict the presence of AI in critically ill cirrhotics and that impaired adrenal function was associated with the outcome^[4,23,49]. AI was also more apparent in patients with more severe liver disease. It should be mentioned that these studies calculated total cortisol without taking into account the low levels of serum albumin. Some of the studies defined adrenal failure as an independent risk factor for worse outcome^[49,54], while others showed no association^[5,50,51]. Interestingly, there was no correlation between AI and worse outcome in cirrhotic patients with variceal bleeding. Since the number of patients was low, safe conclusions could not be drawn.

Not critically ill cirrhotic patients

AI is present in stable patients with decompensated cirrhosis and its prevalence varies according to the applied diagnostic test (SST or LDSST). The data regarding the prevalence of AI in stable cirrhotic patients are indicated in Table 2.

In a prospective trial^[56], adrenal function was evaluated in 79 stable cirrhotics. All patients underwent LDSST and AI was recorded in 34%, 28%, 30% of patients using as definition the presence of peak total cortisol < 494 nmol/L, peak free cortisol < 33 nmol/L at 30 min after stimulation or FCI < 12, respectively. Similarly to critically ill cirrhotics, total cortisol overestimated AI potentially due to the low levels of CBG and albumin, while FCI was correlated with free cortisol. No significant association was highlighted between the presence of AI and the outcome.

In the study of Acevedo *et al.*^[57], RAI was found in 37 (26%) of the 143 non-critically ill cirrhotics with acute decompensation. SST was also used for RAI determination. Interestingly, patients with RAI had longer duration of hospitalization, higher risk for infections, sepsis and hepatorenal syndrome (HRS) type I and higher mortality (during hospitalization and after three months of follow up) compared to those without RAI. In addition, RAI was not associated with the severity of liver disease and the type of decompensation with exception to type I HRS. The latter group of patients had a trend towards higher frequency of RAI. However, in the study of Kharb *et al.*^[10], AI was more frequent in patients with more severe liver disease as estimated by the Child-Pugh score. Moreover, low HDL cholesterol was associated with the presence of AI.

The LDSST was used in a study with 95 hemo-

dynamically stable cirrhotic patients^[7]. The thresholds were firstly basal serum total cortisol < 138 nmol/L and total cortisol 30 min after stimulation < 440 nmol/L, secondly serum total cortisol < 500 nmol/L at 30 min after LDSST and thirdly delta cortisol < 250 nmol/L. The AI prevalence according to each of the above criteria was 7.4%, 19%, 27.4% and 49.4% respectively. Serum free cortisol was also measured and its levels were significantly associated with mortality. Patients with ascites and more severe liver disease had higher free cortisol (basal and after stimulation). In another study, using the same criteria for AI in 101 stable cirrhotics^[8], AI was reported in 38%, 29% and 60%, respectively. Again, there was a strong relationship between AI and severity of liver disease.

Tan *et al.*^[9] evaluated the presence of AI based on total and free cortisol in 43 stable cirrhotics using SST. AI was found in 39%, 47% and 23% of patients by using peak total cortisol < 500 nmol/L, CIRCI criteria (delta cortisol < 250 nmol/L) and FCI (< 12), respectively. In addition, AI was reported in only 12% of subjects by applying peak plasma free cortisol < 33 nmol/L. Therefore, there was a significant discrepancy of AI proportions by using variant diagnostic criteria. Plasma free cortisol was significantly associated with higher MELD score and mortality. In another study^[36] 88 stable, mainly alcoholic cirrhotics were evaluated with SST. AI was assessed with total cortisol (basal value < 250 nmol/L or peak total cortisol after stimulation < 500 nmol/L or delta cortisol < 250 nmol/L) and with salivary cortisol (basal values < 1.8 ng/mL or peak cortisol at 60 min < 12.7 ng/mL or an increase between these two values < 3 ng/mL). AI was overestimated by using total cortisol, compared to salivary cortisol (33% vs 9%), particularly in patients with albumin < 2.5 mg/dL. Ascites and HDL levels were independently associated with the presence of AI. The relatively low prevalence of AI in this study was attributable to the high proportion of patients with alcoholic cirrhosis. Alcohol caused pseudo-cushing syndrome potentially leading to compensation in regards to AI secondary to cirrhosis.

In total, seven studies^[58-64] confirmed that total cortisol overestimates AI in stable cirrhotics, compared to either FCI^[58] or salivary cortisol^[60] (Table 2). Interestingly, Privitera *et al.*^[63] showed that total cholesterol contributed more to impaired cortisol production, compared to HDL. Nevertheless, in the study of Acevedo *et al.*^[64], RAI (defined by SST as delta cortisol < 250 nmol/L) HDL was significantly associated with severe infections ($P = 0.01$), septic shock ($P = 0.01$) and mortality ($P = 0.04$).

Summarizing the above results, SST was used in 10 studies and LDSST in 4^[7,8,56], although the included population were non-critically ill cirrhotics^[63]. The prevalence of AI ranged from 26% to 80% according to the SST and 7.4% to 38% according to LDSST. When the CIRCI criteria were applied, the presence of AI was overestimated in all studies (46%-70% vs 34.6%-40%^[10], 9.4% vs 7.4%-27.4%^[7], 60% vs

Table 2 Characteristics and outcomes of the included studies in not critically ill cirrhotic patients

Ref.	Study design; study period; country	No. of patients; type of liver disease	Adrenal failure	Other observations	Definition of adrenal failure
Fede <i>et al</i> ^[56]	Prospective, observational; NR; United Kingdom	79 patients; cirrhotics for pretransplantation or decompensation of cirrhosis	TC: 27/79 (34%) FC: 22/79 (28%) [for FC < 25: 15/79 (19%)] FCI: 24/79 (30%)	AI was not correlated with the outcome	LDST AI: Peak TC < 494 nmol/L at 20 or 30 min FC < 33 nmol/L FCI < 12
Acevedo <i>et al</i> ^[57]	Prospective, observational; 2008-2010 Spain	143 patients; acute decompensation of cirrhosis - follow up for 3 mo	37/143 (26%)	RAI was similar between different Child-Pugh scores and various causes of decompensations with the exception of HRS type-1 (trend for higher proportions) RAI was correlated with worse outcome both during hospitalization and in 3 mo period AI was correlated with severity of liver disease	SST RAI: Delta cortisol < 250 nmol/L in patients with basal serum TC < 938 nmol/L
Kharb <i>et al</i> ^[10]	Cross sectional; 2010-2011; India	25 ALD, 50 CLD, 10 post liver transplanted	ALD: 9/25 (34.6%) CLD: 20/50 (40%) (18/30 with child 2, 3 and 2/20 with child 1) Post LT: 4/10 (40%) RAI: ALD: 17/25 (65.4%), CLD: 23/50 (46%), post LT: 7/10 (70%)		SST AI: Basal cortisol levels < 83 nmol/L or a peak cortisol response < 500 nmol/L RAI: Delta cortisol < 250 nmol/L
Thevenot <i>et al</i> ^[7]	Prospective; 2008-2009; France	95 patients; hemodynamically stable cirrhotic mainly alcoholic	18/95 (19%) 26/95 (27.4%) 47/95 (49.4%) (According each threshold) (1) 38/101 (38%) (2) 29/101 (29%) (3) 61/101 (60%) (4) 0/41 (0%)	Patients with Child C cirrhosis and those with ascites had higher non significant rise in basal and stimulated serum FC Serum FC levels were directly associated with the risk of non transplant-related mortality AI was more frequent in hypoalbuminemic patients, according TC and delta cortisol and related with the severity of liver disease TC and cFC were significantly related FCI was lower in patients with AI	LDST AI: (1) basal serum TC < 138 nmol/L and a T30 serum TC < 440 nmol/L; (2) T30 serum TC < 500 nmol/L; (3) delta cortisol < 250 nmol/L LDST, FCI, cFC AI: Peak (1) TC < 500 nmol/L (2) TC < 442 nmol/L (3) Delta cortisol < 250 nmol/L (4) FCI < 12
Fede <i>et al</i> ^[8]	Prospective, observational; NR; United Kingdom	101 patients; stable cirrhosis			
Tan <i>et al</i> ^[9]	Prospective, observational; 2008-2009; Australia	43 patients; stable cirrhosis	(1) 18/43 (39%) (2) 20/43 (47%) (3) 5/43 (12%) (4) 25/43 (58%) (5) 10/43 (23%)	With serum FC criteria, patients with AI had significantly higher MELD score ($P = 0.03$) and mortality ($P = 0.0007$) Serum TC was correlated well with serum FC in pts with albumin both > and < 30 g/L Serum FC correlated significantly with FCI at baseline but less strongly with peak FC Overall survival at 6 and 12 mo was similar between AI and non AI group according TC	SST (1) Standard criteria: peak TC < 500 nmol/L (2) CIRCI criteria: delta cortisol < 250 nmol/L (3) Peak serum FC < 33 nmol/L (4) Any set of criteria (5) FCI < 12
Galbois <i>et al</i> ^[36]	Prospective, observational; 2006-2009; France	88 patients; complication of cirrhosis - alcoholic mainly	TC: 29/88 (33%) SC: 8/88 (9.1%)	There was correlation between cFC and SC Between SC and TC there was correlation for alb > 2.5 mg/dL whereas for alb < 2.5 mg/dL there was correlation for T0 but no for T60 or delta cortisol Acites and HDL were independent risk factors for AI	SST TC: basal TC < 250 nmol/L or in T60 < 500 nmol/L or delta cortisol < 250 nmol/L SC: T0 < 1.8 ng/mL or T60 < 12.7 ng/mL or delta cortisol < 3 ng/mL

Vincent <i>et al</i> ^[58]	Retrospective; NR; NR	26 patients; 15 CLD and 11 ALD	TC: 12/26 (46%) FCI: 3/26 (13%)	SST TC < 550 nmol/L FCI < 12 SST TC < 550 nmol/L
Shin <i>et al</i> ^[62]	Prospective; 2011-2012; South Korea	50 patients; stable cirrhosis	22/50 (44%)	AI was not related with the etiology of cirrhosis or alcohol consumption but only with the severity of liver disease
Privitera <i>et al</i> ^[63]	NR; NR; Italy	82 patients; cirrhotic stable	26/82 (32%)	In cirrhotic with AI, there was significant reduction in total cholesterol, TRG and ApoA1, but not in total HDL, HDL2 and HDL3
Cholongitas <i>et al</i> ^[60]	Prospective; 2010-2012; Greece	89 patients; stable decompensated cirrhosis	TC: 49/89 (55%) SC: 33/89 (37%)	For albumin > 2.5, TC and SC correlated for T0 and T60 Urinary potassium was the only factor significant associated with SC-AI
Acevedo <i>et al</i> ^[59]	Prospective; 2007-2009; Spain	198 patients; 10 with compensated and 188 with decompensated cirrhosis	(1) 120/188 (64%), 8/10 (80%) (2) 51/188 (27%), 2/10 (22%)	No significant difference in mortality between patient SST with or without RAI RAI: Basal TC < 414 nmol/L and/or delta cortisol < 250 nmol/L (criteria 1) or delta cortisol < 250 nmol/L (criteria 2) SST RAI: Delta cortisol < 250 nmol/L
Acevedo <i>et al</i> ^[64]	Prospective; 2007-2010; Spain	166 patients; advanced cirrhosis	43/166 (26%)	Those with RAI had higher degree of circulatory dysfunction, SIRS ($P = 0.01$), septic shock ($P = 0.01$) and hospital mortality ($P = 0.04$) AI was associated with reduced survival ($P = 0.03$)
Risso <i>et al</i> ^[61]	NR; NR; NR	85; stable cirrhotic with ascites	33/85 (39%)	SST RAI: Delta cortisol < 250 nmol/L and/or peak cortisol < 500 nmol/L

NR: Not reported; TC: Total cortisol; FCI: Free cortisol index; LDSST: Low dose short synacthen test; SST: Short synacthen test; AI: Adrenal insufficiency; RAI: Relative adrenal insufficiency; ALD: Acute liver disease; CLD: Chronic liver disease; LT: Liver transplantation; cFC: Calculated free cortisol; HDL: High density lipoprotein; TRG: Triglycerides; CIRCI: Critical illness related adrenal insufficiency; SC: Salivary cortisol; HRS: Hepatorenal syndrome; MELD: Model for end-stage liver disease.

29%-38%^[8], 47% vs 12%-39%^[9]. AI based on plasma free cortisol, FCI and salivary cortisol was detected in 12%-28%, 0%-30% and 9.1%-37%, respectively. Predictive factors for the presence of AI were ascites, HRS-1, total and HDL cholesterol. AI was positively correlated with the severity of liver disease in the vast majority of studies^[8-10,62] and with worse outcome in a few studies^[7,61,64].

When plasma free cortisol was applied, AI was detected in a statistically lower proportion compared to the use of total cortisol^[8,9,36,56,58,60]. However for values of albumin greater than 2.5 mg/dL, total cortisol was consistently correlated with free cortisol^[36,60]. Moreover, plasma free cortisol was associated with FCI, salivary cortisol and calculated free cortisol. FCI is considered more appropriate diagnostic test for AI in stable cirrhotics (when it is available) compared to total cortisol. In critically ill cirrhotics, the CIRCI criteria are recommended for Adrenal function evaluation; in case free cortisol is used, the thresholds are the same with those used in healthy individuals. Nevertheless, their implementation in stable cirrhotics is doubtful, so more studies are needed to define the gold standard method for AI diagnosis in non-critically ill cirrhotics.

Patients after liver transplantation

The results of the studies evaluating the AI prevalence in liver transplant recipients are summarized in Table 3. In the study by Kharb *et al*^[10], AI was presented in 4 (40%) of the 10 liver transplant recipients (defined as basal cortisol levels < 80 nmol/L or levels of peak cortisol after stimulation < 500 nmol/L using SST). Using the criterion of delta cortisol < 250 nmol/L, RAI was detected in 70% of patients. These results indicated the possible need of corticosteroid administration in liver transplant recipients post operatively and until the liver function is fully restored. Moreover, Patel *et al*^[65] proved that the administration of 1000 mg methylprednisolone during the operation

Table 3 Characteristics and outcomes of the included studies in post transplanted patients

Ref.	Study design; study period; country	No. of patients; type of liver disease	Adrenal failure	Definition of adrenal failure
Kharb <i>et al</i> ^[10]	Cross sectional; 2010-2011; India	10; OLT	Post LT: 4/10 (40%) RAI: Post LT: 7/10 (70%)	SST AI: Basal cortisol levels < 83 nmol/L or a peak cortisol response < 500 nmol/L RAI: Delta cortisol < 250 nmol/L LDSST
Marik <i>et al</i> ^[4]	Retrospective; 2002-2004; United States	119 post OLT recently and 51 remote OLT	Recent LT: 109/119 (92%) Remote LT: 31/51 (61%)	AI: (1) a random (stress) cortisol < 552 nmol/L in patients with hypoxemic respiratory failure, hypotension or requiring vasopressor agents and (2) a random level < 414 nmol/L or a 30-min post-low-dose cosyntropin stimulation test level of < 552 nmol/L in non-highly stressed patients
Patel <i>et al</i> ^[65]	Retrospective; NR; United Kingdom	90 patients; ICU post OLT; 45 patients received bolus dose of 1000 ng methylprednisolone intraoperative vs 45 patients not receiving	First group: significant reduced requirements for fluid administration ($P = 0.02$), vasopressors ($P = 0.01$), renal replacement therapy ($P = 0.001$), invasive ventilation ($P = 0.01$), and ICU stay ($P = 0.02$), compared to the second group	

AI: Adrenal insufficiency; RAI: Relative adrenal insufficiency; SST: Short synacthen test; LDSST: Low dose short synacthen test; OLT: Orthotopic liver transplantation; LT: Liver transplantation; ICU: Intensive care unit; NR: Not reported.

was associated with better outcome, less need of vasopressors, invasive ventilation and renal replacement therapy. This supports the assumption that RAI is present in liver transplant patients as well. Marik *et al*^[4] estimated AI by using LDSST in liver transplant recipients post operatively and later after transplantation. AI was reported in 109 (92%) of 119 and in 31 (61%) of 51 subjects, respectively. Liver transplant recipients recorded later after transplantation were treated with steroid-free immunosuppressive regimens. The high prevalence of AI was explained by the fact that the LDSST was the preferred test in stable patients, and thus AI was overestimated in stressed subjects.

Treatment with steroids

The data on corticosteroid administration in critically ill patients, especially in those with septic shock are controversial^[42,66,67]. A recent meta-analysis^[68] showed that low dose hydrocortisone improved shock reversal and short term mortality, but not 28-d mortality. Potential explanations were infections, gastrointestinal bleeding and hyperglycaemia observed during steroid administration. The recent International Guidelines for Management of Severe Sepsis and Septic Shock recommend the administration of low dose hydrocortisone intravenously for septic patients remaining hemodynamically unstable despite fluid resuscitation and vasopressor therapy^[12]. The studies regarding the administration of cortisol in cirrhotics are presented in Table 4. Etogo-Asse *et al*^[49] studied 51 vasopressor depended-critically ill cirrhotics receiving hydrocortisone in a median dose of 200 mg/

d. Interestingly, the mortality rate (65%) was similar between those and the group who did not receive corticosteroid supplementation. The only randomized double blind trial^[48] of three years duration conducted in Saudi Arabi and included 75 cirrhotics with septic shock. Thirty nine patients receiving hydrocortisone (50 mg intravenously every six hours until shock resolution) compared with 36 patients receiving placebo. Although there was improvement in hemodynamic parameters ($P = 0.05$) in the hydrocortisone group, no difference was noticed regarding 28-d, intensive care unit (ICU) and hospital mortality. Controversially, the hydrocortisone group had higher frequency of shock relapse ($P = 0.03$) and gastrointestinal bleeding ($P = 0.02$). Alike, du Cheyron *et al*^[6], found similar 30-d mortality between 14 patients who were treated with stress doses of cortisol and 17 who were not treated (50% vs 70%, respectively, $P = 0.29$).

Fernández *et al*^[53] reported AI in 17 of 25 cirrhotics with septic shock treated with 50 mg hydrocortisone four times per day. This group was compared with a historical group with similar characteristics who was not on hydrocortisone. The hydrocortisone group presented higher rates of shock resolution (96% vs 58%, $P = 0.001$), ICU-survival (68% vs 38%, $P = 0.03$) and hospital-survival (64% vs 32%, $P = 0.003$). In the study of Marik *et al*^[4] hydrocortisone (300 mg/d) administered in 140 vasopressor-dependent cirrhotics with acute liver disease (ALD) and chronic liver disease. The mortality rate was significantly lower in patients on hydrocortisone compared to those not treated with

Table 4 Characteristics and outcomes of the included studies of patients treated with steroids

Ref.	Study design; study period; country	No. of patients; type of liver disease	Hydrocortisone	Outcome
Etogo-Asse <i>et al</i> ^[49]	Prospective, observational; 2007-2009; United Kingdom	51 critical ill cirrhotic patients required vasopressors	31 received hydrocortisone of a median dose of 200 mg/d	Mortality: 13/20 (65%) in those who did not and 20/31 (65%) in those who received corticosteroid
Arabi <i>et al</i> ^[48]	Randomized double blind; 2004-2007; Saudi Arabi	75 patients; septic shock and cirrhosis in ICU	39 patients received 200 mg hydrocortisone <i>iv</i> /d vs 36 patients receiving normal saline until shock resolution	Shock reversal: 24/39 (62%) with hydrocortisone vs 14/36 (39%) with placebo ($P = 0.05$) Shock relapse after tapering: 13/39 (34%) vs 5/36 (14%) ($P = 0.03$) 28 d mortality: 33/39 (85%) vs 26/36 (72%), ($P = 0.19$) Increase in gastrointestinal bleeding ($P = 0.02$) in hydrocortisone group
du Cheyron <i>et al</i> ^[6]	Prospective; 2003-2005; France	31 AOCLD with AI	14 treated with stress doses of cortisol vs 17 not treated	30 d mortality: 7/14 (50%) of those treated vs 12/17 (70%) not treated ($P = 0.29$)
Fernández <i>et al</i> ^[53]	Prospective and retrospective; group 1 2004-2006, group 2 2001-2004	Group 1: 17 patients; cirrhosis and septic shock and AI Group 2: 50 patients; no assessment of adrenal function	17 patients of group 1 treated with 200 mg hydrocortisone/d vs 50 patients not treated	Mortality: group 1 32% vs 62% in group 2 in ICU ($P = 0.03$), 36% vs 68% ($P = 0.003$) in hospital Septic shock resolved in 96% vs 58% in group 2 ($P = 0.001$)
Marik <i>et al</i> ^[4]	Retrospective; 2002-2004; United States	140 patients vasopressor depended with ALD or CLD and AI	300 mg hydrocortisone/d	Reduction in dose of norepinephrin in the 24 h ($P = 0.02$) in those with AI treated with hydrocortisone and increase in those with AI not treated ($P = 0.04$) Mortality: 26% in those treated with steroids and 46% in not treated ($P = 0.002$)
Harry <i>et al</i> ^[69]	Retrospective; 1999-2001; United Kingdom	40 patients with ALD or AOCLD required vasopressors	20 patients treated with 300 mg hydrocortisone/d vs 20 patients not treated	In the group of 20 patients treated, there was reduction in doses of norepinephrin, higher risk of infections and no benefit in survival compared with the 20 patients not treated

CLD: Chronic liver disease; ICU: Intensive care unit; ALD: Acute liver disease; AOCLD: Acute on chronic liver disease; AI: Adrenal insufficiency.

hydrocortisone (26% vs 46%, $P = 0.002$). Furthermore, patients with AI on hydrocortisone required less doses of norepinephrine over the first 24 h ($P = 0.02$) compared to those without AI ($P = 0.62$) while patients with AI not receiving hydrocortisone required increased doses of vasopressors compared also with the non AI group ($P = 0.04$). Finally, Harry *et al*^[69] contrasted 20 cirrhotics with ALD or decompensated cirrhosis, vasopressor dependent on 300 mg/d hydrocortisone with a group of 20 cirrhotics with similar characteristics not treated with steroids. The steroids group required less norepinephrine doses, but showed no benefit in survival and higher bacterial infections.

Summarizing the data of five non-randomized trials, glucocorticoids (200-300 mg/d) were usually administered in vasopressor depended critically ill cirrhotics. In three studies, there was a temporary reduction of vasopressor doses in patients treated with steroids but mortality rates between those treated and those not treated with steroids^[6,49,69] were similar secondary to shock relapse and infection increase. However, opposite results come from two other studies^[4,53], reporting significant improvement in hemodynamic stability and mortality of cirrhotics treated either with 200

mg or 300 mg of corticosteroid.

CONCLUSION

Based on recent data, AI is present in cirrhotics either due to the various parameters associated with the primary disease or as a characteristic of cirrhosis *per se*. The fact that AI prevalence is high not only in critically ill but also in stable cirrhotics further supports these data. So far, there has not been a consensus about the appropriate method for the precise AI diagnosis. The results vary according to each test used to evaluate adrenal function. Furthermore, the thresholds in patients with liver disease might be different from other populations and free cortisol cannot be not easily estimated and is costly. Salivary cortisol could be an alternative approach, although it has limitations as well. Additional double blind randomized studies should be recruited in order to indentify the reliable cortisol cut offs. Moreover the benefits of cortisol administration should be further elucidated towards the appropriate given dose and administrative period in hospitalized patients. Ultimately, extreme caution should be urged and cost effectiveness should be taken into account

before long and supraphysiological corticosteroid doses are applied in patients with severe liver disease.

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Natural interferon-beta treatment for patients with chronic hepatitis C in Japan

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liver cirrhosis and hepatocellular carcinoma (HCC). Several studies have demonstrated that the eradication of HCV reduces the occurrence of HCC. In Japan, as many people live to an advanced age, HCV-infected patients are also getting older, and the age at HCC diagnosis has also increased. Although older HCV-infected patients have a risk of developing HCC, the treatment response to peginterferon-alpha plus ribavirin therapy is relatively poor in these patients because of drop-out or discontinuation of this treatment due to adverse events. It is established that the mechanism of action between interferon-alpha and interferon-beta is slightly different. Short-term natural interferon-beta monotherapy is effective for patients with acute hepatitis C and patients infected with HCV genotype 2 and low viral loads. Natural interferon-beta plus ribavirin for 48 wk or for 24 wk are also effective for some patients with HCV genotype 1 or HCV genotype 2. Natural interferon-beta plus ribavirin has been used for certain "difficult-to-treat" HCV-infected patients. In the era of direct-acting anti-virals, natural interferon-beta plus ribavirin may be one of the therapeutic options for special groups of HCV-infected patients. In the near future, signal transduction pathways of interferon-beta will inform further directions.

Key words: Hepatocellular carcinoma; Hepatitis C virus; Interferon-beta; Interferon resistance; Ribavirin

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Core tip: The use of natural interferon-beta plus ribavirin can eradicate hepatitis C virus (HCV) from non-responders to peginterferon-alpha plus ribavirin treatment. Some of these patients may have anti-interferon-alpha neutralizing antibodies. In Japan, natural interferon-beta plus ribavirin has been used for certain "difficult-to-treat" HCV-infected patients such as elderly patients, patients with mental disorders and patients with lower platelet counts, before the era of

Abstract

Chronic hepatitis C virus (HCV) infection can cause

interferon-free regimens. To eradicate hepatocellular carcinoma and end-stage liver diseases associated with HCV, the use of natural interferon-beta with or without ribavirin should be one of the useful treatment options for HCV-infected patients.

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CHRONIC HEPATITIS C VIRUS INFECTION IS A RISK OF HEPATOCELLULAR CARCINOMA

Chronic hepatitis C virus (HCV) infection is a major health problem, and causes liver cirrhosis and hepatocellular carcinoma (HCC)^[1-3]. Chronic HCV infection is the leading cause of HCC in southern European countries, North America and Japan^[4,5]. Despite the recent progress of treatments for HCC^[6,7], the prognosis of HCV-positive HCC patients is still poor without liver transplantation^[5,8]. It is known that the incubation period until HCC detection is usually shorter when the liver fibrosis is more advanced in patients infected with HCV^[9]. Interferon therapy can reduce the risk for HCC^[10] and improve liver fibrosis^[11] if patients with chronic hepatitis C achieve a sustained virological response (SVR) to this therapy^[12,13].

In the direct-acting antivirals (DAAs) against HCV era, peginterferon-alpha plus ribavirin therapy with or without DAAs, as well as interferon-free regimens, led to the higher SVR rates in patients infected with HCV^[14,15]. However, these treatments also had disadvantages due to specific adverse events. We should have more additional therapeutic options for chronic hepatitis C because standard of care treatments are not always available according to the condition of the patients. In this article, we discuss the recent trends in natural interferon-beta treatment for patients with chronic hepatitis C in Japan.

RECENT TRENDS IN HCV-INFECTED PATIENTS IN JAPAN

There are an estimated 1 million HCV-infected patients in Japan^[16,17]. In Japan, as many people live to an advanced age^[18], HCV-infected patients are also growing older^[16,17,19]. Peginterferon-alpha plus ribavirin may lead to a approximately 50% SVR and approximately 80% SVR, respectively, in HCV genotype 1 treated for 48-72 wk and genotype 2-infected Japanese patients treated for 24 wk^[14]. In male and female HCV genotype 1-infected patients aged ≥ 65 years and treated

with peginterferon-alpha plus ribavirin, an SVR was achieved in 54.8% and 29.5%, respectively^[18]. In male and female HCV genotype 2-infected patients aged ≥ 65 years and treated with peginterferon-alpha plus ribavirin, an SVR was achieved in 54.5% and 66.6%, respectively^[18]. However, several contraindications exist for these treatments involving peginterferon-alpha, with an increasing number of contraindication in older patients.

INTERFERON-BETA IS DIFFERENT FROM INTERFERON-ALPHA

Interferon-alpha/-beta and related molecules are classified as type I interferons, and two other types are type II (interferon-gamma) and type III (interferon-lambda)^[20]. Type I interferons signal through a ubiquitously expressed receptor composed of two chains: interferon-alpha receptor 1 and interferon-alpha receptor 2^[20]. These receptors are linked to JAK-STAT pathways to induce the expression of interferon-stimulated genes^[21,22]. The interferon response to infection is rapid and these cytokines serve as a first-line of defense against many pathogens and diseases^[23]. Whereas the murine interferon-alpha gene subtypes have approximately 90% homology, murine interferon-beta appears to be rather more divergent, with only approximately 55% homology to a murine interferon-alpha consensus sequence^[24]. It is clinically established that interferon-alpha and natural interferon-beta have different actions^[25,26]. Although these mechanisms may rely on differential signaling pathways between interferon-alpha and interferon-beta, it is possible that only interferon-beta is induced directly by viral infection and that interferon-alpha induction is a consequence of this initial interferon-beta expression^[24,27]. Of interest, in mice, interferon-alpha can be induced by interferon-beta, but interferon-beta cannot be induced by interferon-alpha^[28].

NATURAL INTERFERON-BETA FOR PATIENTS WITH ACUTE HEPATITIS C

In 1991, Omata *et al.*^[29] reported that the intravenous administration of natural interferon-beta may prevent patients with acute hepatitis C from developing chronic infection. The researchers performed a prospective controlled trial in 25 patients; 11 were treated for an average of 30 d, with a mean 52 megaunits of natural interferon-beta and 14 patients without. The follow-up at 3 years revealed that serum HCV RNA became undetectable in 10 of 11 treated subjects and in only 1 of 12 untreated controls^[29]. It was also reported that a daily intravenous injection of natural interferon-beta at a dosage of 6 million units for 8 wk eradicated HCV RNA from a 29-year-old nurse with acute hepatitis C genotype 1 caused by a needle-stick injury; she had no severe adverse events^[30]. These studies suggested

Table 1 Results of natural interferon-beta plus ribavirin for patients with chronic hepatitis C in Japan

Ref.	G	No. of patients	Naïve (%)	Age (yr, mean \pm SD)/gender (male) (%)	Formula of treatment	SVR rates (%)
Katamura <i>et al.</i> ^[37]	1	11	4 (36)	57/(64)	Natural interferon-beta plus ribavirin for 48 wk	27
	1	22	8 (36)	54/(64)	Peginterferon-alpha plus ribavirin for 48 wk	41
Arase <i>et al.</i> ^[38]	1	40	12 (30)	51.9 \pm 10.0/(70)	Natural interferon-beta plus ribavirin for 48 wk	38
Arase <i>et al.</i> ^[39]	2	24	12 (50)	55.9 \pm 10.2/(46)	Natural interferon-beta plus ribavirin for 24 wk	88
Arase <i>et al.</i> ^[42]	1	14	0 (0)	62.1 \pm 4.3/(43)	Natural interferon-beta plus ribavirin for 48 wk	38
Arase <i>et al.</i> ^[44]	1	23	11 (48)	68.1 \pm 2.6/(30)	Natural interferon-beta plus reduction-dose-ribavirin for 48 wk	39
	1	22	7 (32)	66.9 \pm 3.0/(68)	Natural interferon-beta plus standard-dose-ribavirin for 48 wk	27
Arase <i>et al.</i> ^[47]	2	33	20 (60)	70.4 \pm 3.7/(24)	Natural interferon-beta plus ribavirin for 24 wk	75
Nomura <i>et al.</i> ^[48]	1	21	21 (100)	71.8 \pm 5.1/(46)	Natural interferon-beta plus ribavirin	29
	2	18	18 (100)		Natural interferon-beta plus ribavirin	72
	1	21	21 (100)	69.1 \pm 3.5/(48)	Peginterferon-alpha plus ribavirin	29
	2	18	18 (100)		Peginterferon-alpha plus ribavirin	86

HCV: Hepatitis C virus; G: HCV genotype; SVR: Sustained virological response.

that natural interferon-beta is a therapeutic option for patients with acute hepatitis C, instead of peginterferon-alpha^[31], although there was a contrary opinion^[32].

NATURAL INTERFERON-BETA FOR PATIENTS WITH CHRONIC HEPATITIS C

Japan's national health insurance approves natural interferon-beta with or without ribavirin for treating patients with chronic hepatitis C or compensated liver cirrhosis, although the natural interferon-beta treatment requires intravenous injection at least three times weekly. It was reported that short-term treatment for 4 wk and low doses of natural interferon-beta has only a temporary effect on controlling the disease activity in patients with post-transfusion non-A, non-B chronic active hepatitis^[33]. However, it was reported that three million units of intravenous natural interferon-beta twice daily for 2 wk reduces the HCV RNA levels by 3 log IU/mL which has a stronger effect against HCV, compared with the combination of peginterferon-alpha plus ribavirin, which reduces the HCV RNA levels by 1-2 log IU/mL^[34]. Thus, it is possible that a 3-million-unit twice-daily natural interferon-beta regimen is more effective for reducing HCV RNA levels. However, the SVR rates of patients infected with HCV genotype 1b and a high viral load, who have been treated by natural interferon-beta monotherapy, are 0% to 11%^[35-37].

Natural interferon-beta plus ribavirin for patients with HCV genotype 1

Katamura *et al.*^[37] reported that treatment with natural interferon-beta plus ribavirin for 48 wk leads to an SVR in 27% (3/11) of Japanese patients infected with HCV

genotype 1b and a high viral load; the researchers also observed that during the treatment, the platelet count increased above the baseline after week 4 in this treatment group but not in the peginterferon-plus-ribavirin group (Table 1). Among the 11 with HCV G1, 7 were re-treated patients, and in 4 of 7 a transient virological response had been observed during the first cycle^[37]. By the end of the second cycle of therapy, a sustained virological response was observed in 3 cases. The study of Arase *et al.*^[38] is retrospective, and the 40 patients treated with natural interferon-beta were recruited over a period ranging from December 2004 to May 2008. They^[38] reported that treatment with natural interferon-beta plus ribavirin for 48 wk led to an SVR in 38% (15/40) of patients infected with HCV genotype 1b and a high viral load; moreover, the SVR rate was 87% (13/15) in patients who were negative for HCV RNA at 8 wk after the commencement of treatment (Table 1). One patient discontinued the treatment due to exacerbation of depression, and another patient discontinued the treatment due to a skin rash^[38].

Natural interferon-beta plus ribavirin for patients with HCV genotype 2

Arase *et al.*^[39] reported that treatment with natural interferon-beta plus ribavirin for 24 wk led to an SVR in 88% (21/24) of patients infected with HCV genotype 2 and a high viral load, and that the SVR rate was 94% (18/19) in patients who were negative for HCV RNA at 8 wk after the commencement of treatment (Table 1). No patients discontinued the treatment due to treatment-related adverse events^[39]. Arase *et al.*^[40] also reported that treatment with natural interferon-beta for only 6-8 wk led to an SVR of 56% (14/25) in cirrhotic patients infected with HCV genotype 2 and a low viral load.

Natural interferon-beta monotherapy for 6-8 wk should be sufficient to eradicate HCV in patients infected with HCV genotype 2 at a low viral load (< 5000 IU/mL).

NATURAL INTERFERON-BETA FOR "DIFFICULT-TO-TREAT" HCV-INFECTED PATIENTS

Natural interferon-beta plus ribavirin for HCV-patients previously treated by peginterferon-alpha plus ribavirin

Montalto *et al.*^[41] reported that natural interferon-beta is well tolerated and yield modest results in white patients with chronic hepatitis C who are non-responders to interferon-alpha. Natural interferon-beta administration was neither interrupted nor its dosage reduced due to side effects^[41]. We also observed a patient infected with HCV genotype 1 who failed to respond to interferon with or without ribavirin seven times but then achieved a SVR using natural interferon-beta plus ribavirin for 48 wk^[25]. Arase *et al.*^[42] observed that re-treatment with natural interferon-beta plus ribavirin for 48 wk led to an SVR in 38% (5/14) of previously treated-patients infected with HCV genotype 1b and a high viral load; moreover, the SVR rates were 100% (4/4) and 83% (5/6) in patients who were negative for HCV RNA at 12 wk and 24 wk, respectively (Table 1).

It has recently been reported that anti-interferon-alpha neutralizing antibody is associated with a non-SVR to peginterferon-alpha plus ribavirin in chronic hepatitis C patients^[43]. The same researchers observed 19 non-SVR patients who were positive for anti-interferon-alpha neutralizing antibodies and found that no anti-interferon-alpha neutralizing antibodies interfered with the antiviral activity of natural interferon-beta; furthermore, the re-treatment of patients carrying anti-interferon-alpha neutralizing antibodies with natural interferon-beta plus ribavirin led to the eradication of HCV^[43].

Natural interferon-beta plus ribavirin for elderly HCV-infected patients

Arase *et al.*^[44] reported that the SVR rate was achieved by natural interferon-beta plus reduction-dose or standard-dose ribavirin, respectively, for 48 wk in 39% (9/23) or 27% (6/22) of patients aged 65 years or older who were infected with HCV genotype 1b and a high viral load. They stressed that natural interferon-beta plus a reduction-dose is a possible treatment for patients aged 65 years or older who are infected with HCV genotype 1b and a high viral load^[44]. In this study^[44], the SVR rates were 44% (15/34) and 0% (0/11) in patients with interleukin-28B (IL28B) rs8099917TT and TG, respectively. The IL28B genotype^[18,45,46] is useful for predicting the treatment results of natural interferon-beta plus ribavirin. Arase *et al.*^[47] reported that the SVR rate was achieved by natural interferon-beta plus reduction-dose or standard-dose ribavirin, respectively, for 24 wk in 72% (13/18) or 80% (12/15) of patients aged 65 years or older who were infected with HCV genotype 2

and a high viral load; additionally, they found that natural interferon-beta plus a reduction-dose is an optional treatment for patients aged 65 years and older who were infected with HCV genotype 2 and a high viral load^[47]. Nomura *et al.*^[48] also reported that natural interferon-beta plus ribavirin therapy is safe in elderly patients and that the SVR rate is similar to that of peginterferon-alpha plus ribavirin (Table 1), although this recent study is also a retrospective, non-randomized trial. Among 66 recruited to treatment with natural interferon-beta and ribavirin, 15 were side effect-related treatment discontinuation, 36 patients were available for final analysis according to these figures, and 15 additional patients were lost during the study^[48]. However, they observed in the group of patients treated with peginterferon-alpha plus ribavirin, the rates of patients who discontinued the treatment for adverse effects is 66% (42/66). Thus, this study in elderly patients exceeds the corresponding rate of withdrawals reported in previous studies^[14].

Natural interferon-beta plus ribavirin for HCV-infected patients with mental disorders

Natural interferon-beta plus ribavirin has been available for mild mental disorders such as mild depression and interferon-induced depression in Japan^[37]. Katamura *et al.*^[37] used natural interferon-beta plus ribavirin for 48 wk or for 24 wk, respectively, in 5 HCV genotype 1b and 3 HCV genotype 2 patients with mental disorders. Of the 5 HCV genotype 1b patients, 3 and 2 had depression and interferon-induced depression, respectively, and of the 3 patients infected with HCV genotype 2, 2 and 1 had depression and interferon-induced depression, respectively. Only one of these patients withdrew from treatment due to the exacerbation of depression at week 32. Only one and all three patients with completed treatment achieved an SVR for HCV genotype 1b and genotype 2, respectively^[37]. Careful attention should also be paid if clinicians use natural interferon-beta plus ribavirin for HCV patients with mental disorders. Consultations with the attending psychiatrist may enable achieving a more successful treatment.

Natural interferon-beta plus ribavirin for HCV-infected patients with lower platelet counts

To examine the effects of natural interferon-beta on the platelet counts, the changes in platelet counts were retrospectively analyzed after beginning treatment in 16 HCV-infected patients treated with natural interferon-beta at Chiba University Hospital (Figure 1). In 3 patients with < 50000/ μ L platelets, no reduction in the platelet counts was observed. Katamura *et al.*^[37] also reported that the platelet count during the administration of natural interferon-beta plus ribavirin increased above the baseline after 4 wk. These results indicate that natural interferon-beta plus ribavirin is a therapeutic option for HCV-infected patients with lower platelet counts. But none of the studies regarding patients with chronic HCV related hepatitis are prospective controlled trials.

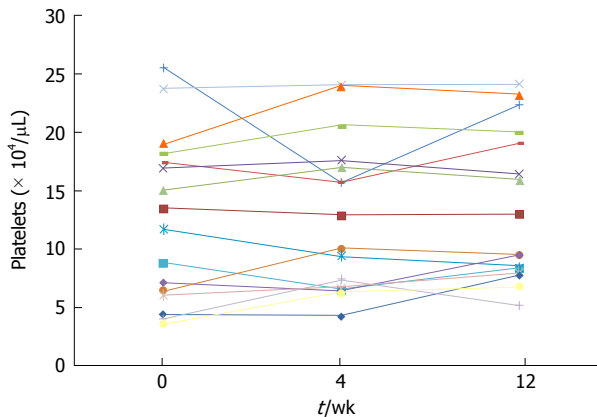


Figure 1 Changes in platelet counts after commencement of treatment with natural interferon-beta in 16 hepatitis C virus-infected patients. This study was approved by the Ethics Committee of Chiba University, School of Medicine (No. 1462).

NATURAL INTERFERON BETA AND ITS RISK FOR HCC

It is still controversy whether interferon-beta therapy can improve the prognosis for HCC^[49,50]. Combination therapy of 5-fluorouracil and interferon-alpha or peginterferon-alpha for advanced HCC still remains challenging^[51-54]. It was previously reported that interferon-beta is more potent than interferon-alpha in inhibition of human hepatoma cell growth with or without combination with anticancer drugs^[55]. Further studies will be needed at this point. In present, we should treat patients infected with HCV as soon as possible, before the occurrence of HCC^[56].

RECOMBINANT INTERFERON-BETA

Natural human interferon-beta is produced by human fibroblasts, and is currently available in Japan. Recombinant human interferon-beta-1a and interferon-beta-1b are produced in mammalian cells or *Escherichia coli*, respectively^[57]. It was reported that recombinant human interferon-beta-1a with or without ribavirin has an excellent safety profile, and after 24-wk-treatment of recombinant human interferon-beta-1a with or without ribavirin, SVR was 21.6% and 27.4% in HCV genotype 1 and genotype 2 patients, respectively^[57-60]. Peginterferon-beta-1a may be beneficial for patients infected with HCV^[61,62].

CONCLUSION

During the preparation of this manuscript, in Japan, since September 2014, interferon-free regimen with daclatasvir plus asunaprevir for 24 wk has been available for treatment of HCV genotype 1 patients who were ineligible, intolerant, or had not responded to prior interferon-based therapy^[63]. In the near future, we might be using all-oral DAAs and interferon-free regimens for

the treatment of all HCV-infected patients^[15].

In summary, natural interferon-beta with or without ribavirin is a treatment option for patients infected with HCV, such as non-responders to peginterferon-alpha plus ribavirin or patients who are unable to use DAAs. In the DAA era, candidates for using natural interferon-beta might exist among special groups, such as "difficult-to-treat" HCV-infected patients including elderly patients, patients with mental disorders and in those with low platelet counts. Because these key messages are supported by current weak data, we may reconfirm this in the further clinical practice. Future studies of the interferon- β signal transduction pathways will inform further directions.

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Antiviral therapies for chronic hepatitis C virus infection with cirrhosis

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recognized as “difficult-to-treat” patients during an era when peginterferon and ribavirin combination therapy is the standard of care. Recent guidelines have clearly stated that treatment should be prioritized in this population to prevent complications such as decompensation and hepatocellular carcinoma. Recent advances in the treatment of chronic hepatitis C have been achieved through the development of direct-acting antiviral agents (DAAs). Boceprevir and telaprevir are first-generation DAAs that inhibit the HCV NS3/4A protease. Boceprevir or telaprevir, in combination with peginterferon and ribavirin, improved the sustained virological response rates compared with peginterferon and ribavirin alone and were tolerated in patients with HCV genotype 1 infection without cirrhosis or compensated cirrhosis. However, the efficacy is lower especially in prior non-responders with or without cirrhosis. Furthermore, a high incidence of adverse events was observed in patients with advanced liver disease, including cirrhosis, in real-life settings. Current guidelines in the United States and in some European countries no longer recommend these regimens for the treatment of HCV. Next-generation DAAs include second-generation HCV NS3/4A protease inhibitors, HCV NS5A inhibitors and HCV NS5B inhibitors, which have a high efficacy and a lower toxicity. These drugs are used in interferon-free or in interferon-based regimens with or without ribavirin in combination with different classes of DAAs. Interferon-based regimens, such as simeprevir in combination with peginterferon and ribavirin, are well tolerated and are highly effective especially in treatment-naïve patients and in patients who received treatment but who relapsed. The efficacy is less pronounced in null-responders and in patients with cirrhosis. Interferon-free regimens in combination with ribavirin and/or two or more DAAs could be used for treatment-naïve, treatment-experienced and even for interferon-ineligible or interferon-intolerant patients. Some clinical trials have demonstrated promising results, and have shown that the efficacy and safety were not different between patients with and without cirrhosis. There are also promising regimens for genotypes other than genotype 1. Interferon

Abstract

Patients who are infected with hepatitis C virus (HCV) and also have advanced fibrosis or cirrhosis have been

is contraindicated in patients with decompensated cirrhosis, and further studies are needed to establish the optimal treatment regimen for this population. In the future, interferon-free and ribavirin-free regimens with high efficacy and improved safety are expected for HCV-infected patients with advanced liver diseases.

Key words: Hepatitis C virus; Hepatocellular carcinoma; Interferon-free regimen; Liver cirrhosis; Direct-acting antiviral agent

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Core tip: In general, patients with cirrhosis who are infected with hepatitis C virus (HCV) are at a higher risk for the development of hepatocellular carcinoma (HCC) compared with patients without cirrhosis. Antiviral treatments for patients with cirrhosis and HCV may reduce the occurrence of HCC and/or prevent the progression to hepatic failure. In this review, we discussed the sustained virological response (SVR) rates of interferon-containing and interferon-free regimens for these patients. Recent advances in the development of direct-acting antivirals against HCV have improved the SVR rates and have reduced the occurrence of adverse events during treatment. Interferon-free regimens might improve the prognosis of patients with cirrhosis and HCV.

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INTRODUCTION

Patients with hepatitis C virus (HCV)-infection and cirrhosis have been recognized as "difficult-to-treat" patients in the era of peginterferon and ribavirin as the standard of care. Since 2011, new direct-acting antiviral agents (DAAs) have been approved for treatment against HCV infection. Interferon-based triple therapy including telaprevir or boceprevir has been more effective than peginterferon and ribavirin alone even in patients with cirrhosis, although some safety concerns also exist.

According to the current guidelines for the management and treatment of HCV infection in the United States and in the EU^[1,2], all patients with chronic HCV infection with compensated disease have an indication for treatment. Treatment should be prioritized for patients with advanced fibrosis and cirrhosis to prevent complications such as decompensation and hepatocellular carcinoma (HCC). In the HALT-C trial, patients with advanced chronic hepatitis C who achieved sustained virological response (SVR) demonstrated a marked

reduction in death/liver transplantation, and in liver-related morbidity/mortality^[3]. Importantly, individuals with advanced liver disease also require long-term follow-up and surveillance for HCC, regardless of the treatment outcome, because HCV eradication reduces but does not abolish the risk of HCC^[1,2]. However, the treatment response is generally low in patients with advanced fibrosis. In Japan, particular care should be taken in the management of side effects in such patients, who are usually older, have other comorbidities, and have worse tolerance. Currently, little data are available for the treatment of patients with decompensated cirrhosis. Interferon is contraindicated in this population because it may worsen hepatic function. Interferon-free regimens could benefit these patients, although the data are still sparse. In this review, recent data with regards to the efficacy and safety of newly developed DAAs in patients with advanced fibrosis and compensated cirrhosis were collected and analyzed.

PEGINTERFERON/RIBAVIRIN TREATMENT IN PATIENTS WITH CIRRHOSIS

Peginterferon and ribavirin therapy had been the standard of care before the approval of protease inhibitors. The overall SVR rate is 40%-50% for individuals with genotype 1 HCV infection and is 70%-80% for individuals with genotype 2 and 3 infection in patients with chronic hepatitis. In patients with cirrhosis, the SVR rate is reported to be 22% for genotype 1 and 4 infections and 55% for genotype 2 and 3 infections in patients from 11 studies that were included in a systematic review^[4]. In a sub-analysis of 2 randomized studies that compared peginterferon alpha-2a or -2b plus ribavirin with interferon alpha plus ribavirin^[5,6], the SVR of peginterferon plus ribavirin was lower in patients with cirrhosis and a mixed HCV genotype compared with patients with no cirrhosis (43%-44% vs 57%-58%, respectively) (Figure 1). Bruno *et al*^[7] reported that the SVR of patients with genotype 1 infection who were treated with peginterferon and ribavirin therapy was negatively affected by the Ishak fibrosis score; the SVR of score 1 was 61% while that of score 6 was 7% (Figure 1)^[7]. In the analysis of 3 randomized international studies^[8], the efficacy and safety of peginterferon alfa-2a and ribavirin were compared in patients with and without advanced fibrosis. In 341 patients who were infected with genotypes 1 and 4, the SVR was higher (60%) in patients without advanced fibrosis than in those with cirrhosis (33%), while in 818 patients who were infected with genotypes 2 and 3, the SVR was 76% and 57%, respectively (Figure 1). No significant differences were observed between patients with and without advanced fibrosis with respect to the incidence of serious adverse events. However, a statistically significant difference was noted in the incidence of platelet counts less than 50000/mm³ during treatment between patients with and without advanced fibrosis or cirrhosis; this was attributed largely

Table 1 Sustained virological response rates for cirrhotic patients who were treated with direct-acting antiviral agents against hepatitis C virus including peginterferon and ribavirin

Ref. (name of trial)	Regimen; genotype; No. of patients (n)	Tx history	SVR rates	
			Cirrhosis vs Non-cirrhosis	SVR rates for P + R
Jacobson <i>et al</i> ^[10] (ADVANCE)	TVR + P + R; G1; n = 363: P + R; n = 361	-	62% vs 78% ¹	33% vs 47%
Sherman <i>et al</i> ^[11] (ILLUMINATE)	TVR + P + R; G1; n = 540	-	63% vs 75% ¹	
Zeuzem <i>et al</i> ^[12] (REALIZE)	TVR + P + R; G1; n = 530: P + R; n = 132	+	Relapse; 84%-85% vs 83%-90% ¹ PR; 40%-44% vs 70%-75% Null; 22%-28% vs 31%-50%	12% vs 38% 10% vs 18% 5% vs 6%
Poordad <i>et al</i> ^[13] (SPRINT-2)	BOC + P + R; G1; n = 734: P + R; n = 363	-	41%-52% vs 67% ¹	38% vs 38%
Bacon <i>et al</i> ^[14] (RESPOND-2)	BOC + P + R; G1; n = 299: P + R; n = 76	+, relapse or PR	35%-77% vs 64%-66%	0% vs 24%
Jacobson <i>et al</i> ^[17]	SMV + P + R; G1; n = 521: P + R; n = 264	-	60% vs 84% ^{2,3}	34% vs 55%
Manns <i>et al</i> ^[18] (QUEST1/2)	SMV + P + R; G1; n = 260: P + R; n = 133	+, relapse	74% vs 82% ^{2,3}	26% vs 41%
Forns <i>et al</i> ^[19] (PROMISE)	SMV + P + R; G1; n = 199: P + R; n = 66	+	Relapse; 73% vs 95% PR; 82% vs 79% Null; 31% vs 66%	0% vs 56% ² 0% vs 8% 0% vs 23%
Lawitz <i>et al</i> ^[25] (NEUTRINO)	SOF + P + R; G1, 4-6; n = 327	-	80% vs 92% ³	

¹Comparison between cirrhosis/bridging fibrosis vs others, or between F3, 4 vs F0-2 (METAVIR score); ²Comparison between F4 vs F0-2 (METAVIR score);

³Data for SVR12. BOC: Boceprevir; G: Genotype; P: Peginterferon; PR: Partial response; R: Ribavirin; SMV: Simeprevir; SVR: Sustained virological response; TVR: Telaprevir; Tx: Treatment; SOF: Sofosbuvir.

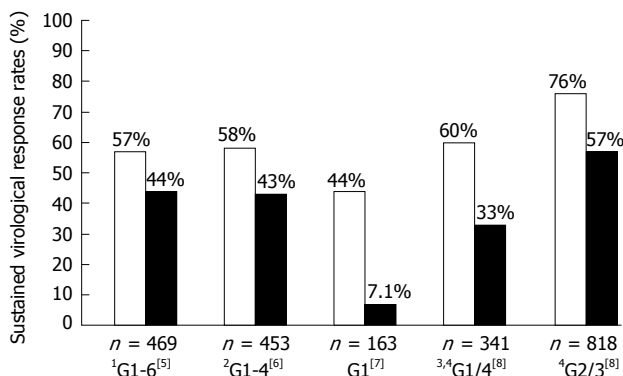


Figure 1 Sustained virological response rates for treatment-naïve patients with cirrhosis who were treated with peginterferon and ribavirin. ¹Comparison between cirrhosis/bridging fibrosis vs others; ²Comparison between an Ishak fibrosis score of 6 vs scores of 0-5; ³Data from 3 studies including the study by Fried *et al*^[6] listed above; ⁴Comparison between cirrhotic patients vs those without advanced fibrosis. White column: Non-cirrhosis (without advanced fibrosis); Black column: Cirrhosis (with advanced fibrosis). G: Genotype.

to a significantly higher incidence of thrombocytopenia in patients with cirrhosis.

FIRST-GENERATION HCV PROTEASE INHIBITORS PLUS PEGINTERFERON/RIBAVIRIN FOR HCV GENOTYPE 1 INFECTION

In 2011, the HCV NS3/4A protease inhibitors telaprevir and boceprevir, in combination with peginterferon and ribavirin were approved for the treatment of HCV. The efficacy and safety of a regimen that comprises first-generation inhibitors for cirrhosis was reviewed in detail by Bourlière *et al*^[9] In the ADVANCE study, 363 treatment-naïve patients were treated with triple therapy including telaprevir for 12 wk (Table 1). Of

these patients, 20% had bridging fibrosis or cirrhosis (METAVIR F3-4)^[10]. The SVR rate in patients with cirrhosis/bridging fibrosis was lower than in the non-cirrhotic patients (62% vs 78%). This in turn was better than the SVR rate for patients who were treated with peginterferon and ribavirin alone - 33% in cirrhotic and 47% in non-cirrhotic patients. Similar results were observed in the ILLUMINATE study^[11]. In the REALIZE study, 530 patients, including 25% of cirrhotic patients who experienced treatment failure after prior therapy, were treated with triple therapy with telaprevir^[12]. The SVR rate was high (84%-90%) in patients who experienced a relapse regardless of the presence of F3/4 fibrosis (44% of patients), while the SVR rate in partial responders or non-responders with F3/4 fibrosis was lower than in patients with F0-2 fibrosis (partial responders: 40%-44% vs 70%-75% for F3/4 and F0-2 fibrosis, respectively; non-responders: 22%-28% vs 31%-50% for F3/4 and F0-2 fibrosis, respectively). In all groups, the SVR rates for triple therapy were higher than the SVR rates for peginterferon and ribavirin regardless of the fibrosis status. Similar differences with respect to the treatment efficacy between cirrhotic and non-cirrhotic patients are observed for regimens that contain boceprevir. In the SPRINT-2 study, the SVR rate in treatment-naïve patients with F3/4 fibrosis was lower than patients with F0-2 fibrosis (41%-52% vs 67% for F3/4 and F0-2, respectively)^[13]. In patients with F3/4 fibrosis, the SVR rate of triple therapy did not differ from that of peginterferon and ribavirin. In patients who experienced a prior relapse and in partial responders (RESPOND-2 study)^[14], the SVR rates in non-cirrhotic patients were comparable or higher than those in cirrhotic patients (64%-66% vs 35%-77%, respectively); this was a better result than that of the cirrhotic patients who was treated with peginterferon and ribavirin alone (0%).

Table 2 Safety data of antiviral treatments for cirrhotic patients infected with hepatitis C virus

Ref. (name of trial)	Regimen; genotypes; No. of patients (<i>n</i>)	Patient characteristics	AE (serious AE) rate; cirrhosis vs non-cirrhosis
Kumada <i>et al</i> ^[27] (AI447026)	ASV + DCV; G1; <i>n</i> = 222	IFN-intolerant/IFN-ineligible or IFN-non-responders	(9% vs 6%)
Forns <i>et al</i> ^[19] (PROMISE)	SMV + P/RBV; G1; <i>n</i> = 260	Treatment experienced; relapse	100% vs 92%-93% (1% vs 1%) ¹
Jacobson <i>et al</i> ^[30] (POSITRON, FUSION)	SOF + RBV; G2-3; <i>n</i> = 408	IFN-ineligible/IFN-intolerant (POSITRON); IFN-failure (FUSION)	97% vs 88% (7% vs 5%)
		12-wk regimen	86% vs 91% (11% vs 2%)
		16-wk regimen	88% vs 88% (6% vs 2%)
Lawitz <i>et al</i> ^[35] (COSMOS)	SMV + SOF ± RBV for 12 or 24 wk; G1; <i>n</i> = 167	F0-2; non-responders	87% vs 88% (5% vs 0%)
		F3-4; non-responders or naïve	

¹Comparison between F3-4 and F0-2 (METAVIR score) during the first 12 wk of therapy. AE: Adverse event; ASV: Asunaprevir; DCV: Daclatasvir; G: Genotype; IFN: Interferon; P: Peginterferon; RBV: Ribavirin; SMV: Simeprevir; SOF: Sofosbuvir.

With regards to safety problems, triple therapy with telaprevir or boceprevir is associated with an increased rate of adverse events such as anemia, dysgeusia or rash compared with peginterferon and ribavirin alone. In the HEP3002 study, 1782 patients with HCV genotype 1 and bridging fibrosis or compensated cirrhosis were treated with triple therapy with telaprevir^[15]. Overall, 31% of the patients developed grade 3-4 anemia, 4% developed grade 3-4 rash, and 12% discontinued telaprevir due to adverse events. Seven patients (0.4%) died, including 6 patients with cirrhosis. The authors concluded that in patients with compensated cirrhosis and advanced liver fibrosis due to HCV genotype 1 who fulfilled the selection criteria of the registration trials, 16 wk of telaprevir triple therapy proved to be safe and well tolerated. However, the results in a real-life setting in France showed that triple therapy in treatment-experienced patients with cirrhosis was related to a high incidence (40%) of serious adverse events and of severe complications and death (6%), especially in patients with a low platelet count and a low serum albumin level^[16]. The authors concluded that patients with cirrhosis require a careful follow-up during treatment due to the increase in side effects that are more common during treatment than in clinical studies^[9].

SECOND-GENERATION HCV PROTEASE INHIBITOR PLUS PEGINTERFERON AND RIBAVIRIN

Simeprevir is a once-daily macrocyclic protease inhibitor that was initially approved in 2013 in the United States. In the QUEST-1/2 study^[17,18], 521 treatment-naïve patients infected with genotype 1 were treated with simeprevir plus peginterferon and ribavirin (Table 1). Of these patients, 9% had cirrhosis. Again, cirrhotic (F4) patients had a lower chance of a SVR than non-cirrhotic (F0-2) patients (60% vs 84%), which was still higher than the SVR rate for those who were treated with peginterferon and ribavirin (34% vs 55%, respectively). In the PROMISE and ASPIRE studies that included

treatment-experienced patients^[19,20], the SVR rate of the simeprevir-containing regimen in patients with cirrhosis was comparable to or lower than that in non-cirrhotic patients - 73%-74% vs 82%-95% for patients who experienced relapse, 82% vs 79% for partial responders, and 31% vs 66% for null responders. The SVR rate for patients with cirrhosis who were treated with triple therapy with simeprevir was greatly improved compared to that in patients with cirrhosis who were treated with peginterferon and ribavirin alone (0%). In the ATAIN study, which compared simeprevir with telaprevir, each in combination with peginterferon and ribavirin, similar SVR rates were observed, although the incidence of adverse events was lower in the simeprevir group than in the telaprevir group^[21].

In clinical trials, adverse events that occur with simeprevir treatment were similar to those with peginterferon and ribavirin alone. In the PROMISE study^[19], adverse events were reported in most patients regardless of fibrosis stage (100% for F3-4 vs 92%-93% for F0-2; Table 2). The incidence of serious adverse events was low in both groups (1% vs 1%). Hepatic impairment is associated with substantial increases in exposure to simeprevir, which is also related to the increased frequency of adverse events; plasma exposure to simeprevir is 2- to 5-fold higher in HCV-uninfected subjects with Child-Pugh B or C cirrhosis than in those with normal hepatic function^[22]. In the AASLD guideline, simeprevir-based treatment is not recommended for patients with decompensated cirrhosis (moderate or severe hepatic impairment; Child Pugh class B or C)^[1].

HCV NS5B POLYMERASE INHIBITOR SOFOSBUVIR PLUS PEGINTERFERON AND RIBAVIRIN

Sofosbuvir is a nucleotide analog HCV NS5B polymerase inhibitor that was approved in 2013 in the United States^[23]. A pharmacokinetic analysis in subjects who were treated with sofosbuvir for 7 d indicated that systemic exposure was approximately 2-fold higher in cirrhotic patients with moderate and severe hepatic

impairment (Child-Pugh B and C) than in non-cirrhotic patients, with minimal change in the primary systemic inactive metabolite GS331007^[24]. The viral decline with sofosbuvir in subjects with moderate (Child-Pugh B) to severe (Child-Pugh C) hepatic impairment was less profound than in non-cirrhotic patients.

In a phase 2 trial, the efficacy of sofosbuvir plus peginterferon alfa-2a and ribavirin were compared to peginterferon and ribavirin in non-cirrhotic, treatment-naïve patients infected with genotype 1 HCV^[25]. The SVR rate at 12 wk (SVR12) in the sofosbuvir-containing arms were higher than in the peginterferon and ribavirin arm (90%-91% vs 58% for sofosbuvir and peginterferon and ribavirin, respectively). In phase 3, 327 treatment-naïve patients (mainly genotype 1, 89%) were treated with sofosbuvir plus peginterferon and ribavirin for 12 wk (NEUTRINO study)^[26]. Of these patients, 17% had cirrhosis. The SVR12 rate in the non-cirrhotic patients was higher than in the cirrhotic patients (92% vs 80%, odds ratio 3). Adverse events were similar regardless of the presence of sofosbuvir. In patients with cirrhosis, only 1 of 54 discontinued the treatment with triple therapy that included sofosbuvir.

It was concluded by the manufacturer that cirrhosis had no clinically relevant effect on the exposure to sofosbuvir and that no dose adjustment was required for patients with mild, moderate or severe hepatic impairment (Child-Pugh A, B or C)^[23]. However, efficacy seems somewhat worse in cirrhotic than in non-cirrhotic patients. Importantly, safety and efficacy have not been established in patients with decompensated cirrhosis. Decompensated cirrhosis has been considered a contraindication to interferon therapy.

INTERFERON-FREE REGIMENS IN CIRRHOTIC PATIENTS

The second-generation HCV NS3/4A protease inhibitor asunaprevir in combination with the HCV NS5A inhibitor daclatasvir was approved in Japan in 2014 for patients infected with HCV genotype 1b including patients who were null-responders to prior treatment, and those who were ineligible or intolerant of interferon. In a phase 3 trial, a total of 222 patients with genotype 1b HCV were treated with this regimen for 24 wk (AI447026, Table 3)^[27]. Of these patients, 10% had cirrhosis. A SVR was achieved by 87% of interferon-ineligible or interferon-intolerant patients and by 81% of previous non-responders. A subgroup analysis indicated that the SVR rates in patients with cirrhosis and in those without cirrhosis were comparable (91% vs 84%).

In the HALLMARK-DUAL study (Table 3), 203 treatment-naïve, 205 interferon-non-responder, and 235 interferon-ineligible or interferon-intolerant patients infected with HCV genotype 1b were treated with this regimen^[28]. Of these patients, 16%, 31% and 47% of treatment-naïve patients, interferon non-responders and interferon-ineligible/interferon-intolerant patients

had cirrhosis, respectively. Overall, the SVR rate of the treatment-naïve group was slightly higher than that of the interferon-non-responder or interferon-ineligible/interferon-intolerant group (90% vs 82%). The SVR rates were similar in patients with (84%) and without cirrhosis (85%), irrespective of the patient group. The SVR rate in patients with baseline platelet counts between 50000/mm³ and less than 90000/mm³ was high (71%), but was slightly lower than that in patients without thrombocytopenia (86%). The most commonly observed adverse events were headache, fatigue, diarrhea, nausea, and asthenia. Serious adverse events that occurred during treatment were reported in 39 patients (6%), and similar incidences were reported across the different patient groups. Adverse events that led to the discontinuation of treatment occurred in 10 (2%) patients, and were mostly associated with higher transaminase levels (7 patients). Patients with and without cirrhosis had similar frequencies of alanine transaminase (1% vs 3%) and aspartate transaminase (1% vs 2%) that were increases greater than five times the upper limit of normal. In the AI447026 study, serious adverse events were observed in 9% of cirrhotic and 6% of non-cirrhotic patients (Table 3). Exposure to asunaprevir is 10- to 30-fold higher in HCV-infected subjects with moderate and severe hepatic impairment (Child-Pugh B and C) compared with those with normal hepatic function. Asunaprevir-containing regimen is not recommended in patients with decompensated cirrhosis.

SOFOSBUVIR-CONTAINING REGIMENS

Sofosbuvir and ribavirin

In a phase 2 trial, Osinusi *et al.*^[29] reported the efficacy of sofosbuvir plus ribavirin treatment for 24 wk in treatment-naïve patients who were infected with genotype 1 HCV^[29] (Table 3). The SVR rate of the 37 patients with F0-2 fibrosis was 65%, while that of the 13 patients with F3-4 fibrosis was 38%. Advanced liver disease was associated with treatment relapse. In contrast, in patients who were infected with genotype 2 or 3 HCV, sofosbuvir and ribavirin therapy was shown to be highly effective. In the FISSION study, treatment-naïve patients who were infected with genotype 2 or 3 HCV were treated with this regimen for 12 wk. In 73 patients with genotype 2 infections who were treated with this regimen, the SVR rate was higher than in the patients who were treated with peginterferon plus ribavirin (95% vs 78%)^[26]. Liver cirrhosis was present in 16% of the patients in the sofosbuvir arm, and 83% of them achieved a SVR.

In patients with genotype 3 HCV infections, the results of the 12-wk regimen was comparable to those after peginterferon and ribavirin treatment for 24 wk; however, the SVR rate was lower than in the patients with genotype 2 HCV infections. A total of 183 patients were treated with sofosbuvir and ribavirin. Of these patients, 16% had cirrhosis. The SVR rate in cirrhosis

Table 3 Sustained virological response rates for cirrhotic patients who were treated with interferon-free regimens

Ref. (name of trial)	Regimen; genotypes; No. of patients (n)	Patient characteristics	SVR rates: cirrhosis vs non-cirrhosis
Kumada <i>et al</i> ^[27] (AI447026)	ASV + DCV; G1; n = 222	IFN-intolerant/IFN-ineligible	91% vs 87%
Manns <i>et al</i> ^[28] (HALLMARK-DUAL)	ASV + DCV; G1; n = 645	IFN-non-response ¹	91% vs 79%
		Treatment naïve	91% vs 89% ³
		IFN-non-response ¹	87% vs 80%
Lawitz <i>et al</i> ^[32] (LONESTAR)	SOF + LDV ± RBV; G1; n = 40	IFN-intolerant/IFN-ineligible	79% vs 84%
		Treatment experienced ²	91% vs 100% ^{3,4}
			100% vs 100% ⁵
Osinusi <i>et al</i> ^[29]	SOF + RBV; G1; n = 50	Treatment naïve	38% vs 65% ⁶
Lawitz <i>et al</i> ^[26] (FISSION)	SOF + RBV; G2-3; n = 256	Treatment naïve; 12-wk regimen	G2; 83% vs 97% ³
			G3; 34% vs 61% ³
Jacobson <i>et al</i> ^[30] (POSITRON, FUSION)	SOF + RBV; G2-3; n = 408	IFN-ineligible/IFN-intolerant (POSITRON); 12-wk regimen	G2; 94% vs 92% ³
		IFN-failure ⁷ (FUSION)	G3; 21% vs 68% ³
		12-wk regimen	G2; 60% vs 96% (90%)
			G3; 19% vs 37%
		16-wk regimen	G2; 78% vs 100% (92%)
			G3; 61% vs 63%
Zeuzem <i>et al</i> ^[31] (VALENCE)	SOF + RBV; G2-3; n = 323	Treatment-naïve	
		12-wk regimen	G2; 100% vs 97% ³
		24-wk regimen	G3; 92% vs 93% ³
		Treatment-experienced	
		12-wk regimen	G2; 88% vs 91%
		24-wk regimen	G3; 60% vs 85%
Lawitz <i>et al</i> ^[35] (COSMOS)	SMV + SOF ± RBV for 12 or 24 wk; G1; n = 167	F0-2; non-responders	F0-2; 90% ³
		F3-4; non-responders or naïve	F3-4; 94%
Poordad <i>et al</i> ^[36] (TURQUOISE-II)	Paritaprevir, ritonavir, ombitasvir + dasabuvir + RBV 12 or 24 wk; G1; n = 380	Treatment-naïve	94% ^{3,8}
		Treatment-experienced	
		Relapse	98%
		Partial response	96%
		Null response	91%

¹Null or partial response during previous treatment; ²Patients experienced triple therapy including telaprevir or boceprevir; ³Data for SVR12; ⁴SVR rates for ribavirin-containing regimens; ⁵SVR rates for regimens without ribavirin; ⁶Comparison between F3-4 and F0-1 (Knodel score); ⁷Relapse, null or partial response to previous treatment; ⁸All patients included in the study had cirrhosis. ASV: Asunaprevir; DCV: Daclatasvir; G: Genotype; IFN: Interferon; LDV: Ledipasvir; RBV: Ribavirin; SOF: Sofosbuvir; SVR: Sustained virological response; SMV: Simeprevir.

was 34% while that in non-cirrhosis was 61%. For the peginterferon and ribavirin arm, the SVR rate in patients with cirrhosis was 30% and that in patients without cirrhosis was 71%.

In the POSITRON study, interferon-ineligible or interferon-intolerant patients were treated with sofosbuvir and ribavirin for 12 wk^[30]. The result was similar to that of the FISSION study. In patients infected with genotype 2 HCV, the SVR rates were high in both the cirrhotic and non-cirrhotic patients (94% and 92%, respectively). In genotype 3 HCV-infected patients, only 21% of cirrhotic patients and 68% of non-cirrhotic patients achieved a SVR.

In the FUSION study, patients who did not achieve a SVR after prior therapy were treated with sofosbuvir and ribavirin for 12 or 16 wk^[30]. In patients infected with genotype 2 HCV, the SVR rate was 82% for the 12-wk arm and 89% for the 16-wk arm. In an analysis of the small fraction of cirrhotic patients, 6 out of 10 (60%) patients in the 12-wk arm and 7 out of 9 (78%) patients in the 16-wk arm achieved a SVR. In patients infected with genotype 3 HCV, the SVR rate in the 12-wk arm was low in both cirrhotic and non-cirrhotic patients (19% and 37%, respectively), while that in the 16-wk arm was 61% and 63% in cirrhotic and non-

cirrhotic patients, respectively.

In the VALENCE study, patients infected with genotype 2 HCV were treated with sofosbuvir and ribavirin for 12 wk while patients infected with genotype 3 HCV were treated for 24 wk^[31]. Of the treatment-naïve patients, more than 90% achieved a SVR among the 32 genotype 2- and 105 genotype 3-infected patients. Two of the 2 genotype 2-infected patients with cirrhosis and 12 of 13 genotype 3-infected patients with cirrhosis achieved a SVR. In treatment-experienced patients, 90% of 41 patients with genotype 2 infections and 77% of 145 patients with genotype 3 infections achieved a SVR. In genotype 2-infected patients, 7 of 8 (87%) cirrhotic patients achieved a SVR. In genotype 3-infected patients, 27 of 45 (60%) cirrhotic patients achieved a SVR.

The most common adverse events ($\geq 20\%$) that were observed after sofosbuvir plus ribavirin combination therapy were fatigue and headache. The discontinuation of treatment due to adverse events was uncommon - one patient in the FUSION study and 2% in the POSITRON study^[30]. In the POSITRON study, the incidences of adverse events and laboratory abnormalities among patients with cirrhosis who received sofosbuvir and ribavirin were similar to those among patients without

cirrhosis (Table 2). In the FUSION study, treatment-emergent serious adverse events were slightly higher in cirrhotic patients than in non-cirrhotic patients (11% vs 2% for 12-wk regimen and 6% vs 2% for 16-wk regimen, respectively).

Sofosbuvir plus ledipasvir with or without ribavirin

In a phase 2 trial referred to as the "LONESTAR" study (Table 3), the efficacy of a fixed-dose combination of sofosbuvir (400 mg) and the HCV NS5A inhibitor ledipasvir (90 mg), with and without ribavirin, was examined in patients with genotype 1 infection who were treatment-naïve ($n = 60$) or who were previously treated with a protease-inhibitor regimen ($n = 40$)^[32]. Among them, 22 of the treatment-experienced patients had cirrhosis. The results showed that more than 90% of patients achieved a SVR irrespective of their treatment history or the presence of compensated cirrhosis. In the phase 3 ION-1 study^[33], 865 genotype 1-infected treatment-naïve patients including 16% of patients with cirrhosis, were treated with this regimen. More than 90% of the patients achieved a SVR regardless of the presence of cirrhosis, inclusion of ribavirin, or treatment duration (12 wk or 24 wk). The ION-2 study consisted of 440 treatment-experienced patients who were infected with genotype 1 HCV, 20% of whom had cirrhosis^[34]. In non-cirrhotic patients, the SVR rate was higher than 90% irrespective of the treatment duration and the inclusion of ribavirin. In cirrhotic patients, the SVR rate for the 12-wk regimen was 82%-86%, which was lower than that for the 24-wk regimen (95%-100%). A multivariate logistic regression analysis identified cirrhosis as the only factor associated with treatment response. The most commonly observed adverse events included fatigue, headache, nausea, and insomnia. Serious adverse events were observed in 2%-3% of patients who were treated with a 12-wk regimen and in 3%-8% who were treated with a 24-wk regimen.

Sofosbuvir plus simeprevir with or without ribavirin

In the COSMOS study^[35], the sofosbuvir and simeprevir combination therapy with or without ribavirin for 12 or 24 wk was tested in 81 previous non-responders with F0-2 fibrosis and in 87 treatment-naïve or previous non-responders with F3-4 fibrosis (Table 3). The SVR12 rate was high regardless of the fibrosis stage - 87% for patients with F0-1, 91% for patients with F2, 96% for patients with F3, and 93% for patients with F4 fibrosis. The most commonly observed adverse events were fatigue, headache, and nausea. Four patients (5%) in the 24-wk group discontinued treatment due to adverse events while no patients in the 12-wk group discontinued treatment. Serious adverse events that occurred during treatment were observed in 4 patients (5%) with F3-4 fibrosis and in 0 patients with F0-2 fibrosis. All 4 patients were treated with the 24-wk regimen, and 1 died. All serious adverse events and death were deemed unrelated to the treatment.

PARITAPREVR, RITONAVIR, OMBITASVIR AND DASABUVIR WITH RIBAVIRIN FOR CIRRHOTIC PATIENTS

In the TURQUOISE-II trial (Table 3), the interferon-free combination of the following drugs was studied: the HCV protease inhibitor paritaprevir (ABT-450), the human immunodeficiency virus protease inhibitor ritonavir, which was used as pharmacologic booster, the HCV NS5A inhibitor ombitasvir (ABT-267), the non-nucleoside HCV polymerase inhibitor dasabuvir (ABT-333), and ribavirin. This combination therapy was studied in 160 previously untreated and 220 previously treated adults with HCV genotype 1 infection and compensated cirrhosis (Child A, METAVIR score > 3 or Ishak score > 4)^[36]. Overall, the SVR12 rate was 92% for the 12-wk regimen and 96% for the 24-wk regimen. The results were superior to the historical control rate of 47% (95%CI: 41%-54%), calculated from the telaprevir-based regimen and weighted to reflect the population. Among the patients who were infected with genotype 1a HCV and who were prior null responders, 80% of the patients in the 12-wk group achieved a SVR. A multivariate logistic-regression analysis showed that a prior null response and genotype 1a infection were independently associated with a lower likelihood of SVR. The most common adverse events were fatigue, headache and nausea. Serious adverse events occurred in 5%-6% of the patients. Seven to 10% of the patients had hemoglobin levels of less than 10 g/dL. Overall, 2% of patients discontinued the treatment due to adverse events. The pharmacokinetics of each drug in HCV-uninfected subjects with hepatic impairment are complex. Among them, paritaprevir exposure is 1.6- to 10-fold higher in patients with moderate to severe hepatic impairment (Child-Pugh B, C) compared with patients with normal hepatic function. Therefore, paritaprevir-containing combination therapy is not recommended in these patients^[37].

CONCLUSION

The EASL guidelines recommend an interferon-free regimen over an interferon-containing regimen in patients with compensated cirrhosis, while the AASLD guidelines recommend that treatment-naïve patients with compensated cirrhosis should receive the same treatment as that given to patients without cirrhosis^[1,2]. In patients with advanced liver fibrosis or cirrhosis, the treatment should be prioritized and should not be delayed to prevent disease progression. The initially approved DAAs telaprevir and boceprevir are currently not recommended for the treatment of HCV due to the higher rate of adverse events associated with these drugs^[1,2,38]. Currently, second-stream DAAs including second-generation HCV protease inhibitors such as simeprevir and asunaprevir, the HCV NS5B inhibitor

sofosbuvir, and the HCV NS5A inhibitor daclatasvir have been approved; in addition, various combination regimens that include interferon and ribavirin have been developed. These DAAs-containing regimens improved the treatment efficacy in patients with both early and advanced liver disease. Furthermore, some regimens showed comparable efficacies and safety profiles between patients with and without cirrhosis. Interferon is contraindicated in patients with decompensated cirrhosis, and further studies are needed to establish optimal treatments for this population. In the future, interferon-free and ribavirin-free regimens with high efficacy and improved safety are expected for patients with advanced liver disease^[39].

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Management of recurrent hepatocellular carcinoma after liver transplant

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Abstract

Hepatocellular carcinoma (HCC) is the leading cause of deaths in patients with hepatitis B or C, and its incidence has increased considerably over the past decade and is still on the rise. Liver transplantation (LT) provides the best chance of cure for patients with HCC and liver cirrhosis. With the implementation of the MELD exception system for patients with HCC waitlisted for LT, the number of recipients of LT is increasing, so is the number of patients who have recurrence of HCC after LT. Treatments for intrahepatic recurrence after transplantation and after other kinds of surgery are more or less the same, but long-term cure of posttransplant recurrence is rarely seen as it is a "systemic" disease. Nonetheless, surgical

resection has been shown to be effective in prolonging patient survival despite the technical difficulty in resecting graft livers. Besides surgical resection, different kinds of treatment are also in use, including transarterial chemoembolization, radiofrequency ablation, high-intensity focused ultrasound ablation, and stereotactic body radiation therapy. Targeted therapy and modulation of immunosuppressants are also adopted to treat the deadly disease.

Key words: Hepatocellular carcinoma; Recurrence; Transarterial chemoembolization; Liver transplantation; Targeted therapy; Resection; Radiofrequency ablation; Transarterial radioembolization; Immunosuppression; Stereotactic body radiation therapy

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Core tip: The management of recurrent hepatocellular carcinoma (HCC) after liver transplantation (LT) seems to be a losing battle. Nonetheless, tremendous efforts have been made to combat this deadly disease. Intrahepatic recurrence may be treated by resection, which has some survival benefits as shown by small clinical trials. Other kinds of therapy including high-intensity focused ultrasound (HIFU) ablation, radiofrequency ablation (RFA) and transarterial chemoembolization (TACE) are also in use. HIFU ablation has been shown to produce better results when compared with RFA and TACE. The efficacy of systemic and targeted therapies for multiple recurrences is under investigation. Early results have suggested that the combination of sorafenib with mammalian target of rapamycin inhibitors may be useful for treating recurrent HCC after LT.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor, the third leading cause of cancer-related deaths, and the first leading cause of deaths in patients with hepatitis B or C, and its incidence has increased considerably over the past decade and is still on the rise^[1-3]. There are different modalities for treating HCC and underlying liver cirrhosis, but liver transplantation (LT) is the ultimate solution^[4]. Various patient selection criteria for LT have been introduced with the hope that as many patients as possible can benefit from the treatment while patient survival is not compromised. Mazzaferro *et al*^[5] introduced the Milan criteria (solitary tumor ≤ 5 cm, or ≤ 3 tumors with each measuring < 3 cm) on the basis of a retrospective study of 48 patients who received LT for HCC. In the study, a 75% overall survival and an 83% recurrence-free survival were achieved in LT recipients chosen according to the Milan criteria at 4 years after transplantation. A set of modestly expanded criteria was developed by the University of California, San Francisco (UCSF). Yao *et al*^[6] showed that HCC patients selected for LT according to the UCSF criteria (solitary tumor ≤ 6.5 cm, or ≤ 3 nodules with the largest lesion ≤ 4.5 cm and a total tumor diameter ≤ 8 cm) had survival rates of 90% and 75.2% at 1 year and 5 years respectively. However, discrepancy between radiological results and pathological results of tumor characteristics is not uncommon. A 30%-50% discrepancy rate has been reported^[6,7].

In Hong Kong, about 8% of the population are carriers of hepatitis B virus (HBV) and most of the cases of HCC are caused by HBV. A survey found that about 10.4% of male adults and 7.7% of female adults were positive of hepatitis B surface antigen (surveillance of viral hepatitis in Hong Kong - 2010 update report. Hong Kong SAR: Department of Health, 2011). On the other hand, the numbers of carriers of hepatitis C virus (HCV) are rising in Japan and the United States. In these places where hepatitis C is epidemic, there is a surge of HCV-related liver cirrhosis and HCC^[8,9].

Even though HCC patients are selected for LT according to standard criteria, 10%-60% of them will have disease recurrence. Some of them will develop recurrence 2 years or even 5 years after transplantation^[10]. With the adoption of the MELD exception system for HCC patients waitlisted for LT, more LTs are performed for HCC. Hong Kong adopted the system in 2009^[11], and nowadays HCC accounts for one third of LTs in Hong Kong. As a corollary, the incidence of HCC recurrence after LT is on the increase in places where the system is adopted. Recurrence of HCC after LT is notoriously difficult to manage. Here is a review of the treatment options available for this challenging situation, trying to shed some light on its management.

RISK FACTORS FOR HCC RECURRENCE

Post-LT HCC recurrence occurs at a rate of 13%-27%^[10,12].

It was reported that 5% of patients developed late (after 5 years) recurrence^[10]. Most patient selection criteria for LT, including the Milan and the UCSF criteria, use tumor size and tumor number as surrogate markers. A meta-analysis by Sotiropoulos *et al*^[13] identified a number of risk factors for poorer patient survival after LT, which were venous invasion, poor tumor cell differentiation, tumor size and stage beyond the Milan criteria, and a high pretransplant serum α -fetoprotein level. Since radiological results and pathological results of tumor characteristics may differ, some centers use pretransplant serum α -fetoprotein level and biopsy to determine tumor cell differentiation and use it as a biological surrogate marker in patient selection criteria^[14,15]. However, preoperative biopsy may cause tumor seeding and bleeding. Saborido *et al*^[16] reported that a significantly higher chance of HCC recurrence came with fine-needle aspiration biopsy before LT (31.8% vs 5.9%, $P = 0.003$). In Hong Kong, contrast computed tomography (CT)^[17] is used for tumor staging. Sometimes positron emission tomography (PET) using both radiotracers of ^{11}C -acetate and ^{18}F -FDG is also employed. In a report, dual-tracer PET had an overall sensitivity of 96.8% and an overall specificity of 91.7%, which are significantly higher than those of contrast CT (41.9% and 33.0% respectively; $P < 0.05$ in both cases)^[18]. It was found that sources of error for contrast CT were related to liver cirrhosis or previous treatment, and there was difficulty in differentiating cirrhotic nodules from HCCs (39%) and in the estimation of tumor size (14%). There was infrequent overstaging of vascular invasion (4.6%) or extrahepatic metastasis (4.6%). Dual-tracer PET and contrast CT had a 4.7% rate of false-negative results. PET using the radiotracer ^{18}F -FDG seems effective in detecting ^{18}F -FDG-avid lesions and thus can be used as an adjunct to detect microvascular invasion^[19]. Nonetheless, such use is still at its infancy and more large-scale trials are needed for its validation.

Deceased-donor LT vs living-donor LT

Living-donor LT (LDLT) has the most significant impact in Asia, where the issue of organ shortage is most extreme. The availability of LDLT has provided the driving force for a drastic increase in cases of LT in recent years. The number of LDLTs performed in Asia each year has increased tremendously. In 2005, LDLT accounted for 90% of the 1497 LTs performed in Asia (excluding mainland China)^[20]. In Hong Kong, about half of the LTs are LDLTs, and more than half are for HCC.

To justify LDLT for HCC, it should have a survival outcome comparable to that of deceased-donor LT (DDLT). Roayaie *et al*^[21] reported a tendency for early tumor recurrence after LDLT (mean: 8.7 mo) when compared with DDLT (mean: 19.6 mo) in a cohort of 311 patients with histologically confirmed HCC after LT. Another multicenter LDLT cohort study (A2ALL) of 106 HCC patients reported a significantly higher 3-year tumor recurrence rate after LDLT (29%) compared with that after DDLT (0%)^[22]. In Hong Kong, a retrospective study

has been conducted to compare LDLT and DDLT in terms of treatment outcomes in 60 HCC patients^[23]. Given the standard patient selection criteria based on radiological tumor size and number according to the UCSF criteria, there was an obvious selection bias for some important clinical characteristics in the LDLT group. Patients having LDLT for HCC had fewer incidental tumors, a lower rate of preoperative transarterial chemoembolization (TACE), a lower rate of salvage transplantation (with pretransplant resection or ablation), shorter waiting time on list, and a lower graft-weight-to-standard-liver-weight ratio. The inferior oncological outcomes in the LDLT group were possibly caused by more aggressive tumor behavior and small-for-size graft injury and regeneration^[24]. Although the overall survival rates were comparable between the LDLT and DDLT groups, the cumulative 5-year HCC recurrence rate was significantly higher in LDLT group (29% vs 0%). Thus, selection of patients with early HCC based on standard tumor size and number for LDLT and DDLT may eventually result in different clinical outcomes. When considering a patient for an LDLT, besides a certain set of patient selection criteria, there are more factors to be taken into account, which include the unique nature of a living-donor graft as a dedicated gift to the recipient and potential donor risks, and additional clinical characteristics should also be considered and good preoperative counseling should be given to the donor and patient. In Hong Kong, the policy of "6-mo-wait" before salvage transplantation does not apply to LDLT, since both donors and recipients willingly accept the relatively higher recurrence rate with the realization that LDLT is their only option.

TREATMENTS FOR HCC RECURRENCE

Theoretically, all modalities for treating HCC can be used to treat its recurrence. Aggressive treatments can usually be given to patients who have satisfactory liver function and no widespread tumor cell dissemination. However, HCC recurrence after LT is considered a "systemic disease", and the efficacy of locoregional treatment for a systemic disease is doubtful. For LT recipients, the use of immunosuppressants may hinder wound healing and thus lead to a higher chance of infective complications. Variable vascular anatomy in a graft liver or dense adhesion at the hilum may cause damage to important structures during dissection. Difficulties may be encountered in interventional radiological procedures like TACE when the catheter is negotiating through the arterial anastomosis. The use of targeted agents for post-LT HCC recurrence has not been validated by any large randomized trials and it may have adverse effects on immunocompromised patients. A multidisciplinary approach with the involvement of hepatologists, surgeons, radiologists, oncologists and radiation oncologists is definitely for the best interest of this group of patients.

Liver resection and local ablative therapy for intrahepatic recurrence

Catalano *et al*^[25] reported the initial results of graft liver resection for graft ischemic damage in 12 patients. The perioperative mortality rate was high at 66.6%, manifesting the difficulty of graft liver resection in the presence of sepsis. On the other hand, Sommacale *et al*^[26] reported that graft liver resection for intrahepatic recurrence achieved a low mortality rate and satisfactory long-term survival with a median follow-up of 92 mo. Nonetheless, there were only 3 patients in the series. According to unpublished data from the only LT center in Hong Kong, in 252 patients who underwent LT for HCC, 35 had disease recurrence. Three patients had only intrahepatic recurrence and underwent aggressive resection. This very small series had a 66.7% 3-year survival and 0% mortality. Actually, all reported series were small and the studies had a retrospective nature with significant selection biases. Hence, more evidence is needed to support graft liver resection as a good treatment for HCC recurrence.

Radiofrequency ablation (RFA), a local ablative treatment, is the established treatment option for resectable and unresectable HCCs. Its efficacy has been shown to be comparable to that of partial liver resection in treating small HCCs^[27]. It would be reasonable to extrapolate that RFA can be an option for treating post-LT intrahepatic recurrence of HCC too. A case report showed that percutaneous RFA achieved 2-year disease-free survival in a 65-year-old patient who had a solitary recurrent HCC inside the graft liver^[28].

Stereotactic body radiation therapy and intra-arterial infusion of yttrium-90 microspheres for intrahepatic recurrence

Numerous advances in external-beam radiation therapy have allowed more accurate targeting and made aggressive dose-fractionation strategies possible with techniques such as stereotactic body radiation therapy (SBRT). As a kind of radiosurgery, SBRT was originally developed to treat intracranial malignancies. It has since been adopted to treat extracranial diseases. The use of SBRT as treatment of HCC has yet to be established, but it is tested by a number of clinical trials for its efficacy in treating unresectable and unablatable HCCs. Initial results showed that it achieved a local control rate of 87%-100%^[17,29-31].

Intra-arterial infusion of yttrium-90 microspheres (Y-90 SIR) is an established treatment for unresectable HCCs^[32] and has gained popularity in recent years. It is often used to treat advanced HCC, especially in patients with a large tumor burden, suboptimal performance status, or lobar portal vein thrombosis^[33]. Chan *et al*^[34] reported that in the treatment of primary HCC, it achieved a 38%-65% partial response rate and a median survival duration of 23 mo, which is 2.6-4.7 times the duration seen in historic controls. In a recent study of 20 patients with unresectable HCCs, it achieved

an overall survival rate of 90% at a median follow-up period of 275 d (range: 32-677 d)^[33]. However, the data on the use of SBRT and intra-arterial infusion of Y-90 SIR for recurrent HCC after LT are extremely scarce. In the only two case reports, complete tumor necrosis was observed in a 52-year-old and a 42-year-old patient with solitary intrahepatic recurrence of HCC after a course of SBRT and intra-arterial infusion of Y-90 SIR respectively^[35,36].

TACE for intrahepatic recurrence

TACE is often used as a bridging therapy for waitlisted patients and its results are satisfactory. Lo *et al*^[37] reported that it resulted in marked tumor response, and the actuarial survival was significantly better in the TACE group (1 year: 57%, 2 years: 31%, 3 years: 26%) compared with the control group (1 year: 32%, 2 years: 11%, 3 years: 3%, $P = 0.002$). When adjustments for baseline variables that were prognostic on univariate analysis were made with a multivariate Cox model, the survival benefit of TACE remained significant (relative risk of death: 0.49; 95%CI: 0.29-0.81; $P = 0.006$).

Chok *et al*^[38] compared TACE and RFA for unresectable HCCs and found that they were comparable in terms of time to disease progression ($P = 0.95$) and overall survival ($P = 0.02$).

Successful outcomes of TACE therapy (with and without the use of iodized oil) for the treatment of recurrent intrahepatic HCC after LT have been reported^[39,40] although the studies were small and retrospective in nature. As said before, the transcatheter procedure can be technically demanding in the presence of distorted vasculature in a post-LT setting.

New therapy for intrahepatic recurrence

High-intensity focused ultrasound (HIFU) ablation is a relatively new totally extracorporeal treatment for unresectable HCCs. Ng *et al*^[41] in their initial research reported that it achieved a primary effective treatment rate of 79.5% and 1-year and 3-year overall survival rates of 87.7% and 62.4% respectively.

Cheung *et al*^[42] compared HIFU ablation with TACE and reported that HIFU ablation achieved rates of complete tumor response, partial tumor response, stable disease and progressive disease (in accordance with the modified Response Evaluation Criteria in Solid Tumors) of 50%, 7.7%, 25.6% and 7.7% respectively. As with TACE, the corresponding rates were 0%, 21.2%, 63.5% and 15.4% respectively ($P < 0.0001$). The 1-year, 3-year and 5-year survival rates achieved by HIFU ablation were 84.6%, 49.2% and 32.3% respectively, and those by TACE were 69.2%, 29.8% and 2.3% respectively ($P = 0.001$).

Chan *et al*^[43] compared HIFU ablation with RFA in terms of survival. The two kinds of ablative treatment produced similar results. The 1-year, 2-year, and 3-year disease-free survival rates were 37.0%, 25.9% and 18.5% respectively in the HIFU group, and 48.6%,

32.1% and 26.5% respectively in the RFA group ($P = 0.61$). The 1-year, 2-year, and 3-year overall survival rates were 96.3%, 81.5% and 69.8% respectively in the former, and 92.1%, 76.1% and 64.2% respectively in the latter ($P = 0.19$).

In the pilot study on HIFU ablation as a bridging therapy for HCC patients waitlisted for LT conducted at the only LT center in Hong Kong, it was found that with the availability of HIFU ablation, the rate of receiving bridging therapy increased dramatically from 39.2% to 80.4%. HIFU ablation and TACE achieved similar percentages of tumor necrosis as seen in excised livers ($P = 0.353$), and both treatments resulted in significantly higher necrosis rates than that in the best medical treatment group ($P = 0.010$ and 0.020)^[44]. As HIFU ablation has been shown to be a useful bridging therapy, it should have great potential in the management of recurrent HCC after LT.

Treatment for multiple recurrence

Mammalian target of rapamycin (mTOR) inhibitors have been shown to have a direct antitumorigenic effect and to be able to inhibit cell growth^[45-47]. In experimental models of HCC, the mTOR pathway was aberrantly activated in up to half of the cases. Although the currently available data came from retrospective studies and are premature, there is the hope that mTOR-based immunosuppressive therapy after LT will one day come into use^[48]. The use of sorafenib, an inhibitor of multiple tyrosine kinases (including c-Raf and b-Raf), has been approved as a first-line treatment for advanced HCC^[49]. Activation of the Ras/mitogen-activated protein kinase pathway is a common finding in neoplastic processes (including in HCC) and is a determinant for promoting cell proliferation and the survival of tumor cells. This makes sorafenib an interesting drug; its use as a treatment for unresectable HCCs and as an adjuvant treatment before and after HCC recurrence is being investigated^[50]. A study from Spain demonstrated that combination therapy resulted in an overall response (in accordance with the Response Evaluation Criteria in Solid Tumors) rate of 3.8% (1/26), and there was sustained stabilization of disease in 13 additional cases (50.0%)^[42]. The median overall survival was 19.3 mo (95%CI: 13.4-25.1 mo), and the median time to progression was 6.77 mo (95%CI: 2.3-11.1 mo). Although a few studies have shown that there is some evidence of synergistic anticancer activity, early-phase clinical studies of mTOR inhibitors plus sorafenib for advanced HCC reported ambivalent findings, which were the results of increased toxicity (e.g., hand-foot syndrome) in combination therapy^[51,52]. In a recent study from Italy, the outcomes of sorafenib treatment for post-LT HCC recurrence were significantly better than those of best medical care [median patient survival from recurrence: 21.3 mo vs 11.8 mo, hazard ratio (HR) = 5.2, $P = 0.0009$; median patient survival from untreatable presentation or progression: 10.6 mo vs 2.2 mo, HR = 21.1, $P <$

0.0001]. The only factor associated with survival found by multivariate analysis was treatment with sorafenib (HR = 4.0, $P = 0.0325$). No severe adverse event was registered^[53]. Individualized treatment should be tailor-made for individual recipients, and input from oncologists would be of great value. However, drug toxicity is a major concern as shown in many studies, and their recommendations should not be overlooked.

Use of different immunosuppressants

It has been suggested that immunosuppressive therapy should switch from using non-mTOR inhibitors to using mTOR inhibitors. Another suggestion is that mTOR inhibitors can be used as an add-on. Monaco^[54] found that the use of mTOR inhibitors might decrease the incidence of new malignancy after transplantation, mainly skin cancer.

A clinical trial by Alamo *et al*^[55] comparing calcineurin inhibitors with everolimus and sirolimus for patients who received LT for oncological disease reported that the HCC recurrence rate was significantly lower and survival significantly prolonged in patients receiving either everolimus or sirolimus. A meta-analysis by Liang *et al*^[56] endorsed the safety and efficacy of sirolimus-based immunosuppression for patients who received LT for HCC. Pooled results of the five studies eligible for evaluation showed that sirolimus-based regimens prolonged overall survival (OR = 2.47; 95%CI: 1.72-3.55) and decreased tumor recurrence (OR = 0.42; 95%CI: 0.21-0.83), with no significant differences in acute rejection and hepatic artery thrombosis.

A United States study compared sirolimus-based maintenance therapy with calcineurin inhibitor treatment for recipients of LT for HCC and found that overall survival was better in the sirolimus arm^[57]. Clinical trials examining the anticancer effects of mTOR inhibitors in recipients of LT for HCC have shown encouraging results^[58]. On multivariate analysis in a large Canadian trial, sirolimus-based maintenance therapy was one of the factors associated with improved survival after LT for HCC (HR = 0.53, 95%CI: 0.31-0.92, $P \leq 0.05$)^[59].

The reported results of using these relatively new agents has suggested that they may prevent or reduce the incidence of HCC recurrence after LT, but a definite answer from large randomized controlled trials is still lacking.

CONCLUSION

Recurrence of HCC after LT is a deadly disease. Although there are a variety of treatment approaches, long-term cure is rarely seen. One of the reasons is that the disease is "systemic" in most of the cases, even if the recurrence is intrahepatic only. Effective adjuvant or systemic therapy has yet to be identified. A multidisciplinary approach with fine-tuning of treatment goals and objectives will definitely be beneficial, and development of new drugs or modification of current systemic agents is urgently needed.

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Basic Study

Normal liver stiffness: A study in living donors with normal liver histology

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Author contributions: Alsebaey A performed the research; Allam N wrote the paper; Alswat K contributed research tools and contributed to the writing of the paper; Waked I supervised the study.

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Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest: Imam Waked is a speaker for Hoffman La Roche, MSD, BMS, GSK, Bayer, Gilead, and Minapharm, has sat on advisory boards of Janssen, Hoffman La Roche, MSD, and GSK, and has acted as investigator in clinical trials for Hoffman La Roche, BMS, GSK, Bayer, and Minapharm. All authors declare no conflicts of interest related to this work.

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Abstract

AIM: To define the normal range of liver stiffness (LS) values using transient elastography in living-related liver transplantation candidate donors with normal liver histology.

METHODS: LS was measured using Fibroscan in 50 (16 women, 34 men) healthy potential donors (mean age 28.4 ± 5.9 years) who were being evaluated for liver donation for their relatives at the National Liver Institute, Menoufeya University, Egypt. All potential donors had normal liver tests and were negative for hepatitis B or C virus infection. Abdominal ultrasounds showed normal findings. None of the subjects had diabetes, hypertension, renal impairment, heart disease, or body mass index $> 30 \text{ kg/m}^2$. All subjects had normal liver histology upon liver biopsy. They all donated the right lobe of their liver with successful outcomes.

RESULTS: The mean LS was $4.3 \pm 1.2 \text{ kPa}$ (range: $1.8\text{--}7.1 \text{ kPa}$). The 5th and 95th percentiles of normal LS were 2.6 kPa and 6.8 kPa , respectively, with a median of 4 kPa ; the interquartile range was 0.6 ± 0.4 . LS measurements were not significantly different between men and women ($4.4 \pm 1.1 \text{ kPa}$ vs $3.9 \pm 1.3 \text{ kPa}$) and did not correlate with age. However, stiffness values were significantly lower in subjects with a body mass index $< 26 \text{ kg/m}^2$ compared to those with an index $\geq 26 \text{ kg/m}^2$ ($4.0 \pm 1.1 \text{ kPa}$ vs $4.6 \pm 1.2 \text{ kPa}$; $P < 0.05$). There were no differences in hospital stay or postoperative bilirubin, albumin, alanine and aspartate transaminases, or creatinine levels (at discharge) between donors with liver stiffness $\leq 4 \text{ kPa}$ and those with stiffness $> 4 \text{ kPa}$.

CONCLUSION: Healthy donors with normal liver histology have a median LS of 4 kPa . Stiffness values are elevated relative to increase in body mass index.

Key words: Fibroscan; Liver stiffness; Living donors;

Normal liver histology; Transient elastography

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Core tip: Although some studies have measured liver stiffness by transient elastography in healthy populations, few reports evaluate these with respect to liver biopsy results. This study adds to the knowledge of liver stiffness values in clinically and histologically normal livers of an Arab population, which may form the basis for future clinical practice. The results of this study suggest a new normal level of liver stiffness for this particular population, which differs from other populations reported in the literature.

Alsebaey A, Allam N, Alswat K, Waked I. Normal liver stiffness: A study in living donors with normal liver histology. *World J Hepatol* 2015; 7(8): 1149-1153 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i8/1149.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i8.1149>

INTRODUCTION

Liver stiffness (LS) measurement (LSM) is a noninvasive method for the evaluation of liver fibrosis, and is used in clinical practice for the diagnosis and follow-up of liver diseases^[1,2]. As liver fibrosis may develop slowly in subjects showing persistently normal liver tests, identifying subjects with normal liver histology without fibrosis or undiagnosed histologic changes is of paramount importance in defining the true normal range of LS values. However, most studies to date have focused on LSM in patients with different chronic liver diseases^[3-11]. A few European studies have addressed LSM in apparently healthy subjects, though these did not correlate LS with liver histology^[12-14]. Hence, the primary aim of this study was to define the normal range of LS values using transient elastography in individuals with normal liver histology as determined by liver biopsy during evaluation as candidate donors for living-related liver transplantation. Furthermore, LS values are examined with respect to age, gender, and body mass index (BMI).

MATERIALS AND METHODS

Subjects

This study involved candidate donors for living-related liver transplantation who passed all stages of evaluation for liver donation for their relatives at the National Liver Institute, Menoufeya University during the period from June 2012 to January 2014. They all had normal liver blood tests and blood pictures, were negative for autoimmune markers and hepatitis B and C virus infection, and had normal abdominal imaging studies. None of the subjects had diabetes, hypertension, renal

impairment, heart disease, or BMI > 30 kg/m². Only the LS of the subjects who had normal histology on liver biopsy were included in analyses.

All subjects provided signed informed consent prior to study enrollment. This study was approved by the Institutional Review Board of the National Liver Institute, Menoufeya University (in 2012), Egypt, and conformed to the ethical guidelines of the 1975 declaration of Helsinki.

LSM

LS was measured by transient elastography using a FibroScan machine device (EchoSens, Paris, France) according to a previously described method^[15]. The procedure was performed in the morning before obtaining a liver biopsy. The physician performing the procedure was blinded to clinical and biochemical data. The median value of ten successful measurements was recorded as the representative LS value, and is representative of the elastic modulus of the liver^[15]. The success rate was calculated as the number of valid measurements divided by the total number of measurements. The interquartile range (IQR) was defined as an index of the intrinsic variability of LSM, corresponding to the interval of LSM results containing 50% of the valid measurements between the 25th and 75th percentiles. The results were considered unreliable if fewer than ten valid readings were obtained, success rate was < 60%, or IQR/LS value was > 30%. LSM failure was recorded when no value was obtained after ten measurements.

Statistical analysis

Continuous data were compared using the Student's *t*-test and categorical data were compared using the Fisher's exact test. The Mann-Whitney *U* test was used to compare non-parametric variables. A Pearson's test was used for correlational analysis. All two-sided *P* < 0.05 were considered significant. Statistical analyses were performed using SPSS version 17 for Windows (SPSS Inc., Chicago IL, United States). Data are presented as mean ± SD.

RESULTS

A total of 128 healthy subjects underwent liver biopsy for evaluation as potential liver donors for their relatives. Subjects excluded from donation due to histologic changes (*n* = 20) or with minimal histologic changes (*n* = 58) that did not prevent donation were not included in this analysis. Fifty individuals between 19 and 42 years of age were finally included in the study. The baseline characteristics of the fifty recruited subjects are shown in Table 1.

LSM was performed with a 100% success rate. IQR was 0.6 ± 0.4. LS values ranged from 1.8 kPa to 7.1 kPa (Figure 1), with a mean of 4.3 ± 1.2 kPa. The 5th and 95th percentiles of LS were 2.6 kPa and 6.8 kPa, respectively, with a median of 4 kPa. There was no

Table 1 Baseline characteristics of the enrolled donors (*n* = 50)

Characteristic	Value
Age (yr)	28.4 ± 5.9 (range: 19-42)
Sex (male/female)	34/16
BMI (kg/m ²)	25.9 ± 2.8 (range: 18-30)
Total bilirubin (mg/dL)	0.6 ± 0.3
ALT (U/L)	16.8 ± 7.2
AST (U/L)	18.8 ± 3.9
Albumin (g/dL)	4.7 ± 0.3
Alkaline phosphatase (U/L)	76.5 ± 17.7

Data are presented as mean ± SD unless otherwise indicated. ALT: Alanine transaminase; AST: Aspartate transaminase; BMI: Body mass index.

Table 2 Donor characteristics according to liver stiffness

Characteristic	Stiffness < 4 kPa (<i>n</i> = 21)	Stiffness ≥ 4 kPa (<i>n</i> = 29)	<i>P</i> -value
Sex (male/female)	14/7	21/8	0.58
Age (yr)	28.70 ± 6.38	28.24 ± 5.81	0.79
BMI	25.00 ± 3.34	26.42 ± 2.26	0.11
Hospital stay (d)	10.00 ± 2.89	10.50 ± 4.40	0.80
Bilirubin (mg/dL)	0.42 ± 0.39	0.57 ± 0.46	0.60
Albumin (g/dL)	3.68 ± 0.36	3.74 ± 0.22	0.75
AST (U/L)	37.25 ± 27.28	39.6 ± 28.99	0.90
ALT (U/L)	57.25 ± 39.43	55.6 ± 48.29	0.96
Creatinine (mg/dL)	0.79 ± 0.12	0.67 ± 0.09	0.05
INR	1.09 ± 0.14	1.04 ± 0.02	0.42

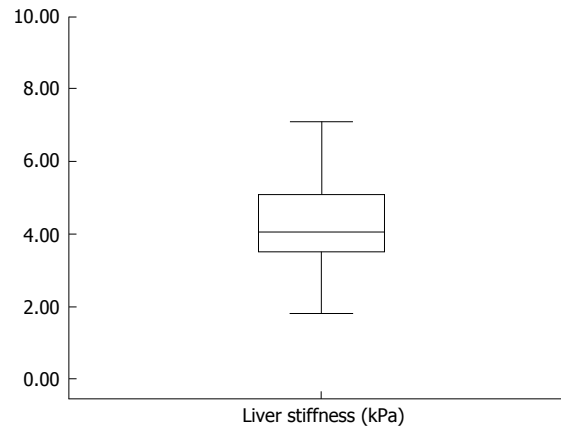
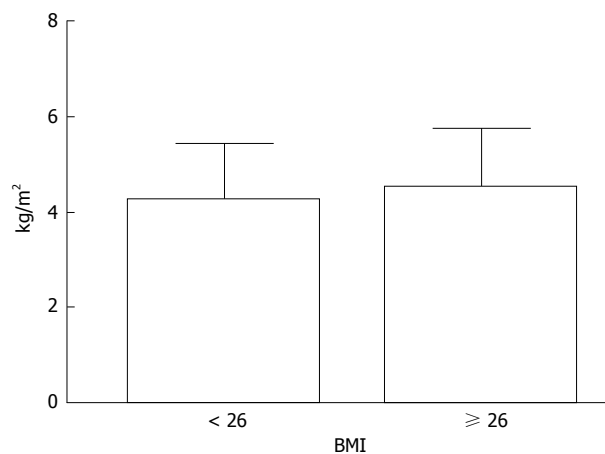
Data are presented as mean ± SD unless otherwise indicated. ALT: Alanine transaminase; AST: Aspartate transaminase; BMI: Body mass index; INR: International normalized ratio.

significant difference in LS between men and women (4.4 ± 1.1 kPa vs 3.9 ± 1.3 kPa). Moreover, LS did not correlate with age. Stiffness values were significantly lower in subjects with BMI < 26 kg/m² than those with BMI ≥ 26 kg/m² (4 ± 1.07 kPa vs 4.6 ± 1.2 kPa; *P* < 0.05) (Figure 2).

The donors donated their right liver lobes. The duration of their hospital stay and postoperative bilirubin, albumin, alanine and aspartate transaminase levels, and creatinine results (on discharge) were recorded. Using the median LS value to divide the donors into two groups, there were no significant differences found in any of these measures (Table 2).

DISCUSSION

The possibility of using LSM as a screening tool for liver disease in the general population has been raised^[16], but true normal LS values have not been well-identified, especially among various populations. Using the 5th and 95th percentiles from a non-obese population, the present study tentatively estimates a healthy liver stiffness range of 2.6 kPa to 6.8 kPa, with a median stiffness of 4 kPa within an Egyptian population. This is lower than that established by Roulot *et al*^[13] (3.3-7.8 kPa in women and 3.8-8.0 kPa in men). However, in their study, patients with potential liver disease were


Figure 1 Boxplot diagram of the liver stiffness measures of the potential donors.

Figure 2 Stiffness values in donors with body mass index < or ≥ 26 kg/m². BMI: Body mass index.

excluded based only on clinical and laboratory data, and no imaging studies or biopsies were performed. Furthermore, there may have been a selection bias, as their study recruited participants from a free health check, and subjects may have had symptoms that triggered their participation. In contrast, a wider range (2.3-8.8 kPa) was reported in another study conducted in 144 normal Romanian subjects^[17]. However, that study comprised a large proportion (about 60%) of subjects that did not receive any laboratory testing or imaging studies, thus their definition of normal was less stringent.

In the present study, normal subjects were selected based on a healthy liver histology, without evidence of fatty liver or fibrosis. Similarly, Kim *et al*^[18] conducted LSM in 12 biopsied healthy donors and reported a lower range of 3.9 kPa to 5.3 kPa. However, their study was in an East-Asian population, with 84.8% of the subjects having a BMI < 25 kg/m². The present study includes a large proportion (46%) of individuals with a BMI of 27-30 kg/m², and shows that LS is higher in individuals with a BMI > 26 kg/m². Importantly, the biopsies did not reveal steatosis, which may influence LSM. Hence,

the potential mechanism for the high LS values in healthy subjects with a higher BMI (without histologic changes of steatosis) remains speculative. The increase of LS with BMI was also reported in the study by Roulot *et al.*^[13] and Wong *et al.*^[19], with higher LS values in subjects with BMI > 30 kg/m².

Some studies observed higher LS values in healthy men than in women^[12,13]. However, consistent with reports by Kim *et al.*^[18] and Fung *et al.*^[20], this study shows no significant sex effect. However, the lack of significance may be due to the small sample size. There are intrinsic differences between men and women in the density of the extracellular matrix of the liver^[21-23], and normal ranges need to be established for each sex in larger studies using the same stringent selection utilized in the present study.

In the current study, age had no significant impact on the LS value. However, the age range is narrow (19-42 years), as older persons are seldom accepted as living liver donors. Sirli *et al.*^[17] also found no difference in LS with age within a wider age group (18-69 years), which is consistent with results from other studies in France, South Korea, and India^[12,18,24]. On the other hand, a study in a Chinese population demonstrated a decline in median LS in the older age group, from 4.2 kPa in those < 25 years of age to 3.4 kPa for those > 55 years^[25].

Although racial differences have not been reported, it is speculated that different cutoff values for normal ranges are needed for various populations^[16]. The distribution of body fat varies with race^[26-30], and this may affect rates of successful LSM acquisition. This has implications for the normal values used in areas of high ethnic diversity. All previous studies that included biopsies (and reported lower LS values) were performed in the FarEast; Fung *et al.*^[20] reported a median LS of 4.6 kPa (all < 7.2 kPa), and Kim *et al.*^[18] reported values all < 5.3 kPa. Consequently, the present study is important because it suggests a new normal level of LS for an Arab population, and provides further evidence that normal LS values should be defined for various populations.

Despite having a small sample size, the present study has considerable strengths. The subjects were living-related liver transplantation donors who were extensively evaluated clinically, chemically, radiologically, and histologically, making this the largest reported cohort of histologically normal livers. The healthy condition of the livers in our subjects was further confirmed intraoperatively during and postdonation. Another important aspect to consider is the large range of LS values obtained in studies that did not rely on histology to define normal liver; studies that include liver histology show a narrower range (< 7.2 kPa)^[18,20]. A stiff liver is rarely found in the absence of any pathology. Hence, transient elastography may be used to screen the general population and to identify those that require further evaluation. The LS threshold requires further investigation and should take into account the population demographics as well as the likely prevalence of the

condition to be screened for.

COMMENTS

Background

Liver stiffness (LS) measurement (LSM) is a noninvasive method for the evaluation of liver fibrosis, and is used in clinical practice for the diagnosis and follow-up of liver diseases. Identification of the true normal LS value is an important prerequisite for widespread application of LSM.

Research frontiers

Although some studies have investigated LS as measured by transient elastography in healthy populations, few have correlated these values with results of liver biopsy in normal individuals. Therefore, the stiffness of livers with normal histology needs further assessment.

Innovations and breakthroughs

This study adds to the knowledge of LS values in a clinically and histologically normal liver population, which may form the basis for future clinical practice.

Applications

Transient elastography may be used in screening the general population and subsequent selection of sub-populations that require further evaluation.

Peer-review

This article presents LS values from healthy livers that were evaluated for living-related liver transplantation in an Arab population. The results are useful in establishing the normal range of LS values in a specific population, which can be used as a reference for further clinical applications.

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